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Effect of Krüppel-Like Factor 3 on Glucose Metabolism

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A thesis submitted in fulfillment of the requirements for the degree of
Masters of Science

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Declaration

The work presented in this thesis is the result of my own research, except where otherwise acknowledged. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

Thilini Jayasinghe  28.03.2013

Thilini Jayasinghe  Date
Dedication

This thesis is dedicated to my ever loving parents MV Jayasinghe and Thilaka Jayasinghe who always believe in me and have given me the opportunity to get great education.
Abstract

Type 2 diabetes is an adult-onset condition in which the body is not able to tightly regulate the amount of glucose in the blood and it results from a combination of causes including genetic, behavioral and environmental factors.

Krüppel-like factor 3 (KLF3) is a zinc finger transcription factor encoded by the klf3 gene. It has three Krüppel-like zinc fingers that bind to DNA. KLF3 inhibits adipogenesis in vitro through its ability to inhibit an adipogenic master regulator, C/EBPα expression (Sue et al., 2008). klf3−/− mice have fewer and smaller fat cells in adipose tissue. Reasons for different phenotype of the klf3−/− mice than the in vitro results could be prevention of expansion of adipocyte progenitors by premature expression of C/EBPα, likelihood of indirect processes influencing fat pad size in vivo (Sue et al., 2008) and less food intake of klf3−/− mice. klf3−/− mice have increased fasting blood glucose level (Unpublished data, Bell-Anderson Lab).

This variation in fasting blood glucose level suggests a role for KLF3 in glucose metabolism. Therefore, this study was designed to investigate the mechanism underlying high fasting blood glucose levels observed in klf3−/− mice.

Mice were sacrificed at 13 weeks of age at 5pm in a naturally fasted state and 9pm in a fed state. Blood was collected and blood glucose and plasma glucagon levels were measured. Liver and red quadriceps muscles were collected in order to measure glycogen and glucose-6-phosphatase (G6Pase) activity.

Body weight of klf3−/− mice was significantly lighter than that of klf3+/+ mice (p=0.003). We observed that klf3−/− mice had significantly higher fasting blood glucose level than klf3+/+ mice (9.8mmol/L ± 0.89 vs 8.7mmol/L ± 0.71 respectively) (p<0.05). klf3+/+ mice had significantly higher liver glycogen level than KLF3 deficient mice in the fed state (233.47µmol/g ± 54.48 vs 189.68µmol/g ± 38.33 respectively) (p=0.027). There was no significant difference in muscle glycogen levels in klf3−/− and klf3+/+ mice in both fed and fasted states. klf3−/− mice had significantly higher G6Pase activity compared to klf3+/+ mice in the fed state (p=0.008). In the fasted state and fed state, klf3−/− mice had more or less similar G6Pase activities which were respectively 14.1 and 14.9 µmol/g/10 minutes. There was no significant difference in plasma glucagon levels of klf3−/− mice and klf3+/+ mice in the fasted state. Insulin levels unchanged in KLF3 knockout and wild type mice in fed state but KLF3 knockout mice had significantly higher insulin level in fasted state (185.6µU/mL ± 57.5 vs
112.4μU/mL ± 35.7 (p=0.004). Food intake data showed that KLF3 knockout mice eat more food in the afternoon in which we considered as a fasted state. Therefore, the higher fasting blood glucose levels observed in klf3−/− mice is not a result of changes in glycogen levels, G6Pase activity, plasma glucagon and could be due to higher food intake and increased insulin level. Results showed that KLF3 could potentially have an impact on glucose metabolism in the fed and fasted state but further analysis on other glucose metabolic pathways like gluconeogenesis should be performed to confirm the underlying mechanisms for increased fasting blood glucose level of klf3−/− mice.

Key words: Krüppel-Like Factor 3, glucose metabolism, adipogenesis, diabetes mellitus
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1. Introduction

Type 2 diabetes is an adult-onset condition in which the body is not able to tightly regulate the amount of glucose in the blood. This develops as a result of insulin resistance, a lack of response to the hormone, insulin, at the cellular level. It is known that obesity is invariably associated with insulin resistance. Type 2 diabetes results from a combination of causes and contributing factors including genetics, behavioral and environmental factors. Much human research is being conducted on type 2 diabetes mellitus and associated disorders to address clinical questions, test therapeutic interventions and prevention, but are limited in contributing to our understanding of the molecular mechanisms underlying insulin resistance and obesity. The use of animal models allows investigation of the involvement of specific genes in fat formation and related processes. This can be used to identify targets for prevention or treatment of obesity, and insulin resistance and diabetes mellitus.

Krüppel-like factor 3 (KLF3), also known as basic Krüppel-like Factor (BKLF), is a member of the Krüppel-like factor family of transcription factors. It is a zinc finger protein encoded by the klf3 gene. The protein contains three Krüppel-like zinc fingers that bind to DNA (McConnell and Yang, 2010). KLF3 is a transcriptional repressor that associates with the cofactor C-terminal binding protein (CtBP) (Pearson et al., 2008). Initially KLF3 was thought to function as a transcriptional activator, but was later revealed to be a strong repressor (Turner and Crossley, 1988). The tissue distribution of KLF3 in the adult mouse includes adipose tissue, liver, lung, muscle, brain and hematopoietic tissue. Sue et al. (2008) demonstrated that KLF3 knockout mice have fewer and smaller fat cells in adipose tissue. Furthermore, they showed that KLF3 and CtBP play a role in the control of adipogenesis (Sue et al., 2008). Levels of KLF3 are higher in preadipocytes and reduced upon differentiation into adipocytes (Sue et al., 2008). Over expression of KLF3 in vitro blocks adipocyte differentiation partly through KLF3’s ability to inhibit an adipogenic master regulator, C/EBPα expression (Sue et al., 2008). Mouse embryonic fibroblasts that lack KLF3 are more prone to differentiate into adipocytes, suggesting that KLF3 inhibits adipogenesis in vivo (Sue et al., 2008).

Thus, one might expect that in the absence of this inhibitor of differentiation, we would see increased adipocyte formation and increased white adipose tissue (WAT) mass in KLF3 knockout mice. However KLF3 knockout mice are lean and smaller in size compared
to their wild type litter mates. Sue et al. (2008) showed that KLF3 knockout mice have reduced WAT mass, despite elevated levels of C/EBPα, C/EBPβ, C/EBPδ and PPARγ.

Sue et al. (2008) has proposed two possible explanations for this unexpected observation that the KLF3 knockout mouse has less fat, whereas in culture KLF3 appears to have an inhibitory role and KLF3 knockout fibroblasts differentiate into adipocytes more readily than normal fibroblasts.

C/EBPα is known to be antimitotic (Turner and Crossley, 1998); thus, it is possible that in culture in vitro its derepression and premature accumulation result in an increase in lipid-accumulating cells, but in mice in vivo the premature expression of this antimitotic factor prevents expansion of adipocyte progenitors and results in a smaller fat pad (Sue et al., 2008). It is also likely that indirect processes influence fat pad size in vivo. KLF3 is broadly expressed, and there may be subtle defects in many tissues that affect physiology and/or behavior (Sue et al., 2008). Moreover, results of Bell-Anderson lab suggest that the knockouts may feed less, so this could well partly explain the phenotype in vivo. Although there are likely to be multiple factors influencing the ultimate in vivo phenotype, the results from 3T3-L1 cells, taken together with the altered adipogenesis in KLF3 knockout fibroblasts in culture, suggest that KLF3 does play a direct role in adipogenesis and that derepression of C/ebpα is one contribution to the phenotype observed in the mice (Sue et al., 2008).

KLF3 deficient mice also show some variations in glucose metabolic parameters suggesting a role for KLF3 in glucose metabolism. KLF3 could potentially be targeted for treatments of insulin resistance and type 2 diabetes.
1.1 Aims of the project

Main objective

• To investigate the mechanism underlying high fasting blood glucose levels in KLF3 deficient mice.

Specific objectives

• To assess whether there are any alterations in glucose storage capacity in KLF3 knockout mice.

• To examine whether there is any change in the activity of liver glucose 6 phosphatase, a rate limiting enzyme in the endogenous production of glucose.

• To assess changes in levels of hormones involved in glucose homeostasis.
2. Literature Review

2.1 The Krüppel-Like Factor (KLF) family

Krüppel-Like factors (KLFs) are transcription factors (McConnell and Yang, 2010) that contain three characteristic zinc fingers near their C terminus (Turner and Crossley, 1999 and Suske et al., 2005). KLFs have very similar zinc finger domains to Sp1 and its family members (Kaczynski et al., 2003 and Suzuki et al., 2005) and may therefore be classified as part of the Sp1/KLF family (McConnell and Yang, 2010). Seventeen KLFs have been identified to date (Suzuki et al., 2005 and McConnell and Yang, 2010).

2.1.1 Structure of KLFs

All members of the KLF family have three highly conserved zinc finger motifs at the carboxy-terminal end of the protein (McConnell and Yang, 2010). Zinc finger domains are common motifs in transcription factors. The most commonly seen zinc finger motif is the C2H2 type (Suzuki et al., 2005), consisting of a zinc atom tetrahedrally coordinated by two conserved cysteine and histidine residues that allow the domain to fold into a ββα structure (Brayer and Segal, 2008). The domains responsible for activation/repression are found at the N-terminus of the molecule. Zinc finger location within KLF protein structures are shown in Figure 01.

The zinc fingers in KLF proteins are linked by a “TGERP”-like motif, which assist in binding to target DNA. (Moreover, the zinc fingers are connected by the characteristic Krüppel link, a seven amino acid spacer TGERP) (Dang et al., 2000). The first and second zinc fingers contain 25 amino acids, and the third contains 23 amino acids. Each zinc finger recognizes three base pairs in the DNA sequence and interacts with nine base pairs in total (Nagai et al., 2009).

![Figure 01: KLF molecule structure.](Source: Pearson et al., 2007).
DNA binding sites are similar among the KLF proteins and they include GC-rich sequences with a preference for the 5’-CACCC-3’ core motif, which is present in the β-globin gene promoter recognized by KLF1 (Miller and Bieker, 1993). KLF proteins exhibit homology in their carboxy-terminal zinc finger domains that allow KLFs to bind GC-rich sites in promoter and enhancer regions of the genes they regulate. Because of these structural similarities, their transcriptional targets can be overlapped. But KLF proteins have unique amino terminal sequences for interaction with specific binding partners (McConnell and Yang, 2010).

**Figure 02:** Protein structure of human KLF family members.

(Source: McConnell and Yang, 2010).
2.1.2 Groups of KLF family proteins

KLF proteins are grouped according to common structural and functional domains. KLFs are highly homologous in their carboxy-terminal DNA-binding regions, which contain three C2H2 zinc finger motifs, but can differ significantly in their N-terminal sequence. The KLF family members are grouped based on the following:

1) the ability to bind acetylases (KLFs 1, 2, 4, 5, 6, and 7).
2) the presence of a CtBP-binding site (KLFs 3, 8, and 12).
3) the presence of a Sin3A-binding site (KLFs 9, 10, 11, 13, 14, and 16). Established sites of acetylation are marked by stars (Figure 02).

**Group 1**
This group includes KLFs 3, 8, and 12 (McConnell and Yang, 2010). They serve as transcriptional repressors through their interaction with the carboxy-terminal binding protein (CtBP).

**Group 2**
Family members in group include KLFs 1, 2, 4, 5, 6 and 7) function predominantly as transcriptional activators (McConnell and Yang, 2010).

**Group 3**
KLFs 9, 10, 11, 13 and 14 are in this group. They have repressor activity through their interaction with the common transcriptional corepressor Sin3A (McConnell and Yang, 2010).

2.1.3 Tissue expression of KLFs

Tissue expression of the KLFs varies. Some family members are expressed in all places in the body (KLF6, 10 and 11) whilst other KLFs are expressed in specific tissues (McConnell and Yang, 2010). For example, KLF1 is expressed primarily in erythroid cells whilst KLF2 is highly expressed in lung. KLFs 4 and 5 are very plentiful in the gastrointestinal tract (Pearson et al., 2008). At the same time, KLFs can regulate the expression of each other. For instance, KLF3 expression is regulated by KLF1 in erythroid cells (Funnell et al., 2007).
2.1.4 Functions of KLFs

The amino-terminal regions of KLFs vary significantly and allow them to bind different coactivators, corepressors, and modifiers, resulting in functional diversity and specificity. Fundamentally, KLFs regulate proliferation, differentiation, development, and programmed cell death (McConnell and Yang, 2010). Furthermore, KLFs have roles in the physiology and pathophysiology of many organ systems including cardiovascular, respiratory, digestive, hematological, and immune systems (McConnell and Yang, 2010).

New biological and pathobiological roles of these KLFs are still being discovered. Structural homologies of KLFs correlate with functional similarities.

a. Cell based functions of KLFs

I. Proliferation

Many KLFs are involved in proliferation. KLF4 and 5 are well-characterized examples (McConnell and Yang, 2010) and are mostly expressed in intestinal tissues. KLF4 has been shown to inhibit cell cycle progression and is expressed at very low levels in actively proliferating cells (Shields et al., 1996). KLF5 participates in several growth factor signaling pathways (Dong and Chen, 2009). KLF 6 negatively (Bianchi et al., 2004) and 8 positively (Zhao et al., 2003) regulate cell cycle progression (McConnell and Yang, 2010). KLF 10 and 11 play important roles in cell growth control and differentiation by inhibiting cell cycle progression (Johnsen et al., 2002 and Buck et al., 2006). Therefore KLFs regulate cell proliferation in a variety of ways by controlling cell cycle components.

II. Differentiation

KLFs also play major roles in cell differentiation. KLFs 2, 3, 4, 5, 6, 7, 11 and 15 have been reported to function as negative or positive regulators of adipocyte differentiation (McConnell and Yang, 2010). KLF1 regulates differentiation during erythropoiesis (Funnell et al., 2007 and McConnell and Yang, 2010) and controls the switch from expression of fetal γ-globin to adult β-globin during the maturation of erythroid cells (Drissen et al., 2005,
Hodge et al., 2006, Pilon et al., 2008 and McConnell and Yang, 2010). KLF4 has also been shown to regulate differentiation in epithelial cells of the intestinal mucosa while KLF 5 regulates differentiation of the cells at the base of the villi (Pearson et al., 2008). Katz et al. (2002) have also shown the specific role of KLF4 in goblet cell differentiation in klf4−/− mouse studies.

III. Apoptosis

Many studies have reported the role of KLFs in apoptosis. Mostly KLF 4, 5 and 6 function in apoptosis. KLF4 is a tumor suppressor due to its ability to induce cell cycle arrest (McConnell and Yang, 2010). KLF5 is also known to be a clear suppressor of apoptosis. Zhu et al. (2006) have observed the low levels of KLF5 in EU-8 leukemia cells and induced expression of IAP (inhibitor-of-apoptosis protein) with introduction of ectopic KLF5. KLF6 is also a tumor suppressor (McConnell and Yang, 2010). Moreover, KLF6 is mutated or deleted in human prostate tumors (Narla et al., 2001) but ectopic expression of KLF6 in prostate cancer cells or non-small cell lung cancer induces apoptosis (Ito et al., 2004 and Huang et al., 2008).

b. Systems based functions of KLFs

I. The cardiovascular system

During embryonic development, KLF2 is expressed in vascular endothelial cells in reduced levels (Anderson et al., 1995). Moreover, klf2−/− mice die in utero from intra-embryonic and intra-amniotic hemorrhage (Kuo et al., 1997, Wani et al., 1998 and Lee et al., 2006). KLF5 promotes fibrosis (McConnell and Yang, 2010). Analysis of klf5+/− heterozygote mouse revealed defects in angiogenesis and blood vessel response to injury (Shindo et al., 2002). KLF10 is also important during development of the heart, male klf10−/− mice develop cardiac hypertrophy by the age of 16 months (Pearson et al., 2008 and Rajamannan et al., 2007). KLF13 is highly expressed in fetal heart (Fisch et al., 2007) and is required for cardiac development (Martin et al., 2001 and Pearson et al., 2008). Moreover, KLF13 knockout mice have enlarged hearts and increased susceptibility to cardiac vacuolar lesions (Gordon et al.,}
Fisch et al. (2007) report that expression of KLF15 in the heart increases significantly after birth and becomes highest in the adult heart. Klf15−/− mice develop fibrosis and deposition of excessive amounts of collagen in the heart (Wang et al., 2008) resulting in cardiac hypertrophy (Fisch et al., 2007) demonstrating that KLF15 is a negative regulator of cardiac fibrosis.

II. The respiratory system

Formerly known as lung KLF (LKL), KLF2 is expressed mostly in the lung (Anderson et al., 1995 and Dang et al., 2000). KLF2 knockout mice die in utero between embryonic day 11.5 and 13.5 of embryonic life making it difficult to study the function of KLF2 in lung development (Wani et al., 1998). However, results of some invitro studies where lung buds embryonic stem cells were used, indicate that KLF2 is an important regulator of late-stage lung development (Wani et al., 1999). KLF5 is expressed at high levels in the bronchiolar epithelium and epithelial lining of the trachea (Ohnishi et al., 2000). Similarly to klf2−/− mice, klf5−/−mice also die early in their life, therefore the exact role of KLF5 on lung development has not been determined (Shindo et al., 2002). However Wan et al. (2008) have revealed that KLF5 is needed for potential maturation of lung morphology and function during the saccular stage of lung development such as regulating surfactant lipid and protein homeostasis, vasculogenesis, maturation of the respiratory epithelium that is required for lung function after birth. In their study, transgenic mice were generated in which the klf5 was conditionally deleted in respiratory epithelial cells in the fetal lung and klf5−/− mice died of respiratory distress immediately after birth (Wan et al., 2008). KLF4 may also play a role in the pathophysiology of lung diseases such as pulmonary fibrosis and inflammatory airway disease (McConnell and Yang, 2010).

III. Hematopoiesis

KLFs regulate erythropoiesis, lymphopoiesis, and the formation and functions of monocytes and macrophages (McConnell and Yang, 2010). Erythropoiesis requires the highly regulated expression of β-globin and related proteins that constitute hemoglobin. KLF1 (erythroid KLF) plays a role in erythropoiesis by activating β-globin transcription and its expression is
restricted to hematopoietic organs such as bone marrow and spleen (Miller et al., 1993). KLF1 knockout mice develop fatal anemia during early fetal life when hematopoiesis starts to occur in the liver (Nuez et al., 1995 and Perkins et al., 1995). Furthermore klf1^−/− mice produce enucleated erythrocytes which are deficient in β-globin (Nuez et al., 1995 and Perkins et al., 1995). In addition to regulation of erythrocyte maturation, KLF1 also controls the development of megakaryocytes (McConnell and Yang, 2010). Frontelo et al. (2007) suggest KLF1 inhibits the formation of megakaryocytes while stimulating erythrocyte differentiation.

Other KLFs also have been shown to regulate erythropoiesis. Expression of KLFs 2, 4, 5, and 12 increase significantly during erythroid differentiation (McConnell and Yang, 2010). KLF2 knockout mice die in utero from intra-embryonic hemorrhage (Kuo et al., 1997 and Wani et al., 1998) and studies from Basu et al. (2005) suggest that KLF2 regulates the maturation and/or the stability of erythroid cells in the yolk sac. Klf2^−/− embryos have significantly higher number of primitive erythroid cells that undergo apoptotic cell death (Basu et al., 2005).

KLF4 is expressed in ES cells and is one of four pluripotency genes and is capable of reprogramming fibroblasts into ES-like cells (Wernig et al., 2007). KLF6 also appears to be an important regulator of ES cell differentiation and hematopoiesis (Matsumoto et al., 2006). KLF6 knockout mice die from a failure of erythropoiesis and yolk sac vascularisation (Matsumoto et al., 2006). While KLF 2, 4 and 13 activate, KLF8 represses the γ-globin promoter through binding to a CACCC element (Zhang et al., 2005). Interestingly KLF11 has no effect on hematopoiesis but KLF11 is expressed in erythroid cells and can regulate the embryonic and fetal globin expression in vitro (Emery et al., 2007).

IV. The immune system and inflammatory responses

KLFs have also been implicated in T cell function. KLF2 is down-regulated upon activation of T-cells in vivo (Dang et al., 2000). Kuo et al. (1997) found that KLF2 is highly expressed in the thymus and medulla like lymphoid organs and that KLF2 inhibits activation, growth, and proliferation of T-cells (Kuo et al., 1997). KLF10 is also important in regulating T cells (Fontenot et al., 2005). Not only KLF2 and 10, but KLF13 also plays a role in T cell activation, survival and development (McConnell and Yang, 2010). Klf13^−/− mice have enlarged thymuses and spleens as a result of decreased T-cell apoptosis (Pearson et al., 2007).
and Zhou et al., 2007). Outram et al. (2008) also suggest that KLF13 influences multiple stages of B- and T-cell development in vivo.

As far as B-cells are concerned, KLF4 plays an important role in B-cell function as a positive regulator of B-cell proliferation (McConnell and Yang, 2010). Van Zelm et al. (2005) show that KLF4 is expressed in early stage B-cell precursors, at the time of immunoglobin gene rearrangement and expression continuously increases until B-cells become matured (Klaewsongkram et al., 2007).

It has also been found that KLFs regulate signaling of macrophages in response to inflammatory cytokines such as IFN-γ, LPS, or TNF-α thereby mediating the development of acute and chronic inflammatory disorders (McConnell and Yang, 2010). KLFs also play an important role in monocyte differentiation. For instance, KLF4 regulates monocyte differentiation in vitro and in vivo (Feinberg et al., 2007 and Alder et al., 2008). In contrast, KLF2 inhibit monocyte activation (Das et al., 2006). Liao et al. (2011) report that KLF4 function as an important regulator of macrophage polarization. Macrophage KLF4 expression was highly induced in M2 macrophages and strongly reduced in M1 macrophages (Liao et al., 2011). M1 macrophages are proinflammatory while M2 macrophages are anti-inflammatory. Liao et al. (2011) report that KLF4 knockout mice exhibited delayed wound healing.

V. The digestive system

It has been revealed that KLFs 4 and 5 play an important role in maintenance of intestinal epithelial homeostasis (McConnell et al., 2007 and Ghaleb et al., 2005) and are expressed highly in adult intestinal epithelial cells (Ton-That et al., 1997, Ohnishi et al., 2000 and Dang et al., 2007). KLF4 inhibits cell proliferation and KLF5 promotes it (Chanchevalap et al., 2004, Ghaleb et al., 2005, McConnell et al., 2007 and Shields et al., 2006). Zhang et al. (2007) demonstrate that KLF5 also mediates the induction of proliferation of colon cancer cells by lysophosphatidic acid (LPA).

It has been revealed that KLF6 regulates the early stages of fibrotic response during liver injury (McConnell and Yang, 2010). Hepatic stellate cells (HSCs) are the major source of extracellular matrix during liver fibrosis and they are activated upon liver injury and during subsequent wound healing (Friedman, 2008). Using rat models it has been shown that
KLF6 is induced during early phase of HSC activation (Lalazar et al., 1997 and Ratziu et al., 1998).

KLF9 regulates intestinal morphogenesis (McConnell and Yang, 2010) while expressed in smooth muscle cells (SMCs) of the small intestine and colon (Simmen et al., 2007). Furthermore Simmen et al. (2007) showed that the jejunum of klf9−/− mice has shorter villi and reduced proliferation of crypt cells.

KLF11 is highly expressed in the pancreatic beta cells. KLF11 regulates the β-cell function by playing a role in glucose signaling in pancreatic beta cells (Neve et al., 2005). Neve et al. (2005) report that KLF11 is inducible by glucose and up-regulates levels of insulin expression in pancreatic beta cells. In addition, KLF11 represses key genes encoding scavengers of oxidative stress such as SOD2 (Superoxide dismutase 2) and catalase resulting in oxidative stress. This observation is extremely important because a tight control of oxidative stress is critical for maintaining the homeostasis of pancreatic beta cells (Neve et al., 2005).

VI. Bone metabolism

KLF10 is the only known KLF family member to date shown to regulate bone metabolism (McConnell and Yang, 2010). Subramaniam et al. (2005) demonstrated that KLF10 knockout mice have a greater number of osteoblasts, but not osteoclasts during bone formation than wild type mice. However, osteoblasts from klf10−/− mice fail to mineralize and support the differentiation of osteoclasts (Subramaniam et al., 2005). As a result, KLF10 knockout mice have defects in bone and tendon strength and microarchitecture (Subramaniam et al., 2005 and Bensamoun et al., 2006). Moreover, Yerges et al. (2010) showed that KLF10 was one of five genes whose expression was associated with volumetric bone density in a SNP study of genes related to bone metabolism.

VII. The nervous system and neuronal morphogenesis

KLFs also have a role in neuronal morphogenesis (McConnell and Yang, 2010). KLF4 was recently identified as an important factor regulating the functions of neurons in the CNS (Moore et al., 2009). It has been shown that retinal ganglion cells (RGCs) lacking KLF4
have increased axon growth both *in vitro* and after optic nerve injury *in vivo*. Thus KLFs act as positive and negative regulators within the nervous system.

KLF7 is also highly expressed in neuronal tissues and KLF7 knockout mice are neonatally lethal (Laub *et al*., 2005).

KLF9 is necessary for some neuron development and neurogenesis. It has been found that KLF9 is expressed in a region called the dentate granule (DG) of the mammalian brain in which neurogenesis occurs in adulthood (Scobie *et al*., 2009). Furthermore, DG neurons from klf9−/− mice showed delayed maturation, and adult KLF9 knockout mice have impaired differentiation of adult-born neurons (Scobie *et al*., 2009).

### VIII. Tumor biology

Many KLFs are involved in the pathology of cancer *in vivo* (McConnell and Yang, 2010). KLF4 is known to be a tumor suppressor of cancers in colon (Zhao *et al*., 2003), esophagus (Wang *et al*., 2002), stomach (Cho *et al*., 2007), pancreas (Wei *et al*., 2008), lung (Hu *et al*., 2009), prostate (Foster *et al*., 2000) and the urinary bladder (Ohnishi *et al*., 2003). At the same time, there are controversial data suggesting that KLF4 can promote tumor formation. For instance high levels of KLF4 mRNA and protein are present in breast tumor samples (Foster *et al*., 2000). KLF4 is present in squamous cells of skin and oral cavity and it involves in pathogenesis of carcinoma in squamous cells (Foster *et al*., 2000). KLF6 is also deregulated in prostate (Narla *et al*., 2001) and liver cancer (Bureau *et al*., 2008).

### IX. Metabolic regulation

The most reported roles for KLFs involved in metabolism are in the regulation of the differentiation of fat cells or adipocytes (McConnell and Yang, 2010). Brey *et al*. (2009) propose that KLFs are an important part of the regulatory cascade that leads to adipogenesis. KLF family members contribute to the transcriptional control of adipogenesis both positively and negatively (figure 03). Table 01 summarizes the effect of KLFs on adipogenesis.
**Figure 03**: Contribution of KLFs in regulating adipocytes differentiation. (Source: Nagai *et al.*, 2009).

**Table 01**: Functions of KLFs in relation to adipogenesis.

<table>
<thead>
<tr>
<th>Type of KLF</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLF2 (LKLF)</td>
<td>Inhibit adipocytes differentiation, expression is higher in undifferentiated preadipocytes and reduced upon differentiation into adipocytes. Overexpression blocks adipocyte differentiation partly through KLF2’s ability to repress PPARγ expression (PPARγ is a master regulator of adipocyte differentiation). Mouse embryonic fibroblasts that lack klf2 are more prone to differentiate into adipocytes, indicating that KLF2 inhibits adipogenesis <em>in vitro</em>.</td>
<td>Banerjee <em>et al.</em>, 2003, Wu <em>et al.</em>, 2005 Banerjee <em>et al.</em>, 2003 Wu <em>et al.</em>, 2005</td>
</tr>
<tr>
<td>KLF3</td>
<td>Inhibits adipocyte differentiation <em>in vitro</em>, expression is higher in preadipocytes and reduced upon differentiation into adipocytes. Over expression blocks adipocyte differentiation partly through KLF3’s ability to inhibit C/EBPα expression. Mouse embryonic fibroblasts that lack klf3 are more prone to differentiate into adipocytes, indicating that KLF3 inhibit adipogenesis <em>in vitro</em>. Over expression of KLF3 blocks differentiation of 3T3-L1 cells in a CtBP dependent manner. CtBP binds NADH, and is a putative metabolic sensor for KLF3-</td>
<td>Sue <em>et al.</em>, 2008 Sue <em>et al.</em>, 2008 McConnell and Yang, 2010</td>
</tr>
<tr>
<td>KLF4</td>
<td>Promotes adipogenesis. Knockdown of KLF4 inhibits adipogenesis and reduces the C/EBP β levels in 3T3-L1 cell cells.</td>
<td>Birsoy et al., 2008</td>
</tr>
<tr>
<td>------</td>
<td>----------------------------------------------------------------------------------------------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>KLF5</td>
<td>Promotes adipogenesis. Induces adipocyte differentiation.</td>
<td>Oishi et al., 2005</td>
</tr>
<tr>
<td>KLF6</td>
<td>Promotes adipogenesis. Delta-like 1 (Dlk-1) or preadipocyte factor-1 (Pref-1) regulates adipogenesis by preventing differentiation of 3T3-L1 cells into adipocytes. KLF6 represses Dlk-1 expression and thereby stimulates 3T3-L1 differentiation. Knockdown of KLF6 prevents adipogenesis.</td>
<td>Smas and Sul, 1993</td>
</tr>
<tr>
<td>KLF7</td>
<td>Expression of KLF7 is decreased upon 3T3-L1 adipocyte differentiation and over expression of KLF7 inhibits adipogenesis. Expression of KLF7 in human preadipocytes inhibited their differentiation and was associated with a reduction in expression of C/EBPα and PPARγ. Over expression in differentiated adipocytes reduced the level of adipokines viz. leptin and adiponectin.</td>
<td>Kanazawa et al., 2005</td>
</tr>
<tr>
<td>KLF11</td>
<td>KLF11 shown to regulate cholesterol-mediated gene expression. In that KLF11 represses the caveolin-1 gene that is involved in cholesterol homeostasis.</td>
<td>Cao et al., 2005</td>
</tr>
<tr>
<td>KLF15</td>
<td>KLF15 regulates adipocyte differentiation through its regulation of PPARγ expression. Its expression is highly promoted during differentiation of 3T3-L1 preadipocytes into adipocytes. RNA interference of KLF15 reduces expression of PPARγ and block the adipogenesis of 3T3-L1 preadipocytes. KLF15 and C/EBPα act synergistically to increase the activity of PPARγ2 in 3T3-L1 adipocytes.</td>
<td>Mori et al., 2005</td>
</tr>
</tbody>
</table>
2.2 Krüppel-Like factor 3 (KLF3)

Krüppel-like factor 3 is a protein in humans that is encoded by the KLF3 gene (Sue et al., 2008). KLF3 was first identified in an erythroid screen for factors related to KLF1 (Crossley et al., 1996). Initially KLF3 was thought to function as a transcriptional activator, but later it was revealed that KLF3 is a strong repressor of transcription (Turner and Crossley, 1998).

In a yeast two-hybrid screen, KLF3 was found to interact with the transcriptional co-repressor C-terminal binding protein (CtBP) (McConnell and Yang, 2010). KLF3 is a more potent transcriptional repressor when bound to CtBP. KLF3's PVDLT (amino acids 61-65) domain, present in its N-terminal variable region, is necessary for its interaction with CtBP (Sue et al., 2008). KLF3 binds CtBP in its NADH-activated state, suggesting that KLF3 regulates transcription in response to the redox state of the cell (Sue et al., 2008).

2.2.1 Tissue distribution of KLF3

KLF3 is broadly expressed in tissues of the mouse, including liver (Funnell et al., 2007), lung, skeletal muscle (Himeda et al., 2010), brain and adipose tissue (Sue et al., 2008) but is particularly abundant in hematopoietic tissues (Funnell et al., 2007).

KLF3 deficient mice are smaller in size compared to wild type mice (Figure 04) and KLF3 knockout mice have smaller and fewer fat cells (adipocytes). Additionally, most organs are proportionally smaller in klf3−/− mice compared to wild type mice (Sue et al., 2008).

![Figure 04: Wild type mouse (left) and KLF3 knock out mouse (right).](image)

(Source: Bell-Anderson lab, School of Molecular Bioscience, The University of Sydney).
2.2.2 Functions of KLF3

The physiological roles of KLF3 are mostly unknown. KLF3 has been shown to bind in vitro to CACCC elements in the promoter regions of many erythroid genes, such as adult β-globin, fetal γ-globin, Gata-1, carbonic anhydrase I, porphobilinogen deaminase (Pbgd), and pyruvate kinase (Funnell et al., 2007). Since CACCC-binding activity of KLF3 is more readily detected in yolk sac and fetal liver, it has been suggested that KLF3 has a role in hematopoiesis (Funnell et al., 2007).

As previously mentioned, KLF3 can also regulate the adipogenic process. klf3 mRNA and protein levels are high initially but decline as adipogenesis progresses (Sue et al., 2008). Microarray analyses have previously suggested that CttBP may be involved in repressing c/ebpa (Grooteclaes et al., 2003).

The PPAR and C/ebp family of transcription factors are master regulators of adipogenesis especially during terminal differentiation and maturation of adipocytes (Sue et al., 2008). As KLF3 expression diminishes, C/ebpα expression also increases. Forced overexpression of KLF3 in the 3T3-L1 system blocks adipogenesis, but a mutant form of KLF3 that cannot bind the co-repressor CtBP is ineffective. This result implicates a CtBP-dependent role for KLF3 in the control of adipogenesis (Sue et al., 2008). Sue et al. (2008) also demonstrated that KLF3 and CtBP repress the C/ebpα promoter. This is consistent with the fact that functional CACCC boxes have been identified in the C/ebpα promoter and that C/ebpα has previously been listed as a gene that is depressed in CtBP knockout cells (Grooteclaes et al., 2003).

KLF3 is also a transcriptional regulator of skeletal muscle genes (Himeda et al., 2010). This research group showed that KLF3 transcripts and protein increase during muscle differentiation and KLF3 is enriched at the promoters of endogenous muscle genes. In that KLF3 and SRF (serum response factor) synergize in trans-activating the MCK (muscle creatine kinase) promoter. But KLF3 knockout mice have no apparent muscle defects (Himeda et al., 2010).

Vu et al. (2011) have revealed a function of KLF3 in B cell development by studying B lymphopoiesis in a klf3–/– mouse model. B cell differentiation is significantly impaired in the bone marrow, spleen, and peritoneal cavity of KLF3 null mice (Vu et al., 2011).
2.2.3 Role of KLF3 in energy metabolism

As previously described, KLF3 has a role in adipogenesis. Furthermore, a role for KLF3 in glucose metabolism has also begun to unravel. According to the unpublished data of Bell-Anderson lab, KLF3 knockout mice have increased fasting blood glucose levels compared to their wild type littermates. However at this stage, mechanisms underlying these observations are not clear.

![Male Fasting (15h) Blood Glucose](image)

**Figure 05:** Blood glucose levels in male mice fasted for 15h, preceding glucose tolerance test. The effect of genotype by ANOVA across all groups is p<0.01.
(Source: Bell-Anderson lab, School of Molecular Bioscience, The University of Sydney).

2.3 Carbohydrate metabolism

![Carbohydrate metabolism](image)

**Figure 06:** Summary of carbohydrate metabolism in the liver.
(Source: Ophardt, 2003)
2.3.1 Glucose as an energy source of the body

Glucose is a monosaccharide which represents an essential biological energy source, enabling the generation of ATP following glycolysis. Although many tissues can also use fats and protein as an energy source, the brain and red blood cells can only use glucose. Glucose is stored in the body, importantly in the liver, as glycogen.

2.3.2 Glycolysis

Glycolysis is a two stage catabolic pathway in which each glucose molecule is converted to two molecules of pyruvate.

Stage 01: Glucose is phosphorylated and cleaved to form two molecules of Glyceradehyde-3-phosphate (G-3-P). Two ATP molecules are consumed during this step.

Stage 02: G-3-P is converted to pyruvate. Four ATP and two NADH molecules are produced in this step. Since two molecules of ATP are consumed in stage 01, net production of ATP per glucose molecule is two.

2.3.2.1 The reactions of the glycolytic pathway

The reactions of the glycolysis pathway are shown in Figure 07 in green color arrows. Glycolysis is stimulated as the energy charge falls down (Berg et al., 2002).

In metabolic pathways, enzymes catalyzing essentially irreversible reactions are potential sites of control. In glycolysis, the reactions catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase are virtually irreversible. Therefore these enzymes would be expected to have regulatory as well as catalytic roles. The inhibition and activation of these enzymes and how those will affect on glycolysis will be discussed in 2.3.6 section in detail.
2.3.2.2 Fate of pyruvate

Pyruvate, the product of glycolysis, is an energy rich molecule. Pyruvate can be metabolized in the following pathways in order to produce energy.

(a) Under aerobic conditions, most cells in the body convert pyruvate into acetyl-CoA which is then entersthe citric acid (TCA) cycle resulting in complete oxidization to CO₂ and H₂O.

(b) In muscle cells, red blood cells and certain bacterial species, pyruvate is converted to lactate in the lactate dehydrogenase reaction (Tornheim and Ruderman, 2011).

\[
\text{Pyruvate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{Lactate} + \text{NAD}^+
\]

In rapidly contracting muscle cells, the demand for energy is high. After depletion of oxygen, lactic acid fermentation provides sufficient regeneration of NAD⁺ to allow glycolysis to continue.

2.3.3 Gluconeogenesis

Maintenance of blood glucose levels during fasting and prolonged exercise are essential for proper functioning of the brain and nervous system. During brief fasting, the blood glucose concentration is topped up through the breakdown of liver glycogen. When fasting is prolonged and glycogen levels are depleted, glucose is formed from non-carbohydrate sources such as lactate, pyruvate, glycerol and other amino acids in a process known as gluconeogenesis. This mainly takes place in the liver. Renal gluconeogenesis contributes to systemic glucose pool only during periods of extreme starvation (Aronoff et al., 2004). Although most tissues have the ability to hydrolyze glycogen, only the kidney and liver contain glucose-6-phosphatase, the enzyme- responsible for releasing glucose back into the circulation.

Gluconeogenesis uses many of the same reactions as glycolysis in reverse (Tornheim and Ruderman, 2011) and the reactions of gluconeogenesis are shown in Figure 07 in blue color arrows.
Figure 07: The reactions of glycolysis and gluconeogenesis.

(Source: Berg et al., 2002)
2.3.3.1 Regulation of gluconeogenesis

Gluconeogenesis is an energy consuming process. The energy for this obviously cannot come from glycolysis. The synthesis of a glucose molecule requires the release of six high energy phosphate bonds; two each at the reactions catalyzed by pyruvate carboxylase, PEPCK and phosphoglycerate kinase.

As with other metabolic pathways, the rate of gluconeogenesis is affected by substrate availability, allosteric effectors and hormones. Gluconeogenesis is stimulated by high concentrations of lactate, glycerol and amino acids. A high-fat diet, starvation and prolonged fasting make large quantities of these molecules available (Felig et al., 1969).

Control of net gluconeogenesis involves regulation of key glycolytic enzymes and the opposing gluconeogenic enzymes (Tornheim and Ruderman, 2011). The four enzymes specific to gluconeogenesis (pyruvate carboxylase, PEPCK, fructose-1,6-bisphosphatase and glucose-6-phosphatase) are regulated by allosteric modulators such as acetyl CoA, level of ATP and AMP (Thabrew and Ayling, 2001).

Hormones also regulate gluconeogenesis by altering synthesis of key rate limiting enzymes (Tornheim and Ruderman, 2011). Importantly, whether either glycolysis or gluconeogenesis is active is largely determined by ratio of insulin to glucagon. After a carbohydrate meal, the insulin/glucagon ratio is high and glycolysis in the liver predominates over gluconeogenesis (Tornheim and Ruderman, 2011). After a period of fasting or following a high fat, low carbohydrate meal, the insulin/glucagon ratio is low and gluconeogenesis in liver predominates over glycolysis. Glucagon, a signal of low glucose levels, causes phosphorylation of Fructose-2,6-bisphosphate Into Fructose-6-phosphate and promotes net gluconeogenesis. Glucagon depresses the synthesis of fructose-2,6-bisphosphate, which releases the inhibition of fructose-1,6-bisphosphatase and inactivates pyruvate kinase (Tornheim and Ruderman, 2011). Furthermore glucagon favors gluconeogenesis and leads to additional synthesis of PEPCK, fructose-1,6-bisphosphatase and glucose-6-phosphatase. Insulin depresses the synthesis of PEPCK, fructose-1,6-bisphosphatase and glucose-6-phosphatase.

Pyruvate kinase is allosterically inhibited by ATP and alanine. The availability of ATP is also an important regulator in the control of gluconeogenesis or glycolysis. At high levels of AMP, a low-energy hydrolysis product of ATP, flux through glycolysis is increased at the expense of gluconeogenesis, conversely, low levels of AMP increase the flux through gluconeogenesis at the expense of glycolysis (McKee and McKee, 2011).
2.3.4 Glycogen as a source of stored energy in the body

Glycogen stores provide readily available glucose to supply the tissues with an oxidizable energy source. Glycogen is a polymer of glucose residues linked by α-(1,4)- and α-(1,6)-glycosidic bonds (Figure 08). Glycogen stores are found principally in the liver. A second major source of stored glucose is the glycogen of skeletal muscle. While liver has a greater concentration of glycogen per gram of weight, total glycogen is higher in muscle because of its larger tissue mass. The purpose of liver glycogen reserve is to break down when glucose is needed and to supply to other tissues, particularly the brain. Muscle glycogen supplies local glycolytic fuel for muscle contraction. However, muscle glycogen is not generally available to other tissues, because muscle lacks the enzyme glucose-6-phosphatase.

![Figure 8: Structure of glycogen.](image)

2.3.5 Glycogen metabolism

The synthesis and degradation of glycogen are regulated in order to provide sufficient glucose for the body’s needs. Both glycogenesis and glycogenolysis are primarily controlled by three hormones: insulin, glucagon and epinephrine (McKee and McKee, 2011).

2.3.5.1 Glycogenesis

Glycogen is synthesized when blood glucose levels are high after a meal, and especially after a carbohydrate rich meal. The synthesis of glycogen involves the following reactions illustrated in Figure 09 (arrows at top in maroon color). Glucagon hormone can inhibit the glycogen synthase stopping the formation of glycogen.
2.3.5.2 Glycogenolysis

This is the breakdown of glycogen to glucose. During the first 8-12 hours of fasting, glycogenolysis in the liver plays a major role in maintaining blood glucose levels by releasing glucose into the blood stream. Reactions of glycogenolysis are shown in Figure 09 (arrows at bottom in orange color).

In glycogenolysis, glycogen stored in liver and muscles, is first converted into glucose-1-phosphate (G-1-PO₄) by breaking α(1,4) linkages (Tornheim and Ruderman, 2011). Glucose-1-phosphate, the major product of glycogenolysis is diverted to glycolysis in muscle cells to generate energy for muscle contraction (McKee and McKee, 2011). In hepatocytes, glucose-1-phosphate is converted to glucose by phosphoglucomutase and glucose-6-phosphatase (McKee and McKee, 2011).

The rate of glycogenolysis is under the influence of the hormones glucagon and insulin from the pancreas and epinephrine from the adrenal glands. Whilst glucagon and epinephrine trigger glycogenolysis, insulin suppresses it.
2.3.6 Key enzymes involved in glucose metabolism

(1) Glucose-6-phosphatase (G6Pase)

G6Pase is an enzyme present in liver that plays an important role in glucose homeostasis in the body. It hydrolyzes glucose-6-phosphate resulting in a phosphate group and free glucose during starvation (Schaftingen and Gerin, 2002), therefore it is an enzyme involved in hepatic glucose production. Moreover, both gluconeogenesis and glycogenolysis result in glucose-6-phosphate, which is hydrolyzed by G6Pase (Nordlie and Sukalski, 1985). G6Pase is a membrane-bound enzyme associated with the endoplasmic reticulum (Schaftingen and Gerin, 2002). In humans, there are three isozymes, G6PC, G6PC2, and G6PC3. G6Pase deficiency is responsible for glycogen storage disease type I (GSD I) (Cori and Cori, 1952 and Arion et al., 1975).

(a) Role of Glucose 6-phosphatase in energy metabolism

Recent studies have indicated that G6Pase has a key role in the regulation of hepatic glucose production. G6Pase does not act on glucose-1-phosphate (G-1-P) or fructose-6-phosphate (F-6-P) (Cori and Cori, 1952). Both must first be converted to glucose-6-phosphate (G-6-P) the only hexose monophosphate that is split by G6Pase.

\[
\text{Glycogen} \xrightarrow{\text{Phosphorylases}} \text{G-1-P} \xrightarrow{\text{Mutase}} \text{G-6-P} \xrightarrow{\text{G6Pase}} \text{Glucose}
\]

The production of glucose from non carbohydrate sources includes the following reactions as final steps (Swanson, 1949).

\[
\text{Triosephosphates} \xrightarrow{\text{Aldolase}} \text{HDP} \xrightarrow{\text{Phosphatases}} \text{F-6-P} \xrightarrow{\text{Isomerases}} \text{G-6-P} \xrightarrow{\text{G6Pas}} \text{Glucose}
\]

(2) Phosphofructokinase

Phosphofructokinase (PFK) catalyses the addition of phosphate to fructose-6-phosphate to form fructose-1,6-bisphosphate. This reaction is an important control point in glycolysis. PFK is inhibited by high level of ATP, a fall of pH, and activated by ADP, AMP, P, fructose-1,6-bisphosphate, fructose-2,6-bisphosphate and excessive formation of lactic acid (Thabrew and
Ayling, 2001). Recently it has been found that phosphofructokinase-M (muscle type) deficient mice have greatly reduced fat stores, despite the presence of the other two isoforms (liver and platelet types) in fat. This suggests the possible importance of glycolytic oscillations for glycerol-3-phosphate generation as precursors for triglyceride synthesis (Getty-Kaushik et al., 2010).

(3) Hexokinase

Hexokinase is usually considered as the first enzyme in glycolysis. Hexokinase can follow the lead of PFK because hexokinase is inhibited by glucose 6-phosphate (Tornheim and Ruderman, 2011). Hexokinase, but not glucokinase, is inhibited by glucose-6-phosphate. Thus, this allows hexokinase to be responsive to the demand for glucose-6-phosphate in the cell. Thus, if PFK is inhibited, the concentration of glucose-6-phosphate will rise and inhibit hexokinase, on the other hand, when PFK is activated, such as by muscular contraction, and fructose-6-phosphate levels drop, the concentration of glucose-6-phosphate will also fall and hexokinase will be de-inhibited. Use of glucose-6-phosphate for glycogen synthesis would also alleviate the inhibition of hexokinase (Tornheim and Ruderman, 2011).

(4) Pyruvate dehydrogenase (PDH)

Pyruvate dehydrogenase is an enzyme responsible for the oxidation of pyruvate to acetyl CoA using NAD and CoA producing NADH and CO₂. Regulation of this step is very important because, although acetyl CoA can be incorporated into fatty acids or made from fatty acids, carbons at this stage cannot be converted back to glucose. The regulation of PDH is on two levels.

1. Acetyl-CoA and NADH inhibit PDH at active sites, in competition with the respective substrates, CoA and NAD. So Acetyl CoA/CoA and NADH/NAD ratios are the important inhibitory parameters.

2. The PDH complex is inactivated by phosphorylation by PDH kinase
PDH is inhibited if there is already plenty of acetyl CoA to enter the citric acid cycle, or NADH for the electron transport chain and oxidative phosphorylation, or ATP. On the other hand, if there is a lot of pyruvate or high blood glucose levels, there is no need to spare the pyruvate as indicated by high plasma insulin levels. Sugden et al. (1995) demonstrated that insulin activates PDH.

2.3.7 Glucose homeostasis

Plasma glucose concentration is a function of the rate of glucose entering the circulation balanced by the rate of glucose removal from the circulation. Blood glucose is derived from three sources:

- intestinal absorption during the fed state (dependent on rate of gastric emptying).
- glycogenolysis.
- gluconeogenesis.

2.3.7.1 Imbalance of glucose - diabetes mellitus

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs (WHO, 1999).

There are three main types of diabetes mellitus (DM).

Type 1 DM results from the body's failure to produce insulin, and currently requires the person to inject insulin or wear an insulin pump (WHO, 1999).

Type 2 DM results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency (WHO, 1999).

The third main form, gestational diabetes, occurs when pregnant women without a previous diagnosis of diabetes develop a high blood glucose level. It may precede development of type 2 DM (WHO, 1999).
**Figure 10:** Glucose homeostasis at fed state in a non-diabetic (left) and diabetic subject (right).

(Source: Aronoff et al., 2004).
(a) **High blood glucose level (fed state)**

After a meal, glucose is released into the blood stream resulting in higher blood glucose levels. At the immediate post meal stage, insulin is secreted into the circulation by β-cells in the islets of Langerhans which make up the endocrine pancreas. Insulin binds to insulin receptors in plasma membrane of target tissues. Activation of insulin signal transduction facilitates the movement of glucose transporter vesicles to the plasma membrane to increase glucose uptake into the cell and reduce blood glucose levels. Intracellularly, insulin stimulates the conversion of glucose into glycogen and fat (Figure 10, left diagram). In the fed state, endogenous glucose production in the liver is also suppressed by,

- the direct action of insulin, delivered via the portal vein, on the liver.
- the direct communication within the pancreas between β- and α-cells, resulting in suppression of glucagon synthesis and secretion.

**Figure 11:** Balance between glucose appearance in the circulation and glucose disappearance or uptake. Glucose appearance is a function of hepatic(endogenous) glucose production and meal-derived sources and is regulated by pancreatic and gut hormones. (Source: Aronoff *et al.*, 2004).

In diabetic patients this process is defective because of a lack of insulin and/or insulin resistance, whereby the cells do not respond to insulin. When blood glucose levels are high, lack of insulin function fails to suppress glucagon secretion and directly inhibit liver glucose production. As a result, the appearance of glucose exceeds the disappearance of glucose in the blood stream. Eventually this leads to postprandial hyperglycemia (Figure 10, right diagram).
(b) Low blood glucose level (fasted state)

In the fasting state, the blood glucose level decreases continually because glucose is in constant demand by the brain, red blood cells etc. To keep pace with the glucose disappearance, endogenous glucose production is necessary. The sole source of endogenous glucose production is the liver. The kidneys also contribute to the blood glucose pool during severe starvation by renal gluconeogenesis.

When the blood glucose level is lower than normal, pancreatic α-cells in the islets of Langerhans detect the drop and secrete the hormone glucagon. Glucagon activates the enzymes in the liver which convert glycogen into glucose and stimulates the formation of glucose from other substances, such as amino acids, via gluconeogenesis. This glucose is passed out into the blood raising blood glucose levels back to normal level (Figure 12). While most tissues have the ability to hydrolyze glycogen to glucose, only the liver and kidneys express G-6-Pase, which can release glucose into the circulation. The enzyme G-6-Pase can hydrolyze G-6-P into a phosphate group and a free glucose. A free glucose molecule can diffuse back to the plasma through a GLUT4 portal allowing release of glucose for use by all the cells in the body.

In the diabetic person in the fasting state, plasma glucose is derived from glycogenolysis and gluconeogenesis under the influence of glucagon. Importantly, exogenous administration of insulin can restore normal postprandial insulin concentrations in the portal vein and to suppress glucagon through a paracrine effect (Figure 12).
Figure 12: Glucose homeostasis at fasted state in a non-diabetic (left) and diabetic subject (right).

(Source: Aronoff et al., 2004).
2.3.8 Bi-hormonal model of glucose homeostasis

In the bi-hormonal model of glucose homeostasis, insulin is responsible for glucose disappearance and glucagon is responsible for glucose appearance. After reaching a post meal peak, blood glucose levels slowly decrease during the next several hours, eventually returning to fasting levels. In the immediate post-feeding state, glucose removal to adipose tissue and skeletal muscle is driven by insulin.

(a) Insulin

This is a key anabolic hormone secreted by β-cells in response to increased blood glucose levels. The primary action of insulin is to stimulate the glucose disappearance. Insulin is a small protein composed of two polypeptide chains containing 51 amino acids. Like many other hormones insulin also carries out its function through binding to specific receptors present on many cells of the body including fat, liver and muscle cells. Insulin helps to control postprandial glucose in three ways.

1. Initially insulin signals the cells of insulin-sensitive peripheral tissues, primarily skeletal muscle, to increase their uptake of glucose.
2. Insulin acts on the liver to promote glycogenesis.
3. Insulin simultaneously inhibits glucagon secretion from pancreatic α-cells, thus signaling the liver to stop producing glucose via glycogenolysis and gluconeogenesis.

Altogether all of these actions reduce the blood glucose level. Other actions of insulin include stimulation of fat synthesis, promotion of triglyceride storage in fat cells, promotion of protein synthesis in the liver and muscle and proliferation of cell growth (Cryer, 1992).

Insulin action is carefully regulated in response to circulating glucose concentrations. Insulin is not secreted if the blood glucose concentration is ≤ 3.3mmol/L, but is secreted in increasing amounts as glucose concentration increases beyond this threshold (Gerich, 1993).

Postprandially the secretion of insulin occurs in two phases: an initial rapid release of preformed insulin followed by increased insulin synthesis and release in response to blood
glucose. Long term release of insulin occurs if glucose concentrations remain high (Cryer, 1992 and Gerich, 1993). Diabetic subjects either lack insulin or are resistant to insulin, resulting in high blood glucose levels due to impaired glucose disposal (primarily into muscle glycogen) and unrestrained hepatic glucose output. While glucose is the most potent stimulus of insulin, other factors stimulate insulin secretion such as increased plasma secretion of some amino acids, specially arginine, leucine, and lysine, GLP-1 and GIP released from the gut following a meal and parasympathetic stimulation via the vagus nerve (Drucker, 2001 and Holst, 1994).

(b) Glucagon

Glucagon is secreted by the α-cells of the islets of Langerhans in response to low blood glucose levels. Glucagon is a key catabolic hormone and it consists of 29 amino acids. Glucagon was characterized as opposing the effects of insulin (Unger, 1971). Glucagon plays a major role sustaining plasma glucose level during the fasting state by stimulating hepatic glucose production.

When plasma glucose levels fall, glucagon secretion increases promoting hepatic glycogen breakdown and gluconeogenesis as well as adipose tissue lipolysis. This returns the plasma glucose to the normal range (Gerich et al., 1979 and Orci et al., 1975). This endogenous source of glucose is not needed during and immediately following a meal and therefore glucagon secretion is suppressed. When coupled with insulin’s direct effect on the liver, glucagon suppression results in a near-total suppression of hepatic glucose output (Figure 13) in the fed state. Glucagon is presumed to have no effect on skeletal muscle glucose metabolism as muscle is reported not to express glucagon receptors (Tornheim and Ruderman, 2011). In the diabetic state, there is inadequate suppression of postprandial glucagon secretion by insulin resulting in elevated glucose production output (Figure 13) Cryer, 1981 and Dinneen et al., 1995).
2.3.9 Other hormones involved in glucose homeostasis

Although initially it was understood that circulating levels of glucose are controlled by insulin and glucagon, later on it was revealed that other hormones play important roles in glucose metabolism such as amylin, epinephrine, cortisol, growth hormone and the incretin hormones: glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) (Aronoff et al., 2004). Those hormones and their glucoregulatory effects have been listed in Table 02.
Table 02: Effects of primary glucoregulatory hormones.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Hormone</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas (α-cells)</td>
<td>Glucagon</td>
<td>Stimulates the breakdown of stored liver glycogen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Promotes hepatic gluconeogenesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Promotes hepatic ketogenesis</td>
</tr>
<tr>
<td>Pancreas (β-cells)</td>
<td>Insulin</td>
<td>Affects glucose metabolism and storage of ingested nutrients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Promotes glucose uptake by cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Suppresses postprandial glucagon secretion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Promotes protein and fat synthesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Promotes use of glucose as an energy source</td>
</tr>
<tr>
<td></td>
<td>Amylin</td>
<td>Suppresses postprandial glucagon secretion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slows gastric emptying</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduces food intake and body weight</td>
</tr>
<tr>
<td>Intestine (L-cells)</td>
<td>GLP-1</td>
<td>Enhances glucose-dependant insulin secretion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Suppresses postprandial glucagon secretion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slows gastric emptying</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduces food intake and body weight</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Promotes β-cell health</td>
</tr>
<tr>
<td>Intestine (K-cells)</td>
<td>GIP</td>
<td>Induce insulin secretion</td>
</tr>
<tr>
<td>Adrenal medulla</td>
<td>Epinephrine</td>
<td>Enhances release of glucose from glycogen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhances release of fatty acids from adipose tissues</td>
</tr>
<tr>
<td>Adrenal cortex</td>
<td>Cortisol</td>
<td>Enhances gluconeogenesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antagonizes insulin</td>
</tr>
<tr>
<td>Anterior pituitary</td>
<td>Growth hormone</td>
<td>Antagonizes insulin</td>
</tr>
</tbody>
</table>

(Sources: Sherwin et al., 1980, Aronoff et al., 2004, Cho and Kieffer, 2010)
(a) Amylin

Amylin is secreted by pancreatic β-cells in response to nutrient stimuli (Cooper et al., 1987, Koda et al., 1992, Moore and Cooper, 1991, Ogawa et al., 1990). Amylin is a 37-amino acid peptide and works along with insulin to coordinate the rate of glucose appearance and disappearance in the circulation, thereby preventing an abnormal rise in glucose concentrations. Studies in humans have demonstrated that the secretory and plasma concentration profiles of insulin and amylin are similar with low fasting concentrations and increases in response to nutrient intake (Koda et al., 1995 and Fineman et al., 1996). In subjects with diabetes, amylin is deficient in type 1 and impaired in type 2 diabetes (Kruger et al., 1999 and Young, 1997).

(b) Incretin hormones: GLP-1 and GIP

Several incretin hormones have been identified and the dominant ones involved in glucose homeostasis are GIP and GLP-1 (Aronoff et al., 2004). GIP stimulates insulin secretion and regulates fat metabolism, but does not inhibit glucagon secretion or gastric emptying (Yip and Wolfe, 2000). GIP levels are normal or slightly elevated in people with type 2 diabetes (Vilsboll et al., 2001). While GIP is a more potent incretin hormone, GLP-1 is secreted in greater concentrations and is more physiologically relevant in humans (Nauck et al., 1993).

GLP-1 is synthesized and secreted by the L-cells found mainly in the ileum and colon (Aronoff et al., 2004). GLP-1 also stimulates glucose-dependent insulin secretion but is significantly reduced postprandially in people with type 2 diabetes or impaired glucose tolerance (Lugari et al., 2002 and Vilsboll et al., 2001). GLP-1 is secreted when gastrointestinal tract glucose levels are high. Both GLP-1 and GIP are effectively stimulated by ingestion of a mixed meal or meals enriched with fat and carbohydrates (Herrmann et al., 1995 and Elliott et al., 1993). In contrast to GIP, GLP-1 inhibits glucagon secretion and slows gastric emptying (Matsuyama et al., 1988). GLP-1 has plasma half life of about 2 minutes (Aronoff et al., 2004). GLP-1 has many physiological effects and they have been listed in Table 02.
2.4 Effect of Krüppel-Like factors on glucose metabolism

It is a well known fact that disturbance of energy metabolism can result in conditions like obesity and insulin resistance and eventually type 2 diabetes mellitus. A novel area of research is the effect of the KLF group of transcriptional factors on body metabolism. The most well characterized role of KLFs in metabolism is through regulation of adipocyte differentiation (McConnell and Yang, 2010). For example in the process of adipogenesis, KLF2 is needed for maintenance of preadipocytes but it is down regulated as differentiation proceeds (Pearson et al., 2008). In contrast, KLF5 and KLF 15 are positive regulators of adipogenesis.

Glucose metabolism and fat metabolism are interconnected in the human body. KLFs also have a role in glucose metabolism and research is ongoing in order to understand the effect of KLFs in glucose homeostasis. Table 03 presents the published findings describing the role of KLFs in glucose metabolism in the body particularly in liver and skeletal muscle to understand their unique functions and actions in the glucose metabolism in the body.

Table 03: Functions of KLFs on glucose metabolism.

<table>
<thead>
<tr>
<th>Type of KLF</th>
<th>Function/ Observation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLF2</td>
<td>Human umbilical vein endothelial cells (HUVECs) cultured at high concentrations of glucose and observed that KLF2 expression significantly decreased in a dose-dependent manner. Endothelial nitric oxide synthase (eNOS) expression was also suppressed. According to in vivo studies using insulin resistant, type 2 diabetic rats, KLF2 was also suppressed in the vessels showing that results were consistent with in vitro findings. Suppression of KLF2 in diabetic rats may increase neointimal formation and intraluminal thrombus formation. Replenishment of KLF2 may be effective for preventing diabetic vascular dysfunction.</td>
<td>Lee et al., 2012</td>
</tr>
<tr>
<td>KLF3</td>
<td>According to the experiments done in Bell-Anderson lab it has been found that KLF3 knockout mice had increased</td>
<td>Unpublished data, Bell-</td>
</tr>
<tr>
<td>KLF4</td>
<td>Evidence suggests that M2 machophages can protect against insulin resistance and the IL-4 mediated induction of characteristic M2 marker genes was significantly attenuated in KLF4 deficient macrophages indicating that KLF4 is required for optimal M2 activation. Insulin and glucose tolerance were measured in HFD (high fat diet)-fed myeloid specific (Mye) wild type and knockout mice. Mye-deficient mice were more resistant to glucose-lowering effect of exogenous insulin. In keeping with an insulin-resistant state, phosphorylation of Akt (protein kinase B) was reduced in liver and skeletal muscle of Mye-deficient mice after HFD.</td>
<td>Liao et al., 2011</td>
</tr>
<tr>
<td>---</td>
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<td>---</td>
</tr>
<tr>
<td>KLF5</td>
<td>KLF5 regulates the expression of SMemb (Embryonic form of myosin heavy chain) in human endothelial cells. SMemb is increased by high glucose concentration. Glucose → MEK 1 → KLF5 → SMemb klf5+/– mice were resistant to high-fat diet-induced obesity and protected from dyslipidemia, glucose intolerance and hepatic steatosis, indicating that klf5+/– mice were less susceptible to metabolic syndrome.</td>
<td>Fukuda et al., 2007</td>
</tr>
<tr>
<td>KLF6</td>
<td>KLF6 was induced by high glucose in proximal tubule kidney cells and up-regulated in the kidney of the diabetic Ren-2 rat. Seven binding sites of KLF6 and three binding sites of peroxisome proliferator-activated receptor-γ (PPAR-γ) located in the Txnip (Thioredoxin-interacting protein) promoter region. Therefore Qi et al also hypothesized that KLF6 and PPAR-γ may also regulate the Txnip</td>
<td>Holian et al., 2008 Qi et al., 2009</td>
</tr>
</tbody>
</table>
expression and promoter activity in hyperglycemic conditions. Txnip is a critical regulator of glucose metabolism in liver and heart and demonstrated to play a main role in global glucose homeostasis.

<table>
<thead>
<tr>
<th>KLF 7</th>
<th>KLF7 has been identified as a new susceptibility gene for type 2 diabetes. It is hypothesized that KLF7 contributes to the pathogenesis of diabetes by inhibiting insulin synthesis and secretion from pancreatic β-cells. The role of KLF7 in an insulin-secreting cell line (HIT-T15) has been examined and it was shown that expression of the insulin gene was significantly reduced in cells over-expressing KLF7 in a dose dependent manner. The mRNA expression of the hexokinase 2 gene in KLF7-overexpressing L6 cells was significantly reduced compared with that in the control cells, whereas the change in expression of GLUT1, GLUT4, and glycogen synthase was not statistically significant between the control cells and KLF7-overexpressing cell.</th>
<th>Kanazawa et al., 2006, McConnell and yang, 2010, Kawamura et al., 2006</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLF 10</td>
<td>Loss of KLF10 changes the expression of genes involved in lipogenesis, gluconeogenesis and glycolysis. KLF10 is a modulator of carbohydrate metabolism in the liver. klf10−/− male mice exhibit hyperglycemia. The effect has been attributed to the direct repression of the proximal PEPCK gene promoter by KLF10.</td>
<td>Guillaumond et al., 2010</td>
</tr>
<tr>
<td>KLF 11</td>
<td>KLF11 transgenic mice have a considerably smaller exocrine pancreas than controls due to reduced proliferation and increased apoptosis. Endogenous KLF11 mRNA levels were reduced by high glucose in INS-1E beta cells. KLF11 plays a role in glucose metabolism by regulating the β-cell function in the pancreas and further it is</td>
<td>Niu et al., 2007, Neve et al., 2005</td>
</tr>
</tbody>
</table>
associated with type 2 diabetes. β-cells cultured in high levels of glucose have higher levels of KLF11, which activates the insulin promoter.

### KLF 15

KLF15 was the first KLF to be found to be involved in glucose metabolism i.e. gluconeogenesis.

KLF15 regulates the expression of insulin-sensitive glucose transporter GLUT4 in both adipose tissue and muscle tissue.

It has been shown that $klf15^{-/-}$ mice exhibit severe hypoglycemia after an overnight (18 hours) fast in both male and female mice because of decreased availability of amino acids-derived substrates necessary for liver gluconeogenesis.

At the same time, male and female KLF15 deficient mice had statistically significant lower blood glucose level compared to $klf15^{+/+}$ mice in the ad libitum-fed state.

KLF15 knockout mice also had increased hepatic insulin sensitivity.

In the fasted state, $klf15^{-/-}$ mice had around 3.5 fold decreased amount of glycogen compared to wild type mice.

Glucagon levels were increased in $klf15^{-/-}$ mice in both the fed and fasted state but no difference in insulin level was observed.

$klf15^{-/-}$ mice liver and muscle show markedly reduced mRNA expression of amino acid degrading enzymes e.g. alanine amino transferase.

Above table summarizes the functions of KLFs in glucose metabolism that have been discovered so far. Krüppel-like factor 2, 3 and 10 have negative effects on glucose level whilst there is a positive effects from KLF 4, 6, 7, 11 and 15.
3. Methods

3.1 Animals

Mice on the background FVB/NJ strain were used in this study. Ethical approval for the use of mice was obtained from The University of Sydney Animal Care and Ethics Committee (ACEC). Plasma and tissue samples were obtained from male mice (n=40) as part of a cohort managed by Mr. Hanapi Mat Jusoh (PhD candidate, Bell-Anderson lab, School of Molecular Bioscience). Mice were housed in the School of Molecular Bioscience animal house (The University of Sydney, NSW, Australia). They were maintained on a 12 hour dark/light cycle (lights on at 6am) at a constant temperature of 22°C ± 1°C with ad libitum access to water and standard chow diet (Glenn Forest Specialty Feeds, WA). Percentage energy from protein, fat and carbohydrate of standard chow diet consisted of 18 %, 9 % and 73% respectively.

3.2 Identification of mice by ear-marking

At three weeks of age, mice were anaesthetized with isoflurane and given one of six unique ear punches for identification. Tail tips (~5 mm) were taken for genotyping.

3.3 Genotyping

3.3.1 Tail digest

Tail tips were digested in 150µL Direct PCR lysis reagent with 0.13 µL proteinase K overnight at 55°C. Samples were heated to 85°C for 45 minutes to inactivate proteinase K. After pulse centrifugation in a capsule HF-120 (Tomy digital biology Co., Ltd, Tokyo, Japan) the lysate was removed for amplification of sequences of interest by polymerase chain reaction (PCR).

3.3.2 PCR

Mice were genotyped to identify the presence of klf3 and/or Neo sequences. The primer pairs used for the amplification reaction are shown in Table 04. For each PCR run, three positive
controls and a non-template control (NTC) were included. The positive controls consisted of DNA from $klf3^{+/+}$, $klf3^{-/-}$ and $klf3^{-/-}$ mice. Isolated genomic DNA (2-20ng) or Baxter H$_2$O (1µL) was mixed with 1×ThermoPol reaction buffer, 0.2mM dNTP and 0.8 µM of primer ($Neo$ and $klf3$ fwd/rev). Finally, Taq polymerase (0.02 U/mL) was added and tubes were centrifuged for 10 seconds in a C1301-230V centrifuge (Bio-Rad Laboratories (Pacific) Pty Ltd, Gladesville, NSW, Australia) before being loaded into a Veriti™ 96-well thermal cycler (Applied Biosystems, Mulgrave, VIC, Australia). The PCR cycle is shown in Figure 14.

Table 04: $klf3$ group primer composition.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Klf3$ Fwd</td>
<td>AAATGCACCTGGGAAGGCTGCAC</td>
</tr>
<tr>
<td>$Klf3$ Rev</td>
<td>CAGACTAGCATGTGGCGTTTCCTG</td>
</tr>
<tr>
<td>$Neo$ Fwd</td>
<td>TGATGCAATGCGGCGCTGCATAC</td>
</tr>
<tr>
<td>$Neo$ Rev</td>
<td>CAGAGAACTCGTCAAGAAGGCGA</td>
</tr>
</tbody>
</table>

*Forward primer, †Reverse primer

Figure 14: PCR cycle.

Temperatures are shown above the line and times are shown below the line (minutes: seconds).
3.3.3 Gel electrophoresis

Loading dye was added to PCR products to a final concentration of 25% (v/v) glycerol, 0.25 % (w/v) bromophenol blue, 6mM EDTA pH 8.0. Approximately 10 µL of samples were loaded onto a 2.0% (w/v) agarose gel made in tris acetate EDTA (TAE) buffer (40 mM Tris Acetate, 1mM EDTA, pH 8.0) and containing 0.3µg/mL ethidium bromide. The gel was run at 80V for approximately 50 minutes in TAE and then visualized and photographed by UV transillumination using Quantity One® software (4.1.1 Gel-Doc 2000, Bio-Rad). Gel schematic is shown in Figure 15.

**Figure 15:** Schematic of *klf3* genotyping.

The smaller band (170bp) represents the undisrupted *klf3* gene, present in *klf3*+/+ and *klf3*+/- mice. The larger band (450bp) represents the Neocassette inserted at the *Klf3* gene locus, and present only in *klf3*+/- and *klf3*−/− mice.
3.4 Experimental design

In this experimental study, 20 knockouts and 20 wild type male mice were used (in some instances, number per each group was slightly deviated depending on the available data). Each group was divided into two in order to be in a fed or fasted state (Figure 16). We followed the natural eating pattern of mice when selecting the time of sacrifice for the fed and fasted state. According to the literature the eating pattern of mice follows a diurnal rhythm, mice eat the most during the dark phase and less during the light phase. Therefore, mice were sacrificed at 9pm and 5pm considered as fed and fasted respectively.

Figure 16: Experimental design of the study.
3.5 Mouse sacrifice

Body weights were recorded to the nearest 0.01g and mice were sacrificed at 13 weeks of age. Mice were sacrificed by intraperitoneal injection of 100μL of “Lethobarb Euthansia injection” (sodium pentobarbitone, 260mg/kg body weight). Animals were observed until they stopped moving and death was confirmed by testing corneal reflex and absence of respiratory movement before proceeding.

3.6 Blood collection

Blood was collected to EDTA tubes by cardiac puncture using a 26G × ½” needle with a 1mL syringe. Blood was separated by centrifugation (10 000rpm, 10 minutes at 25°C temperature) using a Hereaus Biofuge fresco centrifuge (Thermo Fisher Scientific, Waltham, MA, USA). Plasma was transferred to fresh tubes and kept frozen at -80°C until required.

3.7 Analysis of blood glucose

Immediately after blood was collected, blood glucose was measured by Accu-Chek® Performaglucometer (Roche Diagnostics, Australia).

3.8 Tissue collection

Immediately after sacrifice of mice, liver, kidney, lung, red quadriceps muscle, white adipose tissue (subcutaneous, retroperitoneal and gonadal) and brown adipose tissue were collected. All were freeze clamped in liquid nitrogen and stored at -80°C until required. Liver and red muscles were used for analyzing glycogen. Liver samples were used to analyze glucose-6-phosphatase in this study.
3.9 Glycogen analysis of liver and red skeletal muscle

Glycogen was measured using an enzymatic assay. This assay determines glycogen by two main reactions. First glycogen is isolated and broken down to glucose and then that glucose is measured using a glucose oxidase based method.

A known amount of liver and muscle (approximately 20mg) were measured in to 2mL eppendorf tubes. 1M KOH (200 µL) was added and tubes were incubated at 80°C for 25 minutes while shaking. Following this, saturated Na₂SO₄ (75µL) and 95% ethanol (1.725 mL) were added. After vortexing, tubes were centrifuged at 13000rpm for 15 minutes at 4°C. Subsequently, the supernatant was discarded slowly by slanting the tubes. The pellet was resuspended in distilled H₂O (200µL) and incubated at 80°C for 15 minutes while shaking until the precipitate was completely digested. Then, 1 mL and 250 µL of prepared amyloglucosidase enzyme was added to liver samples and muscle samples respectively (enzyme prepared fresh by mixing 3mg of amyloglucosidase with 10mL of 0.25M, pH 4.75 acetate buffer). Finally samples were incubated overnight at 37°C while shaking.

\[
\text{Glycogen} \xrightarrow{\text{Amyloglucosidase}} n \text{ glucose}
\]

1000uL or 500uL of supernatant, from muscle or liver respectively, was taken for glucose analysis using a glucose oxidase method.

3.9.1 Glucose analysis by glucose oxidase method

A series of standards were made with known concentration of glucose (0, 2, 4, 8, 12, 16 and 20mM/L) by using 5mM glucose stock solution. Of the unknown liver and muscle samples, 5 µL and 10 µL were used respectively. Standards (0-20 mM/L) and liver/muscle samples were added in duplicate to each well of a 96-well plate followed by 300 µL of assay buffer. Assay buffer was a mixture of phosphate buffer (25 µL), dye (50 µL), peroxidase (25 µL) and glucose oxidase (125µL). The covered plate was incubated at 37°C for about 20 minutes to allow sufficient time for the reaction to complete. Readings were taken at 492nm by a plate reader (Thermoscientific, Waltham, MA, USA).
3.9.2 Optimization of glycogen assay

To determine the optimal reaction conditions in order to have confidence in our results, different volumes of the same liver homogenate were used while maintaining all other conditions constant.

Figure 17 shows liver glycogen content determined in different volumes of liver extract from the same liver sample. With the increase of volume of extract, liver glycogen content increased. There was a strong correlation between the volume of liver extract and liver glycogen content. Therefore, the minimum volume of 5µL was chosen as the volume of extract to be assayed. As a first step, liver glycogen content of KLF3 knockout and wild type mice in the fed and fasted state were determined. As mentioned in the methodology, an enzymatic assay was used to measure liver and muscle glycogen levels. This methodology was optimized using spare liver samples before the assay was performed on liver and muscle from klf3⁻/⁻ and klf3⁺/+ animals.

![Correlation graph between volume of liver samples and liver glycogen contents.](image-url)

**Figure 17:** Correlation graph between volume of liver samples and liver glycogen contents.
3.10 Glucose-6-phosphatase activity

Glucose-6-phosphatase activity was determined using a radioactive isotope method in which a known amount of radioactive glucose-6-phosphate ([U-\(^{14}\)C]G-6-P) was used to react with the glucose-6-phosphatase enzyme present in liver to form glucose.

Approximately 50mg of liver tissue was homogenized using a Polytron (Kinematica INC, Luceerne, Switzerland) at 13,000rpm speed in 20 volumes of homogenizing buffer. Homogenizing buffer was composed of Triethanolamine HCl (TRA) (100mM), magnesium chloride (5mM), EDTA (5mM), potassium chloride (100mM), DTT (2mM) dissolved in distilled water. Assay buffer (50µL) and homogenate (50µL) were incubated together at 30\(^{\circ}\)C. The assay buffer contained MoPS (50mM), [U-\(^{14}\)C] G-6-PO\(_4\) and cold glucose-6-phosphate (5mM). After 10 minutes, the reaction was terminated by adding FeCl\(_3\) (60mM, pH=6) and centrifuged for 5 minutes at 12000rpm to precipitate proteins and G-6-PO\(_4\). Finally, the supernatant was taken for radioactivity counting and G-6-PO\(_4\)ase activity was measured as µmol of glucose from G-6-P per gram of liver tissue.

3.10.1 Optimization of Glucose-6-phosphatase activity assay

As mentioned in the methodology, glucose-6-phosphatase activity was measured using a radioactive labeled isotope protocol. Before applying the methodology to measure glucose-6-phosphatase enzyme activity in actual liver samples, the conditions were optimized using a test liver sample. The aim of optimization was to determine the optimal reaction time in order to obtain consistent and reliable results. The graph below shows the results of initial glucose-6-phosphatase activity measurements.
Figure 18: Glucose-6-phosphatase activity versus reaction time.

3.11 Plasma glucagon analysis

Plasma glucagon concentration was determined using Alpco® rat, mouse and human glucagon EIA (Enzyme Immunoassay) (Alpco Diagnostics Inc, Salem, NH, USA). The EIA kit for determination of rat, mouse or human pancreatic glucagon in plasma samples is based on a competitive enzyme immunoassay using a combination of a highly specific antibody to glucagon and a biotin-avidin affinity system (Figure 19).

Figure 19: Schematic of EIA.
1. Antigen-specific polyclonal antibody is attached to a solid-phase surface, 2. Plasma is added, which contains antigen (glucagon) in the plasma and this will bind to the antibody, 3. An enzyme-labeled antibody specific to the antigen is added (conjugate), 4. Chromogenic substrate is added, which in the presence of the enzyme, changes color.

First, undiluted plasma, standards (0-10000pg/mL) were added in duplicate to each well of a 96-well plate. The 96-well plate was precoated with a rabbit anti-glucagon antibody. Next, labeled antigen (50µL) was added to the wells for competitive immunoreaction. The plate was incubated at 4°C for 48 hours to allow glucagon to bind to the polyclonal antibodies on the plate surface. The plate was washed three times with a wash solution provided (approximately 0.35 mL/well) and tapped to dry and remove unbound antigen.

After incubation and plate washing, HRP labeled streptavidin (SA-HRP) was added followed by one hour incubation while shaking at room temperature to form HRP labeled streptavidin-biotinylated pancreatic glucagon-antibody complex on the surface of the wells. Then wells were again washed three times with the wash solution provided. Finally, a substrate solution containing O-phenylenediamine dihydrochloride (OPD) (100µL) was added to the wells and incubated for 20 minutes at room temperature and the reaction terminated by addition of a stop solution. The optical absorbance of the wells was read at 490 nm immediately after stopping the color reaction. Finally, HRP enzyme activity was determined by OPD and the concentration of mouse pancreatic glucagon was calculated. Standard curve used for the glucagon analysis is shown in the Figure 20.

![Figure 20: Glucagon standard curve.](image)
3.12 Statistical analysis

The mean and standard deviation values were calculated for all data obtained. Values lower and higher than two standard deviations above or below the mean were excluded from the analysis which is considered as a quantitative method of exclusion of outliers. StatView version 4.57 (Abacus concepts Inc, Berkeley, CA, USA) was used for statistical analysis. Unpaired, two tailed \( t \) tests were used to determine significant differences between groups. A P value < 0.05 was considered statistically significant.
4. Results

4.1 KLF3 knockout mice have significantly lower body weight than wild type mice at the time of sacrifice.

![Figure 21: Comparison of body weight of KLF3 knockout mice and wild type mice. Values are the mean ± s.e.m of 24-27 animals. *P < 0.05 as compared with wild type mice.](image)

We observed that KLF3 knockout mice had significantly lower body weight at the time of sacrifice (p value = 0.003). Wild type mice had mean body weight of 32.0 ± 1.9 g whereas KLF3 knockout mice had 28.1 ± 2.5 g. We also observed that KLF3 knockout mice are smaller in size compared to their wild type litter mates.

4.2 Food intake data of wild type and KLF3 knockout mice in the fed and fasted state.

Figure 22 shows food intake within 12 hours of wild type and KLF3 deficient mice for fed and fasted state. Food intake of wild type mice was significantly lower during natural fasting state than that of night time (fed state) food intake. There was no difference in food intake data between the fed and fasted state of KLF3 knockout mice.
Figure 22: Comparison of food intake of KLF3 knockout mice and wild type mice at fed and fasted state. Values are the mean ± s.e.m of 9-10 animals. *P < 0.05 as compared with fed state.

4.3 KLF3 knockout mice have higher fasting blood glucose level in the fasted state.

We compared the blood glucose levels of KLF3 knockout mice and wild type mice in this study. Mice maintained their normal eating pattern in which we considered 4-5pm as a fasted state and 9pm as a fed state. KLF3 deficient mice had significantly higher blood glucose levels in the fasted state compared with wild type mice (Figure 23). Further experiments were designed to investigate mechanisms underlying the higher blood glucose levels in KLF3 mice. We initially hypothesized that the higher fasting blood glucose levels in KLF3 knockout mice could be due to increased glycogen breakdown or increased gluconeogenesis in the fasted state.
**Figure 23:** Comparison of blood glucose levels in KLF3 knockout and wild type mice in the fed and fasted state. Values are the mean ± s.e.m of 9-11 animals. *P < 0.05 as compared with wild type mice.

### 4.4 Insulin levels are unchanged in KLF3 knockout mice in the fed state.

Figure 24 shows the plasma insulin levels of KLF3 knockout and wild type mice in fed and fasted state. No differences were seen in insulin levels in fed state however KLF3 knockout mice had significantly higher insulin level in fasted state.

**Figure 24:** Comparison of plasma insulin levels in KLF3**** and KLF3****/**** mice in the fed state. Values are the mean ± s.e.m of 10-13 animals. *P < 0.05 as compared with wild type mice.
4.5 *Klf3*<sup>−/−</sup> mice have lower liver glycogen levels in the fed state.

Liver glycogen content in wild type and KLF3 knockout mice in the fed and fasted state were measured and the results are shown in Figure 25. Liver glycogen in the fed state in KLF3 knockout mice was lower than that of wild type mice suggesting that wild type mice may have higher liver glycogen storing capacity. There was no significant difference in glycogen content of KLF3 knockout mice and wild type mice in the fasted state.

![Liver glycogen content](image)

**Figure 25:** Comparison of liver glycogen in *klf3*<sup>−/−</sup> and *klf3*<sup>+/+</sup> mice in the fed and fasted state. Values are the mean ± s.e.m of 12-15 animals. *P* < 0.05 as compared with wild type mice.

4.6 Muscle glycogen levels in KLF3 knock out and wild type mice in fed and fasted state are unchanged.

Figure 26 shows the muscle glycogen content of KLF3 knockout mice and wild type mice. We found no significant difference in the glycogen content of skeletal muscle of knock out and wild type mice either in the fed or fasted state.
Figure 26: Comparison of muscle glycogen in \textit{klf3}^{−/−} and wild type mice in fed and fasted state. Values are the mean ± s.e.m of 9-10 animals.

4.7 KLF3 knockout mice have higher liver glucose-6-phophatase activity compared to wild type mice in the fed state.

The following graph shows the liver glucose-6-phosphatase activity in wild type and KLF3 knockout mice in the fed and fasted state.

Figure 27: Comparison of liver G-6-Pase activity in \textit{klf3}^{−/−} and \textit{klf3}^{+/+} mice in the fed and fasted state. Values are the mean ± s.e.m of 9-11 animals. *\textit{P} < 0.05 as compared with wild type mice.
Figure 27 illustrates that there is a significant difference in the glucose-6-phosphatase activity between wild type and KLF3 knockout mice in the fed state but not in the fasting state. In the fed state, KLF3 knockout mice had significantly higher glucose-6-phosphatase activity compared to their wild type litter mates.

4.8 Plasma glucagon levels of KLF3 knockout and wild type mice in the fasted state are not different.

Glucagon is a pancreatic hormone that acts to increase blood glucose levels when the blood glucose level falls. Plasma glucagon levels were measured by using an Enzyme Immunoassay (EIA) kit as mentioned in the methodology.

As shown in Figure 28, there was no significant difference in the plasma glucagon content of wild type and KLF3 knockout mice.

Figure 28: Plasma glucagon content of wild type and KLF3 knockout mice in the fasted state. Values are the mean ± s.e.m of 9-11 animals.
5. Discussion

We compared the blood glucose levels of KLF3 knockout mice and wild type mice in this study and observed that KLF3 deficient mice had significantly higher blood glucose levels in the fasted state. In the fed and fasted states in these experiments, mice were not food restricted and continued their normal diurnal food consumption pattern. Usually mice eat more during the night time and less during the day time. Therefore, mice sacrificed between 9pm and 10pm were considered as in the fed state whilst 4-5pm in a naturally fasted state. Our food intake data showed that there is a significant difference of food intake of wild type mice during the day time and night time. Wild type mice eat a significantly greater amount of food during the night time (fed state) than day time (fasted state). There is no significance difference of food intake of KLF3 knockout mice in fed and fasted state and they eat more food in fasted state than that of wild type mice.

According to our results, plasma insulin levels in KLF3 deficient mice are not significantly different from wild type mice in the fed state but both groups had higher insulin level than their fasted state. KLF3 lacking mice had higher plasma insulin level in the fasted state. Insulin hormone action is arguably more important during the fed state as it is responsible for lowering blood glucose levels to normal after a meal. The reason for having significantly higher plasma insulin level in KLF3 knockout mice in fasted state could be because they eat more food in the afternoon which we considered as a fasted state in natural feeding. Higher food intake can increase the plasma insulin level in order to reduce the glucose level. Therefore higher food intake of KLF3 knockout mice in afternoon (fasted state), can lead to higher insulin level responding to their higher blood glucose level. By this way, our insulin results of KLF3 knockout mice comply the observation of higher fasting plasma glucose level of them. We also investigated other mechanisms that could potentially result in increased fasting glucose levels. Therefore we hypothesized that the higher fasting blood glucose levels in KLF3 knockout mice could be due to increased glycogen breakdown, increased gluconeogenesis or increased glucagon levels in the fasted state.

Results from this study demonstrated that KLF3 knockout mice are smaller in body weight than that of wild type mice. Moreover, according to previous findings of our lab, KLF3 deficient mice have smaller organs as a percentage of body weight compared to wild type mice.
According to our results, liver glycogen in the fed state in KLF3 knockout mice was lower than that of wild type mice suggesting that wild type mice may have higher liver glycogen storing capacity. There was no significant difference in glycogen content of KLF3 knockout mice and wild type mice in the fasted state but KLF3 knockout mice eat more during afternoon where we considered as the fasted state. Even though knockout mice have smaller body weight, still the results remain unchanged as the values shown here are corrected for liver weight. The lower glycogen content of KLF3 knockout mice reflects that they may have reduced storage capacity of glycogen in the liver also. Other reasons that may explain these results are that KLF3 knockout mice break down liver glycogen at a faster rate than wild type mice or wild type mice eat more and store more than knockout mice during the fed state and have higher activity of glycogen synthetic enzymes like glycogen synthase or lower activity of glycogen breakdown enzymes such as glycogen phosphorylase or lower glucagon levels responsible for glycogen breakdown.

In studies by Chen et al. (1992), mice followed a diurnal eating pattern and both lean and gold thiogluucose induced (GTG) obese mice had more liver glycogen during the night time lowering towards morning and day time irrespective of the fact that they were lean or obese. Our ‘fed’ glycogen stores may not be at same capacity, although the levels seem consistent with the findings of the Chen et al. (1992) study. Our results may differ because of a slight change and time shift for our results (lights of Animal house of School of Molecular Bioscience are off at 6pm, theirs are at 8pm).

There may be a different picture of liver glycogen levels at complete 16hour fasted state in wild type and knockout mice. In a completely fasted state (16hr in which no food is available), liver glycogen stores would be depleting at faster rate and we could observe how fast wild type and knockout mice use liver glycogen. In a completely fasted state and the point where food consumption is lower, the body is physiologically in two different conditions which can affect the switch between different metabolic pathways.

Diurnal glycogen depletion and glucose release of the body can be affected by signals from the central nervous system. La Fleur (2003) has reported that the daily rhythm of plasma glucose concentration depends on the biological clock, which is located in the hypothalamic suprachiasmatic nucleus (SCN). Therefore the daily fluctuation in plasma glucose concentration describes a clear 24-hr rhythm (La Fleur, 2003). These fluctuations depend on food intake,
hepatic glucose production and/or changes in glucose tolerance. Mice, like other rodents, are active at night and their plasma glucose and insulin levels are higher during the night corresponding to greater food intake (La Fleur, 2003).

Unlike in the liver, we found no significant difference in red skeletal muscle glycogen content in KLF3 knock out and wild type mice either in the fed or fasted state. According to Chen et al. (1992) experiments performed in GTG obese and lean mice, there was no significant difference in muscle glycogen content at any point of study despite the constant hyperinsulinemia or hyperglycemia. This observation could be because this is the maximal capacity of which muscle can store glycogen or that muscle glycogen metabolism is independent of insulin and glucose concentration (insulin resistance) (Chen et al., 1992). However, in both groups muscle glycogen content demonstrated a strong diurnal rhythm that related to the pattern of food consumption in lean animals and the glycogen content in muscle from lean and GTG obese mice decreased significantly during the post absorptive phase (12:00h – 18:00h) (Chen et al., 1992).

As previously mentioned, in these experiments, fasted state was considered at the time point when mice consume least food compared to rest of the day. So that was not the complete fasted state in which mice depleted most of blood glucose, liver glycogen and switch to the peripheral muscle glycogen metabolism as a source of glucose for muscle. Therefore it is not a surprise that both knockout and wild type mice have more or less similar glycogen levels at the fed and fasted state.

Next we aimed to measure liver glucose-6-phosphatase activity, because this is the enzyme which breaks down glucose-6-phosphate to glucose in gluconeogenesis and glycogenolysis processes resulting in an increased release of glucose into the circulation. This enzyme is present in the liver and kidneys. As mentioned in the methodology, glucose-6-phosphatase activity was measured using a radioactive labeled isotope protocol. Before applying the methodology to measure glucose-6-phosphatase enzyme activity in actual liver samples, the conditions were optimized using different volumes of a test liver sample. The aim of optimization was to determine the optimal reaction time in order to obtain consistent and reliable results. As shown in Figure 18 (3.10.1 section), as the reaction time increased, glucose-6-phosphatase enzyme activity markedly increased and became more or less constant after 10 minutes. At the minus 5 minute time point, the reaction terminating solution (FeCl₃) was added to samples 5 minutes before the
assay buffer. Therefore the reaction was unable to proceed. Moreover, the zero time point indicates the point at which FeCl₃, and assay buffer were added to sample at the same time again not having time for the reaction to begin. Our assay results indicate that maximum activity of the enzyme is measurable after 10 min, therefore, in the experiments we allowed the assay to proceed for 10 minutes in order to obtain accurate and consistent glucose-6-phosphatase activity measurements.

There was a significant difference in the glucose-6-phosphatase activity between wild type and KLF3 knockout mice in the fed state but not in the fasting state. In the fed state, KLF3 knockout mice had significantly higher glucose-6-phosphatase activity. This result complements our liver glycogen content findings (4.5 section) because in the fed state, liver glycogen content of wild type mice was significantly higher than that of KLF3 deficient mice. Glucose-6-phosphatase is an enzyme which breaks down glucose-6-phosphate into glucose when glucose is needed in the fasted state. Our results suggest that KLF3 knockout mice have lost the ability to lower glucose-6-phosphatase enzyme activity appropriately in the fed state, and this may be a contributing factor to the lower glycogen levels.

Gaedner et al. (1993) demonstrated that inhibition of glucose-6-phosphatase results from the action of insulin to control hepatic glucose production. According to our measurement of plasma insulin, we found no significant difference in plasma levels between KLF3 deficient and wild type mice in the fed state. In the fed state insulin is the responsible hormone, which stimulates postprandial blood glucose levels to return to normal. In the fed state, there is no or little activation of glucose-6-phosphatase under normal conditions, because glucose-6-phosphatase in an enzyme which facilitates glycogen breakdown. This is also reflected by our results. At fasted state, wild type mice have higher enzyme activity while at fed state they have lower enzyme activity which is as expected although not significantly different.

Not only glucose-6-phosphatase but other rate limiting enzyme such as PEPCK and pyruvate dehydrogenase are involved in the different glucose metabolic pathways such as gluconeogenesis and glycogenolysis. Guillaumond et al. (2010) reported that KLF10 knock out male mice exhibit hyperglycemia and the effect has been attributed to the direct repression of the proximal PEPCK gene promoter by KLF10.
There was no significant difference in the plasma glucagon content of wild type and KLF3 knockout mice in 16 hour fasted state. This finding confirms that the fasting blood glucose level of KLF3 knockout mice was not due to changed glucagon level.

When analyzing all above parameters in KLF3 knock out and wild type mice, it seems that the higher fasting blood glucose level along with increased plasma insulin level is not due to increased glycogen content, increased glucose-6-phosphatase activity or glucagon levels in the KLF3 knockout mice. Therefore there can be other mechanisms resulting in increased glucose level in the blood stream during the fasted state of KLF3 knockout mice. A potential mechanism could be gluconeogenesis which releases glucose into blood stream from non carbohydrate sources during fasted state. This could be tested by measuring gluconeogenesis precursors such as pyruvate, glycerol, lactate or rate limiting enzymes of gluconeogenesis such as pyruvate carboxylase, PEPCK and phosphoglycerate kinase. In overall, there is an effect of KLF3 on glucose metabolism in the fed state but in order to get a clear picture, further analysis should be done.

One of the main constraints of our study was the substantial time period needed to generate the required number of KLF3 knockout mice due to the fact that only ~17 percent of offspring are knockouts due to low survival rate because of developmental problems in the womb. If our sample size is too small, it would be difficult to find significant relationships from the data, as statistical tests normally require a considerable sample size to ensure a representative distribution of the population however, we were able to get minimum number of KLF3 knockout mice for the study after considerable time.

Lack of available and/or reliable data on the studies of Krüppel like factor 3 was another limitation for the study. Investigation of KLF3 function is a relatively new area or research and availability of literature is fairly limited. Sometimes, a lack of data or of reliable data would likely require us to limit the scope of our analysis or it could be a significant obstacle in finding a trend and a meaningful relationship. Furthermore, lack of prior research studies on the topic limits citing prior research studies and reduces the foundation for understanding the research problem under investigation. Due to time and financial constraints, I regret not including data on some other parameters listed under the future directions of research section, which are required for a better understanding to address the research question. There is need in future research to revise some methods for gathering data such as complete fasting over natural
fasting or both in order to show that there are any differences between those conditions on parameters measured. We also had to rely on some available pre-existing data which can be a constraint for the study. Longitudinal effect is another problem of our study. The time available to investigate a research problem and to measure change or stability within a sample is constrained by limited time.

Aim of the study was to find the underlying mechanism for increased fasting blood glucose level observed in KLF3 knockout mice. Results of the study showed that increased fasting glucose level is not resulted from increased hepatic glycogen content, increased glucose-6-phosgatase or increased glucagon hormone level. However, increased fasting blood glucose level can be resulted from increased gluconeogenesis. Therefore other possible parameters that can be analyzed in future research can include analyzing of different enzymes (PEPCK) (activity/concentration) and substrates (glycerol, pyruvate, and lactate) present during gluconeogenesis. Moreover, another area could be looking at them RNA expression levels of key enzymes involved in fatty acid oxidation [CPT 1 (carnitine palmitoyltransferase) and MCAD (medium-chain acyl-CoA dehydrogenase)] and gluconeogenesis [phosphoenolpyruvate carboxykinase (PEPCK) which converts oxaloacetate into phosphoenolpyruvate and carbon dioxide].

Glucose metabolism is a very complex inter-related metabolic process in the body and it can be affected by so many other factors at different levels such as GLUT4, antioxidant status, inflammatory factors (M1 and M2 macrophage phenotypes). KLF is a group of transcriptional factors and there could be inhibitory and activation effects of other KLFs on KLF3 which might be interesting in relation to glucose metabolism. Therefore other future research could be based on examining synergetic and antagonist effects of other transcriptional factors in KLF group on KLF3 in regulation of glucose metabolism.
6. Conclusions

These results suggest that KLF3 may play a role in glucose metabolism and contributes to the regulation of glycogen synthesis and/or degradation in the liver in the fed state.

However there is no difference in glycogen content of KLF3 knockout and wild type mice in the fasted state suggesting that increased fasting blood glucose levels in KLF3 deficient mice is not associated with changes in glycogen content of the liver. Since KLF3 knockout mice have significantly lower glycogen content in their livers in the fed state than that of wild type mice, we conclude that KLF3 knockout mice have poor glycogen storage capacity and KLF3 may alter liver glycogen storage in the fed state. In addition, KLF3 does not appear to have any effect on skeletal muscle glycogen storage in the fed and fasted state.

Furthermore, KLF3 knockout mice have significantly higher glucose-6-phosphatase enzyme activity in the fed state than that of wild type mice implying that KLF3 may regulate the glucose-6-phosphatase activity in the fed state. Klf3−/− mice have more or less same glucose-6-phosphatase enzyme activity in the fed and fasted status indicating that glucose-6-phosphatase system is not suppressed in the fasted state in KLF3 knockout mice.

Plasma glucagon hormone responsible for glucose homeostasis was not affected by KLF3 deficiency. However, increased insulin level in KLF3 knockout mice in fasted state partly explained by the higher food intake. Therefore overall, increased fasting blood glucose level of KLF3 knockout mice does not appear to be associated with increased liver glycogen content, increased glucose-6-phosphatase enzyme activity and/or increased glucagon level.
References


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