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The consequences of the loss of Glyoxalase 1 on cellular metabolism

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Table of Contents

1. Introduction

1.1 Diabetes

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia which arises from defects in insulin action, insulin secretion, or both. It has reached epidemic proportions and its prevalence has risen substantially over the years accounting for 537 million people living with diabetes worldwide in 2021 (International Diabetes Federation, 2021).

The first standardized classification and definition of diabetes mellitus was made by the National Diabetes Data Group in 1979. This classification classified diabetes in two major types: insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM). The discovery of other types of diabetes forced this classification to be updated identifying four types of diabetes: type 1 diabetes (formerly IDDM), type 2 diabetes (formerly NIDDM), gestational diabetes and “other types” (Mayfield, 1998; Solis-Herrera et al., 2000).

Type 1 diabetes (T1D) is considered an autoimmune disease in which insulin-producing pancreatic β-cells are destroyed resulting in an absolute deficiency in insulin production (Daneman, 2006; Guthrie & Guthrie, 2004). Type 2 diabetes (T2D) is characterized by the inability to respond to insulin effectively and unlike T1D, is more prevalent in patients over 45 years of age. It is highly related to the diet and lifestyle of an individual and typically develops in obese individuals with a body mass index (BMI) of greater than 30kg/m^2. As the global rates of obesity have tripled since 1975, so have the incidences of T2D, accounting now for 90% of diabetes cases (Vijan, 2019).

1.2 Type 2 Diabetes

1.2.1 Epidemiology and prevention

The increased tendency of obesity, sedentarism, and the “Western-style” diet has resulted in an extraordinary increase in the number of patients with T2D. Incidence and prevalence of T2D can vary according to geographical region with it increasing faster in low-income and middle-income countries as compared to high-income countries (B. Zhou et al., 2016). For example, areas in China and India are suffering for dramatically increases in its prevalence, despite the relatively low prevalence of obesity (Hu, 2011). Epidemiological studies have improved our understanding of the biological and lifestyle risk factors for T2D. The single most important risk factor is increasing adiposity, which is generally associated with increased BMI levels (J. M. Chan et al., 1994; Colditz, 1995). Diet and physical activity are both key behavioural risk factors for the development of T2D (Grøntved et al., 2012; Ley et al., 2014). Genetics also play an important role, as T2D has a tendency to cluster in families (Barnett et al., 1981; Hemminki et al., 2010). However, the strongest risk for the development of T2D is obesity (Y. C. Wang et al., 2011).

1.2.2 Diagnosis and classification

Diagnostic criteria for T2D have been primarily based upon blood glucose levels. Fasting blood glucose ≥100mg/dL or ≥140mg/dL collected two hours after an oral glucose tolerance test are an indicator of a prediabetic state, whilst a fasting blood glucose of ≥126 mg/dL or ≥ 200mg/dL in a glucose tolerance test are indicators of impaired glucose tolerance and established T2D. Glycated haemoglobin or HbA1c has been added as a surrogate marker for long-term glycaemia. In healthy

individuals HbA1c accounts for ≤ 5.7%, whilst in diabetic patients values will go above 6.5% (Khan et al., 2019).

In the last decade, evidence has emerged showing that the considerable heterogeneity within the diabetic patients is not reflected by the traditional classification system. This classification is particularly relevant to T2D, given that its heterogeneous phenotype could result in types of diabetes which cannot be easily assigned to any other diabetic type. Several international studies have performed data driven cluster analysis identifying five diabetic subgroups based on six variables: age at diagnosis, BMI, HbA1c, β-cell function (HOMA2-B; homeostasis model assessment 2), insulin resistance (HOMA2-IR) and glutamate decarboxylase autoantibodies (GADA). These five novel subgroups are: (i) severe autoimmune diabetes (SAID), which includes patients with an early onset of the disease, a low BMI, presence of GADA, insulin deficiency and poor metabolic control; (ii) severe insulin-deficient diabetes (SIDD), which includes patients with similar characteristics of SAID but without the presence of GADA; (iii) severe insulin-resistant diabetes (SIRD), which includes patients with high insulin resistance or HOMA2-IR and a high BMI; (iv) mild obesity-related diabetes (MOD), which includes patients with a high BMI (obese patients) but without insulin resistance; and (v) mild age-related diabetes (MARD), which includes patients with similar characteristics to MOD but with higher age at diagnostics and a possibly lower BMI (Ahlqvist et al., 2018; Dennis et al., 2019; Pigeyre et al., 2022; Slieker et al., 2021; Zaharia et al., 2019; Zou et al., 2019).

1.2.3 Consequences and complications of T2D

The chronic metabolic imbalance in individuals with T2D puts them at high risk for long-term vascular complications, which include macrovascular (cardiovascular comorbidities) and microvascular (nephropathy, retinopathy and neuropathy) complications (DeFronzo et al., 2015; Forbes & Cooper, 2013; Harding et al., 2019).

There is an increased risk of cardiovascular disease (CVD) in diabetes, which includes coronary artery disease, stroke and peripheral arterial disease. The common mechanism in macrovascular complications of diabetes is the process of atherosclerosis, leading to narrower arterial walls (Fowler, 2008). CVD is the most prevalent cause of mortality in diabetic populations (Matheus et al., 2013).

Diabetic nephropathy represents the major cause of end-stage renal failure and it is characterized by albuminuria and a progressive decline in renal function, showed by a decreased in the glomerular filtration rate. Diabetic nephropathy is reported to occur in 20-50% of diabetic patients and it is typically associated with hypertension and increased cardiovascular morbidity and mortality (Selby & Taal, 2020).

Diabetic retinopathy is characterized by vascular abnormalities in the retina and it can be divided in two stages: non-proliferative diabetic retinopathy (NPDR) or early stage; and proliferative diabetic retinopathy (PDR) or late-stage. NPDR is characterized by increased vascular permeability and capillary occlusion, leading to haemorrhages and microaneurysms, whilst PDR is characterized by neovascularization, leading to severe vision impairments (W. Wang & Lo, 2018).

Diabetic neuropathy is defined as a neurodegenerative disorder in which the peripheral and autonomic nervous systems are damaged leading to nerve dysfunction. It is normally characterized by numbness, pain, tingling and weakness (Feldman et al., 2019).

The medical and socioeconomic burden of diabetes is a consequence of these complications. For instance, diabetic retinopathy is the leading cause of blindness in adults and diabetic nephropathy accounts to over half of patients in dialysis or qualified for kidney transplants (Guthrie & Guthrie, 2004), whilst diabetic neuropathy is leading cause of foot ulceration and lower-limb amputation, which are independently associated with an increased risk of mortality (Sloan et al., 2021).

The development of long-term complications of diabetes can be delayed or prevented by the management of hyperglycaemia.

1.2.4 Management and treatment

The treatment of T2D ranges from lifestyle measures to pharmacological management with insulin or antidiabetic drugs, with the common goal of lower blood glucose levels. The management of T2D is complicated by multiple pathophysiological disturbances, resulting in the need of a combination therapy of different antidiabetic agents and a patient-centred approach.

Lifestyle measures range from dietary to exercise recommendations. Dietary approaches focus on the introduction of Mediterranean diets or an almost vegetarian diet. Increased vegetables and dietary fibre, as well as the reduced intake of refined sugars, saturated fats (especially animal fats) and industrial meals are the most recommended dietary changes. Reduced alcoholic intake and the cessation of smoking are also part of the guidelines. Apart from dietary measurements, T2D treatment is also based on an increase in physical activity. The type of exercise recommended is decided on an individual basis, but it encloses aerobic endurance training and strength training to build and maintain musculature. The recommended amount of exercise for individuals suffering from T2D is 150 minutes of moderate intensity exercise per week (Asif, 2014; Landgraf et al., 2019; Pfeiffer & Klein, 2014). Even though lifestyle measurements help to improve glucose levels and cardiometabolic health, they normally have to be accompanied by pharmacological therapy.

Antidiabetic medications normally used for the treatment of T2D are: (i) metformin, which acts by suppressing hepatic glucose production; (ii) sulfonylureas and meglitinides, which act increasing insulin secretion; (iii) thiazolidinediones, which act as insulin sensitizers by enhancing insulin action in different tissues; (iv) dipeptidyl peptidase 4 (DPP4) inhibitors and glucagon-like peptide 1 (GLP1) receptor agonists, which act modulating GLP1 levels; (v) alpha-glucosidase inhibitors (AGIs), which slow the rate of carbohydrate absorption in the intestine; (vi) sodium/glucose co-transporter 2 (SGLT2) inhibitors, which block renal glucose absorption; and (vii) insulin, which is utilize when the already mentioned antidiabetic drugs fail to normalize hyperglycaemia (DeFronzo et al., 2015).

A disadvantage of combination therapy is the risk of side effects. Some of the side effects from antidiabetic drugs include hypoglycaemia, increased body weight, lactic acidosis, fluid retention, nausea and vomiting. The complexity and adverse effects of diabetes pharmacological therapies can substantially reduce the quality of life (DeFronzo et al., 2015). A more patient-centred approach and the develop of drugs that restore normoglycaemia by targeting specific pathogenic defects could improve the clinical management of the disease, but a deeper understanding of the molecular mechanisms of T2D and its complications is needed to establish novel pharmacological concepts (Stumvoll et al., 2005).

1.3 Pathophysiology of T2D

T2D is categorized as a multifactorial disorder associated with genetic and environmental factors. It is characterized by β-cell dysfunction, insulin resistance, decreased glucose utilization, abnormal hepatic glucose production and chronic inflammation (Rehman & Akash, 2017).

It is well defined that T2D clusters in families (Meigs et al., 2000; Poulsen et al., 1999). The concordance rate of T2D in monozygotic twins is about 70%, while in dizygotic twins is 20-30% (Kaprio et al., 1992). The lifetime risk for the development of T2D for an individual with one parent suffering for T2D is 40% and it rises to 70% when both parents are affected by the disease (Tillil & Köbberling, 1987). The prevalence of T2D varies widely among populations and, although the ethnic variability could be explained by genetic differences, it is significantly influenced by environmental and cultural factors. For example, a significant proportion of the previously explained heritability could be attributed to the heritability of obesity rather than diabetes.

Regardless of the genetic or environmental factors, the first abnormality seen in individuals who develop T2D is insulin resistance (Martin et al., 1992). However, the onset of T2D will only occur if β-cells are unable to produce enough insulin to overcome insulin resistance (Ferrannini & Mari, 2014). It has been reported that patients with T2D show a decrease of about 40-60% in β-cell mass (Butler et al., 2003; Rahier et al., 2008). However, it remains unknown how much β-cell mass is needed to maintain glycaemia. Removal of half the pancreas has been shown to have only a small effect on glucose tolerance (Menge et al., 2008), thereby suggesting that even though β-cell mass plays a role in the development of T2D, the actual loss in β-cell function is the most critical factor.

Obesity, inflammation, lipotoxicity and glucotoxicity are some of the factors that could led to β-cell dysfunction (Cerf, 2013). Inflammatory responses, from proinflammatory cytokines or hyperglycaemia-induced oxidative stress, can lead to β-cell death and dysfunction by targeting the mitochondria and energy production (Gurgul-Convey et al., 2011; Lenzen, 2008; Simmons, 2007). Long-term exposure to free fatty acids or saturated fat can also lead to β-cell loss and dysfunction (Yaney & Corkey, 2003). Obesity is characterized by inflammation, increased adiposity and the ability to desensitize glucose recipient organs to insulin, therefore it is strongly associated to β-cell dysfunction. Moreover, because it also increases insulin demand, it can lead to the hyperfunction and exhaustion of β-cells, resulting in the loss of function (Eguchi et al., 2012).

The progressive decline in insulin secretion as a result of β-cell dysfunction and the reduced insulin sensitivity caused by obesity in insulin-responsive tissues, such as liver, skeletal muscle or adipose tissue, can lead to insulin resistance. Multiple abnormalities can contribute to insulin resistance in different tissues. In muscle, it is driven by defects in insulin signalling, glucose phosphorylation or transport, pyruvate dehydrogenase complex activity, mitochondrial oxidative activity and glycogen synthesis (Samuel & Shulman, 2012). In the liver, increased gluconeogenesis would play the major role in the increase of basal glucose production and fasting hyperglycaemia, contributing to insulin resistance (Magnusson et al., 1992).

1.4 Insulin signalling and resistance

In order to understand how insulin resistance develops in patients with T2D, it is critical to understand the effects of insulin and its signalling pathway. Insulin was first discovered in Toronto in 1921 and was accompanied by the first successful treatment of a T2D in 1922 (Fralick &

Zinman, 2021). Insulin is a potent anabolic agent that will promote the uptake, storage and synthesis of nutrients and will block their catabolism. In general terms, insulin promotes protein and lipid synthesis and the storage of glucose as glycogen. On the other hand, it inhibits lipolysis and gluconeogenesis. Insulin also participates in the regulation of gene expression, proliferation and survival (Saltiel, 2021).

Insulin binds to the α subunits of the insulin receptor (IR) causing a conformational change in the β subunits that will result in tyrosine kinase activation, starting a phosphorylation cascade that transduces the insulin signal within the cells. Insulin will activate two main signalling cascades: the phosphatidylinositol-3-kinase (PI3k)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) pathway and the mitogen-activated protein kinase (MAPK) signalling pathway. The PI3k/Akt/mTOR pathway leads to the metabolic effects of insulin, whilst activation of the MAPK signalling pathway regulates the mitogenic effects of insulin (Ortiz-Huidobro et al., 2021; Saltiel, 2021).

Akt acts as the central node in the insulin signalling cascade. The complete activation of Akt requires its phosphorylation in two different sites: Threonine (Thr) 308 and Serine (Ser) 473. Phosphoinositide-dependent kinase-1(PDK1), a downstream target of PI3k and phosphatidylinositol (3,4,5)-trisphosphate (PIP3), phosphorylates Akt in the Thr308 residue, and mTORC2 (mTOR complex 2), is responsible of the phosphorylation in the Ser473 residue. Once activated, Akt participates in the phosphorylation and inhibition of glycogen synthase kinase-3 beta (GSK3β) which prevents it from phosphorylating and inhibiting glycogen synthase, thereby stimulating glycogen synthesis. Through the inhibition of GSK3, Akt also inhibits the degradation of sterol regulatory element-binding proteins (SREBPs), enhancing lipid production. Another direct target of Akt is mTORC1 (mTOR complex 1), which is activated by the Akt-induced degradation of TSC (tuberous sclerosis complex)-2 and TSC-1 and the Akt phosphorylation and inhibition of PRAS40 (proline-rich Akt substrate of 40 kDa), an inhibitor of mTORC1. mTORC1 then phosphorylates and inhibits 4EBP-1 (eukaryotic translation initiation factor 4E-binding protein 1) and activates protein S6 kinase (p70S6k), resulting in increased protein synthesis. P70S6k can also activate SREBP1, thereby promoting lipid synthesis. Akt can also phosphorylate and activate phosphodiesterase 3B (PDE3B), resulting in decreased cyclic adenosine monophosphate (cAMP) levels and decreased lipolysis. Akt can phosphorylate the members of the forkhead box protein O (FoxO) family in several sites, which leads to the exclusion of the FoxO proteins from the nucleus blocking its transcriptional activity, which can lead to a decrease in hepatic glucose production. To further increase the complexity of the insulin signalling transduction pathway, there has been extensively research showing that there is a crosstalk between other signalling pathways. For example, Akt can activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and can block Erk (extracellular signal-regulated kinase) signalling through the inhibition of cRaf (Boucher et al., 2014; Manning & Cantley, 2007) promoting cell growth and survival (***Figure 1***).

Akt can also phosphorylate 5'-adenosine monophosphate-activated protein kinase (AMPK) α1 subunit in Ser485 promoting its inhibition (Hawley et al., 2014). AMPK acts as a major regulator of metabolism by modulating glucose metabolism, inflammation, ageing, redox regulation, lipid metabolism and protein synthesis (Jeon, 2016). AMPK inhibits lipid anabolism (de novo fatty acid, cholesterol and triglycerides synthesis) and activates lipid catabolism (promoting fatty acid uptake and inhibiting lipolysis) (CARLING et al., 1989; Habets et al., 2009; D. G. Hardie & Pan, 2002; Y. Li et al., 2011; Muoio et al., 1999). AMPK stimulates muscle glucose uptake, inhibits glycogen

synthesis and inhibits gluconeogenesis (Hunter et al., 2011; Koo et al., 2005; Zheng et al., 2001). AMPK inhibits protein synthesis by inhibiting cap-dependent translation or downregulating ribosomal RNA synthesis (Hoppe et al., 2009). AMPK can also regulate mitochondrial biogenesis by activating PGC1α, a cofactor that promotes the transcription of mitochondrial genes (O'Neill et al., 2011). AMPK can also regulate antioxidant defence during oxidative stress by upregulating several antioxidant genes such as the ones encoding superoxide dismutase and uncoupling protein 2 (Greer et al., 2007). Therefore, AMPK is an important target in the regulation of metabolism, but it can be severely dysregulated in chronic diseases such as obesity, inflammation, diabetes or cancer, depending upon the activation/inhibition status. The most well-defined mechanism for the activation of AMPK is via phosphorylation of Thr172 by AMP or ADP. ATP inhibits the binding of both AMP and ADP, and thereby AMPK can act as a sensor of AMP/ATP or ADP/ATP ratios. The activating phosphorylation on Thr172 is regulated by several kinases, such as liver kinase B1, protein phosphatase 2A and 2C, calcium/calmodulin-dependent kinase kinase 2, TGFβ-activated kinase 1 and Mg^{2+}/Mn^{2+}-dependent protein phosphatase 1E (D. Grahame Hardie et al., 2012; Joseph et al., 2015; S. Y. Kim et al., 2012; Voss et al., 2011). The inhibition of AMPK will occur by phosphorylation on Ser485. Diacylglycerol or DAG (whose levels increase in obesity or diabetes) can inhibit AMPK via Ser485 phosphorylation through protein kinase C (PKC) (Coughlan et al., 2016).

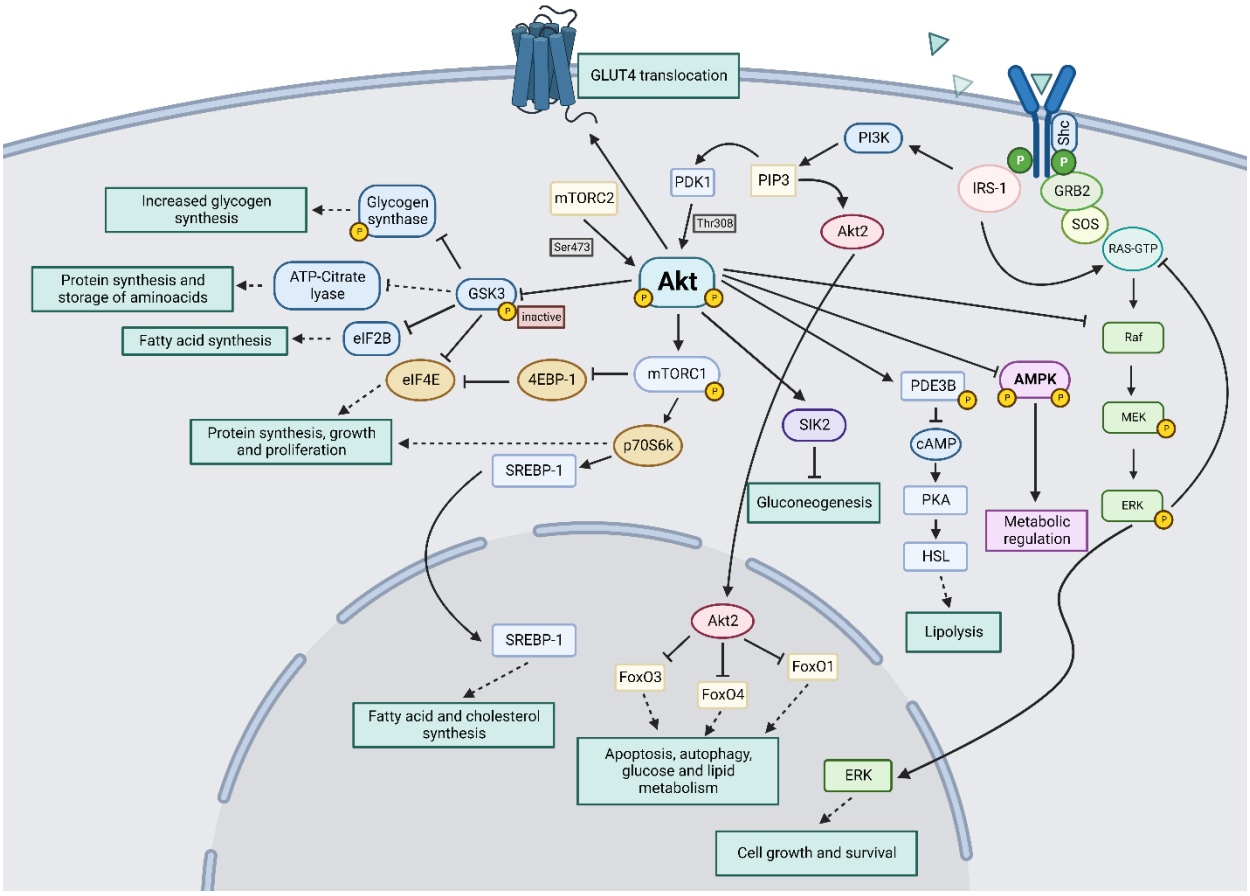


Figure 1. The insulin signalling pathway.

1.4.1 Molecular mechanisms of insulin resistance

The metabolic functions of insulin are directed by the PI3K/Akt pathway and therefore, it is the pathway associated with insulin resistance in T2D (Cusi et al., 2000). Insulin receptor substrate (IRS) proteins can be phosphorylated either on tyrosine residues, leading to activation, or in serine residues, leading to inhibition. An increase in serine phosphorylation of IRS is associated with insulin resistance (Copps & White, 2012). Several factors can contribute to this increase, including mitochondrial dysfunction, inflammation, activation of PKCs by ectopic fat and endoplasmic reticulum (ER) stress (**Figure 2**).

1.4.1.1 Mitochondrial dysfunction

The mitochondria is a double-membrane bound organelle found in eukaryotic cells. They play a number of vital roles, among which the most important is the production of energy, in the form of ATP, during oxidative phosphorylation. The heavily folded inner membranes of the mitochondria are called cristae and accommodate many copies of the respiratory chain components, which consist of NADH dehydrogenase (Complex I), succinate dehydrogenase (Complex II), cytochrome c oxidoreductase (Complex III) and cytochrome c oxidase (Complex IV). These complexes, together with ATP synthase (Complex V) form what is known as the electron transport chain (ETC), and is the machinery by which ATP is produced in a process known as oxidative phosphorylation. Briefly, the electron carrying substrates NADH and $FADH_2$ generated by citric acid cycle, enter the ETC via Complex I and II and are reduced to NAD and FAD, respectively. The electrons are then transferred to ubiquinone (Q), a soluble electron carrier found within the intermembrane space of the mitochondria. Ubiquinone is reduced to ubiquinol (QH_2) by Complex III and the electrons are transferred to cytochrome c, a water-soluble carrier. Complex IV then catalyses the final transfer of electrons to produce molecular oxygen (O_2) and water (H_2O). During this series of electron transfer, protons are translocated from the interior of the mitochondria or matrix into the intermembrane space, thereby creating an electrochemical proton gradient across the mitochondrial inner membrane, which is then used by complex V to produce ATP from ADP.

The ETC is an efficient system, however, the alternating one-electron oxidation-reduction reactions can lead to side-reactions with molecular oxygen and the production of the reactive oxygen species (ROS), such as superoxide, hydrogen peroxide and the hydroxyl radical, which can damage proteins, lipids and DNA. Cellular antioxidant defences, like glutathione peroxidase, glutathione reductase, superoxide dismutase (SOD) or catalase, maintain ROS levels and prevent oxidation of cellular molecules. Excess ROS can occur when ROS production overwhelms the cellular antioxidant capacity, leading to oxidative damage to the proteins, DNA and lipids in mitochondria (Schieber & Chandel, 2014). This will result in impaired enzymatic functions in the ETC and ultimately in mitochondrial dysfunction and reduced mitochondrial biogenesis, which has been associated with the development of cardiovascular (Ide et al., 1999; Madamanchi & Runge, 2007; Tsutsui et al., 2001), neurodegenerative (Reddy, 2008) and metabolic disorders (Cantó & Auwerx, 2009; Houstis et al., 2006; Mootha et al., 2003)

The association between impaired mitochondrial oxidative capacity and insulin resistance has been extensively reported, with T2D patients showing impaired mitochondrial function, decreased lipid metabolism and reduced $NADH_2$-O_2 oxidoreductase activity (Kelley et al., 2002; J.-Y. Kim et al., 2000). It has also been reported that in T2D patients, there is a downregulation of genes

involved in oxidative metabolism, such as the ones under the control of PGC1α (Mootha et al., 2003) and a decrease of 35% in ADP-stimulated mitochondrial respiration (Phielix et al., 2008). One possible cause-effect link between mitochondrial dysfunction and insulin resistance could be attributed to the impairment in metabolic fuel oxidation as a result of this decrease in mitochondrial oxidative capacity, leading to the accumulation of lipotoxic intermediates and the development of insulin resistance.

Another mechanism for this association is represented by ROS. ROS can directly affect insulin signalling by activating redox-sensitive serine kinases that phosphorylate and inhibit IRS proteins (Rains & Jain, 2011).

Altered mitochondrial function has also been associated with decreased levels of adiponectin. Adiponectin is a cytokine produced by adipocytes recognized as an insulin sensitizer, improving insulin sensitivity in major insulin target tissues as liver or muscle. The underlying mechanisms for this association involve the capacity of adiponectin to reduce plasma glucose levels, supress gluconeogenesis and activating AMPK (Berg et al., 2002; Yamauchi et al., 2002). Defective mitochondria can lead to a decreased expression of GLUT4 and decreased secretion of adiponectin, contributing to insulin resistance (C. Wang et al., 2013).

Although there have been several studies supporting the association between mitochondrial dysfunction and insulin resistance, what type of mitochondrial dysfunction is the direct cause or result of insulin resistance remains a topic of debate.

1.4.1.2 Inflammation

Systemic inflammation is a well-documented contributor to insulin resistance. Epidemiological studies have shown a correlation between increased levels of inflammation markers as fibrinogen, plasminogen activator inhibitor-1 (PAI-1), C-reactive protein (CRP), sialic acid, IL-6 and white cell count with the development of insulin resistance in T2D (Festa et al., 2002; Pradhan, 2001; Prince et al., 1981; Schmidt et al., 1999; Vozarova et al., 2002).

Investigating the intracellular pathways activated by inflammation, has shown that the majority of pro-inflammatory stimuli can activate JNK1 (JUN amino-terminal kinase 1) and IKKβ (IκB kinase-β) pathways. Toll-like receptors (TLRs), which are an important component of innate immune response are activated by altered lipid metabolism, can also activate JNK1 and IKKβ pathways. Activation of JNK1 and IKKβ/NF-κB pathways can lead to insulin resistance by the phosphorylation of serine residues and the consequent inhibition of IRS proteins (Aguirre et al., 2000; Cai et al., 2005; Hirosumi et al., 2002). Concordantly, the genetic or pharmacological inhibition of JNK1 or IKKβ/NF-κB pathways can improve insulin resistance and improve glycaemic control in vitro and in vivo (Arkan et al., 2005; Goldfine, 2010; Yuan et al., 2001).

1.4.1.3 Lipid-induced insulin resistance

The two most studied mediators for lipid-induced insulin resistance are DAG and ceramides. Increased DAG levels can induce insulin resistance by activation of members of the PKC family in muscle (PKCθ) and liver (PKCδ and PKCε). PKC can directly phosphorylate serine residues of the IRS, especially of IRS-1, resulting in its inhibition and reduced downstream signalling (Schmitz-Peiffer & Whitehead, 2003). PKC can also enhance JNK1 and IKKβ, further increasing the serine phosphorylation of IRS (Jaeschke & Davis, 2007; Khoshnan et al., 2000). The levels of the PKC isoforms in muscle and liver have been reported to be increased in T2D and obesity

(Bezy et al., 2011; Morino, 2005; Szendroedi et al., 2014). Consistently, the partial or complete loss of PKC ameliorates lipid-induce insulin resistance (Gassaway et al., 2018; J. K. Kim et al., 2004; Y. Li et al., 2004).

Increased ceramides have also been associated with insulin resistance. Ceramides can impair insulin signaling by decreasing the activation of Akt. Ceramides can increase phosphatase-2A activity, a known phosphatase in charge of the dephosphorylation and inhibition of Akt, and can impair Akt translocation resulting in a decrease activation on the insulin signalling cascade (Schubert et al., 2000; Stratford et al., 2004).

1.4.1.4 ER stress

The ER is the major site of synthesis, folding and transport of proteins and its main responsibility is to ensure that proteins achieve the adequate structure. Normally, unfolded or misfolded proteins are degraded by the ER, but when the folding capacity of the ER cannot overcome the high load of unfolded or misfolded proteins, it leads to ER stress and the activation of the unfolded protein response (UPR). The UPR involves the activation of three transmembrane proteins: inositol-requiring enzyme 1 (IRE-1), protein kinase R-like ER kinase (PERK) and activating transcription factor 6 (ATF6). The activation of these three proteins and their downstream pathways ultimately results in increased gene expression of chaperones or genes involved in the degradation or folding of proteins, apoptosis and decreased protein synthesis (Salvadó et al., 2015).

ER stress has been reported to lead to insulin resistance in different tissues by different mechanisms. In adipose tissue, increased levels of IRE-1, JNK and X-box binding protein 1 (XBP1s; which increase the transcription of UPR genes), are observed in obese patients (Boden et al., 2008). In in vivo models of HFD-induced inflammation, non-esterified fatty acids (NEFAs) can trigger ER stress, leading to the PERK-dependent increased expression of pro-inflammatory cytokines like TNFα or IL-6 contributing to the development of insulin resistance (Jiao et al., 2011; Kawasaki et al., 2012).

In the liver, ER stress contributes to insulin resistance by modulation of gluconeogenesis and lipogenesis. ER stress can activate gluconeogenesis though activation of the transcription factor CHREBH (carbohydrate-responsive element-binding protein), an ATF6 liver homolog, leading to an increase in the expression of G6Pase and PEPCK whereas activation of XBP1, FOXO1 is degraded and gluconeogenesis is reduced (Lee et al., 2010; Y. Zhou et al., 2011). Lipogenesis is activated by the PERK pathway, which activates SREBP1 and leads to lipid accumulation resulting in insulin resistance. mTOR, a regulator of SREBP1 expression, can also activate ER stress and promote insulin resistance and hepatic steatosis (Kammoun et al., 2009; H. Li et al., 2014).

In skeletal muscle, ER stress can promote insulin resistance by IRE1/JNK-dependent IRS serine phosphorylation (Hwang et al., 2012) and by NEFA-induced inflammation (Peng et al., 2011). Other mechanisms found to contribute to insulin resistance in skeletal muscle involve tribbles 3 (TRB3) and protein phosphatase 1B (PTP1B). TRB3 and PTP1B are both induced by ER stress and participate in the inhibition of the insulin signalling cascade by binding to Akt and blocking its activation (Du et al., 2003) and dephosphorylating insulin receptor β and IRS-1 (Dadke et al., 2000), respectively.

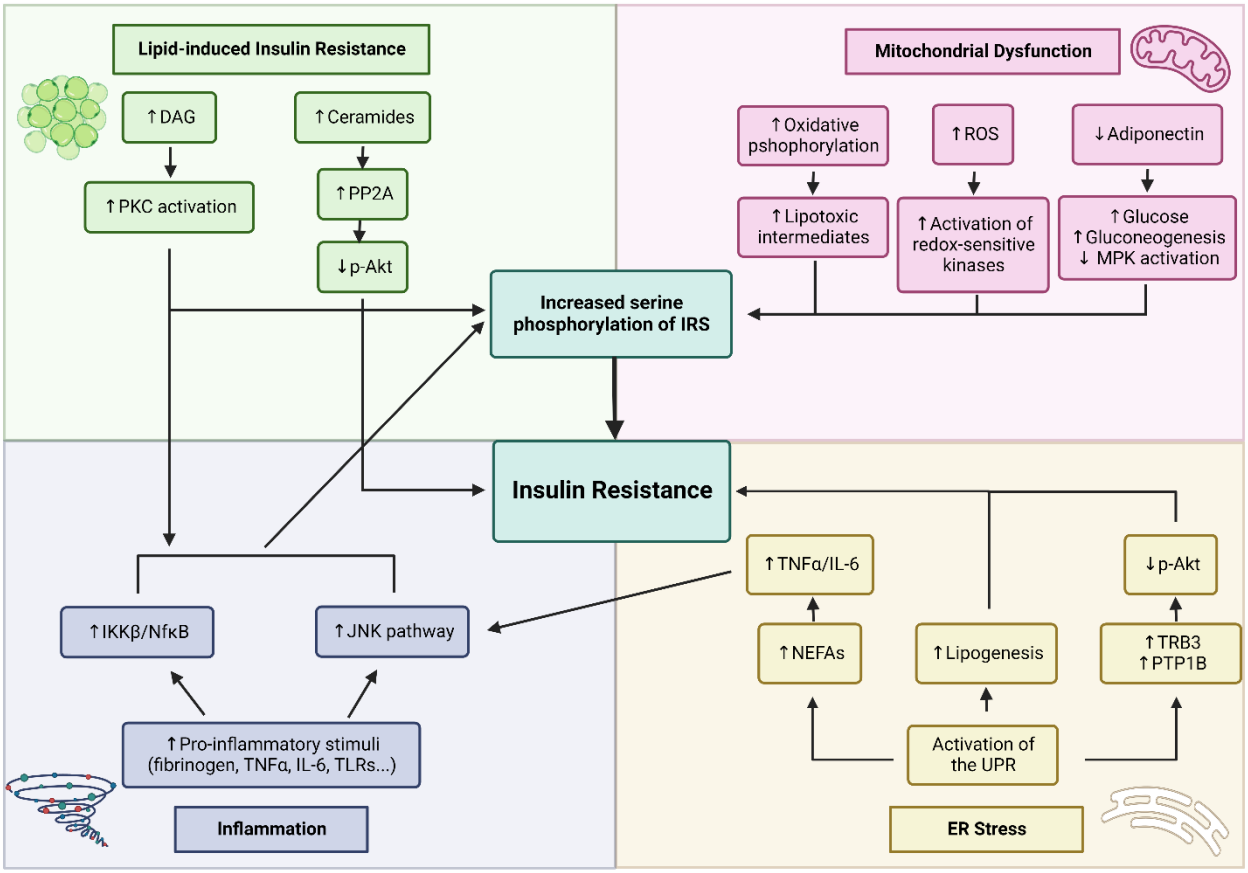


Figure 2. Mechanisms of insulin resistance in T2D. Created with BioRender.com.

1.4.1.5 Methylglyoxal as the driving force for insulin resistance

Even though it is generally accepted that these four factors can contribute to the development of insulin resistance, it is not yet known what happens at the onset of diabetes, that could lead to the activation of these pathways and consequently to insulin resistance. In the last decades several studies have focused on the study of methylglyoxal (MG), a highly reactive by-product of glycolysis which has been reported to be elevated in clinical and experimental models of diabetes. Interestingly, MG has been associated with inflammation, mitochondrial dysfunction, PKC activation and ER stress and thus could be the driving force at the onset of diabetes for insulin resistance.

1.5 Methylglyoxal in health and disease

1.5.1 Methylglyoxal formation and action

MG or 2-oxopropanal is an α-oxoaldehyde present in physiological conditions in all living cells (Paul J. Thornalley, 1993). MG is a highly reactive metabolite and it is considered the most potent glycating agent in humans (Glomb & Monnier, 1995; Moritsugu Shinohara et al., 1998; Smuda & Glomb, 2013). It is mainly formed as a by-product of glycolysis by the non-enzymatic degradation of the triose phosphates glyceraldehyde-3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP) (Phillips & Thornalley, 1993; Sousa Silva et al., 2013). The non-enzymatic formation of MG occurs in all cells and organisms under physiological conditions and it constitutes only the

0.1% of the glucotriose flux (Phillips & Thornalley, 1993; P J Thornalley, 1988). MG is present in three forms in aqueous solution: unhydrated (1%), monohydrate (71%) and dihydrate (28%), which are in dynamic equilibrium (Paul J. Thornalley, 1996). However, the majority of MG is found bound to proteins, peptides or amino acids (Lo et al., 1994).

The glycating ability of MG results in the modification of proteins, specifically arginine, lysine and cysteine residues, leading to the formation of advanced glycation endproducts (AGEs). The glycation of proteins is a complex of serial reactions commonly referred as the "Maillard Reaction". The major protein modification by MG is of arginine residues, forming the hydroimidazolone N_{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine or MG-H1, which accounts for >90% of AGEs (Rabbani & Thornalley, 2012; Westwood & Thornalley, 1995). MG-H1 protein modifications are often directed to functionally important arginine residues and therefore, contribute to loss of function (Ahmed et al., 2003; Rabbani & Thornalley, 2012). AGEs can also interact with the receptor of AGEs (RAGE), initiating cellular signals that lead to the activation of NF-κB and the transcription of proinflammatory factors (Daroux et al., 2010; T. H. Fleming et al., 2010; Grillo & Colombatto, 2008). MG can also react with deoxyguanosine in DNA, forming N^2-carboxyethyl-2'-deoxyguanosine (CeDG), the major MG-DNA adduct (Shuck et al., 2018).

1.5.2 Methylglyoxal in diabetes

In the context of diabetes, MG has been linked to the development of vascular dysfunction and microvascular complications in both experimental and clinical studies. Endothelial dysfunction in diabetes is characterized by inflammation, changes in vasoregulation, increased production of ROS and altered barrier function (Eringa et al., 2013). It has been reported that exogenous administration of MG in endothelial cells can induce diabetes-like vascular changes (Berlanga et al., 2005; Sena et al., 2012).

Elevated levels of MG have been associated with the onset and progression of diabetic kidney disease (Beisswenger et al., 2005; Hanssen et al., 2019; Lu et al., 2011; Nakayama et al., 2008; Rabbani & Thornalley, 2014a) and markers of renal function such as low eGFR or albuminuria (Hanssen et al., 2017; Jensen et al., 2016). MG has also been shown to play a significant role in the pathogenesis of diabetic retinopathy, with several studies linking elevated MG to the induction of apoptosis in pericytes and endothelial cells (Bento et al., 2010; C.-M. Chan et al., 2016; Padayatti et al., 2001). In the field of diabetic neuropathy, it has been found that MG-H1 levels are higher in STZ-induced diabetes sciatic nerves (Paul J. Thornalley et al., 2003) and the ADDITION-Denmark study proposed MG as a risk factor for the development of diabetic neuropathy (Andersen et al., 2018). Moreover, MG has been associated to promote diabetes-induced hyperalgesia (Bierhaus et al., 2012) and to induced apoptosis in Schwann cells by the activation of p38 MAPK (Fukunaga et al., 2004). The implication of MG in diabetes, diabetic complications and other age-related diseases has been extensively reviewed by Schalkwijk and Stehouwer in 2020 (Schalkwijk & Stehouwer, 2020).

1.5.2.1 Methylglyoxal and insulin resistance

Several studies have also reported that elevated MG levels can result in decreased insulin action. In vitro studies have shown that the administration of extracellular MG could directly inhibit the insulin dependent activation of PI3k, Akt and ERK, mainly by the inhibition of the tyrosine phosphorylation of IRS-1 (Deshmukh et al., 2017; Fiory et al., 2011; Jia & Wu, 2007; Nigro et al., 2014; Riboulet-Chavey et al., 2006)

MG has also been proposed as an inducer of mitochondrial dysfunction. MG administration in different cell types has shown to contribute to the loss of mitochondrial integrity by different mechanisms. MG administration can result in the increase of ROS and to oxidative stress (Hirosumi et al., 2002; Yuan et al., 2001), induce mitochondrial permeability transition (Jaeschke & Davis, 2007), reduce mitochondrial membrane potential and ATP production (Hirosumi et al., 2002; Schmitz-Peiffer & Whitehead, 2003) and directly decrease the activity and expression of several complexes from the electron transport chain (Cai et al., 2005; Prince et al., 1981). MG-induced glycation of the mitochondrial proteome and the subsequent functional impairment of mitochondrial proteins, has been proposed as the potential underlying mechanism linking elevated levels of MG with mitochondrial dysfunction and oxidative stress (Rabbani & Thornalley, 2008).

MG can contribute to inflammation and its effect in vascular inflammation in endothelial dysfunction has been extensively reviewed. MG can lead to inflammation by activation of JNK and p-38 pathways (Aguirre et al., 2000; Vozarova et al., 2002) and increased levels of cytokines as IL-6 have been observed under MG exposure (Festa et al., 2002). Exposure to MG can result in increased concentrations of intracellular Ca^{2+}, leading to the activation of classical PKC isoforms as PKCα or PKCβ by promoting their translocation to the plasma membrane (Hadas et al., 2013). Moreover, MG-associated AGEs can also lead to increased membrane activity of PKC (Ge et al., 2009).

Another detrimental effect of MG is its ability to induce ER stress. It has been reported that MG can stimulate the three pathways associated with ER stress and the activation of the UPR. MG can increase the expression and activation of PERK, IRE1 and ATF6, while also participating in inducing heat shock response, combinedly leading to ER stress (Irshad et al., 2019; Kırça & Yeşilkaya, 2022; Palsamy et al., 2014).

The capacities of MG to interact and activate molecular pathways which lead to the development of insulin resistance, would suggest that increased levels of MG could be a driving force for the development of insulin resistance in T2D (**Figure 3**).

1.5.3 Metabolism and detoxification of methylglyoxal

Due to the association of MG with the development of diabetes and associated complications (T. Fleming et al., 2012; Han et al., 2007; Kold-Christensen & Johannsen, 2020; Maessen et al., 2015; Matafome et al., 2017), as well as obesity (Hernandez-Castillo & Shuck, 2021; Matafome et al., 2013) and cancer (Bellahcène et al., 2018; Bellier et al., 2019; Paul J. Thornalley & Rabbani, 2011), the understanding of the metabolism and detoxification of MG has gained significant importance in the last decade. MG can be detoxified through different enzymatic networks such as aldo-keto reductases (AKRs) or aldehyde dehydrogenases (ALDHs), but the glyoxalase system is still considered the major enzymatic detoxification system for MG (***Figure 4***; Racker, 1951; Paul J. Thornalley, 1993).

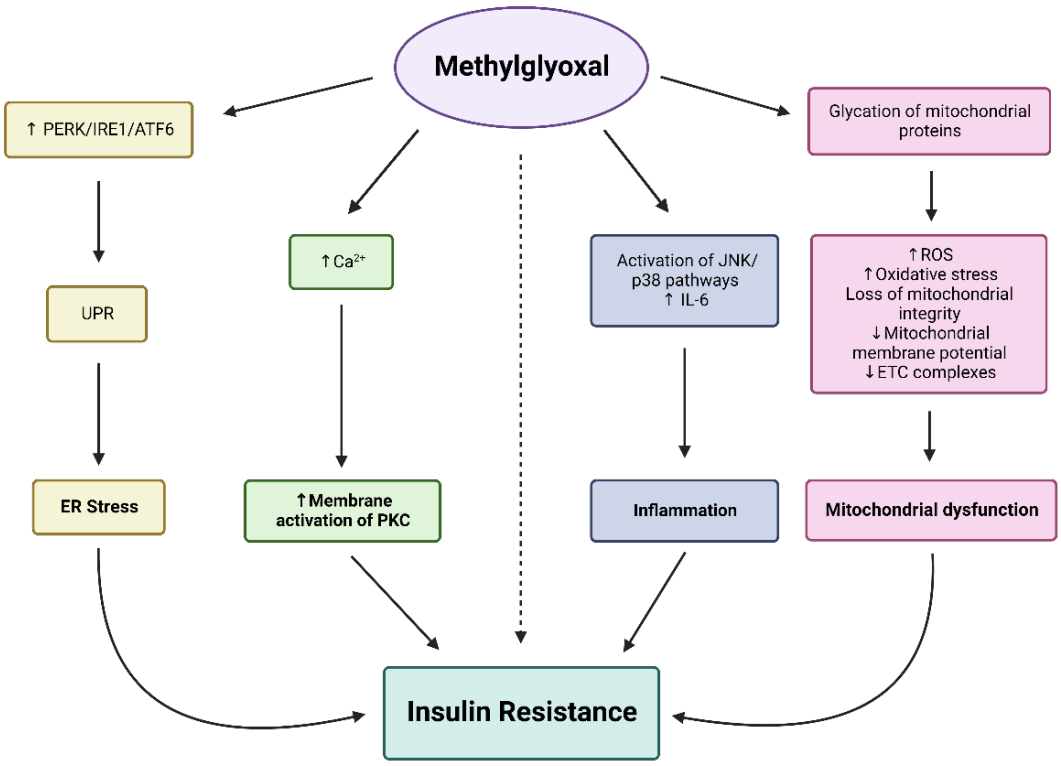


Figure 3. Methylglyoxal as a driving force for insulin resistance. Created with BioRender.com.

1.5.3.1 The Glyoxalase System

The glyoxalase system was first discovered independently by Dakin and Dudley (1913) and Neuberg (1913) and it was described as only one enzyme catalysing the conversion of MG to lactate (Dakin & Dudley, 1913; Neuberg, 1913). Further studies showed that the glyoxalase system is a ubiquitous enzymatic network that comprises two enzymes: glyoxalase 1 (Glo1) and glyoxalase 2 (Glo2), requiring a catalytic amount of reduced glutathione (GSH) (Carrington & Douglas, 1986; Racker, 1951; P. J. Thornalley, 1990). Glo1 catalyses the formation of S-D-lactoylglutathione from hemithioacetal, a compound formed spontaneously from MG and GSH (Mannervik, 1980). Glo2 catalyses the hydrolysis of S-D-lactoylglutathione to D-lactate, regenerating the GSH consumed in the reaction catalysed by Glo1 (Uotila, 1989). Both Glo1 and Glo2 are considered to be metalloproteins dependent on zinc in their active sites. However, it has been reported that Glo1 could act with alternative metals under physiological conditions (Mannervik, 2008).

Glo1 has been found to exhibit genetic polymorphism and Glo1 allele frequency has shown geographic variations which has been associated with the incidence of diabetes and its complications (Paul J. Thornalley, 1991). Glo1 has several regulatory elements: a metal-response element (MRE), an insulin-response element (IRE), an early gene 2 factor isoform 4 regulatory element (E2F4), an activating enhancer-binding protein 2α regulatory element (AP-2α) and an antioxidant response-element (ARE). Nuclear factor erythroid 2-related factor 2 (Nrf2) can regulate Glo1 expression by the ARE and it has been reported that Nrf2 activators such as

sulforaphane or resveratrol, can induce Glo1 activity. On the contrary, Glo1 expression can be downregulated by the activation of RAGE (He et al., 2020; Rabbani et al., 2014). Recent studies have highlighted the importance of Glo1 post-translational modifications, being able to show how phosphorylation of T107 in the Glo1 protein by the calcium/calmodulin-dependent Protein Kinase Type II delta (CamKIIδ) could be a precise and quick mechanism for the regulation of Glo1 activity (Morgenstern, Katz, et al., 2020).

The glyoxalase system is present in the cytosol of all living cells and its widespread distribution suggests that it could fulfil a fundamental function to biological life (Carrington & Douglas, 1986). However, recent reviews focusing on the complete loss of Glo1 in various model organisms and the alternative detoxification pathways of methylglyoxal have questioned the essential role of Glo1 on cellular metabolism (Morgenstern, Campos Campos, et al., 2020).

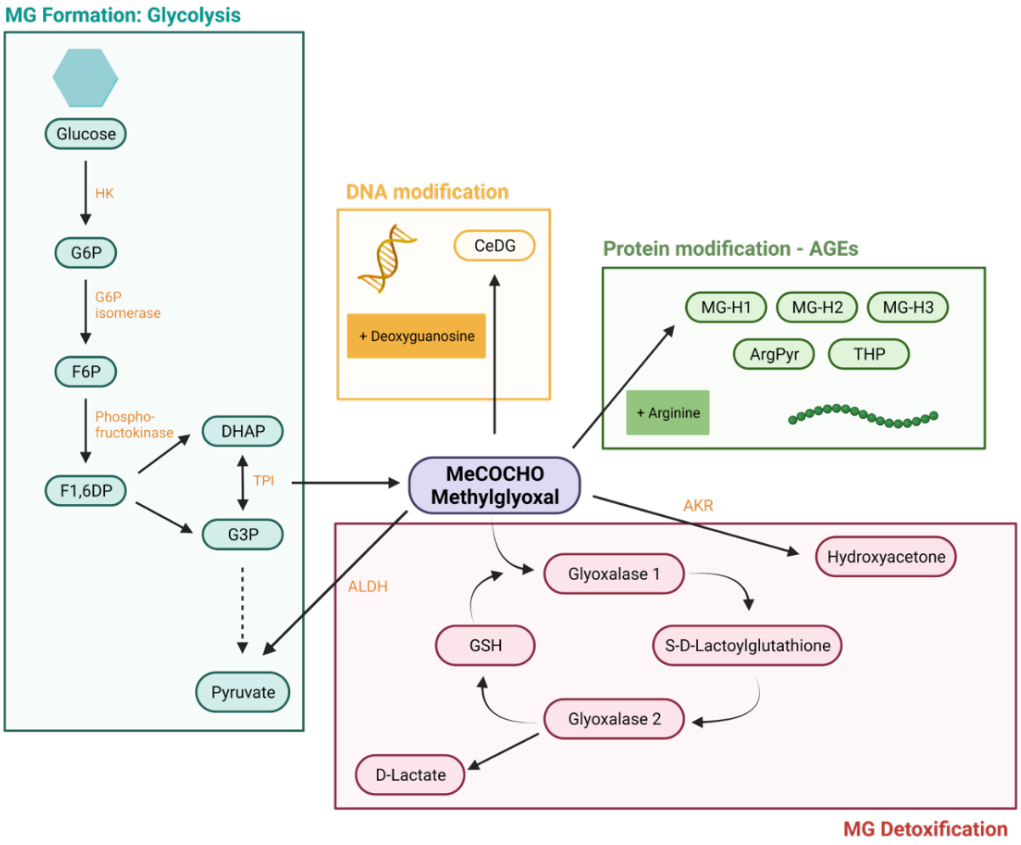


Figure 4. Summary of the main enzymatic pathways of MG formation, detoxification and reactivity to form glycation adducts. Created with BioRender.com.

1.5.3.2 Alternative detoxification pathways

There are three other pathways which have been reported to contribute to the detoxification of MG: (1) the AKR family; (2) the ALDH family, and (3) MG reductase (Vander Jagt et al., 1992). The majority of the studies have focused in AKR and ALDH families. AKR is a family of NADPH-dependent oxidoreductases that reduce various aldehydes and ketones. In mammalian organisms, AKR1 and AKR7 have shown the capacity to detoxify MG, resulting in 95%

hydroxyacetone and 5% D-lactaldehyde (Ko et al., 2005; Vander Jagt et al., 1992; Vander Jagt & Hunsaker, 2003). The ALDH family can catalyse the conversion of MG to pyruvate (Izaguirre et al., 1998; Monder, 1967; Vander Jagt & Hunsaker, 2003). However, these alternative pathways seem to gain importance only when the glyoxalase system is not capable to properly detoxify MG (Morgenstern et al., 2017; Schumacher et al., 2018).

1.6 Pathophysiology of Glyoxalase 1

Glo1, as the rate-limiting step of the glyoxalase system, has been extensively studied as a potential therapeutic target in age-related diseases. Glo1 activity and expression have been associated with the development of diabetes and diabetic complications, implying that a dysfunction in the glyoxalase system may play a role in the onset of the disease (Maessen et al., 2015; Rabbani & Thornalley, 2011). In models of T1D, Glo1 activity has shown to be modified in several tissues and blood when compared to control non-diabetic animals (Phillips et al., 1993). In T2D patients, skeletal muscle biopsies exhibit increased carbonyl stress and decreased Glo1 activity, which inversely correlated with BMI and HOMA-IR. This was also accompanied with lower Nrf2 expression, a positive regulator of Glo1 activity and expression (Mey et al., 2018).

Apart from its role in diabetes, Glo1 has also been described as a potential oncogene. Several studies showed an increased activity and expression of Glo1 in different tumour tissues, suggesting a positive correlation between Glo1 expression and increased tumour grade (FONSECA-SÁNCHEZ et al., 2012; Hosoda et al., 2015; Ranganathan & Tew, 1993). A possible explanation of the increased activity of Glo1 in cancer is as a consequence of the high glycolytic rates and therefore high MG production (Paul J. Thornalley & Rabbani, 2011). As such, Glo1 has been postulated as a novel diagnostic marker in cancer (Rounds et al., 2021). In order to understand the physiological relevance of Glo1 in different disease states, several studies have modulated Glo1 expression and activity in vitro and in vivo, using either pharmacological or genetic approaches.

In the context of cancer, Glo1 inhibitors have been used as potential chemotherapy because of the association of increased Glo1 activity and increased tumour progression. Al-Balas et al. recently reviewed the actual knowledge about available human Glo1 inhibitors. Glutathione based inhibitors were the first generation of inhibitors proposed by Vince et al. in 1969. Flavonoids, as quercetin or naringin, TLSC702, anthocyanidins, curcumin derivatives or nonsteroidal anti-inflammatory drugs, as indomethacin or zopolrestat, have been also reported as potent Glo1 inhibitors (Al-Balas et al., 2018). In a recent study performed in late-stage breast cancer patients, the inhibition of Glo1 by TLSC702 reduced cell viability and tumour-sphere formation improving cancer progression (Motomura et al., 2021). Other studies have shown that the inhibition of Glo1 could increase sensitivity to TRAIL, a cytokine playing a role in immune surveillance for cancer, and suggest that a combined therapy with TRAIL and MG or Glo1 inhibitors may be a useful novel chemotherapy (Taniguchi et al., 2012).

In contrast, in diabetes and vascular damage, the opposite approach of inducting Glo1 has been taken. Dicarbonyl stress plays a role as a driver of cardiovascular disease and therefore, the overexpression or induction of Glo1 has been postulated as a potential therapeutic treatment (Aragonès et al., 2020). Several studies have reported that the overexpression of Glo1 could prevent hyperglycaemia-induced AGE formation and macromolecular exocytosis in bovine endothelial cells (M Shinohara et al., 1998), prevent impaired arteriogenesis in diabetic rats (Brouwers et al., 2014) and improve cerebrovascular remodelling in basilar artery tissue during

hypertension (Gao et al., 2019). Several inducers have also been used in order to increase Glo1 activity. In a study performed in obese subjects, the co-administration of trans-resveratrol and hesperetin increased Glo1 activity by a 27%, decreased MG plasma levels by a 37% and total body MG-protein glycation by a 14%. It could also decrease plasma glucose by a 5% and increased oral glucose insulin sensitivity index and therefore was described as a suitable treatment for improved metabolic and vascular health in obese patients (Xue et al., 2016).

Several studies have also investigated the effects resulting from the knock-down (KD) or knock-out (KO) of Glo1 to observe the consequences of this defect and clarify its mechanism of action. The KD of Glo1 in endothelial cells resulted in significant accumulation of MG and MG-modified proteins which was associated with impaired angiogenesis, dysfunction and vascular damage (Nigro et al., 2019; Stratmann et al., 2016). In spontaneously hypertensive rats, the downregulation of Glo1 resulted in increased MG levels and dicarbonyl stress associated with increased AMPK activation and metabolic effects (Šilhavý et al., 2020). In mice, the KD of Glo1 could mimic diabetic nephropathy showing increased MG levels and MG modification of glomerular proteins, which were directly related to alterations in kidney morphology indistinguishable from the ones caused by diabetes (Giacco et al., 2014).

The complete KO of Glo1 however, produced controversial results. In *Drosophila melanogaster* the complete loss of Glo1, meaning no detectable activity or protein expression, produced a 50% increase in the levels of MG and MG-H1, increased glycogen storage and a 3-fold increase in circulating insulin. This was accompanied by impaired p70S6K phosphorylation resulting in insulin resistance, increased FASN activity and hyperglycaemia, but only after 5 weeks of age. The loss of Glo1, however, in flies did not result in increased AKR or ALDH activity or expression (Moraru et al., 2018). A comparable T2D-like phenotype was found in the Glo1-KO model generated in zebrafish. Glo1-KO zebrafish showed increased MG levels (1.3-fold), increased blood glucose and impaired insulin signalling by increased phosphorylation of p70S6k; but only after feeding and not in fasting conditions. They also studied the implication of overfeeding for 8 weeks, which resulted in increased fasting blood glucose, impaired insulin signalling by reduced phosphorylation of p70S6k and increased MG levels in postprandial conditions, which culminated in retinal alterations. Unlike in flies, this study could observe that ALDH could partially compensate for the loss of Glo1 in zebrafish (Lodd et al., 2019).

Both studies suggested that the complete loss of Glo1 recapitulates a T2D-like phenotype, with increased MG levels and insulin resistance. However, when the KO of Glo1 was studied in mammalian cells or mice, the effects were strikingly different. The loss of Glo1 was first studied in murine Schwann cells and it showed neither increased MG nor increased MG-H1 levels. In fact, this study revealed how in mammalian systems the loss of Glo1 could be compensated by an Akr1b3-efficient MG detoxification. In the Glo1-KO cells, it was shown that Akr1b3 could convert MG to hydroxyacetone or lactaldehyde (Morgenstern et al., 2017). Following this study, the loss of Glo1 was studied in mice and similar results were obtained; Glo1-KO mice did not show any significant increase in MG or MG-H1 levels and just as in Schwann cells, with the loss of Glo1 being compensated for by an increase in Akr1b3, in the kidney, and ALDH1a3, in the liver. Consistent with these changes, elevated levels of hydroxyacetone and decreased levels of D-lactate were found in the kidneys and the liver of Glo1-KO mice (Schumacher et al., 2018). Another study utilizing Glo1-KO mice had in focus the study of Glo1-related psychiatric disorders. In this study, they could observe a 2-fold increase in MG levels but only in liver and not in other tissues like brain (Jang et al., 2017).

The controversial results found between Glo1-KO species and also when comparing the effects of the KD of Glo1 and the total loss of Glo1 (***Table 1***) have put Glo1 in the centre of interest, as its effects and especially its actual implications and importance for cellular viability are not completely understood.

Organism	 *Nigro et al., 2019, BBA; Stratmann et al., 2016, Sci. Rep.*	 *Šilhavý et al., 2020, Antioxidants*	 *Giacco et al., 2014, Diabetes*	 *Moraru et al., 2018, Cell Metab.*	 *Lodd et al., 2019, JCI Insight*	 *Morgenstern et al., 2017, J Biol Chem.*	 *Schumacher et al., 2018, Mol Metab.*
Glo1 genotype	Glo1 KD	Glo1 KD	Glo1 KD	Glo1 KO	Glo1 KO	Glo1 KO	Glo1 KO
MG metabolism	MG and MG-H1 levels ↑3 fold	MG levels ↑2 fold in myocardium	MG-H1 levels ↑5 fold in kidney	MG levels ↑1.4 fold, MG-H1 levels ↑2 fold	MG levels ↑1.3 fold	Unaltered MG or MG-H1	Unaltered MG or MG-H1
Phenotype	Increased collagen expression, endothelial inflammation and dysfunction and apoptosis.	Reduced adiposity and ectopic fat accumulation, mediated by AMPK activation in the heart.	MG modification of glomerular proteins and oxidative stress. Mimics diabetic nephropathy.	Young flies: increased insulin. Adult flies: obese and hyperglycemic.	Hyperglycemia after diet induced-obesity Compensation Glo1 loss by ALDH	No obvious alterations Compensation of Glo1 loss by Akr and ALDH	No obvious alterations Compensation of Glo1 loss by Akr and ALDH

Table 1. Consequences of the KD or KO of Glo1 in different model organisms.

1.7 Aim of the study

Type-2 diabetes (T2D) is a heterogeneous and multifactorial disorder whose pathophysiology is not completely understood. The first impairment seen in individuals suffering from T2D is insulin resistance resulting from: (i) mitochondrial dysfunction; (ii) lipid-induced insulin resistance, (iii) endoplasmic reticulum stress; and (iv) inflammation. MG, a potent-glycating agent produced in physiological conditions as a by-product of glycolysis, has shown to be associated with the activation of the four above-mentioned impairments leading to insulin resistance. The effects of MG on cellular metabolism have been studied extensively and the modulation of its main detoxification enzyme, Glo1, has been used as a strategy to resemble increased levels of MG in diabetes. The knockdown of Glo1 in experimental murine models has been linked to the development of diabetic nephropathy and has shown increased MG levels. Moreover, the complete knockout of Glo1 (Glo1-KO) in organisms such as *Drosophila melanogaster* or zebrafish, produced a T2D-phenoype with insulin resistance, potentially as a cause of increased levels of MG. The aim of this study is to determine whether the loss of Glo1 in mice can also recapitulate at T2D phenotype. In order to address this aim, Glo1-KO mice will be compared to a wild-type subjected to high-fat diet, as model of T2D, and physiological parameters related to the onset on T2D studied.

2. Materials & Methods

2.1 Animals

2.1.1 Generation of CRISPR/Cas9-induced Glo1 Knock-out mice

Glo1 knockout (Glo1-KO) mice were generated using CRISPR-Cas9-mediated genome editing as previously described (Schumacher et al., 2018). Briefly, sgRNA 171 (Sigma) was injected into the cytoplasm of C57BL/6N zygotes together with Cas9 mRNA. Mice with an allele exhibiting an 8-bp deletion (Δ8) and a 7-bp deletion (Δ7) in the Glo1 gene were bred to obtain $Glo1_{\Delta 8/\Delta 8}$ and $Glo1_{\Delta 7/\Delta 7}$ homozygous mice. The absence of Glo1 activity in blood was also measured to ensure the correct genotyping.

2.1.2 Experimental Setup

Wild-type (WT) C57BL/6N mice were purchased from Charles River Laboratories (Wilmington, MA, USA). All mouse stains were maintained under standard laboratory conditions of 12 h light/12 h dark cycle (22°C, 55% humidity) and had access to food and water ad libitum. The WT mice were subject to either a standard chow diet or to a high-fat diet (HFD) (60 kcal% fat, #D12492, Research Diets Inc.), whilst the Glo1-KO mice reached a standard chow diet. Mice were kept under these dietary conditions for a period of 12 months. During this period, blood glucose and bodyweight were measured on a weekly basis. Blood glucose was measured from blood taken from the tail vein using an Accu-Check Aviva III (Roche) glucometer, according to the manufacturer's instructions. Intraperitoneal glucose and insulin tolerance tests were performed on a monthly basis. Mice (were starved for 12 or 4 h, respectively, then glucose (2 g/g body wt dissolved in 0.9% NaCl) or insulin (0.0005 units/g body wt dissolved in 0.9% NaCl) were injected intraperitoneally. Blood glucose was measured from tail vein blood at baseline and at 15 min, 30 min, 60 min, and 120 min after the intraperitoneal injection. After 12 months of monitoring, WT mice on the standard chow, WT mice on the HFD, and the Glo1-KO mice on the standard chow, were fasted overnight (ca.16hrs) and injected intraperitoneally with either PBS or insulin glargin (0.0005 units/g body weight dissolved in 0.9% NaCl, Lantus®, Sanofi). After 15 minutes, the mice were sacrificed using carbon dioxide and perfused with ice-cold PBS. Major organs were dissected and weighted and either flash-frozen in liquid nitrogen or preserved in paraformaldehyde (PFA) for further analysis. Blood samples were collected in EDTA tubes for isolation of plasma. All procedures were approved by the Animal Care and Use Committee at the regional authority in Karlsruhe, Germany (35-9185,81/G-295/15).

2.2 Measurement of Insulin Levels by ELISA

Plasma insulin was measured using the mouse ultrasensitive insulin ELISA commercial kit (Alpco) following manufacturer's instructions. Briefly, 5µl of plasma sample were added to each well of a 96-well plate pre-coated with a monoclonal antibody specific for insulin. Calibration curve was performed adding 5µl of standard insulin samples of known concentration (0.188 ng/ml, 0.5 ng/ml, 1.25 ng/ml, 3.75 ng/ml and 6.9 ng/ml). 75µl of Working Strength Conjugate was added to each well and the plate was covered and incubated for 2 hours at RT in an orbital shaker. Wells´ content was decanted and the microplate was washed 6 times with 300µl or washing buffer. Then, 100µl of TMB substrate were added into each well and the plate was sealed and incubated at RT for 30 minutes in an orbital shaker. Immediately afterwards, 100µl of stop solution were added to each well and the absorbance at 450nm was measured in a FLUOstar Omega microplate reader (BMG Labtech). Calibration curve was adjusted to a 5-parameter logistic fit and concentrations of

plasma samples were extrapolated. Data analysis was performed using MARS Data Analysis Software (v 3.01 R2, BMG Labtech).

2.3 Measurement of HbA1c by HPLC

Glycated haemoglobin (HbA1c) was determined by cation-exchange chromatography on a PolyCAT A column with absorbance detection. The relative amount of HbA1c was expressed as a percentage of haemoglobin, based upon the area under the respective peaks.

2.4 Assessment of Kidney Function

Urine was collected for 4 hrs using metabolic cages, and the total volume was determined. The albumin content of urine was measured using ICL Mouse Albumin ELISA, according to the manufacturer's instructions. Urine creatinine was determined by colorimetric assay (BioVision, Milpitas, CA) and ACR was subsequently calculated (Qi et al., 2005).

2.5 Tissue Histology

Tissue (liver, kidney and pancreas) were fixated in 4% formalin at 4°C for 24h. The fixated tissues were then embedded into paraffin and tissue sections (5µm) cut using a HM 340E Rotary Microtome (Thermo Scientific Fisher). The tissue sections were stained with haematoxylin and eosin (H&E) following the standard operating protocol (SOP) of the Pathology Institute of Heidelberg University Hospital. Morphology and potential differences between the groups were examined using an upright Zeiss AXIO Imager M2 (Carl Zeiss Microscopy GmbH, Germany). Representative images were taken at a 20x magnification and acquired using Zen 2.3 pro (Carl Zeiss Microscopy GmbH, Germany). For the liver, the size and number of lipid droplets was quantified from the H&E-stained tissue using Fiji v2.1.0 (Schindelin et al., 2012). Briefly, 8-bit converted images were thresholded and lipid droplets were highlighted in white. Analyze particles function was then used to count the number of droplets as well as determined the area. For the pancreas, whole slide images were also taken using a Hamamatsu NanoZoomer Digital Pathology system at a 20x magnification. Islet number, size and distribution was quantified using the QuPath software v.0.2.3 (Queen's University, Belfast, Northern Ireland).

2.6 Immunofluorescence Analysis of the Pancreas

Immunofluorescence of paraffin-embedded pancreas slides was performed to examine insulin and glucagon expression. Tissue sections were deparaffinized and rehydrated by immersing the slides first in xylol and then in different percentages of ethanol. Antigen retrieval was then performed by immersing the slides in citric acid buffer (Tri-sodium citrate, distilled water, 0.05% Tween-20, pH 6.0) with subsequent microwave heat cycles (4 x 3-5 minutes). Samples were then cooled down at RT for ca. 30 minutes and then washed three times (5-minute washes) with TBS containing 0.1% Tween 20 (TBS-T). Slides were then blocked with 5% BSA in TBS-T for 1 hour at RT followed by the incubation with the properly diluted primary antibodies in 5% BSA in TBS-T against insulin and glucagon at 4°C in the dark overnight (**Table 2**). Slides were then washed three times with TBS-T and incubated for 1h at room temperature in the dark with the appropriate fluorescence labelled secondary antibodies diluted in 5% BSA in TBST (**Table 2**). After 3 washes with TBS-T, slides were incubated with DAPI 500ng/ml in 5% BSA in TBS-T for 15 minutes at room temperature in the dark. Slides were then washed with TBS-T and mounted for imaging and analysis. Immunofluorescence images were taken using an upright Zeiss AXIO Imager M2 (Carl Zeiss Microscopy GmbH, Germany) at a 20x magnification and acquired using Zen 2.3 pro (Carl Zeiss Microscopy GmbH, Germany). Fluorescence intensity of insulin and glucagon was measured using Fiji v2.1.0 (Schindelin et al., 2012).

Antibody	Type	Isotype	Cat. Nr, Company	Dilution	Blocking Buffer
Insulin	Primary	Guinea Pig	#ab7842, Abcam	1:100	5% BSA in TBS-T
Glucagon	Primary	Rabbit	#ab92517, Abcam	1:2000	5% BSA in TBS-T
Alexa Fluor® 488 anti-Guinea Pig	Secondary	Goat	#A11073, Invitrogen	1:500	5% BSA in TBS-T
Alexa Fluor® 555 anti-rabbit	Secondary	Donkey	#406412, BioLegend	1:500	5% BSA in TBS.T

Table 2. Antibody list for Immunofluorescence

2.7 Isolation of Total RNA from the Liver

Frozen liver samples were grounded into a fine powder using an ice-cold mortar in liquid nitrogen (Morgenstern, Kliemank, et al., 2020). Total RNA was then extracted from ca.20mg of the pulverized tissue using the peqGOLD MicroSpin Total RNA kit (Peqlab) and according to manufacturer´s instructions. RNA quantification was performed spectrophotometrically using NanoDropTM 2000 (Thermo Fischer Scientific).

2.8 Gene Expression Profiling

Gene expression profiling from total RNA extracted from each of the livers was performed by the Microarray Unit of the Genomics and Proteomics Core Facility of the German Cancer Research Center (DKFZ), using the Clariom S Mouse Array (Affymetrix GeneChips®). Expression data analysis was performed using R (R Core Team, 2022) and RStudio (RStudio Team, 2022) and pathway analysis was conducted using the pathfindR package (Ulgen et al., 2019).

2.9 Immunoblotting

2.9.1 Protein Extracts

Frozen liver samples were grounded into a fine powder using an ice-cold mortar in liquid nitrogen (Morgenstern, Kliemank, et al., 2020). Cytoplasmic and nuclear extracts were obtained from liver pulverized tissue using NE-PER™ Nuclear and Cytoplasmic extraction reagents (Thermo Fischer Scientific), according to the manufacturer's instructions. All extraction buffers were supplemented with protease and phosphatase inhibitors immediately prior to use (Halt™ Protease and Phosphatase Inhibitor Cocktail (100X), Thermo Fischer Scientific). The protein concentration of the different extracts was measured using the Bradford assay (Bradford, 1976). Extracts were stored at -80°C.

2.9.2 MG-H1 antibody

A rat, monoclonal antibody against MG-H1 was developed in collaboration with the monoclonal antibody facility at Helmholtz Centre in Munich (Morgenstern et al., 2017). Briefly, a peptide-based antigen was synthesized which contained a single, chemical defined MG-H1 residue (T. Wang et al., 2012). Immunization and hybridomas were generated according to the standard procedures.

Hybridomas were screened for reactivity towards the MG-H1-peptide antigen, and human serum albumin modified minimally with MG (Ahmed et al., 2005) by ELISA and western blotting. Positive wells were established and cloned twice by limiting dilution. One anti-MG-H1 monoclonal antibody recognizing MG-H1 was designated 6D7 was used subsequently purified from the culture supernatant by affinity chromatography. To improve detection, the antibody was conjugated [PEG]4-Biotin using EZ-Link™ NHS-PEG4 biotinylation Kit (Thermo Fischer Scientific; #21455), according the manufacturer's instructions.

2.9.3 Western Blot

Protein extracts (ca.30 µg) were incubated with 5x Pierce™ Lane Marker Reducing Sample Buffer (Thermo Fischer Scientific) for 12 minutes at 98°C. Protein extracts were separated by SDS-PAGE (4 - 20 % Mini-PROTEAN® TGX, Bio-rad) applying 200V for 5 minutes and 170 for ca. 40 minutes. Proteins were then transfer onto nitrocellulose membranes using the Trans-Blot Turbo Transfer System (Bio-rad) using the Bio-rad pre-defined mixed molecular weight transfer programme (7 minutes). The quality of the transfer was evaluated using Ponceau S Staining Solution (Thermo Fischer Scientific). Membranes were washed and then blocked for 1h at room temperature with the appropriate blocking buffer. Membranes were then incubated overnight at 4°C with the diluted primary antibody (**Table 3**) in appropriate blocking buffer. After washing with TBS-T, the membranes were then incubated with the appropriate secondary antibodies diluted in blocking buffer (**Table 3**) for one hour at room temperature. Membranes were visualized with either the ChemiDoc MP imager (Bio-rad) for the horseradish-conjugated secondary antibodies, or with an Odyssey® DLx scanner (LI-COR) using Odyssey® imaging software (LI-COR), for the fluorescent conjugated secondary antibodies. Protein expression was quantified by densitometry using Fiji v2.1.0 (Schindelin et al., 2012) and normalized to the stated house-keeper.

Antibody	Type	Isotype	Catalogue Nr, Company	Dilution	Blocking Buffer
OXPHOS	Primary	Mouse	#ab110413, Abcam	1:500	5% MP in TBST
MG-H1	Primary	Rat	-	-	Pierce® protein-free blocking buffer (Thermo Fischer Scientific)
AMPK	Primary	Rabbit	#5831, Cell Signaling Technology	1:1000	5% BSA in TBST
p-AMPK (Thr172)	Primary	Rabbit	#2535, Cell Signaling Technology	1:1000	5% BSA in TBST
p-AMPK (Ser485)	Primary	Rabbit	#4185, Cell Signaling Technology	1:1000	5% BSA in TBST
Akt	Primary	Rabbit	#9272, Cell Signaling Technology	1:1000	5% MP in TBS-T
p-Akt (Ser473)	Primary	Rabbit	#4060, Cell Signaling Technology	1:1000	5% MP in TBS-T

p-Akt (Thr308)	Primary	Rabbit	#9275, Cell Signaling Technology	1:1000	5% MP in TBS-T
β-Actin	Primary	Rabbit	#4970, Cell Signaling Technology	1:1000	5% MP in TBS-T
β-Actin	Primary	Mouse	#3700, Cell Signaling Technology	1:1000	5% MP in TBS-T
GAPDH	Primary	Mouse	#97166, Cell Signaling Technology	1:1000	5% MP in TBS-T
Anti-rabbit IgG	Secondary	Goat	#7074S, Cell Signaling Technology	1:2000	Same as primary
Anti-rabbit IgG (H+L) (DyLight™ 800 4X PEG Conjugate)	Secondary	Goat	#5151, Cell Signaling Technology	1:10000	Same as primary
anti-Mouse IgG IRDye® 680RD	Secondary	Goat	#926-68070, LI-COR	1:10000	Same as primary
IRDye® 800CW Streptavidin	Secondary	Goat	#926-32230, LI-COR	1:5000	Same as primary

Table 3. Antibody list for western blot analysis

2.10 cDNA synthesis & Quantitative PCR (qPCR)

Total RNA from the liver (1-2µg) was used for cDNA synthesis using the High-capacity cDNA Reverse Transcription Kit (Thermo Fischer Scientific) according to manufacturer´s instructions. The cDNA synthesis was performed in a T3 Thermocycler (Biometra) following the steps in **Table 4**.

Step	**1**	**2**	**3**	**4**
Temperature (°C)	25	37	85	4
Time (minutes)	10	120	5	∞

Table 4. cDNA synthesis thermocycler programme

cDNA was diluted 1:10 with water and the qPCR was performed using DyNAmo ColorFlash SYBR Green qPCR Master Mix (Thermo Fisher Scientific). Briefly, 2µl of diluted cDNA were transferred in duplicate to a 96-well qPCR plate with the subsequent addition of 18µl of master mix containing 1 x SYBR Green Mastermix, 200nM forward primer and 200nM reverse primer (Eurofins Genomics) (**Table 5**).

Gene		Sequence 5'→ 3'
Cyp2c38	for	CACGGCCCATTGTTGTATTGC
	rev	TGAACCGTCTTGTCTCTTTCCA
Cyp2c65	for	ACAACCCAAAGACGGAGTTTAC
	rev	CTGTCCCAGCAGCAAACAGAT
Cyp2c67	for	CGATATGTTGACCTTGGCCC
	rev	TCAAACACCTCTGGGTTGGG
Cyp2b9	for	GCTCATTCTCTGGTCAGATGTTT
	rev	CGCTTGTGGTCTCAGTTCCA
Cyp2b13	for	TTTTCTTCCAGTGTGTTACAGCC
	rev	AACGCAGGAACTGTTCATCTG
Ephx2	for	GCGTTCGACCTTGACGGAG
	rev	TGTAGCTTTCATCCATGAGTGGT
PGC1α	for	TTCATCTGAGTATGGAGTCGCT
	rev	GGGGGTGAAACCACTTTTGTAA
Nrf1	for	TATGGCGGAAGTAATGAAAGACG
	rev	CAACGTAAGCTCTGCCTTGTT
Tfam	for	ATTCCGAAGTGTTTTTCCAGCA
	rev	TCTGAAAGTTTTGCATCTGGGT
Tfb2m	for	GGCCCATCTTGCATTCTAGGG
	rev	CAGGCAACGGCTCTATATTGAAG
TOMM7	for	CGACGGTTCAGGCATTCCA
	rev	ATCCGCTGGGGCTTTATTCC
TIMM17	for	TGCCCCTGGCGAATTGTAG
	rev	CTGTCAAACTTCCTCGGAGTC
TIMM23	for	GAAGGTGGCGGAAGAAGTAGC
	rev	GGGGGTTCATACCAGTCAGC
Actin	for	GGCTCTATTCCCCTCCATCG
	rev	CCAGTTGGTAACAATGCCATGT
GAPDH	for	AGGTCGGTGTGAACGGATTTG
	rev	TGTAGACCATGTAGTTGAGGTCA

Table 5. Primers

The Plates were then sealed and centrifuged briefly (2 minutes, 1000 rpm, 4°C). qPCR analysis was performed using a LightCycler® 480 Instrument II (Roche Applied Science) following the programme in **Table 6**. Signals of amplified products were verified using melting curve analysis, and mRNA levels were normalized to the geometric mean of Actin and GAPDH. Relative expression levels were calculated using the Ct method (Livak & Schmittgen, 2001).

Step	Temperature (°C)	Time	Number of cycles
Pre-incubation	95	7 min	1
Amplification	95	10 sec	45-50
	60	20 sec	
	72	1 sec	
Melting curve	95	5 sec	1
	65	1 min	
	97	5 acquisitions per °C	
Cooling	4	∞	1

Table 6. qPCR protocol

2.11 Metabolite Measurements

2.11.1 Dicarbonyls

Dicarbonyls (glyoxal, methylglyoxal and 3-deoxyglucosone) in the liver tissue was determined by isotope dilution, tandem mass spectroscopy, following derivatization with 1,2-diaminobenzene (Rabbani & Thornalley, 2014c). Briefly, pre-weighted amounts of tissue (ca.10mg) were homogenized in ice-cold 20% (wt/vol) trichloroacetic acid in 0.9% (wt/vol) sodium chloride (20 µl) and water (80 µl). An aliquot (5 µl) of the internal standard (13C3-MG; 400 nM) was then added and the samples vortexed mixed. Following centrifugation (14000 rpm; 5 mins @ 4 °C), 35 µl of the supernatant was transferred to an HPLC vials containing a 200 µl glass interest. An aliquot (5 µl) of 3 % sodium azide (wt/vol) was then added to each sample followed by 10 µl of 0.5 mM DB in 200 mM HCl containing 0.5 mM diethylenetriaminepentaacetic acid (DETAPAC) in water. The samples were then incubated for 4hrs at room temperature, protected from the light. Samples were then analyzed by LC-MS/MS using an ACQUITY™ ultra-high-performance liquid chromatography system with a Xevo-TQS LC-MS/MS mass spectrometer (Waters, Manchester, UK). The column was a Waters BEH C18 (100 x 2.1 mm) and guard column (5 x 2.1 mm). The mobile phase was 0.1 % formic acid in water with a linear gradient of 0–100 % 0.1 % formic acid in 50 % Acetonitrile:water over 0-10mins; the flow rate was 0.2ml/min and column temperature was 5°C. The capillary voltage was 0.5 kV, the cone voltage 20 V, the interscan delay time 100 ms, the source and desolvation gas temperatures 150 and 350 °C, respectively, and the cone gas and desolvation gas flows were 150 and 800 l/h, respectively. Mass transitions (parent ion>fragment ion; collision energy), retention time, limit of detection, and recoveries were as follows: Glyoxal, 130.9 > 77.1, 22eV, 5.28 min, 1.15 pmol, 99%; MG, 145.0 > 77.1, 24eV, 5.93 min, 0.52 pmol, 98%; 3-deoxyglucosone, 235.0 > 199.1, 14eV, 4.09 min, 0.36 pmol, 95%. Acquisition and quantification were completed with MassLynx 4.1 and TargetLynx 2.7 (Waters®). Calibration curves were constructed by plotting peak area ratio of analyte/isotopic standard against analyte concentration (0-20pmol).

2.11.2 GSH & GSSG

Reduced glutathione (GSH), oxidized glutathione (GSSG) and S-D-lactoylglutathione (SDL) was measured by stable dilution, liquid chromatography tandem mass spectrometry. Briefly, pulverised liver was homogenized in ice-cold 3% (wt/vol) trichloroacetic acid, 0.25% sodium azide (wt/vol) and 0.5mM diethylenetriaminepentaacetic acid (DETAPAC) in 0.9% (wt/vol) sodium chloride (100µl). Following centrifugation (14000 rpm; 5 mins @ 4 °C), 20µl of the supernatant was transferred to an HPLC vial containing 20µl of the isotopic standards (20pmol [glycine-13C2,15N]-GSH and 10pmol [13C4, 15N2-GSSG]. Samples were then analysed by LC-MS/MS using an ACQUITY™ ultra-high-performance liquid chromatography system with a Xevo-TQS LC-MS/MS mass spectrometer (Waters, Manchester, UK). The column was a Waters BEH C18 (100 x 2.1 mm) and guard column (5 x 2.1 mm). The mobile phase was 0.1 % formic acid in water with a linear gradient of 0–100 % 0.1 % formic acid in 50 % Acetonitrile:water over 0-10mins; the flow rate was 0.2ml/min, column temperature was 5°C and injection volume was 10µl. The capillary voltage was 2.5 kV, the cone voltage 18V, the interscan delay time 3ms, the source and desolvation gas temperatures 150 and 250 °C, respectively, and the cone gas and desolvation gas flows were 150 and 600 l/h, respectively. Mass transitions (retention time, parent ion>fragment ion, collision energy), retention times were as follows: GSH, 2.33mins, 370.9 > 178.7, 12eV; GSSG, 5.02mins, 613.1 > 483.9, 18eV; SDL, 5.19mins 380.1 > 75.5, 36eV. Acquisition and quantification were completed with MassLynx 4.1 and TargetLynx 2.7 (Waters®). Calibration curves were constructed by plotting peak area ratio of analyte/isotopic standard against analyte concentration (GSH: 0-100pmol; GSSG: 0-50pmol; SDL: 0-10pmol).

2.11.3 Adenosine Compounds

Pulverized liver (ca. 10mg) was sent to the Centre for Organismal Studies, Heidelberg and the concentrations of sugars and organic acids were assessed using liquid chromatography coupled fluorescence detection (Centre for Organismal studies by Dr. Gernot Poschet). Briefly, adenosine compounds were extracted from grounded liver tissue with ice-cold 0.1 M HCl in an ultrasonic ice-bath for 10 min. The resulting homogenates were centrifuged (16,400 g, 4°C, 10 min) to remove cell debris. Adenosines were derivatized with chloroacetaldehyde as previously described and separated at 42 °C by reversed phase chromatography on an Acquity BEH C18 column (150 mm x 2.1 mm, 1.7 µm, Waters) connected to an Acquity H-class UPLC system (Bürstenbinder et al., 2007). The column was equilibrated with buffer A (5.7 mM TBAS, 30.5 mM KH2PO4, pH 5.8) at a flow rate of 0.45 ml/min. The separated derivatives were detected by fluorescence (Acquity FLR detector, Waters, excitation: 280 nm, emission: 410 nm) and quantified using ultrapure standards (Sigma). Data acquisition and processing was performed with the Empower3 software suite (Waters).

2.11.4 Sugars & Organic Acids

Pulverized liver (ca. 40mg) was sent to the Centre for Organismal Studies, Heidelberg and the concentrations of sugars and organic acids were assessed using liquid chromatography coupled fluorescence detection (Centre for Organismal studies by Dr. Gernot Poschet). Briefly, organic acids and sugars were extracted from grounded tissue using ultra-pure water and heated at 95°C for 20 min. Organic acids were separated using an IonPac AS11-HC (2 mm, ThermoScientific) column connected to an ICS-5000 system (ThermoScientific) and quantified by conductivity detection after cation suppression (ASRS-300 2 mm, suppressor current 95-120 mA). At 30°C the column was equilibrated with solvent A (ultra-pure water). Separation of anions and organic acids was achieved by increasing the concentration of solvent B (100 mM NaOH) in buffer A (Neubert et al., 2016). Soluble sugars were separated at 25°C on a CarboPac PA1 column (ThermoScientific) connected to the ICS-5000 system and quantified by pulsed amperometric

detection (HPAEC-PAD). The column was equilibrated with solvent A. Baseline separation of carbohydrates was achieved by increasing the concentration of solvent B (300 mM NaOH). Data acquisition and quantification was performed with Chromeleon 7 (ThermoScientific) (Neubert et al., 2016).

2.12 Data and statistical analysis

Data was analysed using GraphPad Prism (version 9.3.1 for macOS, GraphPad Software, San Diego, California USA, www.graphpad.com). All data is expressed as mean value ± SEM of at least three biological replicates. Data was first subjected to normality and lognormality tests (Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test) and subsequently analysed using ordinary one-way ANOVA (with Tukey´s multiple comparisons test) or nonparametric Kruskal-Wallis test (with Dunn´s multiple comparisons test). Differences were considered significant when $p < 0.05$.

3. Results

3.1 Physiological effects of the loss of Glo1 and a long-term HFD

3.1.1 Glucose homeostasis and diabetes parameters

In order to determine if the effects of the loss of Glo1 were similar to the effects of a HFD-induced T2D model, body weight and blood sugar were measured over time and diabetes-related parameters at the end of the 12-month experiment. Body weight was increased by 1.5-fold in the HFD mice as compared to the control mice (46.74 ± 1.74 vs 29.90 ± 0.38g; $p < 0.0001$) or the Glo1-KO mice (46.74 ± 1.74 vs 31.80 ± 0.36g; $p < 0.0001$). Glo1-KO mice did not show an increase in body weight as compared to controls throughout the experiment (Figure 5A). Blood sugar was also increased in the HFD mice as compared to either the control mice (130 ± 1.99 vs 109.8 ± 0.88 mg/dL; $p < 0.0001$) and Glo1-KO mice (130 ± 1.99 vs 117 ± 0.91 mg/dL; $p < 0.0001$), especially within the first half of the experiment, after which an adaptation effect was observed, in which the blood glucose normalized by the end of the experiment (Figure 5B). Glo1-KO mice showed a tendency of increased blood glucose levels in comparison with control mice, but this increase was only of ca. 1-fold (117 ± 0.91 vs 109.8 ± 0.88 mg/dL; $p < 0.001$; Figure 5B). There were no significant differences in the glycated haemoglobin (HbA1c), as a surrogate of long-term glycaemia between any of the groups over the course of the experiment (Figure 5C).

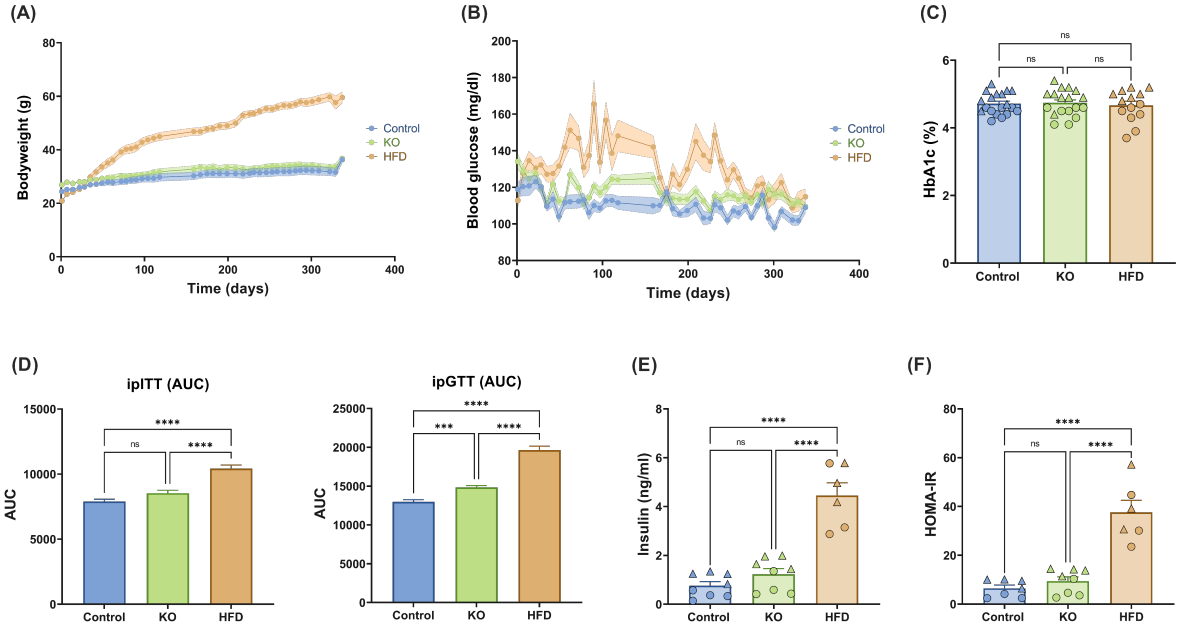


Figure 5. (A) Body weight measurements over time. **(B)** Blood glucose levels measured over time. **(C)** HbA1c measured at the end of the experiment. **(D)** Intraperitoneal glucose (ipGTT) and insulin (ipITT) tolerance tests performed at the end of the experiment and represented by the AUC. **(E)** Insulin plasma levels measured at the end of the experiment. **(F)** HOMA-IR calculated with fasting glucose and insulin levels at the end of the experiment. Data is represented by the Mean ± SEM of biological replicates for Control, Glo1-KO and HFD mice. When individual values are shown, triangles represent male mice and circles represent female mice. *** $p < 0.001$, **** $p < 0.0001$, **ns** $p > 0.05$.

After 12 months, intraperitoneally insulin and glucose tolerance tests were performed as indicators of glucose homeostasis. Increased area under the curve (AUC) in the insulin tolerance test was observed in the HFD mice when compared to control (10433 ± 264 vs 7893 vs 172 AU, $p < 0.0001$) or the Glo1-KO (10433 ± 264 vs 8539 ± 221 AU, $p < 0.0001$) mice (Figure 5D), but

no significant difference was found between control and Glo1-KO mice (Figure 5D). In the glucose tolerance test, HFD mice again showed increased AUC when compared to control (19631 ± 509 vs 12973 ± 284 AU, $p < 0.0001$) and Glo1-KO (19631 ± 509 vs 14828 ± 246 AU, $p < 0.0001$) mice (Figure 1D) and a mild increased AUC was also observed in the Glo1-KO mice when compared to controls ($p < 0.001$, Figure 5D).

Insulin levels were measured in plasma samples at the end of the experiment and they were found to be increased in the HFD mice 6-fold when compared to controls (4.45 ± 0.5 vs 0.76 ± 0.17ng/ml, $p < 0.0001$) and 4-fold as compared to Glo1-KO mice (4.45 ± 0.5 vs 1.23 ± 0.23ng/ml, $p < 0.0001$) mice (Figure 5E). However, no significant increase was found between Glo1-KO and control mice (Figure 5E). HOMA-IR was calculated from glucose and insulin fasting levels and as a parameter of insulin resistance. A similar trend was found, with the HFD mice showing an increased HOMA-IR when compared to controls (37.56 ± 4.97 vs 6.44 ± 1.32, $p < 0.0001$) and Glo1-KO (37.56 ± 4.97 vs 9.385 ± 1.77, $p < 0.0001$) mice (Figure 5F). No significant differences were found between controls and Glo1-KO mice (Figure 5F). This data would suggest that a T2D-insulin resistant phenotype has developed in the HFD mice but not in the Glo1-KO mice.

3.1.2 Pancreatic morphology and hormone expression

Despite not finding significant differences between Glo1-KO mice and controls, a trend to mildly impaired glucose homeostasis could be observed. Therefore, to established whether such trends were associated with changes in insulin production, the histology of pancreas from the mice was examined.

H&E-stained pancreas sections were first analysed with respect to the distribution or size of the islets. Endocrine area was measured by the percentage of the pancreas covered by islets and no significant differences were found between controls and either Glo1-KO mice (0.57 ± 0.06 vs 0.73 ± 0.2%, $p > 0.05$, Figure 6A and 6B) or HFD mice (0.57 ± 0.06 vs 1.26 ± 0.39%, $p > 0.05$, Figure 6A and 6B). Compared to controls, islet distribution was also unchanged in either Glo1-KO mice (5.947e-007 ± 7.483e-008 vs 7.034e-007 ± 1.404e-00 islets/μm^2, $p > 0.05$, Figure 6A and 6B) or HFD mice (5.947e-007 ± 7.483e-008 vs 7.231e-007 ± 1.187e-007 islets/μm^2, $p > 0.05$, Figure 6A and 6B).

Insulin and glucagon expression were measured by immunofluorescence, using fluorescence intensity as a readout. As compared to controls, the ratio insulin expression to β-cell area was not significantly changed between in Glo1-KO (3194 ± 189 vs 3274 ± 164, $p > 0.05$, Figure 6C and 6D) or HFD mice (3194 ± 189 vs 2760 ± 304, $p > 0.05$, Figure 6C and 6D). The same was also found regarding the ratio glucagon expression to α-cell area, with no changes observed from controls in either Glo1-KO (8029 ± 323 vs 6451 ± 680, $p > 0.05$, Figure 6C and 6D) or HFD mice (8029 ± 323 vs 8075 ± 409, $p > 0.05$, Figure 6C and 6D). However, a sex-specific effect was observed in glucagon intensity, with female Glo1-KO mice showing decreased glucagon expression/α-cell area when compared to Glo1-KO males (3353 ± 318.5 vs 9549 ± 476.7, $p < 0.0001$, Figure 6C and 6D). This data would suggest that the physiological results observed in

Glo1-KO and, especially, HFD mice in this study are not caused by morphological changes in the pancreas or impaired expression of pancreatic hormones.

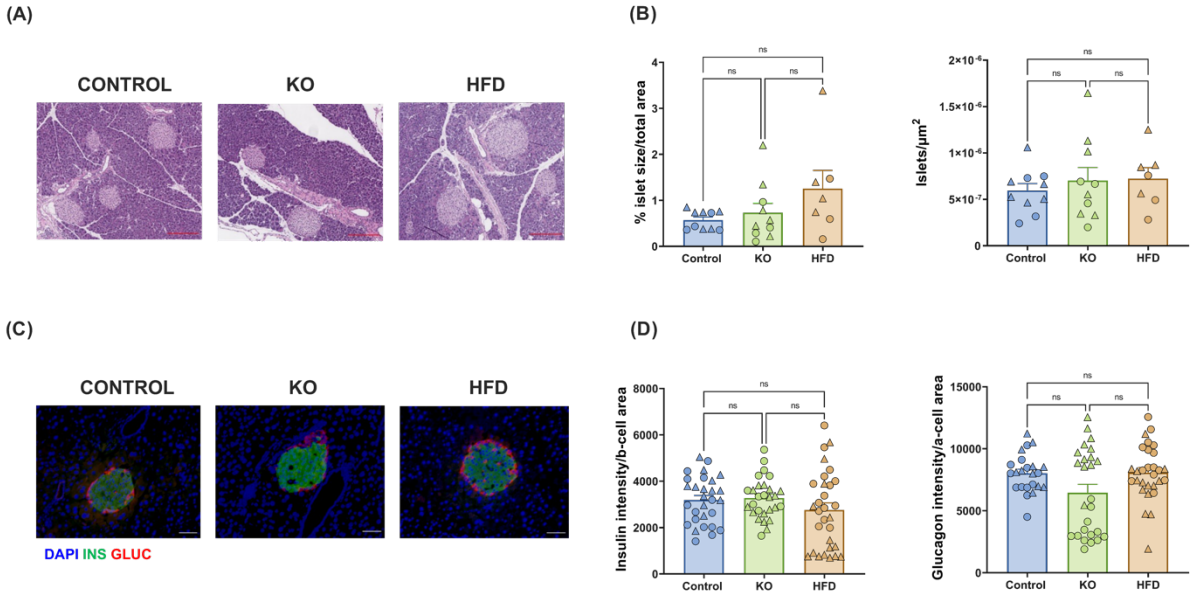


Figure 6. Pancreatic morphology and hormone expression. (A) Representative H&E images of the pancreas at a 20x magnification. Scale bar is 200µm. **(B)** Pancreas analysis of whole slide images representing the % of the pancreas occupied by islets (left) and islet distribution (right). **(C)** Immunofluorescence representative merged images of DAPI, insulin and glucagon signals at a 20x magnification. Scale bar is 50µm. **(D)** Pancreas immunofluorescence analysis representing insulin (left) and glucagon (right) intensity corrected by the respective β-cell or α-cell area. Values represent individual islets. Data is represented by the Mean ± SEM of biological replicates for Control, Glo1-KO and HFD mice. When individual values are shown, triangles represent male mice and circles represent female mice. **ns** $p > 0.05$.

3.1.3 Liver and kidney analysis

As no differences were observed in the pancreas, liver and kidney, the two other organs that can promote and be affected by impaired glucose homeostasis were analysed.

H&E images liver sections showed no morphological differences between the groups apart from an increased fat accumulation in the liver of HFD mice (Figure 7A). The liver weight to body weight ratio was decreased in the HFD mice when compared to controls (0.036 ± 0.002 vs 0.041 ± 0.001, $p < 0.05$, Figure 7B) and Glo1-KO mice (0.036 ± 0.002 0.042 ± 0.001, $p < 0.05$, Figure 7B). This difference in HFD group was due primarily to a decreased ratio in the HFD female mice when compared to males (0.027 ± 0.002 vs 0.044 ± 0.002, $p < 0.01$, Figure 7B), suggesting the accumulation of fat in other parts of the body rather than as visceral fat in females. Further analysis of the lipid droplets of the liver showed an increase in the percentage of the area of the liver occupied by lipids in the HFD mice when compared to controls (17.12 ± 1.2 vs 5.07 ± 1.2% $p < 0.0001$, Figure 7C) and Glo1-KO mice (17.12 ± 1.2 vs 7.23 ± 0.88%, $p < 0.0001$, Figure 7C). Lipid droplet size was also significantly increased by 4.5-fold in the HFD mice when compared to controls (0.07 ± 0.001 vs 0.0155 ± 0.0001 AU, $p < 0.0001$, Figure 7D) and Glo1-KO mice (0.07 ± 0.001 vs 0.0156 ± 0.0001 AU, $p < 0.0001$, Figure 7D). Glo1-KO mice also showed an increase in lipid droplet size as compared to controls, although it was only ca. 1-fold (0.0156 ± 0.0001 vs 0.0155 ± 0.0001 AU, $p < 0.0001$, Figure 7D).

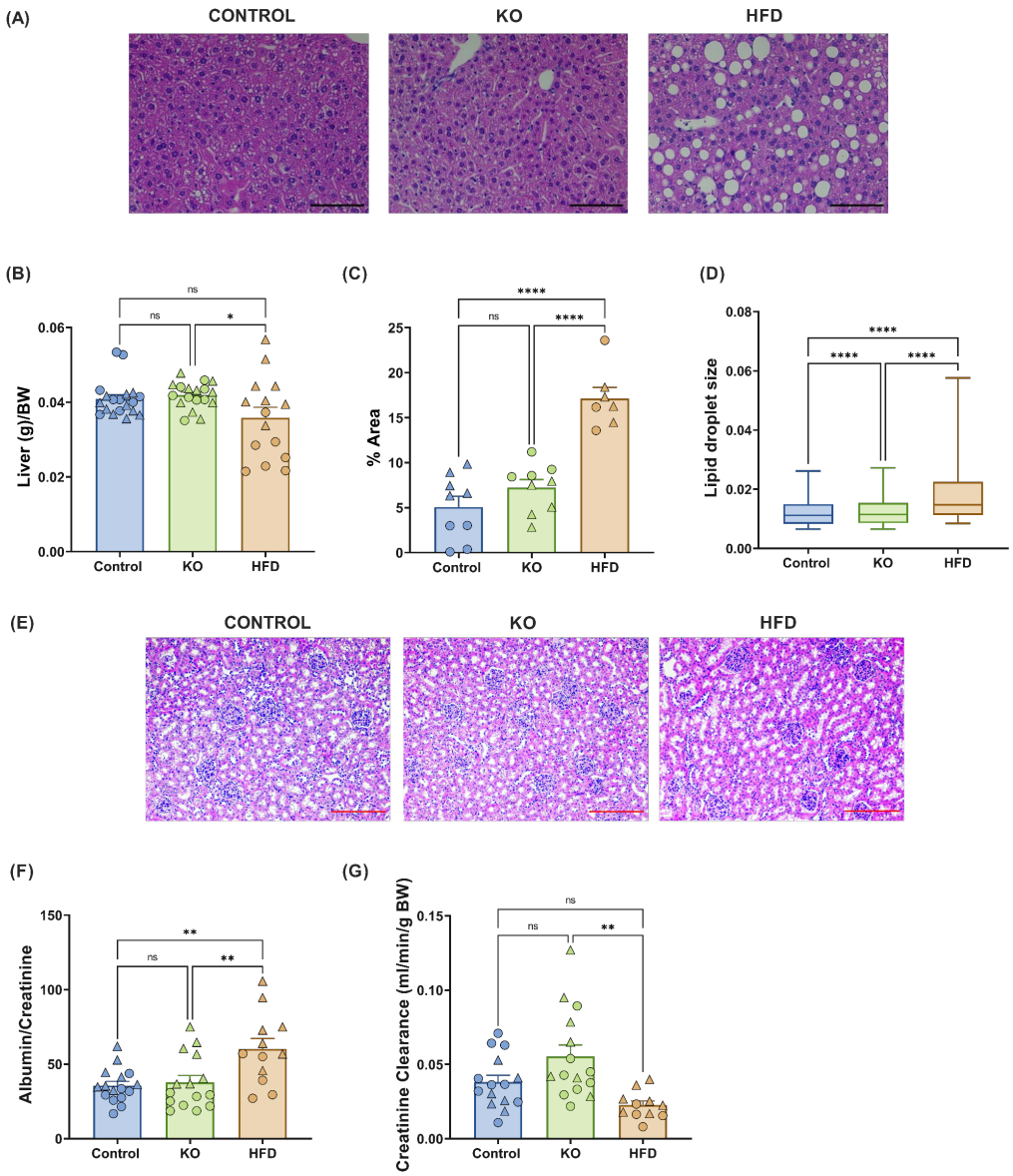


Figure 7. Liver and kidney analysis. (A) Representative H&E images of the liver at a 20x magnification. Scale bar is 100µm. **(B)** Liver weight to body weight ratio measured at the end of the experiment. **(C)** Lipid analysis of the liver representing the % area of the liver occupied by lipid droplets. **(D)** Lipid droplet size measured from representative images of each group. **(E)** H&E representative images of the kidney at a 10x magnification. Scael bar is 1000µm. **(F)** Albumin/creatinine ratio measured in the urine at the end of the experiment. **(G)** Creatinine clearance calculated at the end of the experiment. Data is represented by the Mean ± SEM of biological replicates for Control, Glo1-KO and HFD mice. When individual values are shown, triangles represent male mice and circles represent female mice. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$, **ns** $p > 0.05$.

H&E images of the kidney showed no prominent morphological changes between the groups (Figure 7E). However, an assessment of kidney function showed that the albumin/creatinine ratio was increased in the HFD mice as compared to either the control (60.25 ± 6.96 vs 35.62 ± 2.85, $p < 0.01$, Figure 7F) or Glo1-KO mice (60.25 ± 6.96 vs 37.77 ± 4.67, $p < 0.01$, Figure 7F). No significant differences were observed between the Glo1- KO mice and controls ($p > 0.05$, Figure 7F). Consistently, creatinine clearance was also decreased in the HFD mice as compared to Glo1-KO mice (0.023 ± 0.003 vs 0.055 ± 0.008 ml/min/g body weight, $p < 0.01$, Figure 7G), whilst no significant differences were found between Glo1-KO and control mice (0.055 ± 0.008 vs 0.038 ±

0.004 ml/min/g body weight, $p > 0.05$, Figure 7G). This data suggests that the physiological changes seen in the HFD mice could be triggered by increased fat deposition in the liver and lead to impaired kidney function, which is consistent to what it has been reported in the literature (Nakamura & Terauchi, 2013; Sun et al., 2020). However, the physiological trends observed in the Glo1-KO mice cannot be explained by any changes in the liver or kidneys of the animals, suggesting that the loss of Glo1-KO is not an adequate model for the development of T2D.

3.2 Effects of the loss of Glo1 at a molecular level

3.2.1 Liver Gene Expression Profiling

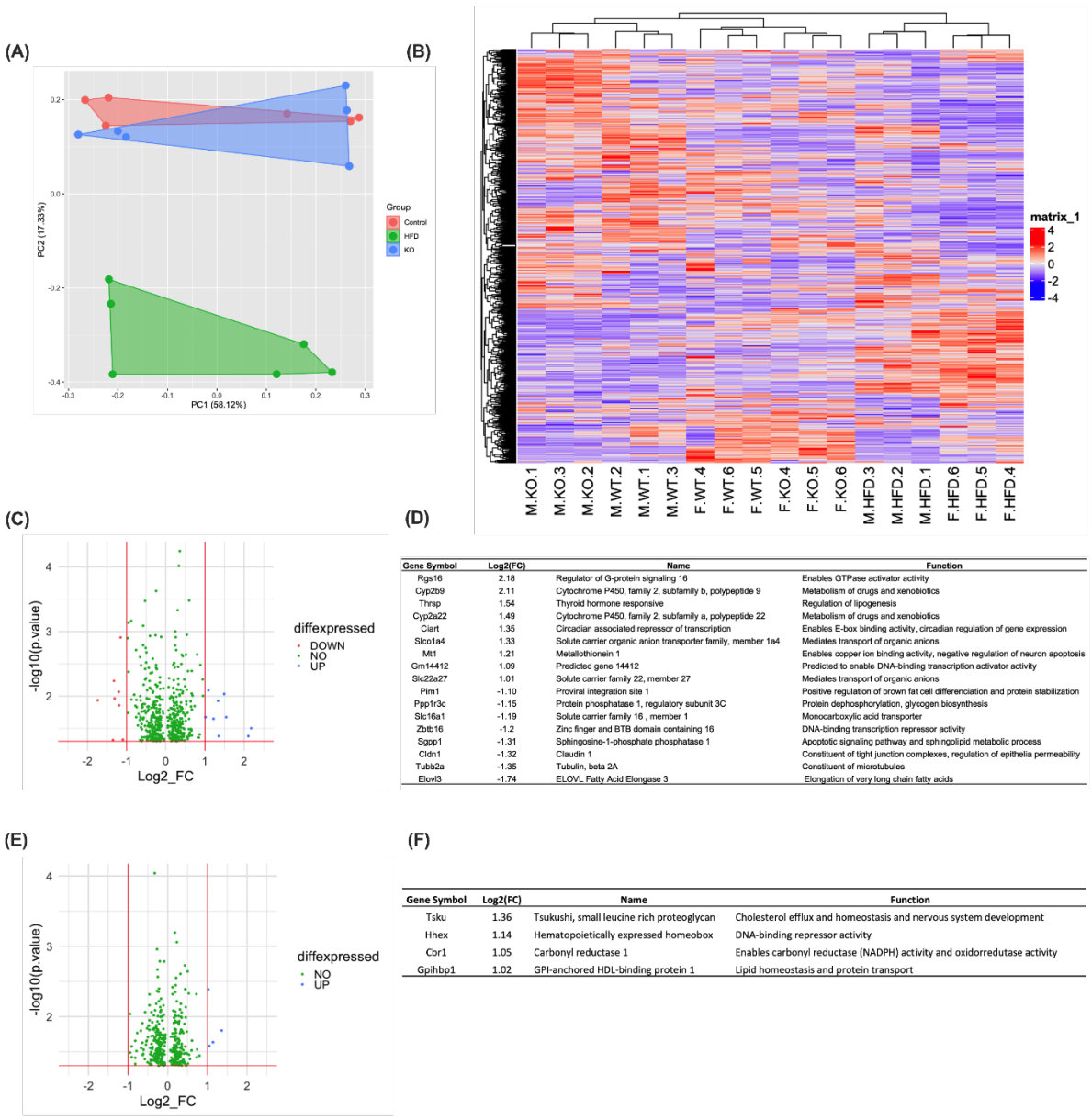
At this point in the study, it could be observed that Glo1-KO mice are not equivalent to T2D phenotype, induced by HFD. However, there were trends observed in the Glo1-KO mice that could not be explained by any changes or functional impairments in major organs. In order to determine the potential molecular differences between the control, Glo1-KO and HFD mice, expression profiling, using Affymetrix microarrays, was performed from the total RNA isolated from the livers of the different experimental groups.

Principal component analysis (PCA) confirmed the previous conclusion from the phenotyping data that the loss of Glo1 does not have the same effects as a long-term HFD. Furthermore, it showed that the gene expression, as compared to the control mice, was also not substantially changed (Figure 8A). Hierarchical clustering showed that across all of the data there were two well-defined clusters comprising of the control and Glo1-KO mice together and the HFD mice separately, indicating the substantial effects that the HFD diet has induced (Figure 8B). Interestingly, for each of these clusters there was a strong differentiation between sexes, especially for the Glo1-KO mice in which the male mice are strongly separated from female mice (Figure 8B). This would suggest, the loss of Glo1 may have a sex-specific effect.

To define the potential sex dimorphism of Glo1, volcano plots between control and Glo1-KO male and female mice were generated to examine the extend of differentially expressed genes. Significantly differentially expressed genes were considered when $p < 0.05$ (or Log(p value) > 1.301) and the $Log2(FC) < -1$ or $Log2(FC) > 1$. In Glo1-KO males, only 17 genes were found to be significantly up- (9 transcripts) or downregulated (8 transcripts) as compared to the male controls/wild-type (Figure 8C and 8D). In contrast, in the Glo1-KO females only 4 genes were found to be upregulated as compared to female controls/wild-types (Figure 8E and 8F). Due to the low number on differentiational changed transcripts, it was difficult to assign specific causal roles that such transcripts might have in the context of gender. As such, functional network enrichment, which categorizes the identified proteins with regard to function, based upon overlap statistics, was performed.

3.2.2 Hepatic mitochondrial function

The enrichment plot showed several pathways that were significantly changed between female and male Glo1-KO mice (Figure 9A). Of these pathways, oxidative phosphorylation, in particularly the complexes of the electron transport chain (ETC) was found to be significantly upregulated in the male Glo1-KO mice (Figure 9B). To confirm whether the increase in the expression of the ETC complexes was translated into an increased protein expression, western blotting for the different complexes was performed.



Gene Symbol	Log2(FC)	Name	Function
Rgs16	2.18	Regulator of G-protein signaling 16	Enables GTPase activator activity
Cyp2b9	2.11	Cytochrome P450, family 2, subfamily b, polypeptide 9	Metabolism of drugs and xenobiotics
Thrsp	1.54	Thyroid hormone responsive	Regulation of lipogenesis
Cyp2a22	1.49	Cytochrome P450, family 2, subfamily a, polypeptide 22	Metabolism of drugs and xenobiotics
Ciart	1.35	Circadian associated repressor of transcription	Enables E-box binding activity, circadian regulation of gene expression
Slco1a4	1.33	Solute carrier organic anion transporter family, member 1a4	Mediates transport of organic anions
Mt1	1.21	Metallothionein 1	Enables copper ion binding activity, negative regulation of neuron apoptosis
Gm14412	1.09	Predicted gene 14412	Predicted to enable DNA-binding transcription activator activity
Slc22a27	1.01	Solute carrier family 22, member 27	Mediates transport of organic anions
Plm1	-1.10	Proviral integration site 1	Positive regulation of brown fat cell differenciation and protein stabilization
Ppp1r3c	-1.15	Protein phosphatase 1, regulatory subunit 3C	Protein dephosphorylation, glycogen biosynthesis
Slc16a1	-1.19	Solute carrier family 16 , member 1	Monocarboxylic acid transporter
Zbtb16	-1.2	Zinc finger and BTB domain containing 16	DNA-binding transcription repressor activity
Sgpp1	-1.31	Sphingosine-1-phosphate phosphatase 1	Apoptotic signaling pathway and sphingolipid metabolic process
Cldn1	-1.32	Claudin 1	Constituent of tight junction complexes, regulation of epithelia permeability
Tubb2a	-1.35	Tubulin, beta 2A	Constituent of microtubules
Elovl3	-1.74	ELOVL Fatty Acid Elongase 3	Elongation of very long chain fatty acids

Gene Symbol	Log2(FC)	Name	Function
Tsku	1.36	Tsukushi, small leucine rich proteoglycan	Cholesterol efflux and homeostasis and nervous system development
Hhex	1.14	Hematopoietically expressed homeobox	DNA-binding repressor activity
Cbr1	1.05	Carbonyl reductase 1	Enables carbonyl reductase (NADPH) activity and oxidorredutase activity
Gpihbp1	1.02	GPI-anchored HDL-binding protein 1	Lipid homeostasis and protein transport

Figure 8. **Hepatic gene expression profiling results and analysis. (A)** PCA plot of hepatic gene expression results between Control, Glo1-KO and HFD mice. **(B)** Heatmap and hierarchical clustering of hepatic gene expression analysis of Control, Glo1-KO and HFD mice represented by its individual biological replicates. **(C)** Volcano plot of differentially expressed genes between Control and Glo1-KO male livers. Vertical red lines represent the threshold of Log2(FC) < -1 and Log2(FC) >1. Horizontal red line represents the threshold in significance of –log(p-value) > 1.301. **(D)** Significantly up or downregulated genes in the livers of Glo1-KO male mice. **(E)** Volcano plot of differentially expressed genes between Control and Glo1-KO female livers. Vertical red lines represent the threshold of Log2(FC) < -1 and Log2(FC) >1. Horizontal red line represents the threshold in significance of –log(p-value) > 1.301. **(F)** Significantly upregulated genes in the livers of Glo1-KO female mice.

In the control mice, there was no difference in the protein expression of the ETC complexes between male and female mice (Figure 9C). However, in Glo1-KO mice there were significant increases in the male mice, as compared to the female mice, for the expression of Complex I (0.305 ± 0.02 vs 0.152 ± 0.02 AU, $p < 0.01$, Figure 9C) and Complex II (0.993 ± 0.001 vs 0.678 ± 0.064 AU, $p > 0.0001$, Figure 9C).

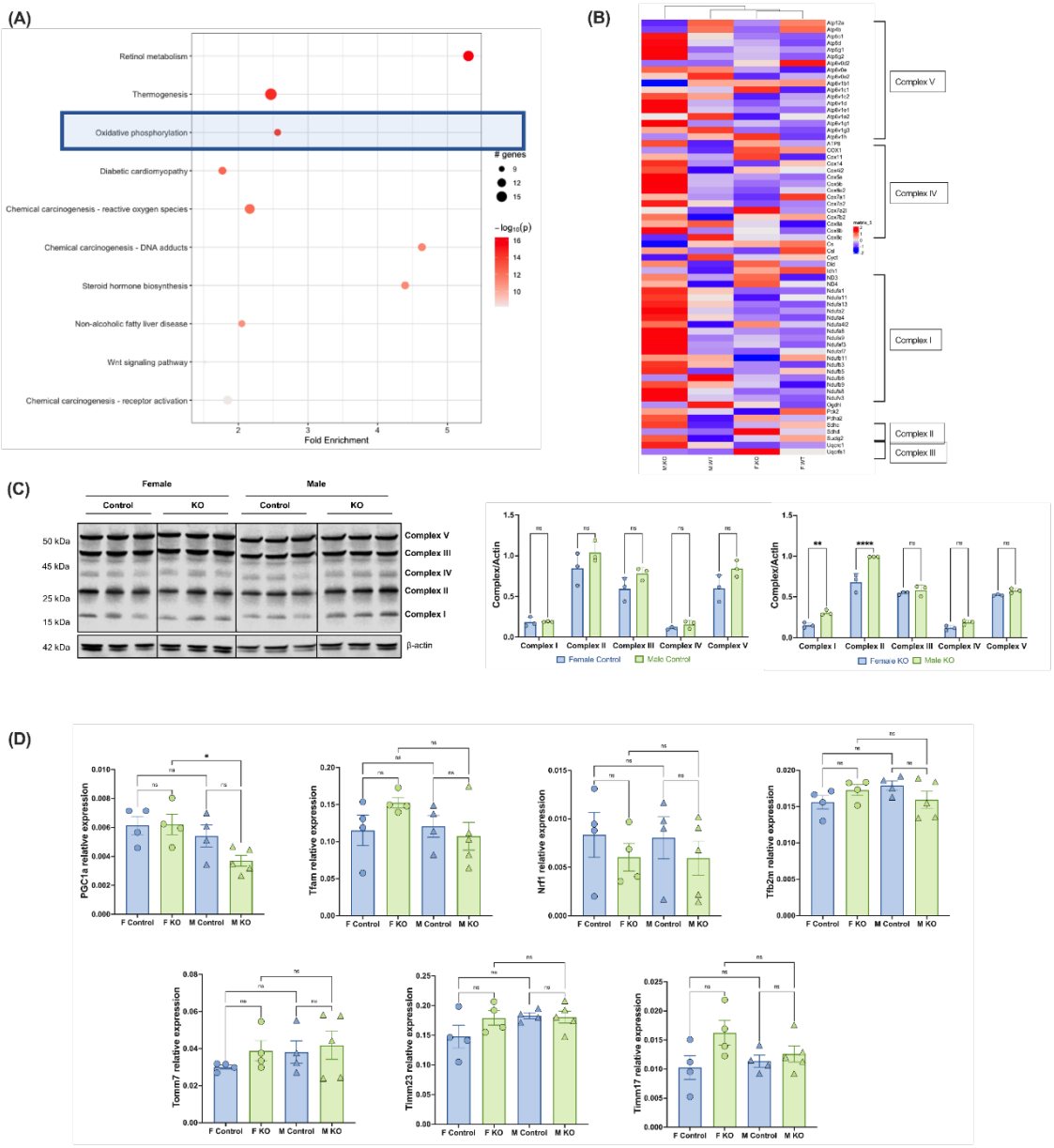


Figure 9. Mitochondrial gene and protein expression and biogenesis. (A) Enrichment plot of most differentially expressed pathways in the livers of female and male Glo1-KO mice after gene expression profiling. **(B)** Heatmap of the expression of genes involved in the different complexes in the mitochondrial electron transport chain in Control and Glo1-KO male and female livers**. (C)** Western blot of electron transport chain complexes in Glo1-KO female and male liver extracts. Followed by the densitometry analysis in Control (left) and Glo1-KO (right) mice**. (D)** Hepatic RNA expression of genes involved in mitochondrial biogenesis measured by qPCR in Control and Glo1-KO female (F) and male (M) mice. Data is represented by the Mean ± SEM of biological replicates. ** $p < 0.01$, **** $p < 0.0001$, **ns** $p > 0.05$.

The increased expression of the ETC complexes in the Glo1-KO male could be the result of increased mitochondrial biogenesis. The expression of genes involved in mitochondrial biogenesis as PGC1α, Tfam, Nrf1, Tfb2m, Tomm7, Timm23 or Timm17 was therefore analysed by qPCR. No differences were found between the groups (Figure 9D), suggesting that the upregulation of ETC complexes in the Glo1-KO males is not due to an increase in the number of mitochondria. Nevertheless, the increased expression of ETC complexes in the Glo1-KO male mice could be an indication of a greater capacity to produce to ATP.

In order to determine whether this was the case, hepatic levels of the different intermediates of the citric acid or tricarboxylic acid (TCA) cycle and glycolysis substrates after insulin stimulation was performed. Under baseline conditions, there was no significant differences between control and Glo1-KO mice females or males (data not shown). However, following insulin stimulation, a sex-specific effect was observed in the fold-change baseline to insulin stimulation levels. Female Glo1-KO mice did not show significant increases in any of the metabolites analysed when compared to control female mice (Figure 10A). However, the Glo1-KO male mice showed significant increases in the levels of the TCA cycle intermediates, as compared to the control male mice. For example, fumaric acid was increased 8-fold (4.32-fold ± 1.71 vs 0.55-fold ± 0.07, $p < 0.01$, Figure 10B), succinic acid was increased 4-fold (2.79-fold ± 0.75 vs 0.36-fold ± 0.03, $p < 0.01$, Figure 10B) and malic acid was increased 6-fold (2.79-fold ± 1.19 vs 0.49-fold ± 0.03, $p < 0.01$, Figure 10B). The Glo1-KO male mice also showed increased levels of pyruvic acid (3.67-fold ± 0.94 vs 0.62-fold ± 0.05, $p < 0.001$, Figure 10B) and D-glucose (1.84-fold ± 0.19 vs 0.63-fold ± 0.04, $p < 0.05$, Figure 10B) as compared to control males, following insulin stimulation.

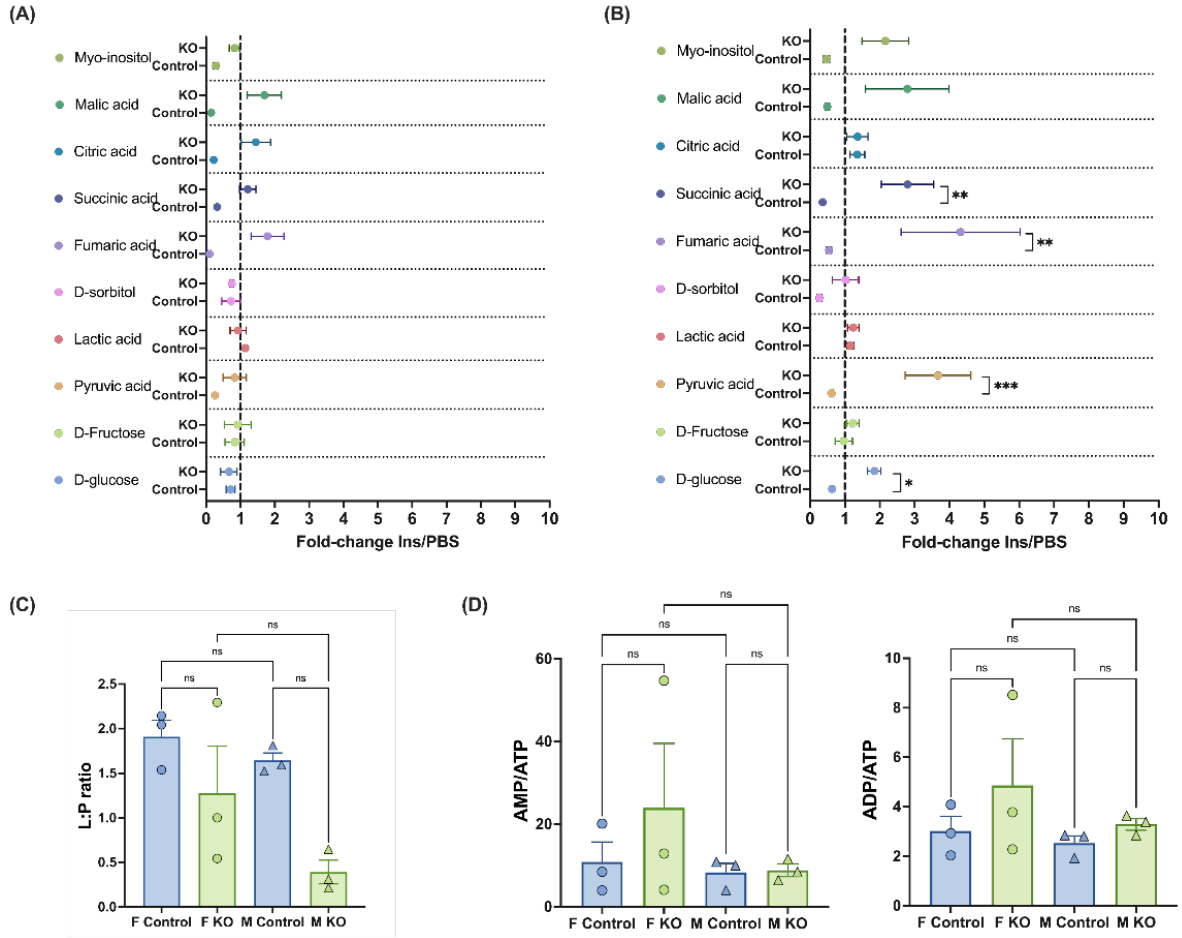


Figure 10. Citric acid cycle and nucleotides. (A) Hepatic levels of the intermediates of the citric acid cycle in female Control and Glo1-KO mice after insulin stimulation. **(B)** Hepatic levels of the intermediates of the citric acid cycle in male Control and Glo1-KO mice after insulin stimulation. **(C)** Lactate to pyruvate ratio after insulin stimulation in Control and Glo1-KO female (F) and male (M) livers. **(D)** AMP/ATP (left) and ADP/ATP (right) ratios in liver samples from Control and Glo1-KO female (F) and male (M) mice. Data is represented by the Mean ± SEM of biological replicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **ns** $p > 0.05$.

The lactate to pyruvate (L:P) ratio showed a non-significant decrease after insulin stimulation in the Glo1-KO males as compared to controls (0.39-fold ± 0.13 vs 1.64-fold ± 0.09, $p = 0.06$, Figure

10C), mainly caused by the increase in pyruvate levels in the Glo1-KO mice after insulin stimulation, suggesting an impairment in mitochondrial function or pyruvate metabolism in Glo1-KO male mice. In contrast, the L:P ratio in the Glo1-KO females was not changed when compared to female controls (1.28-fold ± 0.5 vs 1.91-fold ± 0.19, p = 0.4, Figure 10C).

Another readout for mitochondrial function is the levels of adenine nucleotides: AMP, ADP and ATP; being considered as energy metabolites (D. Grahame Hardie, 2011). Similar trends in the AMP/ATP and ADP/ATP ratios were found in the livers of the different groups, with female Glo1-KO mice showing increased ratios of both AMP/ATP and ADP/ATP when compared to control and Glo1-KO male mice (Figure 10D). However, these differences were non-significant and as such it was difficult to discern what is the relevance of these measurements to the changes already described.

3.2.3 Hepatic AMPK activation and insulin signalling

Most of the processes that are sensitive to cellular energy are mainly control directly or indirectly by AMPK. In the Glo1-KO female mice, activation of AMPK by phosphorylation at Thr172 was significantly increased as compared to the Glo1-KO male mice (0.0084 ± 0.0002 vs 0.0047 ± 0.0008 AU, p < 0.05, Figure 11A and 11B), whilst inactivation of AMPK, by phosphorylation at Ser485, was found to be unchanged between Glo1-KO female and Glo1-KO male mice (0.0018 ± 0.0001 vs 0.0019 ± 0.0003 AU, p = 0.98, Figure 11A and 11B). However, it was increased in the Glo1-KO male mice as compared to the male controls (0.0019 ± 0.0003 vs 0.0009 ± 0.0002 AU, p < 0.05, Figure 11A and 11B). These results would suggest that Glo1-KO male mice have a decreased activation of AMPK when compared to both control male mice and female Glo1-KO mice.

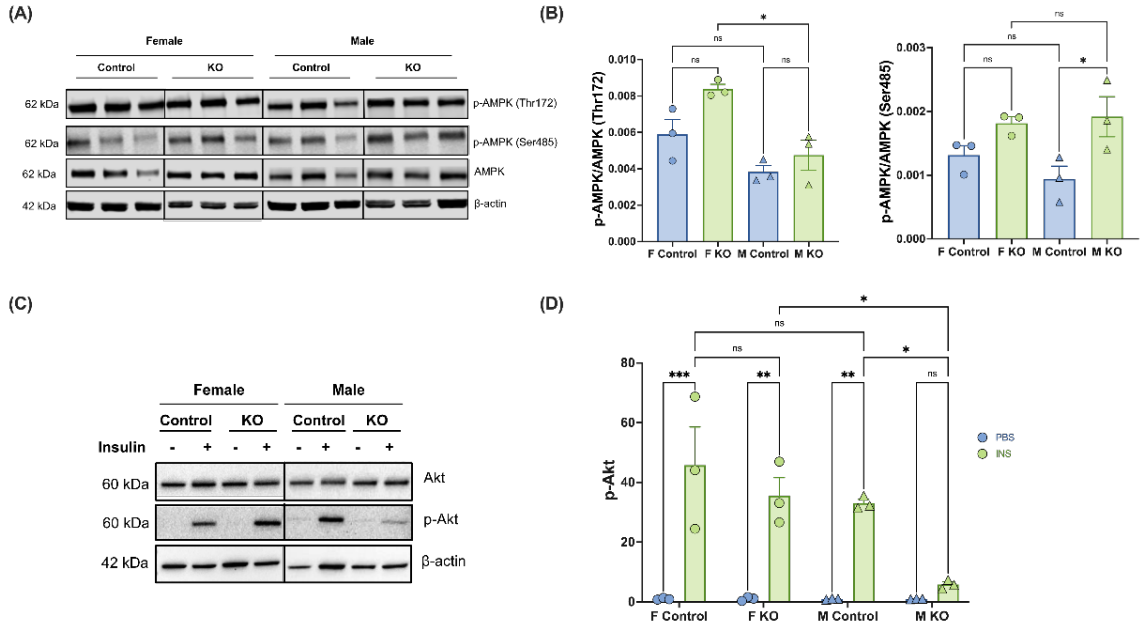


Figure 11. Hepatic AMPK activation and insulin signalling. (A) Western blot of total AMPK and AMPK activating (Thr172) and inhibitory (Ser485) phosphorylations in liver extracts of Control and Glo1-KO female and male mice using **β**-actin as a loading control. **(B)** Densitometry analysis of AMPK western blots of Control and Glo1-KO female (F) and male (M) liver extracts. **(C)** Western blot of total Akt and p-Akt (Thr308 + Ser473) in liver extracts of Control and Glo1-KO female and male mice using **β**-actin as a loading control. **(D)** Densitometry anaylsis of Akt western blot of Control and Glo1-KO female (F) and male (M) liver extracts, represented as the fold-change of INS/PBS. Data is represented by the Mean ± SEM of biological replicates. * p <0.05, ** p < 0.01, *** p < 0.001, **ns** p > 0.05.

To determine the downstream consequences resulting from the differentiation in the phosphorylation of AMPK, the phosphorylation status of Akt was investigated. The fold-change in the total phosphorylation of Akt (Thr308 + Ser473) following insulin stimulation was significantly reduced in the Glo1-KO male mice as compared to the male controls (5.81-fold ± 0.85 vs 33.07-fold ± 1.24, $p < 0.05$, Figure 11C and 11D) and to Glo1-KO female mice (5.81-fold ± 0.85 vs 35.57-fold ± 5.99, $p < 0.05$, Figure 11C and 11D). This data would confirm that an impairment in AMPK phosphorylation in Glo1-KO male mice would lead to a reduced activation of hepatic insulin signalling.

3.2.4 Dicarbonyls and redox stress in the liver

A potential mechanism for the molecular differences observed in the Glo1-KO mice could be increased hepatic MG and MG-H1, as well as increased redox stress. To establish whether this was the case, MG levels and the levels of reduced (GSH) and oxidized (GSSG) glutathione were measured in the liver of the mice by LC-MS/MS. The levels of MG were not significantly increased in the Glo1-KO mice when compared to controls (8.21 ± 0.43 vs 6.46 ± 0.79 pmol/mg, $p = 0.09$, Figure 12A), nor there was any sex difference (Figure 12A). Similarly, redox stress as indicated by the GSH/GSSG ratio, was not significantly changed in Glo1-KO mice as compared to controls (64.21 ± 8.85 vs 51.63 ± 6.56, $p = 0.075$, Figure 12B). The levels of MG-H1, measured by western blot in liver protein extracts showed no significantly elevation in Glo1-KO mice when compared to controls (13.63 ± 1.23 vs 11 ± 1.05 AU, $p = 0.7$, Figure 12C and 12D), nor there were any differences between sexes observed (Figure 12C and 12D). These results suggest that the molecular differences observed in the Glo1-KO male mice are not as a result of increased MG levels or MG-related AGEs, neither they are a consequence of increased hepatic redox stress.

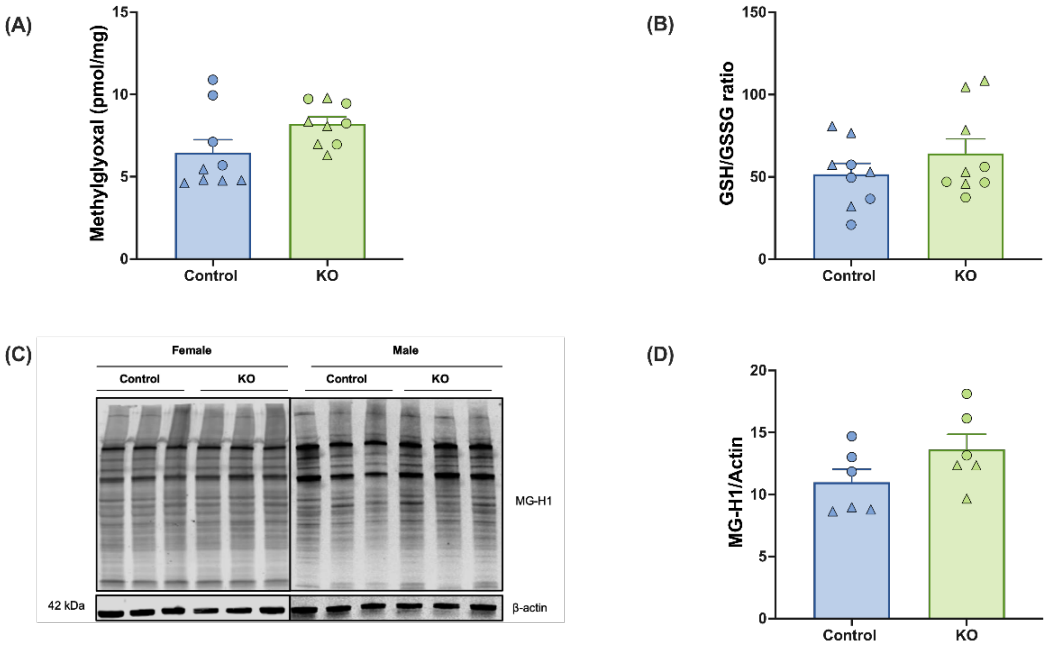


Figure 12. Hepatic levels of MG, MG-H1 and redox stress. (A) MG levels of Control and Glo1-KO mice measured in the liver by LC/MS-MS. **(B)** GSH/GSSG ratio of Control and Glo1-KO mice measured in the liver by LC/MS-MS. **(C)** Western blot of MG-H1 in Control and Glo1-KO liver extracts using **β**-actin as a loading control **(D)** Densitometry analysis of MG-H1 western blot. Data is represented by the Mean ± SEM of biological replicates. **ns** $p > 0.05$.

3.2.5 Hepatic arachidonic acid metabolism

Glo1-KO male mice were found to have decreased responsiveness to insulin, as compared to the Glo1-KO female mice, suggesting that the female mice are protected from the negative consequences resulting from the loss of Glo1. Further analysis of the expression profiling data which compared all of the male and female mice regardless of the genetic background, showed that pathways involved in xenobiotic and drug metabolism, specifically the cytochrome P450 (cyp450) family, was upregulated in the female mice (Figure 13A).

This finding is not unexpected, as it has been previously reported that the expression of cyp450 family to be sex dimorphic, (Renaud et al., 2011). In the context of this study, 17 cyp450 isoenzymes were found to be differentially expressed between male and female mice; ten of which were predominantly expressed in the female mice and seven in male mice (Figure 13B). It was subsequently found by the pathway analysis, that the differentially changed isoenzymes in the male and female mice were involved in the metabolism of arachidonic acid, a polyunsaturated omega-6 fatty acid, to epoxyeicosatrienoic acids (EETs), which are known to have beneficial effects in diseases as diabetes or the metabolic syndrome (dos Santos & Fleming, 2020).

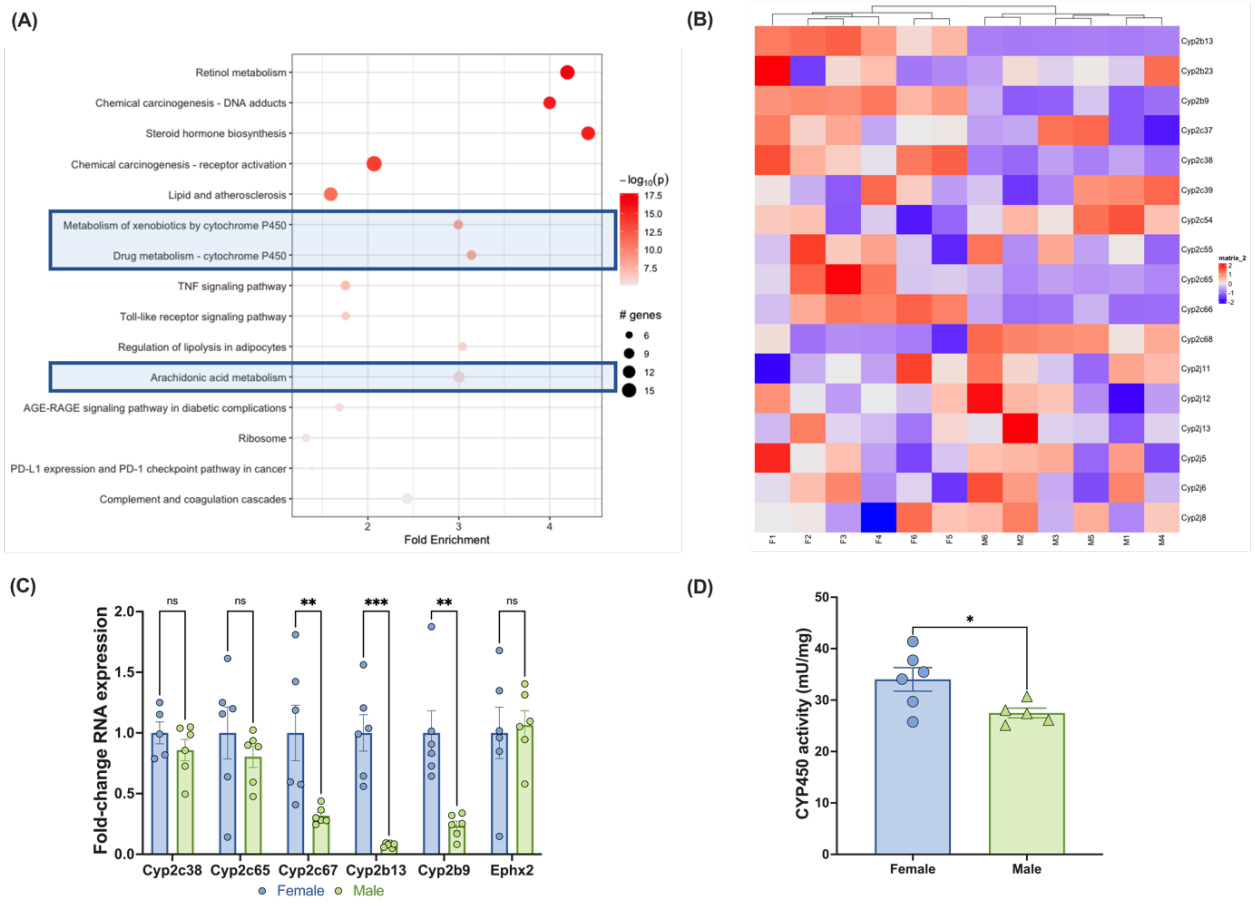


Figure 13. Arachidonic acid metabolism. (A) Enrichment plot of most differentially expressed pathways in the livers of female and male mice after gene expression profiling. (B) Heatmap of the gene expression of Cyp450 isoforms in the livers of female and male mice. **(C)** RNA expression of Cyp450 isoforms involved in arachidonic acid metabolism measured by qPCR in liver samples from female and male mice. Data shows the fold-change of males to females. **(D)** Cyp450 total activity in liver extracts from female and male mice. Data is represented by the Mean ± SEM of biological replicates. * p <0.05, ** p < 0.01, *** p < 0.001, **ns** p >0.05.

qPCR analysis showed that regardless of the loss of Glo1, male mice in comparison to female mice, had a significant reduction in the expression of Cyp3c67 (0.31-fold ± 0.03, $p < 0.01$, Figure 13C), Cyp2b13 (0.07-fold ± 0.01, $p < 0.001$, Figure 13C), and Cyp2b9 (0.23-fold ± 0.04, $p < 0.01$, Figure 13C), which correspond with epoxygenases of the Cyp450 family responsible for the metabolism of arachidonic acid into EETs. Consist with the decrease in expression, a decrease in total liver cyp450 activity was observed in the male mice as compared to the female mice (27.48 ± 0.9 vs 34.02 ± 2.3 mU/mg, $p < 0.05$, Figure 13D).

EEts can be converted into dihydroxyeicosatrienoic acids (DHETs) by the soluble epoxide hydrolase (sEH). The biological actions of DHETs are less well understood, but high concentrations of these diols have been suggested to be detrimental, as they have been related to promote inflammation. Therefore, a lower expression of sEH leading to a reduced conversion of EETs into DHETs has been proposed as a protective mechanism against inflammation and metabolic disorders (dos Santos & Fleming, 2020). The expression of Ephx2, the gene encoding for the expression of sEH, is not significantly changed between females and males in this study (Figure 13C), suggesting that a potential increase in EETs in female mice would be mainly caused by increased metabolism of arachidonic acid by Cyp450 epoxygenases, but not by an decreased expression of sEH.

4. Discussion

4.1 The loss of Glo1 in mice does not recapitulate to a T2D-phenotype

The aim of this study was to determine if the loss of Glo1 could lead to a diet-induced T2D phenotype. However, the results showed that the effects resulting from the loss of Glo1 were not comparable and that physiologically, the Glo1-KO mice showed little or no difference to the aged-matched wild-type mice. This finding is consistent with the previously studies using this Glo1-KO mouse (Schumacher et al., 2018). This mouse line which was created using CRISPR/Cas9 to delete eight nucleotides within the Glo1 gene, lead to the systemic loss of the Glo1 protein and activity (Morgenstern et al., 2017; Schumacher et al., 2018). As have been reported previously, the mice were viable and showed no reproductive phenotype, which is inconsistent with previous claims that the complete loss of Glo1 would be lethal to an organism (Arai et al., 2010; Shafie et al., 2016). Furthermore, the loss in protein/activity was stable, unlike the previous siRNA-lentiviral approach used to generate Glo1 knock-down mouse, in which it has been reported that the partial loss of Glo1 leads to increases in MG and MG-H1, but also symptoms of diabetic nephropathy, independent of hyperglycaemia (Giacco et al., 2014). A Glo1-KO mouse line has also been generated using a gene trapping approach which lead to the complete loss of Glo1 activity in the liver and brain (Jang et al., 2017). It was subsequently claimed that the AGE content within these organs was elevated. However, these measurements were performed by western blotting and no corroborated with quantitative mass spectrometric measurements. The extent of these increases, based upon the presented blot, and which are not reported to be significant, remains questionable. Nevertheless, these mice were also reported to be viable and showed no deficit in reproductive function. With the exception, of the Glo1 knock-down mouse study, there are currently no in vivo studies which would indicate that the loss of Glo1 is detrimental to health. Within the context of clinical diabetes, MG and/or MG-H1 have been reported to be elevated in patients (Rabbani & Thornalley, 2012, 2014b), however, this has yet to be associated with the loss of Glo1; the majority of clinical studies have reported either no differences or an increase in activity, based upon the measurement of activity, in red blood cells (McLellan et al., 1994; Skapare et al., 2013; P.J. Thornalley et al., 1989), or D-lactate, as a stable end-product of the glyoxalase system, in the plasma and urine (Chou et al., 2015; Scheijen et al., 2012).

This raises a critical question of whether Glo1 is truly relevant for regulating MG levels. As it has been shown in other studies, the loss of Glo1 in murine models can be effectively compensated, therefore provided one a means by which the detrimental effects of MG can be migrated (Morgenstern et al., 2017; Schumacher et al., 2018). In this study, the lack of any physiological differences between the Glo1-KO and the wild-type mice, could be the result of MG not becoming elevated, due to alternative detoxification. This may also be an explanation as to why the loss of Glo1 in organisms lower down the phylogenetic tree show more detrimental effects, as their capacity to perform alternative detoxification is reduced by a limited expression of the required aldo-keto reductase and/or aldehyde dehydrogenase isoenzymes (Morgenstern, Campos Campos, et al., 2020).

The relevance of alternative detoxification by aldo-keto reductase in vivo has been demonstrated by Baba et al., which showed that the detoxification of MG was lost in the hearts of Akr1b3 knockout mice (Baba et al., 2009). In type 1 model of diabetes, it was subsequently shown that Akr1b3 knockout mice had increased accumulation of AGEs in the plasma heart, whilst loss of Akr1b3 in apoE-knockout mouse not only increasing AGE accumulation but also the formation of atherosclerotic lesions. A similar approach was performed with the Glo1 knockdown mouse and whilst it was found that Glo1 activity was decreased within the tissues, there was no increase in MG-derived AGEs or enhanced formation of atherosclerosis (Baba et al., 2009). These two

studies together would suggest that the loss of Glo1 alone is insufficient a trigger to elevate MG levels, and potential development of diabetes and its associated complications. The combined loss of Glo1 and Akr1b3 may therefore provide a more suitable model for studying the contribution of MG to diabetes in future studies. However, a limitation to this approach is that all members of the aldo-keto reductase superfamily share similar enzymatic properties (Wortmann et al., 2016). As such, the loss of one member would not necessarily lead to the loss of alternative detoxification, as it could be partly compensated by the other family members (Barski et al., 2008). Furthermore, the physiological relevance of such a model to clinical diabetes would remain unclear, unless it could first be proven that Glo1 activity is reduced and aldo-keto reductase activity is increased in diabetic individuals.

The loss of the Glo1 protein, as has been demonstrated in the various Glo1-KO experimental models, would inevitably lead to the loss of activity. However, a decrease in activity could also result without the loss of the intact protein. For instance, Glo1 is a GSH dependent enzyme, and as such the activity of Glo1 within a given cell or tissue will be dependent not only upon the expression of the protein but will also the cellular concentration of GSH. If the GSH concentration was decrease, then this would also lead to a decrease in activity, without any loss of the protein (Abordo et al., 1999; P.J. Thornalley, 2003; Paul J Thornalley, 1998). Unfortunately, proving whether this is a mechanism of action in vitro or in vivo remains elusive as in situ measurements of Glo1 activity are not possible without using saturation conditions to perform the activity assay (Arai et al., 2014). It has also been shown the post-translational modifications of Glo1 can also affect the activity and affinity of the enzyme without a loss of the protein (Birkenmeier et al., 2010; Cortizo et al., 2022; de Hemptinne et al., 2009; Morgenstern, Katz, et al., 2020). Such modulators of Glo1 would suggest that activity, rather than remaining constant, could fluctuate leading to periods when MG is elevated. If this were the case, then an experimental model in which the expression of Glo1 could be periodically switched on or off, may provide a more physiological relevant model for study. The generation of an inducible knock-out, using the tetracycline-controlled transcriptional activation method (Gossen & Bujard, 1992), would allow for this as the suppression of Glo1 would be reversible, unlike the Cre recombinase expression system which tends to be irreversible once induced. If such an approach was also targeted to a specific organ, such as the liver which has the highest Glo1 activity, it may be possible to get a better understanding of the relationship that Glo1 has to the metabolism of MG, and in turn, the physiological effects induced by elevated MG.

4.2 Sex-specific effects driven by Glo1

The Glo1-KO mice did not recapitulate a T2D phenotype, however, a sex dimorphism was observed at the molecular level, in which male Glo1-KO mice were found to have changed mitochondrial function, decreased responsiveness to insulin, as measured by Akt phosphorylation, and decreased activation of AMPK as compared to the female Glo1-KO mice. This evidence of a sex dimorphism is not unexpected as there is substantial clinical and experiment evidence published in the last decade which showed that there are significant molecular differences between the sexes, particularly with respect to diabetes. For example, epidemiological studies have shown that diabetes is more prevalent in men than in women, especially amongst individuals aged 45-60 years (Peters et al., 2019; B. Zhou et al., 2016). The explanation for such differences in respect to energy partitioning and expenditure, body composition and regulation of glucose homeostasis (Tramunt et al., 2020).

Such sex differences tend to be explained within the context of the expression and action of the sex hormones progestogens, androgens and estrogens. In the context of diabetes, an association between estrogens and the activation of protective effects in skeletal muscle, brains, liver, adipose

tissues and pancreatic ß-cells has been reported. The activation of estrogen receptor signaling pathways have been shown to lead to decreased body weight and adiposity, increased insulin sensitivity and increased glucose tolerance (Ortiz-Huidobro et al., 2021). Such positive sex-hormone effects are supported by the deleterious impact of the menopause and experimental ovariectomies on body composition and glucose homeostasis in female mice, which can be prevented by estrogen treatment (Anagnostis et al., 2019; Handgraaf et al., 2013; Kanaya et al., 2003; Riant et al., 2009). However, the complex mechanism(s) which are responsible for regulating sex-dimorphic dependent metabolism in diabetes are not yet completely understood. This is due in part to many experimental diabetes studies using exclusively male mice, to avoid any potential confounding effects the female sex hormones may have had. As consequence, such studies can only provide insights into the molecular differences relevant to half of the population.

Several studies have also reported sex dimorphisms when knocking out various genes and/or proteins, achieving unexpected results between sexes (Kreiner et al., 2013; Mansukhani et al., 2017; Ramanathan & Siegel, 2014; Sheng et al., 2017; Tangirala et al., 1995; Vomhof-DeKrey et al., 2019). For example, an LDL receptor (LDLR)-KO model in mice revealed that atherosclerosis develops differently between sexes, with females showing more atherosclerotic lesions than males when fed a high fat-low cholesterol diet (Mansukhani et al., 2017). However, in the same LDLR-KO model, a study performed using a diet with a higher cholesterol content reported increased atherosclerotic lesion area in male mice (Tangirala et al., 1995). This demonstrates that sex is a critical variable in experimental studies and that experimental conditions need to be selected carefully, as they may have opposite effects between sexes.

With respect to metabolism, when studying the loss of key regulator genes and/or proteins, male mice are reported to the more affected. For instance, in a study in GABAB1-KO mice, KO-males showed increased fasting insulin and HOMA-IR when compared to KO-females, accompanied by a decreased activation of Akt in KO-males with no observations in KO-females (Bonaventura et al., 2013). Similar results were found when studying proton channel Hv1-KO mice, with KO-males showing impaired hepatic glucose homeostasis with lower expression of glycolytic genes and higher expression gluconeogenic genes when compared to KO-females, leading to increased fasting glucose levels in KO-males (Pang et al., 2021). Hepatic steatosis has been shown to be more severe in male mice when compare to female mice when examining G-protein-coupled bile acid receptor 1 (Gpbar1) and farnesoid X receptor (FXR)-KO mice (Sheng et al., 2017; Vassileva et al., 2010). Similar effects have been reported in the kidney, with Apoe/Integrin chain Itga8-double KO males showing increased renal damage than KO-females (Marek et al., 2017).These studies also demonstrate that whilst sharing a commen genus, the genders of a species have distinct biochemical and molecules differences, in which female mice are protected against metabolic dysfunction. It is therefore critical that all future experimental studies included both female and male mice.

4.3 Females are protected against metabolic impairments

It is well described that females are less susceptible to metabolic disorders, with males more likely to develop hyperglycemia, insulin resistance and obesity (Tramunt et al., 2020). In the case of T2D, males are more frequently diagnosed at a lower BMI and age than females, even though obesity is more common in females than males (Kautzky-Willer et al., 2016; Zhang et al., 2019). One explanation for this could be the differences in which the different genders accumulate fat within the body; females have a larger proportion of fat in relation to body mass, as such, they tend to accumulate fat subcutaneously, whereas males are more likely to deposit this fat in the abdominal region and as visceral fat, which is associated with a greater health risk (Power & Schulkin, 2008). Moreover, when subjected to nutritional challenges, females have been found

to be more resistant to its obesifying effects (Casimiro et al., 2021; Gelineau et al., 2017; Oraha et al., 2022). Females are also considered to be more insulin sensitive than males, with glucose and insulin tolerance tests in healthy individuals showing enhanced insulin sensitivity index, potentially due to a female-specific increase in glucose uptake by skeletal muscle (Tramunt et al., 2020).

The underlying molecular mechanism(s) which give females protection against the effects of obesity remain unclear. Unsurprisingly, sex hormones are considered to play a significant role in this protection. Estrogen signaling, for instance, can regulate lipid and cholesterol metabolism, through activation of the PI3K pathway in the liver (Palmisano et al., 2018). This supports the assumption that estrogen receptors can act by interacting with kinases as PI3K, ERK or AMPK, leading to increased insulin sensitivity (Mauvais-jarvis et al., 2013). Another protective mechanism found in females is increased production of adiponectin (Manieri et al., 2019; Ohman-hanson et al., 2016). Adiponectin, a cytokine produced in the adipose tissue, has been shown to be decreased in obese individuals and is associated with cardiovascular diseases and metabolic disorders. Adiponectin can also reduce inflammatory cytokines (Kobashi et al., 2005; Ouchi et al., 2000) and oxidative stress (Tao et al., 2007), leading to improvements in insulin resistance. Adiponectin can also improve insulin resistance via activation of AMPK and peroxisome proliferator-activated receptor-α (PPAR-α) (Matsuda & Shimomura, 2014). Brown adipose tissue (BAT) mass has also been found to be increased in females, as well as its thermogenic activity (Cypess et al., 2009; Pfannenberg et al., 2010). Brown adipocytes are known to be energy-combusting cells that accelerate substrate oxidation, and therefore the activation of thermogenic metabolism in BAT may improve metabolic health. Consequently, individuals with high BAT activity levels show increased levels of high-density lipoprotein (HDL) cholesterol and reduced levels of HbA1c, glucose and triglycerides (Matsushita et al., 2014; Q. Wang et al., 2015).

Liver metabolism has also been shown to be markedly sex dimorphic. Females have been shown to be less prone to develop liver disease, by virtue of decreased lipid accumulation. It has been shown that females have reduced expression of nuclear receptors, such as PPARα and increased activation of glucocorticoid receptors (GR), leading to reduced inflammation. Moreover, xenobiotic receptors are also increased in female livers as compared to males, leading to increased detoxification of xenobiotic compounds. Increased detoxification and reduced inflammation can result in reduced lipid accumulation and risk of liver disease in females (Rando & Wahli, 2011).

Transcriptional profiling of different organs from male and female mice performed by Rinn et al., showed that the majority of genes with sex-specific expression in the liver were involved in drug and steroid metabolism, corresponding to cyp450 isoenzymes. It was therefore concluded that sex differences in drug and steroid metabolism could have important implications on the physiological differences between females and males (Rinn et al., 2004). In this study, a sex-specific expression of hepatic cyp450 isoenzymes was also observed in which the expression of specific isoenzymes involved in arachidonic acid metabolism were found to be significantly higher in the females as compared to the males. These included Cyp2c and Cyp2b families of epoxygenases, specifically Cyp2c38, Cyp2c65, Cyp2c67, Cyp2b13 and Cyp2b9, which are involved in the metabolism of arachidonic acid to EETs in mice. The increased expression was also reflected by an increase in the total activity of cyp450 in the liver. This would suggest that female mice have a higher capacity to metabolism arachidonic acid to EETs.

EETs have been described to have many beneficial effects particularly as anti-inflammatory agents, with increased levels of EETs being classified as protective in the liver against metabolic syndrome. This protective effect of EETs comes from various mechanisms: (i) promotion of the phosphorylation of the IR, activating insulin signalling; (ii) induction of PPAR activation, promoting

mitochondrial function and a decreased in triglycerides; (iii) inhibition of ER stress; and (iv) the activation of AMPK, resulting in reduced cholesterol synthesis and increased insulin sensitivity (dos Santos & Fleming, 2020).

Based upon theses findings and the results of this study it can be concluded that the mechanism by which the Glo1-KO female mice are protected is through increased production of EETs. Upregulation of cyp450 isoenzymes in the female mice, leads to an increased metabolism of arachidonic acid resulting in increased levels of EETs. The increased production of EETs would then lead to a protection to develop metabolic disorders in females, as a result of the beneficial effects of these eicosanoids.

In order to determine whether this hypothesis was correct, further experiments would be required. In vivo, the levels of polyunsaturated fatty acids (PUFAs) and eicosanoids would need to be measured in male and female, wild-type and Glo1-KO mice to establish whether female mice have higher levels. The measurement of such metabolites is analytically challenging because of the very low concentrations (picograms per milliliter) in blood, urine, and tissues, in vitro metabolism, and autoxidation. Such measurements would therefore need to be performed by liquid-chromatography, tandem mass spectrometry, which has the sensitivity and specificity to detect such analytes. In vitro experiments would also need to be conducted using primary hepatocytes isolated from male and female, wild-type and Glo1-KO mice. The male isolated hepatocytes would be treated with increasing concentrations of EETs and the responsive to insulin, as measured by Akt phosphorylation, measured as to determined whether the deficient in insulin-dependent Akt phosphorylation observed in this study for the Glo1-KO male mice is reversed. Conversely, the female isolated hepatocytes could be treated with cell permeable inhibitors of cyp450, to reduce activity, or transfected with isoenzyme-specific siRNAs to reduce the expression of the cyp450s and normalize EET levels. As with the male hepatocytes, insulin-dependent Akt phosphorylation would be measured as to determine whether the levels of phosphorylation were reduced to a level that was observed in the Glo1-KO male mice. Such in vitro experiments would provide the evidence need to establish whether the increased arachidonic acid metabolism in the female mice was the basis for the protection observed. If this was proven to be the case, then additional in vitro and in vivo studies could be undertaken, to determine the relative contribution of this protective mechanism in the development of diabetes and its associated complications.

4.4 Concluding remarks

In conclusion, this study could show that the loss of Glo1 in mice does not recapitulate a T2D phenotype. However, this study did show that the effects resulting from the loss of Glo1 are sex dimorphic, in which male mice are more susceptible. The increase in arachidonic acid metabolism, in particularly epoxyeicosatrienoic acids (EETs), and the beneficial effects that they induce with respect to inflammation, mitochondrial dysfunction and insulin resistance, is proposed as a mechanism which can protect females from the loss of Glo1. A better understanding of sex dimorphism, particularly within the context of diabetes, may provide the means of developing therapeutic approaches which are effective for all individuals.

5. Summary

Type-2 diabetes (T2D) is a heterogeneous and multifactorial disorder whose pathophysiology is not completely understood. The first impairment seen in individuals suffering from T2D is insulin resistance resulting from: (i) mitochondrial dysfunction; (ii) lipid-induced insulin resistance; (iii) endoplasmic reticulum stress; and (iv) inflammation. Methylglyoxal (MG), a potent-glycating agent produced in physiological conditions as a by-product of glycolysis, has shown to be associated with the activation of the four above-mentioned impairments leading to insulin resistance. The effects of MG on cellular metabolism have been studied extensively and the modulation of its main detoxification enzyme, Glo1, has been used as a strategy to resemble increased levels of MG in diabetes. The knockdown of Glo1 in experimental murine models has been linked to the development of diabetic nephropathy and has shown increased MG levels. Moreover, the complete knockout of Glo1 (Glo1-KO) in Drosophila melanogaster or zebrafish, produced a T2D-phenoype with insulin resistance, potentially as a cause of increased levels of MG. In this study, it was hypothesized that the loss of Glo1 in mice could also recapitulate a T2D-phenotype. To test this hypothesis, Glo1-KO C57BL/6 mice were generated and compared to wildtype mice (WT), as a non-diabetic model, and mice subjected to a long-term high-fat diet (HFD), as a model of T2D. As expected, mice on a HFD showed increased body weight and blood glucose levels, as well as impaired glucose and insulin tolerance as compared to WT-mice. None of these physiological alterations, however, were seen in the Glo1-KO mice. Analysis of the liver and kidneys also showed no changes in the Glo1-KO mice. However, hepatic gene expression profiling showed a sex dimorphism in the Glo1-KO mice. The Glo1-KO male mice showed increased expression in the complexes of the mitochondrial electron transport chain. Further experiments showed a potential impairment in mitochondrial function in male Glo1-KO mice, as shown by significant increases in the levels of the citric acid cycle intermediates, after insulin stimulation, as well as an impaired lactate to pyruvate ratio. The impaired mitochondrial function in Glo1-KO males was also accompanied by reduced AMPK and insulin signalling cascade activation, as measured by Akt phosphorylation. Comparison of the hepatic gene expression from the female and male WT- and Glo1-KO mice showed an increased expression in the cytochrome P450 family in female mice. Specifically, an increase in the expression and activity of the isoenzymes involved in the metabolism of arachidonic acid. In conclusion, this study could show that the loss of Glo1 in mice does not recapitulate a T2D phenotype. However, this study did show that the effects resulting from the loss of Glo1 are sex dimorphic, in which male mice are more susceptible. The increase in arachidonic acid metabolism, in particularly the levels of epoxyeicosatrienoic acids (EETs), and the beneficial effects that they induce with respect to inflammation, mitochondrial dysfunction and insulin resistance, is proposed as a protective mechanism in females from the loss of Glo1.

6. Zusammenfassung

Typ-2 Diabetes (T2D) ist eine heterogene und multifaktorielle Störung deren Pathophysiologie nicht komplett verstanden ist. Die erste feststellbare Einschränkung bei an T2D leidenden Personen ist Insulinresistenz als Folge von: (i) mitochondrialer Dysfunktion, (ii) lipid-induzierter Insulinresistenz, (iii) Stress des endoplasmatischen Retikulums und (iv) Entzündungen. Die Aktivierung aller vier ursächlichen Einflussfaktoren ist mit Methylglyoxal (MG), einem starken Glykationsmittel, welches unter physiologischen Bedingungen als Nebenprodukt der Glykolyse entsteht, assoziiert. Die Effekte von MG auf den zellulären Metabolismus wurden ausführlich untersucht wobei die diabetisch erhöhten Level von MG durch die Beeinflussung seines Haupt-Detoxifizierungsenzyms, Glyoxalase 1 (Glo1), nachgestellt wurden. Es konnte gezeigt werde, dass der Knockdown von Glo1 in experimentellen Mausmodellen mit diabetischer Nephropathie und erhöhten MG-Leveln verbunden ist. Außerdem führt der komplette Knockout von Glo1 (Glo1-KO) in Drosophila melanogaster und Zebrafisch zu einem insulinresistenten T2D-Phänotyp, welcher möglicherweise durch erhöhte MG-Level verursacht wird. Der Hypothese dieser Studie zufolge, könnte der Verlust von Glo1 auch in Mäusen einen T2D-Phänotyp hervorrufen. Um dies zu testen, wurden Glo1-KO C57BL/6-Mäuse generiert und mit Wildtyp-Mäusen (WT), als nicht-diabetische Kontrollgruppe, und Wildtyp-Mäusen mit einer langfristigen fettreichen Diät (HFD), als T2D-Model, verglichen. Wie erwartet zeigten die HFD-Mäuse erhöhte Werte für Körpergewicht und Blutglukoselevel, sowie eingeschränkte Glukose- und Insulintoleranz verglichen zu WT-Mäusen. Keine dieser physiologischen Veränderungen, oder Veränderungen an Leber und Niere konnten in den Glo1-KO-Mäusen beobachtet werden. Allerdings zeigte die Expressionsanalyse hepatischer Gene einen geschlechtsspezifischen Dimorphismus, mit erhöhter Expression der Komplexe der mitochondrialen Elektronentransportkette in männlichen Glo1-KO-Mäusen. Eine potentielle Einschränkung der mitochondrialen Funktion in männlichen Glo1-KO-Mäusen wurde durch signifikant erhöhte Level von Zitronensäurezyklus-Intermediaten, nach Insulinstimulation, und Veränderung des Verhältnisses von Laktat zu Pyruvat nachgewiesen. Außerdem zeigte die Messung der Akt Phosphorylierung, dass die reduzierte mitochondriale Funktion in männliche Glo1-KO-Mäusen mit einer reduzierten Aktivierung der AMPK und Insulinsignalkaskade verbunden ist. In weiblichen Mäusen wurde, unabhängig von Glo1, im Vergleich zu männlichen Mäusen eine erhöhte Expression von Genen der Cytochrom P450 Familie festgestellt. Dies bezieht sich insbesondere auf einen Anstieg der Expression und Aktivität der Isoenzyme welche im Stoffwechsel von Arachidonsäure involviert sind. Zusammengefasst zeigte diese Studie, dass der Verlust von Glo1 in Mäusen nicht in einem T2D-Phänotyp resultiert. Es konnte jedoch gezeigt werden, dass der Verlust von Glo1 geschlechtsspezifische Effekte hat und männliche Mäuse stärker beeinträchtigt werden. Der Anstieg im Arachidonsäure-Stoffwechsel, insbesondere die höheren Level von Epoxyeicosatriensäuren und die damit assoziierten förderlichen Effekte mit Bezug zu Entzündungen, mitochondrialer Dysfunktion und Insulinresistenz, wird daher als schützender Mechanismus beim Verlust von Glo1 in weiblichen Mäusen vorgeschlagen.

7. Reference list

Abordo, E. A., Minhas, H. S., & Thornalley, P. J. (1999). Accumulation of α-oxoaldehydes during oxidative stress: a role in cytotoxicity. *Biochemical Pharmacology*, *58*(4), 641–648. https://doi.org/10.1016/S0006-2952(99)00132-X

Aguirre, V., Uchida, T., Yenush, L., Davis, R., & White, M. F. (2000). The c-Jun NH2-terminal Kinase Promotes Insulin Resistance during Association with Insulin Receptor Substrate-1 and Phosphorylation of Ser307. *Journal of Biological Chemistry*, *275*(12), 9047–9054. https://doi.org/10.1074/jbc.275.12.9047

Ahlqvist, E., Storm, P., Käräjämäki, A., Martinell, M., Dorkhan, M., Carlsson, A., Vikman, P., Prasad, R. B., Aly, D. M., Almgren, P., Wessman, Y., Shaat, N., Spégel, P., Mulder, H., Lindholm, E., Melander, O., Hansson, O., Malmqvist, U., Lernmark, Å., … Groop, L. (2018). Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. *The Lancet Diabetes & Endocrinology*, *6*(5), 361–369. https://doi.org/10.1016/S2213-8587(18)30051-2

Ahmed, N., Dobler, D., Dean, M., & Thornalley, P. J. (2005). Peptide Mapping Identifies Hotspot Site of Modification in Human Serum Albumin by Methylglyoxal Involved in Ligand Binding and Esterase Activity. *Journal of Biological Chemistry*, *280*(7), 5724–5732. https://doi.org/10.1074/jbc.M410973200

Ahmed, N., Thornalley, P. J., Dawczynski, J., Franke, S., Strobel, J., Stein, G., & Haik, G. M. (2003). Methylglyoxal-Derived Hydroimidazolone Advanced Glycation End-Products of Human Lens Proteins. *Investigative Opthalmology & Visual Science*, *44*(12), 5287. https://doi.org/10.1167/iovs.03-0573

Al-Balas, Q. A., Hassan, M. A., Al-Shar'i, N. A., Al Jabal, G. A., & Almaaytah, A. M. (2018). Recent Advances in Glyoxalase-I Inhibition. *Mini-Reviews in Medicinal Chemistry*, *19*(4), 281–291. https://doi.org/10.2174/1389557518666181009141231

Anagnostis, P., Christou, K., Artzouchaltzi, A.-M., Gkekas, N. K., Kosmidou, N., Siolos, P., Paschou, S. A., Potoupnis, M., Kenanidis, E., Tsiridis, E., Lambrinoudaki, I., Stevenson, J. C., & Goulis, D. G. (2019). Early menopause and premature ovarian insufficiency are associated with increased risk of type 2 diabetes: a systematic review and meta-analysis. *European Journal of Endocrinology*, *180*(1), 41–50. https://doi.org/10.1530/EJE-18-0602

Andersen, S. T., Witte, D. R., Dalsgaard, E.-M., Andersen, H., Nawroth, P., Fleming, T., Jensen, T. M., Finnerup, N. B., Jensen, T. S., Lauritzen, T., Feldman, E. L., Callaghan, B. C., & Charles, M. (2018). Risk Factors for Incident Diabetic Polyneuropathy in a Cohort With Screen-Detected Type 2 Diabetes Followed for 13 Years: ADDITION-Denmark. *Diabetes Care*, *41*(5), 1068–1075. https://doi.org/10.2337/dc17-2062

Aragonès, G., Rowan, S., Francisco, S. G., Yang, W., Weinberg, J., Taylor, A., & Bejarano, E. (2020). Glyoxalase system as a therapeutic target against diabetic retinopathy. *Antioxidants*, *9*(11), 1–25. https://doi.org/10.3390/antiox9111062

Arai, M., Nihonmatsu-Kikuchi, N., Itokawa, M., Rabbani, N., & Thornalley, P. J. (2014). Measurement of glyoxalase activities. *Biochemical Society Transactions*, *42*(2), 491–494. https://doi.org/10.1042/BST20140010

Arai, M., Yuzawa, H., Nohara, I., Ohnishi, T., Obata, N., Iwayama, Y., Haga, S., Toyota, T., Ujike, H., Arai, M., Ichikawa, T., Nishida, A., Tanaka, Y., Furukawa, A., Aikawa, Y., Kuroda, O., Niizato, K., Izawa, R., Nakamura, K., … Itokawa, M. (2010). Enhanced Carbonyl Stress in a Subpopulation of Schizophrenia. *Archives of General Psychiatry*, *67*(6), 589. https://doi.org/10.1001/archgenpsychiatry.2010.62

Arkan, M. C., Hevener, A. L., Greten, F. R., Maeda, S., Li, Z.-W., Long, J. M., Wynshaw-Boris, A., Poli, G., Olefsky, J., & Karin, M. (2005). IKK-β links inflammation to obesity-induced insulin resistance. *Nature Medicine*, *11*(2), 191–198. https://doi.org/10.1038/nm1185

Asif, M. (2014). The prevention and control the type-2 diabetes by changing lifestyle and dietary pattern. *Journal of Education and Health Promotion*, *3*(1), 1. https://doi.org/10.4103/2277-9531.127541

Baba, S. P., Barski, O. A., Ahmed, Y., O'Toole, T. E., Conklin, D. J., Bhatnagar, A., & Srivastava, S. (2009). Reductive Metabolism of AGE Precursors: A Metabolic Route for Preventing AGE Accumulation in Cardiovascular Tissue. *Diabetes*, *58*(11), 2486–2497. https://doi.org/10.2337/db09-0375

Barnett, A. H., Eff, C., Leslie, R. D. G., & Pyke, D. A. (1981). Diabetes in identical twins. *Diabetologia*, *20*(2), 87–93. https://doi.org/10.1007/BF00262007

Barski, O. A., Tipparaju, S. M., & Bhatnagar, A. (2008). The Aldo-Keto Reductase Superfamily and its Role in Drug Metabolism and Detoxification. *Drug Metabolism Reviews*, *40*(4), 553–624. https://doi.org/10.1080/03602530802431439

Beisswenger, P. J., Drummond, K. S., Nelson, R. G., Howell, S. K., Szwergold, B. S., & Mauer, M. (2005). Susceptibility to Diabetic Nephropathy Is Related to Dicarbonyl and Oxidative Stress. *Diabetes*, *54*(11), 3274–3281. https://doi.org/10.2337/diabetes.54.11.3274

Bellahcène, A., Nokin, M. J., Castronovo, V., & Schalkwijk, C. (2018). Methylglyoxal-derived stress: An emerging biological factor involved in the onset and progression of cancer. *Seminars in Cancer Biology*, *49*(June 2017), 64–74. https://doi.org/10.1016/j.semcancer.2017.05.010

Bellier, J., Nokin, M. J., Lardé, E., Karoyan, P., Peulen, O., Castronovo, V., & Bellahcène, A. (2019). Methylglyoxal, a potent inducer of AGEs, connects between diabetes and cancer. *Diabetes Research and Clinical Practice*, *148*, 200–211. https://doi.org/10.1016/j.diabres.2019.01.002

Bento, C. F., Fernandes, R., Matafome, P., Sena, C., Seiça, R., & Pereira, P. (2010). Methylglyoxal-induced imbalance in the ratio of vascular endothelial growth factor to angiopoietin 2 secreted by retinal pigment epithelial cells leads to endothelial dysfunction. *Experimental Physiology*, *95*(9), 955–970. https://doi.org/10.1113/expphysiol.2010.053561

Berg, A. H., Combs, T. P., & Scherer, P. E. (2002). ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. *Trends in Endocrinology & Metabolism*, *13*(2), 84–89. https://doi.org/10.1016/S1043-2760(01)00524-0

Berlanga, J., Cibrian, D., Guillén, I., Freyre, F., Alba, J. S., Lopez-Saura, P., Merino, N., ALDAMA, A., QUINTELA, A. M., Triana, M. E., Montequin, J. F., Ajamieh, H., Urquiza, D., Ahmed, N.,

& Thornalley, P. J. (2005). Methylglyoxal administration induces diabetes-like microvascular changes and perturbs the healing process of cutaneous wounds. *Clinical Science*, *109*(1), 83–95. https://doi.org/10.1042/CS20050026

Bezy, O., Tran, T. T., Pihlajamäki, J., Suzuki, R., Emanuelli, B., Winnay, J., Mori, M. A., Haas, J., Biddinger, S. B., Leitges, M., Goldfine, A. B., Patti, M. E., King, G. L., & Kahn, C. R. (2011). PKCδ regulates hepatic insulin sensitivity and hepatosteatosis in mice and humans. *Journal of Clinical Investigation*, *121*(6), 2504–2517. https://doi.org/10.1172/JCI46045

Bierhaus, A., Fleming, T., Stoyanov, S., Leffler, A., Babes, A., Neacsu, C., Sauer, S. K., Eberhardt, M., Schnölzer, M., Lasischka, F., Neuhuber, W. L., Kichko, T. I., Konrade, I., Elvert, R., Mier, W., Pirags, V., Lukic, I. K., Morcos, M., Dehmer, T., … Nawroth, P. P. (2012). Methylglyoxal modification of Na v 1.8 facilitates nociceptive neuron firing and causes hyperalgesia in diabetic neuropathy. *Nature Medicine*, *18*(6), 926–933. https://doi.org/10.1038/nm.2750

Birkenmeier, G., Stegemann, C., Hoffmann, R., Günther, R., Huse, K., & Birkemeyer, C. (2010). Posttranslational Modification of Human Glyoxalase 1 Indicates Redox-Dependent Regulation. *PLoS ONE*, *5*(4), e10399. https://doi.org/10.1371/journal.pone.0010399

Boden, G., Duan, X., Homko, C., Molina, E. J., Song, W., Perez, O., Cheung, P., & Merali, S. (2008). Increase in Endoplasmic Reticulum Stress–Related Proteins and Genes in Adipose Tissue of Obese, Insulin-Resistant Individuals. *Diabetes*, *57*(9), 2438–2444. https://doi.org/10.2337/db08-0604

Bonaventura, M. M., Rodriguez, D., Ferreira, M. L., Crivello, M., Repetto, E. M., Bettler, B., Libertun, C., & Lux-Lantos, V. A. (2013). Sex differences in insulin resistance in GABAB1 knockout mice. *Life Sciences*, *92*(3), 175–182. https://doi.org/10.1016/j.lfs.2012.11.007

Boucher, J., Kleinridders, A., & Kahn, C. R. (2014). Insulin Receptor Signaling in Normal and Insulin-Resistant States. *Cold Spring Harbor Perspectives in Biology*, *6*(1), a009191–a009191. https://doi.org/10.1101/cshperspect.a009191

Bradford, M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry*, *72*(1–2), 248–254. https://doi.org/10.1006/abio.1976.9999

Brouwers, O., Niessen, P. M. G., Miyata, T., Østergaard, J. A., Flyvbjerg, A., Peutz-Kootstra, C. J., Sieber, J., Mundel, P. H., Brownlee, M., Janssen, B. J. A., De Mey, J. G. R., Stehouwer, C. D. A., & Schalkwijk, C. G. (2014). Glyoxalase-1 overexpression reduces endothelial dysfunction and attenuates early renal impairment in a rat model of diabetes. *Diabetologia*, *57*(1), 224–235. https://doi.org/10.1007/s00125-013-3088-5

Bürstenbinder, K., Rzewuski, G., Wirtz, M., Hell, R., & Sauter, M. (2007). The role of methionine recycling for ethylene synthesis in Arabidopsis. *The Plant Journal*, *49*(2), 238–249. https://doi.org/10.1111/j.1365-313X.2006.02942.x

Butler, A. E., Janson, J., Bonner-Weir, S., Ritzel, R., Rizza, R. A., & Butler, P. C. (2003). β-Cell Deficit and Increased β-Cell Apoptosis in Humans With Type 2 Diabetes. *Diabetes*, *52*(1), 102–110. https://doi.org/10.2337/diabetes.52.1.102

Cai, D., Yuan, M., Frantz, D. F., Melendez, P. A., Hansen, L., Lee, J., & Shoelson, S. E. (2005). Local and systemic insulin resistance resulting from hepatic activation of IKK-β and NF-κB. *Nature Medicine*, *11*(2), 183–190. https://doi.org/10.1038/nm1166

Cantó, C., & Auwerx, J. (2009). PGC-1α, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. *Current Opinion in Lipidology*, *20*(2), 98–105. https://doi.org/10.1097/MOL.0b013e328328d0a4

CARLING, D., CLARKE, P. R., ZAMMIT, V. A., & HARDIE, D. G. (1989). Purification and characterization of the AMP-activated protein kinase. Copurification of acetyl-CoA carboxylase kinase and 3-hydroxy-3-methylglutaryl-CoA reductase kinase activities. *European Journal of Biochemistry*, *186*(1–2), 129–136. https://doi.org/10.1111/j.1432-1033.1989.tb15186.x

Carrington, S. J., & Douglas, K. T. (1986). The glyoxalase enigma -- the biological consequences of a ubiquitous enzyme. *IRCS Medical Science*, *14*, 763–768.

Casimiro, I., Stull, N. D., Tersey, S. A., & Mirmira, R. G. (2021). Phenotypic sexual dimorphism in response to dietary fat manipulation in C57BL/6J mice. *Journal of Diabetes and Its Complications*, *35*(2), 107795. https://doi.org/10.1016/j.jdiacomp.2020.107795

Cerf, M. E. (2013). Beta Cell Dysfunction and Insulin Resistance. *Frontiers in Endocrinology*, *4*. https://doi.org/10.3389/fendo.2013.00037

Chan, C.-M., Huang, D.-Y., Huang, Y.-P., Hsu, S.-H., Kang, L.-Y., Shen, C.-M., & Lin, W.-W. (2016). Methylglyoxal induces cell death through endoplasmic reticulum stress-associated ROS production and mitochondrial dysfunction. *Journal of Cellular and Molecular Medicine*, *20*(9), 1749–1760. https://doi.org/10.1111/jcmm.12893

Chan, J. M., Rimm, E. B., Colditz, G. A., Stampfer, M. J., & Willett, W. C. (1994). Obesity, Fat Distribution, and Weight Gain as Risk Factors for Clinical Diabetes in Men. *Diabetes Care*, *17*(9), 961–969. https://doi.org/10.2337/diacare.17.9.961

Chou, C.-K., Lee, Y.-T., Chen, S.-M., Hsieh, C.-W., Huang, T.-C., Li, Y.-C., & Lee, J.-A. (2015). Elevated urinary d-lactate levels in patients with diabetes and microalbuminuria. *Journal of Pharmaceutical and Biomedical Analysis*, *116*, 65–70. https://doi.org/10.1016/j.jpba.2015.06.014

Colditz, G. A. (1995). Weight Gain as a Risk Factor for Clinical Diabetes Mellitus in Women. *Annals of Internal Medicine*, *122*(7), 481. https://doi.org/10.7326/0003-4819-122-7-199504010-00001

Copps, K. D., & White, M. F. (2012). Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2. *Diabetologia*, *55*(10), 2565–2582. https://doi.org/10.1007/s00125-012-2644-8

Cortizo, F. G., Pfaff, D., Wirth, A., Schlotterer, A., Medert, R., Morgenstern, J., Weber, T., Hammes, H.-P., Fleming, T., Nawroth, P. P., Freichel, M., & Teleman, A. A. (2022). The activity of glyoxylase 1 is regulated by glucose-responsive phosphorylation on Tyr136. *Molecular Metabolism*, *55*, 101406. https://doi.org/10.1016/j.molmet.2021.101406

Coughlan, K. A., Valentine, R. J., Sudit, B. S., Allen, K., Dagon, Y., Kahn, B. B., Ruderman, N. B., & Saha, A. K. (2016). PKD1 Inhibits AMPKα2 through Phosphorylation of Serine 491 and Impairs Insulin Signaling in Skeletal Muscle Cells. *Journal of Biological Chemistry*, *291*(11), 5664–5675. https://doi.org/10.1074/jbc.M115.696849

Cusi, K., Maezono, K., Osman, A., Pendergrass, M., Patti, M. E., Pratipanawatr, T., DeFronzo, R. A., Kahn, C. R., & Mandarino, L. J. (2000). Insulin resistance differentially affects the PI 3-kinase– and MAP kinase–mediated signaling in human muscle. *Journal of Clinical Investigation*, *105*(3), 311–320. https://doi.org/10.1172/JCI7535

Cypess, A. M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A. B., Kuo, F. C., Palmer, E. L., Tseng, Y., Doria, A., Kolodny, G. M., & Kahn, C. R. (2009). *Identification and Importance of Brown Adipose Tissue in Adult Humans*. 1509–1517.

Dadke, S., Kusari, J., & Chernoff, J. (2000). Down-regulation of Insulin Signaling by Protein-tyrosine Phosphatase 1B Is Mediated by an N-terminal Binding Region. *Journal of Biological Chemistry*, *275*(31), 23642–23647. https://doi.org/10.1074/jbc.M001063200

Dakin, H. D., & Dudley, H. W. (1913). ON GLYOXALASE. *Journal of Biological Chemistry*, *14*(4), 423–431. https://doi.org/10.1016/S0021-9258(18)88589-1

Daneman, D. (2006). Type 1 diabetes. *Lancet (London, England)*, *367*(9513), 847–858. https://doi.org/10.1016/S0140-6736(06)68341-4

Daroux, M., Prévost, G., Maillard-Lefebvre, H., Gaxatte, C., D'Agati, V. D., Schmidt, A. M., & Boulanger, É. (2010). Advanced glycation end-products: Implications for diabetic and non-diabetic nephropathies. *Diabetes & Metabolism*, *36*(1), 1–10. https://doi.org/10.1016/j.diabet.2009.06.005

de Hemptinne, V., Rondas, D., Toepoel, M., & Vancompernolle, K. (2009). Phosphorylation on Thr-106 and NO-modification of glyoxalase I suppress the TNF-induced transcriptional activity of NF-κB. *Molecular and Cellular Biochemistry*, *325*(1–2), 169–178. https://doi.org/10.1007/s11010-009-0031-7

DeFronzo, R. A., Ferrannini, E., Groop, L., Henry, R. R., Herman, W. H., Holst, J. J., Hu, F. B., Kahn, C. R., Raz, I., Shulman, G. I., Simonson, D. C., Testa, M. A., & Weiss, R. (2015). Type 2 diabetes mellitus. *Nature Reviews Disease Primers*, *1*(1), 15019. https://doi.org/10.1038/nrdp.2015.19

Dennis, J. M., Shields, B. M., Henley, W. E., Jones, A. G., & Hattersley, A. T. (2019). Disease progression and treatment response in data-driven subgroups of type 2 diabetes compared with models based on simple clinical features: an analysis using clinical trial data. *The Lancet Diabetes & Endocrinology*, *7*(6), 442–451. https://doi.org/10.1016/S2213-8587(19)30087-7

Deshmukh, A. B., Bai, S., Aarthy, T., Kazi, R. S., Banarjee, R., Rathore, R., Mv, V., Hv, T., Kumar Bhat, M., & Mj, K. (2017). Methylglyoxal attenuates insulin signaling and downregulates the enzymes involved in cholesterol biosynthesis. *Molecular BioSystems*, *13*(11), 2338–2349. https://doi.org/10.1039/c7mb00305f

dos Santos, L. R. B., & Fleming, I. (2020). Role of cytochrome P450-derived, polyunsaturated fatty acid mediators in diabetes and the metabolic syndrome. *Prostaglandins & Other Lipid*

Mediators, *148*, 106407. https://doi.org/10.1016/j.prostaglandins.2019.106407

Du, K., Herzig, S., Kulkarni, R. N., & Montminy, M. (2003). TRB3: A *tribbles* Homolog That Inhibits Akt/PKB Activation by Insulin in Liver. *Science*, *300*(5625), 1574–1577. https://doi.org/10.1126/science.1079817

Eguchi, K., Manabe, I., Oishi-Tanaka, Y., Ohsugi, M., Kono, N., Ogata, F., Yagi, N., Ohto, U., Kimoto, M., Miyake, K., Tobe, K., Arai, H., Kadowaki, T., & Nagai, R. (2012). Saturated Fatty Acid and TLR Signaling Link β Cell Dysfunction and Islet Inflammation. *Cell Metabolism*, *15*(4), 518–533. https://doi.org/10.1016/j.cmet.2012.01.023

Eringa, E. C., Serne, E. H., Meijer, R. I., Schalkwijk, C. G., Houben, A. J. H. M., Stehouwer, C. D. A., Smulders, Y. M., & van Hinsbergh, V. W. M. (2013). Endothelial dysfunction in (pre)diabetes: Characteristics, causative mechanisms and pathogenic role in type 2 diabetes. *Reviews in Endocrine and Metabolic Disorders*, *14*(1), 39–48. https://doi.org/10.1007/s11154-013-9239-7

Feldman, E. L., Callaghan, B. C., Pop-Busui, R., Zochodne, D. W., Wright, D. E., Bennett, D. L., Bril, V., Russell, J. W., & Viswanathan, V. (2019). Diabetic neuropathy. *Nature Reviews Disease Primers*, *5*(1), 41. https://doi.org/10.1038/s41572-019-0092-1

Ferrannini, E., & Mari, A. (2014). β-Cell function in type 2 diabetes. *Metabolism*, *63*(10), 1217–1227. https://doi.org/10.1016/j.metabol.2014.05.012

Festa, A., D'Agostino, R., Tracy, R. P., & Haffner, S. M. (2002). Elevated Levels of Acute-Phase Proteins and Plasminogen Activator Inhibitor-1 Predict the Development of Type 2 Diabetes. *Diabetes*, *51*(4), 1131–1137. https://doi.org/10.2337/diabetes.51.4.1131

Fiory, F., Lombardi, A., Miele, C., Giudicelli, J., Beguinot, F., & Van Obberghen, E. (2011). Methylglyoxal impairs insulin signalling and insulin action on glucose-induced insulin secretion in the pancreatic beta cell line INS-1E. *Diabetologia*, *54*(11), 2941–2952. https://doi.org/10.1007/s00125-011-2280-8

Fleming, T., Cuny, J., Nawroth, G., Djuric, Z., Humpert, P. M., Zeier, M., Bierhaus, A., & Nawroth, P. P. (2012). Is diabetes an acquired disorder of reactive glucose metabolites and their intermediates? *Diabetologia*, *55*(4), 1151–1155. https://doi.org/10.1007/s00125-012-2452-1

Fleming, T. H., Humpert, P. M., Nawroth, P. P., & Bierhaus, A. (2010). Reactive Metabolites and AGE/RAGE-Mediated Cellular Dysfunction Affect the Aging Process – A Mini-Review. *Gerontology*. https://doi.org/10.1159/000322087

FONSECA-SÁNCHEZ, M. A., CUEVAS, S. R., MENDOZA-HERNÁNDEZ, G., BAUTISTA-PIÑA, V., OCAMPO, E. A., MIRANDA, A. H., JURADO, V. Q., MARCHAT, L. A., ÁLVAREZ-SÁNCHEZ, E., PLASENCIA, C. P., & LÓPEZ-CAMARILLO, C. (2012). Breast cancer proteomics reveals a positive correlation between glyoxalase 1 expression and high tumor grade. *International Journal of Oncology*, *41*(2), 670–680. https://doi.org/10.3892/ijo.2012.1478

Forbes, J. M., & Cooper, M. E. (2013). Mechanisms of diabetic complications. *Physiological Reviews*, *93*(1), 137–188. https://doi.org/10.1152/physrev.00045.2011

Fowler, M. J. (2008). Microvascular and Macrovascular Complications of Diabetes. *Clinical Diabetes*, *26*(2), 77–82. https://doi.org/10.2337/diaclin.26.2.77

Fralick, M., & Zinman, B. (2021). The discovery of insulin in Toronto: beginning a 100 year journey of research and clinical achievement. *Diabetologia*, *64*(5), 947–953. https://doi.org/10.1007/s00125-020-05371-6

Fukunaga, M., Miyata, S., Liu, B. F., Miyazaki, H., Hirota, Y., Higo, S., Hamada, Y., Ueyama, S., & Kasuga, M. (2004). Methylglyoxal induces apoptosis through activation of p38 MAPK in rat Schwann cells. *Biochemical and Biophysical Research Communications*, *320*(3), 689–695. https://doi.org/10.1016/j.bbrc.2004.06.011

Gao, M., Sun, L., Liu, Y. L., Xie, J. W., Qin, L., Xue, J., Wang, Y. T., Guo, K. M., Ma, M. M., & Li, X. Y. (2019). Reduction of glyoxalase 1 (GLO1) aggravates cerebrovascular remodeling via promoting the proliferation of basilar smooth muscle cells in hypertension. *Biochemical and Biophysical Research Communications*, *518*(2), 278–285. https://doi.org/10.1016/j.bbrc.2019.08.047

Gassaway, B. M., Petersen, M. C., Surovtseva, Y. V., Barber, K. W., Sheetz, J. B., Aerni, H. R., Merkel, J. S., Samuel, V. T., Shulman, G. I., & Rinehart, J. (2018). PKCε contributes to lipid-induced insulin resistance through cross talk with p70S6K and through previously unknown regulators of insulin signaling. *Proceedings of the National Academy of Sciences*, *115*(38). https://doi.org/10.1073/pnas.1804379115

Ge, S., Zeng, R., Luo, Y., Liu, L., Wei, H., Zhang, J., Zhou, H., & Xu, G. (2009). Role of protein kinase C in advanced glycation end products-induced epithelial-mesenchymal transition in renal proximal tubular epithelial cells. *Journal of Huazhong University of Science and Technology [Medical Sciences]*, *29*(3), 281–285. https://doi.org/10.1007/s11596-009-0303-1

Gelineau, R. R., Arruda, N. L., Hicks, J. A., Monteiro De Pina, I., Hatzidis, A., & Seggio, J. A. (2017). The behavioral and physiological effects of high-fat diet and alcohol consumption: Sex differences in C57BL6/J mice. *Brain and Behavior*, *7*(6), e00708. https://doi.org/10.1002/brb3.708

Giacco, F., Du, X., D'Agati, V. D., Milne, R., Sui, G., Geoffrion, M., & Brownlee, M. (2014). Knockdown of Glyoxalase 1 Mimics Diabetic Nephropathy in Nondiabetic Mice. *Diabetes*, *63*(1), 291–299. https://doi.org/10.2337/db13-0316

Glomb, M. A., & Monnier, V. M. (1995). Mechanism of Protein Modification by Glyoxal and Glycolaldehyde, Reactive Intermediates of the Maillard Reaction. *Journal of Biological Chemistry*, *270*(17), 10017–10026. https://doi.org/10.1074/jbc.270.17.10017

Goldfine, A. B. (2010). The Effects of Salsalate on Glycemic Control in Patients With Type 2 Diabetes. *Annals of Internal Medicine*, *152*(6), 346. https://doi.org/10.7326/0003-4819-152-6-201003160-00004

Gossen, M., & Bujard, H. (1992). Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proceedings of the National Academy of Sciences*, *89*(12), 5547–5551. https://doi.org/10.1073/pnas.89.12.5547

Greer, E. L., Oskoui, P. R., Banko, M. R., Maniar, J. M., Gygi, M. P., Gygi, S. P., & Brunet, A. (2007). The Energy Sensor AMP-activated Protein Kinase Directly Regulates the Mammalian FOXO3 Transcription Factor. *Journal of Biological Chemistry*, *282*(41), 30107–30119. https://doi.org/10.1074/jbc.M705325200

Grillo, M. A., & Colombatto, S. (2008). Advanced glycation end-products (AGEs): involvement in aging and in neurodegenerative diseases. *Amino Acids*, *35*(1), 29–36. https://doi.org/10.1007/s00726-007-0606-0

Grøntved, A., Rimm, E. B., Willett, W. C., Andersen, L. B., & Hu, F. B. (2012). A Prospective Study of Weight Training and Risk of Type 2 Diabetes Mellitus in Men. *Archives of Internal Medicine*, *172*(17), 1306. https://doi.org/10.1001/archinternmed.2012.3138

Gurgul-Convey, E., Mehmeti, I., Lortz, S., & Lenzen, S. (2011). Cytokine toxicity in insulin-producing cells is mediated by nitro-oxidative stress-induced hydroxyl radical formation in mitochondria. *Journal of Molecular Medicine*, *89*(8), 785–798. https://doi.org/10.1007/s00109-011-0747-1

Guthrie, R. A., & Guthrie, D. W. (2004). Pathophysiology of Diabetes Mellitus. *Critical Care Nursing Quarterly*, *27*(2), 113–125. https://doi.org/10.1097/00002727-200404000-00003

Habets, D. D. J., Coumans, W. A., El Hasnaoui, M., Zarrinpashneh, E., Bertrand, L., Viollet, B., Kiens, B., Jensen, T. E., Richter, E. A., & Bonen, A. (2009). Crucial role for LKB1 to AMPKα2 axis in the regulation of CD36-mediated long-chain fatty acid uptake into cardiomyocytes☆. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1791*(3), 212–219. https://doi.org/10.1016/j.bbalip.2008.12.009

Hadas, K., Randriamboavonjy, V., Elgheznawy, A., Mann, A., & Fleming, I. (2013). Methylglyoxal Induces Platelet Hyperaggregation and Reduces Thrombus Stability by Activating PKC and Inhibiting PI3K/Akt Pathway. *PLoS ONE*, *8*(9), e74401. https://doi.org/10.1371/journal.pone.0074401

Han, Y., Randell, E., Vasdev, S., Gill, V., Gadag, V., Newhook, L. A., Grant, M., & Hagerty, D. (2007). Plasma methylglyoxal and glyoxal are elevated and related to early membrane alteration in young, complication-free patients with Type 1 diabetes. *Molecular and Cellular Biochemistry*, *305*(1–2), 123–131. https://doi.org/10.1007/s11010-007-9535-1

Handgraaf, S., Riant, E., Fabre, A., Waget, A., Burcelin, R., Lière, P., Krust, A., Chambon, P., Arnal, J.-F., & Gourdy, P. (2013). Prevention of Obesity and Insulin Resistance by Estrogens Requires ERα Activation Function-2 (ERαAF-2), Whereas ERαAF-1 Is Dispensable. *Diabetes*, *62*(12), 4098–4108. https://doi.org/10.2337/db13-0282

Hanssen, N. M. J., Scheijen, J. L. J. M., Jorsal, A., Parving, H.-H., Tarnow, L., Rossing, P., Stehouwer, C. D. A., & Schalkwijk, C. G. (2017). Higher Plasma Methylglyoxal Levels Are Associated With Incident Cardiovascular Disease in Individuals With Type 1 Diabetes: A 12-Year Follow-up Study. *Diabetes*, *66*(8), 2278–2283. https://doi.org/10.2337/db16-1578

Hanssen, N. M. J., Stehouwer, C. D. A., & Schalkwijk, C. G. (2019). Methylglyoxal stress, the glyoxalase system, and diabetic chronic kidney disease. *Current Opinion in Nephrology and Hypertension*, *28*(1), 26–33. https://doi.org/10.1097/MNH.0000000000000465

Hardie, D. G., & Pan, D. A. (2002). Regulation of fatty acid synthesis and oxidation by the AMP-activated protein kinase. *Biochemical Society Transactions*, *30*(6), 1064–1070. https://doi.org/10.1042/bst0301064

Hardie, D. Grahame. (2011). AMP-activated protein kinase—an energy sensor that regulates all aspects of cell function. *Genes & Development*, *25*(18), 1895–1908. https://doi.org/10.1101/gad.17420111

Hardie, D. Grahame, Ross, F. A., & Hawley, S. A. (2012). AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nature Reviews Molecular Cell Biology*, *13*(4), 251–262. https://doi.org/10.1038/nrm3311

Harding, J. L., Pavkov, M. E., Magliano, D. J., Shaw, J. E., & Gregg, E. W. (2019). Global trends in diabetes complications: a review of current evidence. *Diabetologia*, *62*(1), 3–16. https://doi.org/10.1007/s00125-018-4711-2

Hawley, S. A., Ross, F. A., Gowans, G. J., Tibarewal, P., Leslie, N. R., & Hardie, D. G. (2014). Phosphorylation by Akt within the ST loop of AMPK-α1 down-regulates its activation in tumour cells. *Biochemical Journal*, *459*(2), 275–287. https://doi.org/10.1042/BJ20131344

He, Y., Zhou, C., Huang, M., Tang, C., Liu, X., Yue, Y., Diao, Q., Zheng, Z., & Liu, D. (2020). Glyoxalase system: A systematic review of its biological activity, related-diseases, screening methods and small molecule regulators. *Biomedicine and Pharmacotherapy*, *131*(August), 110663. https://doi.org/10.1016/j.biopha.2020.110663

Hemminki, K., Li, X., Sundquist, K., & Sundquist, J. (2010). Familial Risks for Type 2 Diabetes in Sweden. *Diabetes Care*, *33*(2), 293–297. https://doi.org/10.2337/dc09-0947

Hernandez-Castillo, C., & Shuck, S. C. (2021). Diet and Obesity-Induced Methylglyoxal Production and Links to Metabolic Disease. *Chemical Research in Toxicology*, *34*(12), 2424–2440. https://doi.org/10.1021/acs.chemrestox.1c00221

Hirosumi, J., Tuncman, G., Chang, L., Görgün, C. Z., Uysal, K. T., Maeda, K., Karin, M., & Hotamisligil, G. S. (2002). A central role for JNK in obesity and insulin resistance. *Nature*, *420*(6913), 333–336. https://doi.org/10.1038/nature01137

Hoppe, S., Bierhoff, H., Cado, I., Weber, A., Tiebe, M., Grummt, I., & Voit, R. (2009). AMP-activated protein kinase adapts rRNA synthesis to cellular energy supply. *Proceedings of the National Academy of Sciences*, *106*(42), 17781–17786. https://doi.org/10.1073/pnas.0909873106

Hosoda, F., Arai, Y., Okada, N., Shimizu, H., Miyamoto, M., Kitagawa, N., Katai, H., Taniguchi, H., Yanagihara, K., Imoto, I., Inazawa, J., Ohki, M., & Shibata, T. (2015). Integrated genomic and functional analyses reveal glyoxalase I as a novel metabolic oncogene in human gastric cancer. *Oncogene*, *34*(9), 1196–1206. https://doi.org/10.1038/onc.2014.57

Houstis, N., Rosen, E. D., & Lander, E. S. (2006). Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature*, *440*(7086), 944–948. https://doi.org/10.1038/nature04634

Hu, F. B. (2011). Globalization of Diabetes. *Diabetes Care*, *34*(6), 1249–1257.

https://doi.org/10.2337/dc11-0442

Hunter, R. W., Treebak, J. T., Wojtaszewski, J. F. P., & Sakamoto, K. (2011). Molecular Mechanism by Which AMP-Activated Protein Kinase Activation Promotes Glycogen Accumulation in Muscle. *Diabetes*, *60*(3), 766–774. https://doi.org/10.2337/db10-1148

Hwang, S.-L., Li, X., Lee, J.-Y., & Chang, H. W. (2012). Improved insulin sensitivity by rapamycin is associated with reduction of mTOR and S6K1 activities in L6 myotubes. *Biochemical and Biophysical Research Communications*, *418*(2), 402–407. https://doi.org/10.1016/j.bbrc.2012.01.038

Ide, T., Tsutsui, H., Kinugawa, S., Utsumi, H., Kang, D., Hattori, N., Uchida, K., Arimura, K., Egashira, K., & Takeshita, A. (1999). Mitochondrial Electron Transport Complex I Is a Potential Source of Oxygen Free Radicals in the Failing Myocardium. *Circulation Research*, *85*(4), 357–363. https://doi.org/10.1161/01.RES.85.4.357

International Diabetes Federation. (2021). *IDF Diabetes Atlas* (10th ed.). https://www.diabetesatlas.org

Irshad, Z., Xue, M., Ashour, A., Larkin, J. R., Thornalley, P. J., & Rabbani, N. (2019). Activation of the unfolded protein response in high glucose treated endothelial cells is mediated by methylglyoxal. *Scientific Reports*, *9*(1), 7889. https://doi.org/10.1038/s41598-019-44358-1

Izaguirre, G., Kikonyogo, A., & Pietruszko, R. (1998). Methylglyoxal as substrate and inhibitor of human aldehyde dehydrogenase: comparison of kinetic properties among the three isozymes. *Comparative Biochemistry and Physiology - Part B: Biochemistry & Molecular Biology*, *119*, 747–754.

Jaeschke, A., & Davis, R. J. (2007). Metabolic Stress Signaling Mediated by Mixed-Lineage Kinases. *Molecular Cell*, *27*(3), 498–508. https://doi.org/10.1016/j.molcel.2007.07.008

Jang, S., Kwon, D. M., Kwon, K., & Park, C. (2017). Generation and characterization of mouse knockout for glyoxalase 1. *Biochemical and Biophysical Research Communications*, *490*(2), 460–465. https://doi.org/10.1016/j.bbrc.2017.06.063

Jensen, T. M., Vistisen, D., Fleming, T., Nawroth, P. P., Rossing, P., Jørgensen, M. E., Lauritzen, T., Sandbaek, A., & Witte, D. R. (2016). Methylglyoxal is associated with changes in kidney function among individuals with screen-detected Type 2 diabetes mellitus. *Diabetic Medicine*, *33*(12), 1625–1631. https://doi.org/10.1111/dme.13201

Jeon, S.-M. (2016). Regulation and function of AMPK in physiology and diseases. *Experimental & Molecular Medicine*, *48*(7), e245–e245. https://doi.org/10.1038/emm.2016.81

Jia, X., & Wu, L. (2007). Accumulation of endogenous methylglyoxal impaired insulin signaling in adipose tissue of fructose-fed rats. *Molecular and Cellular Biochemistry*, *306*(1–2), 133–139. https://doi.org/10.1007/s11010-007-9563-x

Jiao, P., Ma, J., Feng, B., Zhang, H., Alan-Diehl, J., Eugene-Chin, Y., Yan, W., & Xu, H. (2011). FFA-Induced Adipocyte Inflammation and Insulin Resistance: Involvement of ER Stress and IKKβ Pathways. *Obesity*, *19*(3), 483–491. https://doi.org/10.1038/oby.2010.200

Joseph, B. K., Liu, H.-Y., Francisco, J., Pandya, D., Donigan, M., Gallo-Ebert, C., Giordano, C., Bata, A., & Nickels, J. T. (2015). Inhibition of AMP Kinase by the Protein Phosphatase 2A Heterotrimer, PP2APpp2r2d. *Journal of Biological Chemistry*, *290*(17), 10588–10598. https://doi.org/10.1074/jbc.M114.626259

Kammoun, H. L., Chabanon, H., Hainault, I., Luquet, S., Magnan, C., Koike, T., Ferré, P., & Foufelle, F. (2009). GRP78 expression inhibits insulin and ER stress–induced SREBP-1c activation and reduces hepatic steatosis in mice. *Journal of Clinical Investigation*, *119*(5), 1201–1215. https://doi.org/10.1172/JCI37007

Kanaya, A. M., Herrington, D., Vittinghoff, E., Lin, F., Grady, D., Bittner, V., Cauley, J. A., & Barrett-Connor, E. (2003). Glycemic Effects of Postmenopausal Hormone Therapy: The Heart and Estrogen/progestin Replacement Study: A Randomized, Double-Blind, Placebo-Controlled Trial. *Annals of Internal Medicine*, *138*(1), 1. https://doi.org/10.7326/0003-4819-138-1-200301070-00005

Kaprio, J., Tuomilehto, J., Koskenvuo, M., Romanov, K., Reunanen, A., Eriksson, J., Stengård, J., & Kesäniemi, Y. A. (1992). Concordance for Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. *Diabetologia*, *35*(11), 1060–1067. https://doi.org/10.1007/BF02221682

Kautzky-Willer, A., Harreiter, J., & Pacini, G. (2016). Sex and gender differences in risk, pathophysiology and complications of type 2 diabetes mellitus. *Endocrine Reviews*, *37*(3), 278–316. https://doi.org/10.1210/er.2015-1137

Kawasaki, N., Asada, R., Saito, A., Kanemoto, S., & Imaizumi, K. (2012). Obesity-induced endoplasmic reticulum stress causes chronic inflammation in adipose tissue. *Scientific Reports*, *2*(1), 799. https://doi.org/10.1038/srep00799

Kelley, D. E., He, J., Menshikova, E. V., & Ritov, V. B. (2002). Dysfunction of Mitochondria in Human Skeletal Muscle in Type 2 Diabetes. *Diabetes*, *51*(10), 2944–2950. https://doi.org/10.2337/diabetes.51.10.2944

Khan, R., Chua, Z., Tan, J., Yang, Y., Liao, Z., & Zhao, Y. (2019). From Pre-Diabetes to Diabetes: Diagnosis, Treatments and Translational Research. *Medicina*, *55*(9), 546. https://doi.org/10.3390/medicina55090546

Khoshnan, A., Bae, D., Tindell, C. A., & Nel, A. E. (2000). The Physical Association of Protein Kinase Cθ with a Lipid Raft-Associated Inhibitor of κB Factor Kinase (IKK) Complex Plays a Role in the Activation of the NF-κB Cascade by TCR and CD28. *The Journal of Immunology*, *165*(12), 6933–6940. https://doi.org/10.4049/jimmunol.165.12.6933

Kim, J.-Y., Hickner, R. C., Cortright, R. L., Dohm, G. L., & Houmard, J. A. (2000). Lipid oxidation is reduced in obese human skeletal muscle. *American Journal of Physiology-Endocrinology and Metabolism*, *279*(5), E1039–E1044. https://doi.org/10.1152/ajpendo.2000.279.5.E1039

Kim, J. K., Fillmore, J. J., Sunshine, M. J., Albrecht, B., Higashimori, T., Kim, D.-W., Liu, Z.-X., Soos, T. J., Cline, G. W., O'Brien, W. R., Littman, D. R., & Shulman, G. I. (2004). PKC-θ knockout mice are protected from fat-induced insulin resistance. *Journal of Clinical Investigation*, *114*(6), 823–827. https://doi.org/10.1172/JCI22230

Kim, S. Y., Jeong, S., Jung, E., Baik, K.-H., Chang, M. H., Kim, S. A., Shim, J.-H., Chun, E., & Lee, K.-Y. (2012). AMP-activated protein kinase-α1 as an activating kinase of TGF-β-activated kinase 1 has a key role in inflammatory signals. *Cell Death & Disease*, *3*(7), e357–e357. https://doi.org/10.1038/cddis.2012.95

Kırça, M., & Yeşilkaya, A. (2022). Methylglyoxal stimulates endoplasmic reticulum stress in vascular smooth muscle cells. *Journal of Receptors and Signal Transduction*, *42*(3), 279–284. https://doi.org/10.1080/10799893.2021.1918167

Ko, J., Kim, I., Yoo, S., Min, B., Kim, K., & Park, C. (2005). Conversion of methylglyoxal to acetol by Escherichia coli aldo-keto reductases. *Journal of Bacteriology*, *187*(16), 5782–5789. https://doi.org/10.1128/JB.187.16.5782-5789.2005

Kobashi, C., Urakaze, M., Kishida, M., Kibayashi, E., Kobayashi, H., Kihara, S., Funahashi, T., Takata, M., Temaru, R., Sato, A., & Yamazaki, K. (2005). *Adiponectin Inhibits Endothelial Synthesis of Interleukin-8*. https://doi.org/10.1161/01.RES.0000194328.57164.36

Kold-Christensen, R., & Johannsen, M. (2020). Methylglyoxal Metabolism and Aging-Related Disease: Moving from Correlation toward Causation. *Trends in Endocrinology and Metabolism*, *31*(2), 81–92. https://doi.org/10.1016/j.tem.2019.10.003

Koo, S.-H., Flechner, L., Qi, L., Zhang, X., Screaton, R. A., Jeffries, S., Hedrick, S., Xu, W., Boussouar, F., Brindle, P., Takemori, H., & Montminy, M. (2005). The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. *Nature*, *437*(7062), 1109–1114. https://doi.org/10.1038/nature03967

Kreiner, G., Chmielarz, P., Roman, A., & Nalepa, I. (2013). Gender differences in genetic mouse models evaluated for depressive-like and antidepressant behavior. *Pharmacological Reports*, *65*(6), 1580–1590. https://doi.org/10.1016/S1734-1140(13)71519-6

Landgraf, R., Aberle, J., Birkenfeld, A. L., Gallwitz, B., Kellerer, M., Klein, H., Müller-Wieland, D., Nauck, M. A., Reuter, H.-M., & Siegel, E. (2019). Therapy of Type 2 Diabetes. *Experimental and Clinical Endocrinology & Diabetes*, *127*(S 01), S73–S92. https://doi.org/10.1055/a-1018-9106

Lee, M.-W., Chanda, D., Yang, J., Oh, H., Kim, S. S., Yoon, Y.-S., Hong, S., Park, K.-G., Lee, I.-K., Choi, C. S., Hanson, R. W., Choi, H.-S., & Koo, S.-H. (2010). Regulation of Hepatic Gluconeogenesis by an ER-Bound Transcription Factor, CREBH. *Cell Metabolism*, *11*(4), 331–339. https://doi.org/10.1016/j.cmet.2010.02.016

Lenzen, S. (2008). Oxidative stress: the vulnerable β-cell. *Biochemical Society Transactions*, *36*(3), 343–347. https://doi.org/10.1042/BST0360343

Ley, S. H., Hamdy, O., Mohan, V., & Hu, F. B. (2014). Prevention and management of type 2 diabetes: dietary components and nutritional strategies. *The Lancet*, *383*(9933), 1999–2007. https://doi.org/10.1016/S0140-6736(14)60613-9

Li, H., Min, Q., Ouyang, C., Lee, J., He, C., Zou, M.-H., & Xie, Z. (2014). AMPK activation prevents excess nutrient-induced hepatic lipid accumulation by inhibiting mTORC1 signaling and endoplasmic reticulum stress response. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1842*(9), 1844–1854. https://doi.org/10.1016/j.bbadis.2014.07.002

Li, Y., Soos, T. J., Li, X., Wu, J., DeGennaro, M., Sun, X., Littman, D. R., Birnbaum, M. J., & Polakiewicz, R. D. (2004). Protein Kinase C θ Inhibits Insulin Signaling by Phosphorylating IRS1 at Ser1101. *Journal of Biological Chemistry*, *279*(44), 45304–45307. https://doi.org/10.1074/jbc.C400186200

Li, Y., Xu, S., Mihaylova, M. M., Zheng, B., Hou, X., Jiang, B., Park, O., Luo, Z., Lefai, E., Shyy, J. Y.-J., Gao, B., Wierzbicki, M., Verbeuren, T. J., Shaw, R. J., Cohen, R. A., & Zang, M. (2011). AMPK Phosphorylates and Inhibits SREBP Activity to Attenuate Hepatic Steatosis and Atherosclerosis in Diet-Induced Insulin-Resistant Mice. *Cell Metabolism*, *13*(4), 376–388. https://doi.org/10.1016/j.cmet.2011.03.009

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. *Methods*, *25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Lo, T. W., Westwood, M. E., McLellan, A. C., Selwood, T., & Thornalley, P. J. (1994). Binding and modification of proteins by methylglyoxal under physiological conditions. A kinetic and mechanistic study with N alpha-acetylarginine, N alpha-acetylcysteine, and N alpha-acetyllysine, and bovine serum albumin. *Journal of Biological Chemistry*, *269*(51), 32299–32305. https://doi.org/10.1016/S0021-9258(18)31635-1

Lodd, E., Wiggenhauser, L. M., Morgenstern, J., Fleming, T. H., Poschet, G., Büttner, M., Tabler, C. T., Wohlfart, D. P., Nawroth, P. P., & Kroll, J. (2019). The combination of loss of glyoxalase1 and obesity results in hyperglycemia. *JCI Insight*, *4*(12), 1–17. https://doi.org/10.1172/jci.insight.126154

Lu, J., Randell, E., Han, Y., Adeli, K., Krahn, J., & Meng, Q. H. (2011). Increased plasma methylglyoxal level, inflammation, and vascular endothelial dysfunction in diabetic nephropathy. *Clinical Biochemistry*, *44*(4), 307–311. https://doi.org/10.1016/j.clinbiochem.2010.11.004

Madamanchi, N. R., & Runge, M. S. (2007). Mitochondrial Dysfunction in Atherosclerosis. *Circulation Research*, *100*(4), 460–473. https://doi.org/10.1161/01.RES.0000258450.44413.96

Maessen, D. E. M., Stehouwer, C. D. A., & Schalkwijk, C. G. (2015). The role of methylglyoxal and the glyoxalase system in diabetes and other age-related diseases. *Clinical Science*, *128*(12), 839–861. https://doi.org/10.1042/CS20140683

Magnusson, I., Rothman, D. L., Katz, L. D., Shulman, R. G., & Shulman, G. I. (1992). Increased rate of gluconeogenesis in type II diabetes mellitus. A 13C nuclear magnetic resonance study. *Journal of Clinical Investigation*, *90*(4), 1323–1327. https://doi.org/10.1172/JCI115997

Manieri, E., Herrera-melle, L., Mora, A., Tom, A., Leiva-vega, L., Fern, D. I., Rodr, E., Mor, L., Torres, J. L., Seoane, L. M., Cubero, F. J., & Marcos, M. (2019). *Adiponectin accounts for gender differences in hepatocellular carcinoma incidence*. *216*(5), 1108–1119.

Mannervik, B. (1980). Glyoxalase I. In: Enzymatic Basis of Detoxification. *Academic Press, New York (W. Jacoby, Ed.)*, *2*, 263–293.

Mannervik, B. (2008). Molecular enzymology of the glyoxalase system. *Drug Metabolism and*

Drug Interactions, *23*(1–2), 13–27. https://doi.org/10.1515/DMDI.2008.23.1-2.13

Manning, B. D., & Cantley, L. C. (2007). AKT/PKB Signaling: Navigating Downstream. *Cell*, *129*(7), 1261–1274. https://doi.org/10.1016/j.cell.2007.06.009

Mansukhani, N. A., Wang, Z., Shively, V. P., Kelly, M. E., Vercammen, J. M., & Kibbe, M. R. (2017). Sex differences in the LDL receptor knockout mouse model of atherosclerosis. *Artery Research*, *20*(C), 8. https://doi.org/10.1016/j.artres.2017.08.002

Marek, I., Canu, M., Cordasic, N., Rauh, M., Volkert, G., Fahlbusch, F. B., Rascher, W., Hilgers, K. F., Hartner, A., & Menendez-Castro, C. (2017). Sex differences in the development of vascular and renal lesions in mice with a simultaneous deficiency of Apoe and the integrin chain Itga8. *Biology of Sex Differences*, *8*(1), 19. https://doi.org/10.1186/s13293-017-0141-y

Martin, B. C., Warram, J. H., Krolewski, A. S., Soeldner, J. S., Kahn, C. R., Martin, B. C., & Bergman, R. N. (1992). Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. *The Lancet*, *340*(8825), 925–929. https://doi.org/10.1016/0140-6736(92)92814-V

Matafome, P., Rodrigues, T., Sena, C., & Seiça, R. (2017). Methylglyoxal in Metabolic Disorders: Facts, Myths, and Promises. *Medicinal Research Reviews*, *37*(2), 368–403. https://doi.org/10.1002/med.21410

Matafome, P., Sena, C., & Seiça, R. (2013). Methylglyoxal, obesity, and diabetes. *Endocrine*, *43*(3), 472–484. https://doi.org/10.1007/s12020-012-9795-8

Matheus, A. S. de M., Tannus, L. R. M., Cobas, R. A., Palma, C. C. S., Negrato, C. A., & Gomes, M. de B. (2013). Impact of Diabetes on Cardiovascular Disease: An Update. *International Journal of Hypertension*, *2013*, 1–15. https://doi.org/10.1155/2013/653789

Matsuda, M., & Shimomura, I. (2014). *Roles of adiponectin and oxidative stress in obesity-associated metabolic and cardiovascular diseases*. 1–10. https://doi.org/10.1007/s11154-013-9271-7

Matsushita, M., Yoneshiro, T., Aita, S., Kameya, T., Sugie, H., & Saito, M. (2014). Impact of brown adipose tissue on body fatness and glucose metabolism in healthy humans. *International Journal of Obesity*, *July 2013*, 812–817. https://doi.org/10.1038/ijo.2013.206

Mauvais-jarvis, F., Clegg, D. J., & Hevener, A. L. (2013). *The Role of Estrogens in Control of Energy Balance and Glucose Homeostasis*. *34*(June), 309–338. https://doi.org/10.1210/er.2012-1055

Mayfield, J. (1998). Diagnosis and classification of diabetes mellitus: new criteria. *American Family Physician*, *58*(6), 1355–1362, 1369–1370. http://www.ncbi.nlm.nih.gov/pubmed/9803200

McLellan, A. C., Thornalley, P. J., Benn, J., & Sonksen, P. H. (1994). Glyoxalase System in Clinical Diabetes Mellitus and Correlation with Diabetic Complications. *Clinical Science*, *87*(1), 21–29. https://doi.org/10.1042/cs0870021

Meigs, J. B., Cupples, L. A., & Wilson, P. W. (2000). Parental transmission of type 2 diabetes: the Framingham Offspring Study. *Diabetes*, *49*(12), 2201–2207. https://doi.org/10.2337/diabetes.49.12.2201

Menge, B. A., Tannapfel, A., Belyaev, O., Drescher, R., Müller, C., Uhl, W., Schmidt, W. E., & Meier, J. J. (2008). Partial Pancreatectomy in Adult Humans Does Not Provoke β-Cell Regeneration. *Diabetes*, *57*(1), 142–149. https://doi.org/10.2337/db07-1294

Mey, J. T., Blackburn, B. K., Miranda, E. R., Chaves, A. B., Briller, J., Bonini, M. G., & Haus, J. M. (2018). Dicarbonyl stress and glyoxalase enzyme system regulation in human skeletal muscle. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *314*(2), R181–R190. https://doi.org/10.1152/ajpregu.00159.2017

Monder, C. (1967). Alpha-keto aldehyde dehydrogenase, an enzyme that catalyzes the enzymic oxidation of methylglyoxal to pyruvate. *Journal of Biological Chemistry*, *242*(20), 4603–4609. https://doi.org/10.1016/s0021-9258(18)99499-8

Mootha, V. K., Lindgren, C. M., Eriksson, K.-F., Subramanian, A., Sihag, S., Lehar, J., Puigserver, P., Carlsson, E., Ridderstråle, M., Laurila, E., Houstis, N., Daly, M. J., Patterson, N., Mesirov, J. P., Golub, T. R., Tamayo, P., Spiegelman, B., Lander, E. S., Hirschhorn, J. N., … Groop, L. C. (2003). PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nature Genetics*, *34*(3), 267–273. https://doi.org/10.1038/ng1180

Moraru, A., Wiederstein, J., Pfaff, D., Fleming, T., Miller, A. K., Nawroth, P., & Teleman, A. A. (2018). Elevated Levels of the Reactive Metabolite Methylglyoxal Recapitulate Progression of Type 2 Diabetes. *Cell Metabolism*, *27*(4), 926-934.e8. https://doi.org/10.1016/j.cmet.2018.02.003

Morgenstern, J., Campos Campos, M., Nawroth, P., & Fleming, T. (2020). The Glyoxalase System—New Insights into an Ancient Metabolism. *Antioxidants*, *9*(10), 939. https://doi.org/10.3390/antiox9100939

Morgenstern, J., Fleming, T., Schumacher, D., Eckstein, V., Freichel, M., Herzig, S., & Nawroth, P. (2017). Loss of Glyoxalase 1 Induces Compensatory Mechanism to Achieve Dicarbonyl Detoxification in Mammalian Schwann Cells. *Journal of Biological Chemistry*, *292*(8), 3224–3238. https://doi.org/10.1074/jbc.M116.760132

Morgenstern, J., Katz, S., Krebs-Haupenthal, J., Chen, J., Saadatmand, A., Cortizo, F. G., Moraru, A., Zemva, J., Campos, M. C., Teleman, A., Backs, J., Nawroth, P., & Fleming, T. (2020). Phosphorylation of T107 by CamKIIδ Regulates the Detoxification Efficiency and Proteomic Integrity of Glyoxalase 1. *Cell Reports*, *32*(12), 108160. https://doi.org/10.1016/j.celrep.2020.108160

Morgenstern, J., Kliemank, E., Campos, M. C., Nawroth, P., & Fleming, T. (2020). Michaelis-Menten Kinetics Measurements of Aldo-Keto Reductases for Various Substrates in Murine Tissue. *STAR Protocols*, *1*(3), 100206. https://doi.org/10.1016/j.xpro.2020.100206

Morino, K. (2005). Reduced mitochondrial density and increased IRS-1 serine phosphorylation in muscle of insulin-resistant offspring of type 2 diabetic parents. *Journal of Clinical Investigation*, *115*(12), 3587–3593. https://doi.org/10.1172/JCI25151

Motomura, H., Ozaki, A., Tamori, S., Onaga, C., Nozaki, Y., Waki, Y., Takasawa, R., Yoshizawa, K., Mano, Y., Sato, T., Sasaki, K., Ishiguro, H., Miyagi, Y., Nagashima, Y., Yamamoto, K., Sato, K., Hanawa, T., Tanuma, S. I., Ohno, S., & Akimoto, K. (2021). Glyoxalase 1 and protein kinase Cλ as potential therapeutic targets for late-stage breast cancer. *Oncology Letters*, *22*(1), 1–11. https://doi.org/10.3892/OL.2021.12808

Muoio, D. M., Seefeld, K., Witters, L. A., & Coleman, R. A. (1999). AMP-activated kinase reciprocally regulates triacylglycerol synthesis and fatty acid oxidation in liver and muscle: evidence that sn-glycerol-3-phosphate acyltransferase is a novel target. *The Biochemical Journal*, *338 (Pt 3)*, 783–791.

Nakamura, A., & Terauchi, Y. (2013). Lessons from Mouse Models of High-Fat Diet-Induced NAFLD. *International Journal of Molecular Sciences*, *14*(11), 21240–21257. https://doi.org/10.3390/ijms141121240

Nakayama, K., Nakayama, M., Iwabuchi, M., Terawaki, H., Sato, T., Kohno, M., & Ito, S. (2008). Plasma α-Oxoaldehyde Levels in Diabetic and Nondiabetic Chronic Kidney Disease Patients. *American Journal of Nephrology*, *28*(6), 871–878. https://doi.org/10.1159/000139653

Neuberg, C. (1913). The destruction of lactic aldehyde and methylglyoxal by animal organs. *Biochem. Z.*, *49*, 502–506.

Neubert, P., Halim, A., Zauser, M., Essig, A., Joshi, H. J., Zatorska, E., Larsen, I. S. B., Loibl, M., Castells-Ballester, J., Aebi, M., Clausen, H., & Strahl, S. (2016). Mapping the O-Mannose Glycoproteome in Saccharomyces cerevisiae. *Molecular & Cellular Proteomics*, *15*(4), 1323–1337. https://doi.org/10.1074/mcp.M115.057505

Nigro, C., Leone, A., Longo, M., Prevenzano, I., Fleming, T. H., Nicolò, A., Parrillo, L., Spinelli, R., Formisano, P., Nawroth, P. P., Beguinot, F., & Miele, C. (2019). Methylglyoxal accumulation de-regulates HoxA5 expression, thereby impairing angiogenesis in glyoxalase 1 knock-down mouse aortic endothelial cells. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1865*(1), 73–85. https://doi.org/10.1016/j.bbadis.2018.10.014

Nigro, C., Raciti, G. A., Leone, A., Fleming, T. H., Longo, M., Prevenzano, I., Fiory, F., Mirra, P., D'Esposito, V., Ulianich, L., Nawroth, P. P., Formisano, P., Beguinot, F., & Miele, C. (2014). Methylglyoxal impairs endothelial insulin sensitivity both in vitro and in vivo. *Diabetologia*, *57*(7), 1485–1494. https://doi.org/10.1007/s00125-014-3243-7

O'Neill, H. M., Maarbjerg, S. J., Crane, J. D., Jeppesen, J., Jørgensen, S. B., Schertzer, J. D., Shyroka, O., Kiens, B., van Denderen, B. J., Tarnopolsky, M. A., Kemp, B. E., Richter, E. A., & Steinberg, G. R. (2011). AMP-activated protein kinase (AMPK) β1β2 muscle null mice reveal an essential role for AMPK in maintaining mitochondrial content and glucose uptake during exercise. *Proceedings of the National Academy of Sciences*, *108*(38), 16092–16097. https://doi.org/10.1073/pnas.1105062108

Ohman-hanson, R. A., Cree-green, M., Kelsey, M. M., Bessesen, D. H., Sharp, T. A., Pyle, L., Pereira, R. I., & Nadeau, K. J. (2016). *Ethnic and Sex Differences in Adiponectin : From Childhood to Adulthood*. *101*(December), 4808–4815. https://doi.org/10.1210/jc.2016-1137

Oraha, J., Enriquez, R. F., Herzog, H., & Lee, N. J. (2022). Sex-specific changes in metabolism

during the transition from chow to high-fat diet feeding are abolished in response to dieting in C57BL/6J mice. *International Journal of Obesity*. https://doi.org/10.1038/s41366-022-01174-4

Ortiz-Huidobro, R. I., Velasco, M., Larqué, C., Escalona, R., & Hiriart, M. (2021). Molecular Insulin Actions Are Sexually Dimorphic in Lipid Metabolism. *Frontiers in Endocrinology*, *12*. https://doi.org/10.3389/fendo.2021.690484

Ouchi, N., Kihara, S., Arita, Y., & Okamoto, Y. (2000). *Basic Science Reports Endothelial NF- B Signaling Through a*. 1296–1301.

Padayatti, P. S., Jiang, C., Glomb, M. A., Uchida, K., & Nagaraj, R. H. (2001). High concentrations of glucose induce synthesis of argpyrimidine in retinal endothelial cells. *Current Eye Research*, *23*(2), 106–115. https://doi.org/10.1076/ceyr.23.2.106.5472

Palmisano, B. T., Zhu, L., Eckel, R. H., & Stafford, J. M. (2018). Sex differences in lipid and lipoprotein metabolism. *Molecular Metabolism*, *15*, 45–55. https://doi.org/10.1016/j.molmet.2018.05.008

Palsamy, P., Bidasee, K. R., Ayaki, M., Augusteyn, R. C., Chan, J. Y., & Shinohara, T. (2014). Methylglyoxal induces endoplasmic reticulum stress and DNA demethylation in the Keap1 promoter of human lens epithelial cells and age-related cataracts. *Free Radical Biology and Medicine*, *72*, 134–148. https://doi.org/10.1016/j.freeradbiomed.2014.04.010

Pang, H., Li, J., Wang, Y., Su, X., Gao, Y., & Li, S. J. (2021). Mice lacking the proton channel Hv1 exhibit sex-specific differences in glucose homeostasis. *Journal of Biological Chemistry*, *297*(4), 101212. https://doi.org/10.1016/j.jbc.2021.101212

Peng, G., Li, L., Liu, Y., Pu, J., Zhang, S., Yu, J., Zhao, J., & Liu, P. (2011). Oleate Blocks Palmitate-Induced Abnormal Lipid Distribution, Endoplasmic Reticulum Expansion and Stress, and Insulin Resistance in Skeletal Muscle. *Endocrinology*, *152*(6), 2206–2218. https://doi.org/10.1210/en.2010-1369

Peters, S. A. E., Muntner, P., & Woodward, M. (2019). Sex Differences in the Prevalence of, and Trends in, Cardiovascular Risk Factors, Treatment, and Control in the United States, 2001 to 2016. *Circulation*, *139*(8), 1025–1035. https://doi.org/10.1161/CIRCULATIONAHA.118.035550

Pfannenberg, C., Werner, M. K., Ripkens, S., Stef, I., Deckert, A., Schmadl, M., Reimold, M., Ha, H., Claussen, C. D., & Stefan, N. (2010). *Impact of Age on the Relationships of Brown Adipose Tissue With Sex and Adiposity in Humans*. *59*(July), 1789–1793. https://doi.org/10.2337/db10-0004.C.

Pfeiffer, A. F. H., & Klein, H. H. (2014). The Treatment of Type 2 Diabetes. *Deutsches Ärzteblatt International*. https://doi.org/10.3238/arztebl.2014.0069

Phielix, E., Schrauwen-Hinderling, V. B., Mensink, M., Lenaers, E., Meex, R., Hoeks, J., Kooi, M. E., Moonen-Kornips, E., Sels, J.-P., Hesselink, M. K. C., & Schrauwen, P. (2008). Lower Intrinsic ADP-Stimulated Mitochondrial Respiration Underlies In Vivo Mitochondrial Dysfunction in Muscle of Male Type 2 Diabetic Patients. *Diabetes*, *57*(11), 2943–2949. https://doi.org/10.2337/db08-0391

Phillips, S. A., Mirrlees, D., & Thornalley, P. J. (1993). Modification of the glyoxalase system in streptozotocin-induced diabetic rats. *Biochemical Pharmacology*, *46*(5), 805–811. https://doi.org/10.1016/0006-2952(93)90488-I

Phillips, S. A., & Thornalley, P. J. (1993). The formation of methylglyoxal from triose phosphates. Investigation using a specific assay for methylglyoxal. *European Journal of Biochemistry*, *212*(1), 101–105. https://doi.org/10.1111/j.1432-1033.1993.tb17638.x

Pigeyre, M., Hess, S., Gomez, M. F., Asplund, O., Groop, L., Paré, G., & Gerstein, H. (2022). Validation of the classification for type 2 diabetes into five subgroups: a report from the ORIGIN trial. *Diabetologia*, *65*(1), 206–215. https://doi.org/10.1007/s00125-021-05567-4

Poulsen, P., Ohm Kyvik, K., Vaag, A., & Beck-Nielsen, H. (1999). Heritability of Type II (non-insulin-dependent) diabetes mellitus and abnormal glucose tolerance - a population-based twin study. *Diabetologia*, *42*(2), 139–145. https://doi.org/10.1007/s001250051131

Power, M. L., & Schulkin, J. (2008). Sex differences in fat storage, fat metabolism, and the health risks from obesity: possible evolutionary origins. *British Journal of Nutrition*, *99*(5), 931–940. https://doi.org/10.1017/S0007114507853347

Pradhan, A. D. (2001). C-Reactive Protein, Interleukin 6, and Risk of Developing Type 2 Diabetes Mellitus. *JAMA*, *286*(3), 327. https://doi.org/10.1001/jama.286.3.327

Prince, R. L., Larkins, R. G., & Alford, F. P. (1981). The effect of acetylsalicylic acid on plasma glucose and the response of glucose regulatory hormones to intravenous glucose and arginine in insulin treated diabetics and normal subjects. *Metabolism*, *30*(3), 293–298. https://doi.org/10.1016/0026-0495(81)90155-4

Qi, Z., Fujita, H., Jin, J., Davis, L. S., Wang, Y., Fogo, A. B., & Breyer, M. D. (2005). Characterization of Susceptibility of Inbred Mouse Strains to Diabetic Nephropathy. *Diabetes*, *54*(9), 2628–2637. https://doi.org/10.2337/diabetes.54.9.2628

R Core Team. (2022). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.r-project.org

Rabbani, N., & Thornalley, P. J. (2008). Dicarbonyls linked to damage in the powerhouse: glycation of mitochondrial proteins and oxidative stress. *Biochemical Society Transactions*, *36*(5), 1045–1050. https://doi.org/10.1042/BST0361045

Rabbani, N., & Thornalley, P. J. (2011). Glyoxalase in diabetes, obesity and related disorders. *Seminars in Cell and Developmental Biology*, *22*(3), 309–317. https://doi.org/10.1016/j.semcdb.2011.02.015

Rabbani, N., & Thornalley, P. J. (2012). Methylglyoxal, glyoxalase 1 and the dicarbonyl proteome. *Amino Acids*, *42*(4), 1133–1142. https://doi.org/10.1007/s00726-010-0783-0

Rabbani, N., & Thornalley, P. J. (2014a). The Critical Role of Methylglyoxal and Glyoxalase 1 in Diabetic Nephropathy. *Diabetes*, *63*(1), 50–52. https://doi.org/10.2337/db13-1606

Rabbani, N., & Thornalley, P. J. (2014b). Dicarbonyl proteome and genome damage in metabolic and vascular disease. *Biochemical Society Transactions*, *42*(2), 425–432.

https://doi.org/10.1042/BST20140018

Rabbani, N., & Thornalley, P. J. (2014c). Measurement of methylglyoxal by stable isotopic dilution analysis LC-MS/MS with corroborative prediction in physiological samples. *Nature Protocols*, *9*(8), 1969–1979. https://doi.org/10.1038/nprot.2014.129

Rabbani, N., Xue, M., & Thornalley, P. J. (2014). Activity, regulation, copy number and function in the glyoxalase system. *Biochemical Society Transactions*, *42*(2), 419–424. https://doi.org/10.1042/BST20140008

Racker, E. (1951). The mechanism of action of glyoxalase. *The Journal of Biological Chemistry*, *190*(2), 685–696. http://www.ncbi.nlm.nih.gov/pubmed/14841219

Rahier, J., Guiot, Y., Goebbels, R. M., Sempoux, C., & Henquin, J. C. (2008). Pancreatic β-cell mass in European subjects with type 2 diabetes. *Diabetes, Obesity and Metabolism*, *10*, 32–42. https://doi.org/10.1111/j.1463-1326.2008.00969.x

Rains, J. L., & Jain, S. K. (2011). Oxidative stress, insulin signaling, and diabetes. *Free Radical Biology and Medicine*, *50*(5), 567–575. https://doi.org/10.1016/j.freeradbiomed.2010.12.006

Ramanathan, L., & Siegel, J. M. (2014). Gender differences between hypocretin/orexin knockout and wild type mice: age, body weight, body composition, metabolic markers, leptin and insulin resistance. *Journal of Neurochemistry*, *131*(5), 615–624. https://doi.org/10.1111/jnc.12840

Rando, G., & Wahli, W. (2011). Sex differences in nuclear receptor-regulated liver metabolic pathways. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, *1812*(8), 964–973. https://doi.org/10.1016/j.bbadis.2010.12.023

Ranganathan, S., & Tew, K. D. (1993). Analysis of glyoxalase-I from normal and tumor tissue. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1182*(3), 311–316. https://doi.org/10.1016/0925-4439(93)90074-B

Reddy, P. H. (2008). Mitochondrial Medicine for Aging and Neurodegenerative Diseases. *NeuroMolecular Medicine*, *10*(4), 291–315. https://doi.org/10.1007/s12017-008-8044-z

Rehman, K., & Akash, M. S. H. (2017). Mechanism of Generation of Oxidative Stress and Pathophysiology of Type 2 Diabetes Mellitus: How Are They Interlinked? *Journal of Cellular Biochemistry*, *118*(11), 3577–3585. https://doi.org/10.1002/jcb.26097

Renaud, H. J., Cui, J. Y., Khan, M., & Klaassen, C. D. (2011). Tissue Distribution and Gender-Divergent Expression of 78 Cytochrome P450 mRNAs in Mice. *Toxicological Sciences*, *124*(2), 261–277. https://doi.org/10.1093/toxsci/kfr240

Riant, E., Waget, A., Cogo, H., Arnal, J.-F., Burcelin, R., & Gourdy, P. (2009). Estrogens Protect against High-Fat Diet-Induced Insulin Resistance and Glucose Intolerance in Mice. *Endocrinology*, *150*(5), 2109–2117. https://doi.org/10.1210/en.2008-0971

Riboulet-Chavey, A., Pierron, A., Durand, I., Murdaca, J., Giudicelli, J., & Van Obberghen, E. (2006). Methylglyoxal impairs the insulin signaling pathways independently of the formation of intracellular reactive oxygen species. *Diabetes*, *55*(5), 1289–1299.

https://doi.org/10.2337/db05-0857

Rinn, J. L., Rozowsky, J. S., Laurenzi, I. J., Petersen, P. H., Zou, K., Zhong, W., Gerstein, M., & Snyder, M. (2004). Major molecular differences between mammalian sexes are involved in drug metabolism and renal function. *Developmental Cell*, *6*(6), 791–800. https://doi.org/10.1016/j.devcel.2004.05.005

Rounds, L., Nagle, R. B., Muranyi, A., Jandova, J., Gill, S., Vela, E., & Wondrak, G. T. (2021). Glyoxalase 1 Expression as a Novel Diagnostic Marker of High-Grade Prostatic Intraepithelial Neoplasia in Prostate Cancer. *Cancers*, *13*(14), 3608. https://doi.org/10.3390/cancers13143608

RStudio Team. (2022). *RStudio: Integrated Development Environment for R*. RStudio, Inc. http://www.rstudio.com/

Saltiel, A. R. (2021). Insulin signaling in health and disease. *Journal of Clinical Investigation*, *131*(1). https://doi.org/10.1172/JCI142241

Salvadó, L., Palomer, X., Barroso, E., & Vázquez-Carrera, M. (2015). Targeting endoplasmic reticulum stress in insulin resistance. *Trends in Endocrinology & Metabolism*, *26*(8), 438–448. https://doi.org/10.1016/j.tem.2015.05.007

Samuel, V. T., & Shulman, G. I. (2012). Mechanisms for Insulin Resistance: Common Threads and Missing Links. *Cell*, *148*(5), 852–871. https://doi.org/10.1016/j.cell.2012.02.017

Schalkwijk, C. G., & Stehouwer, C. D. A. (2020). Methylglyoxal, a highly reactive dicarbonyl compound, in diabetes, its vascular complications, and other age-related diseases. *Physiological Reviews*, *100*(1), 407–461. https://doi.org/10.1152/physrev.00001.2019

Scheijen, J. L. J. M., Hanssen, N. M. J., Waarenburg, M. P. H. van de, Jonkers, D. M. A. E., Stehouwer, C. D. A., & Schalkwijk, C. G. (2012). L(+) and D(<math> <mo mathvariant="bold">-</mo> </math>) Lactate Are Increased in Plasma and Urine Samples of Type 2 Diabetes as Measured by a Simultaneous Quantification of L(+) and D(<math> <mo mathvariant="bold">-</mo> </math>) Lactate by Reversed-. *Experimental Diabetes Research*, *2012*, 1–10. https://doi.org/10.1155/2012/234812

Schieber, M., & Chandel, N. S. (2014). ROS Function in Redox Signaling and Oxidative Stress. *Current Biology*, *24*(10), R453–R462. https://doi.org/10.1016/j.cub.2014.03.034

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, *9*(7), 676–682. https://doi.org/10.1038/nmeth.2019

Schmidt, M. I., Duncan, B. B., Sharrett, A. R., Lindberg, G., Savage, P. J., Offenbacher, S., Azambuja, M. I., Tracy, R. P., & Heiss, G. (1999). Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. *The Lancet*, *353*(9165), 1649–1652. https://doi.org/10.1016/S0140-6736(99)01046-6

Schmitz-Peiffer, C., & Whitehead, J. (2003). IRS-1 Regulation in Health and Disease. *IUBMB Life (International Union of Biochemistry and Molecular Biology: Life)*, *55*(7), 367–374.

https://doi.org/10.1080/1521654031000138569

Schubert, K. M., Scheid, M. P., & Duronio, V. (2000). Ceramide Inhibits Protein Kinase B/Akt by Promoting Dephosphorylation of Serine 473. *Journal of Biological Chemistry*, *275*(18), 13330–13335. https://doi.org/10.1074/jbc.275.18.13330

Schumacher, D., Morgenstern, J., Oguchi, Y., Volk, N., Kopf, S., Groener, J. B., Nawroth, P. P., Fleming, T., & Freichel, M. (2018). Compensatory mechanisms for methylglyoxal detoxification in experimental & clinical diabetes. *Molecular Metabolism*, *18*(September), 143–152. https://doi.org/10.1016/j.molmet.2018.09.005

Selby, N. M., & Taal, M. W. (2020). An updated overview of diabetic nephropathy: Diagnosis, prognosis, treatment goals and latest guidelines. *Diabetes, Obesity and Metabolism*, *22*(S1), 3–15. https://doi.org/10.1111/dom.14007

Sena, C. M., Matafome, P., Crisóstomo, J., Rodrigues, L., Fernandes, R., Pereira, P., & Seiça, R. M. (2012). Methylglyoxal promotes oxidative stress and endothelial dysfunction. *Pharmacological Research*, *65*(5), 497–506. https://doi.org/10.1016/j.phrs.2012.03.004

Shafie, A., Xue, M., Barker, G., Zehnder, D., Thornalley, P. J., & Rabbani, N. (2016). Reappraisal of putative glyoxalase 1-deficient mouse and dicarbonyl stress on embryonic stem cells in vitro. *Biochemical Journal*, *473*(22), 4255–4270. https://doi.org/10.1042/BCJ20160691

Sheng, L., Jena, P. K., Liu, H.-X., Kalanetra, K. M., Gonzalez, F. J., French, S. W., Krishnan, V. V., Mills, D. A., & Wan, Y.-J. Y. (2017). Gender Differences in Bile Acids and Microbiota in Relationship with Gender Dissimilarity in Steatosis Induced by Diet and FXR Inactivation. *Scientific Reports*, *7*(1), 1748. https://doi.org/10.1038/s41598-017-01576-9

Shinohara, M, Thornalley, P. J., Giardino, I., Beisswenger, P., Thorpe, S. R., Onorato, J., & Brownlee, M. (1998). Overexpression of glyoxalase-I in bovine endothelial cells inhibits intracellular advanced glycation endproduct formation and prevents hyperglycemia-induced increases in macromolecular endocytosis. *Journal of Clinical Investigation*, *101*(5), 1142–1147. https://doi.org/10.1172/JCI119885

Shinohara, Moritsugu, Thornalley, P. J., Giardino, I., Beisswenger, P., Thorpe, S. R., Onorato, J., & Brownlee, M. (1998). Overexpression of glyoxalase-I in bovine endothelial cells inhibits intracellular advanced glycation endproduct formation and prevents hyperglycemia-induced increases in macromolecular endocytosis. *Journal of Clinical Investigation*, *101*(5), 1142–1147. https://doi.org/10.1172/JCI119885

Shuck, S. C., Wuenschell, G. E., & Termini, J. S. (2018). Product Studies and Mechanistic Analysis of the Reaction of Methylglyoxal with Deoxyguanosine. *Chemical Research in Toxicology*, *31*(2), 105–115. https://doi.org/10.1021/acs.chemrestox.7b00274

Šilhavý, J., Malínská, H., Hüttl, M., Marková, I., Oliyarnyk, O., Mlejnek, P., Šimáková, M., Liška, F., Kazdová, L., Moravcová, R., Novotný, J., & Pravenec, M. (2020). Downregulation of the Glo1 Gene Is Associated with Reduced Adiposity and Ectopic Fat Accumulation in Spontaneously Hypertensive Rats. *Antioxidants*, *9*(12), 1179. https://doi.org/10.3390/antiox9121179

Simmons, R. A. (2007). Role of metabolic programming in the pathogenesis of β-cell failure in

postnatal life. *Reviews in Endocrine and Metabolic Disorders*, *8*(2), 95–104. https://doi.org/10.1007/s11154-007-9045-1

Skapare, E., Konrade, I., Liepinsh, E., Strele, I., Makrecka, M., Bierhaus, A., Lejnieks, A., Pirags, V., & Dambrova, M. (2013). Association of reduced glyoxalase 1 activity and painful peripheral diabetic neuropathy in type 1 and 2 diabetes mellitus patients. *Journal of Diabetes and Its Complications*, *27*(3), 262–267. https://doi.org/10.1016/j.jdiacomp.2012.12.002

Slieker, R. C., Donnelly, L. A., Fitipaldi, H., Bouland, G. A., Giordano, G. N., Åkerlund, M., Gerl, M. J., Ahlqvist, E., Ali, A., Dragan, I., Festa, A., Hansen, M. K., Mansour Aly, D., Kim, M., Kuznetsov, D., Mehl, F., Klose, C., Simons, K., Pavo, I., … Pearson, E. R. (2021). Replication and cross-validation of type 2 diabetes subtypes based on clinical variables: an IMI-RHAPSODY study. *Diabetologia*, *64*(9), 1982–1989. https://doi.org/10.1007/s00125-021-05490-8

Sloan, G., Selvarajah, D., & Tesfaye, S. (2021). Pathogenesis, diagnosis and clinical management of diabetic sensorimotor peripheral neuropathy. *Nature Reviews Endocrinology*, *17*(7), 400–420. https://doi.org/10.1038/s41574-021-00496-z

Smuda, M., & Glomb, M. A. (2013). Fragmentation Pathways during Maillard-Induced Carbohydrate Degradation. *Journal of Agricultural and Food Chemistry*, *61*(43), 10198–10208. https://doi.org/10.1021/jf305117s

Solis-Herrera, C., Triplitt, C., Reasner, C., DeFronzo, R. A., & Cersosimo, E. (2000). Classification of Diabetes Mellitus. In *Endotext*. http://www.ncbi.nlm.nih.gov/pubmed/25905343

Sousa Silva, M., Gomes, R. A., Ferreira, A. E. N., Ponces Freire, A., & Cordeiro, C. (2013). The glyoxalase pathway: the first hundred years… and beyond. *Biochemical Journal*, *453*(1), 1–15. https://doi.org/10.1042/BJ20121743

Stratford, S., Hoehn, K. L., Liu, F., & Summers, S. A. (2004). Regulation of Insulin Action by Ceramide. *Journal of Biological Chemistry*, *279*(35), 36608–36615. https://doi.org/10.1074/jbc.M406499200

Stratmann, B., Engelbrecht, B., Espelage, B. C., Klusmeier, N., Tiemann, J., Gawlowski, T., Mattern, Y., Eisenacher, M., Meyer, H. E., Rabbani, N., Thornalley, P. J., Tschoepe, D., Poschmann, G., & Stühler, K. (2016). Glyoxalase 1-knockdown in human aortic endothelial cells – effect on the proteome and endothelial function estimates. *Scientific Reports*, *6*(1), 37737. https://doi.org/10.1038/srep37737

Stumvoll, M., Goldstein, B. J., & van Haeften, T. W. (2005). Type 2 diabetes: principles of pathogenesis and therapy. *The Lancet*, *365*(9467), 1333–1346. https://doi.org/10.1016/S0140-6736(05)61032-X

Sun, Y., Ge, X., Li, X., He, J., Wei, X., Du, J., Sun, J., Li, X., Xun, Z., Liu, W., Zhang, H., Wang, Z.-Y., & Li, Y. C. (2020). High-fat diet promotes renal injury by inducing oxidative stress and mitochondrial dysfunction. *Cell Death & Disease*, *11*(10), 914. https://doi.org/10.1038/s41419-020-03122-4

Szendroedi, J., Yoshimura, T., Phielix, E., Koliaki, C., Marcucci, M., Zhang, D., Jelenik, T., Müller, J., Herder, C., Nowotny, P., Shulman, G. I., & Roden, M. (2014). Role of diacylglycerol

activation of PKCθ in lipid-induced muscle insulin resistance in humans. *Proceedings of the National Academy of Sciences*, *111*(26), 9597–9602. https://doi.org/10.1073/pnas.1409229111

Tangirala, R. K., Rubin, E. M., & Palinski, W. (1995). Quantitation of atherosclerosis in murine models: correlation between lesions in the aortic origin and in the entire aorta, and differences in the extent of lesions between sexes in LDL receptor-deficient and apolipoprotein E-deficient mice. *Journal of Lipid Research*, *36*(11), 2320–2328. http://www.ncbi.nlm.nih.gov/pubmed/8656070

Taniguchi, H., Horinaka, M., Yoshida, T., Yano, K., Goda, A. E., Yasuda, S., Wakada, M., & Sakai, T. (2012). Targeting the glyoxalase pathway enhances TRAIL efficacy in cancer cells by downregulating the expression of antiapoptotic molecules. *Molecular Cancer Therapeutics*, *11*(10), 2294–2300. https://doi.org/10.1158/1535-7163.MCT-12-0031

Tao, L., Gao, E., Jiao, X., Yuan, Y., Li, S., Christopher, T. A., Lopez, B. L., Koch, W., Chan, L., Goldstein, B. J., & Ma, X. L. (2007). *Ischemia / Reperfusion Involves the Reduction of Oxidative / Nitrative Stress*. 1408–1416. https://doi.org/10.1161/CIRCULATIONAHA.106.666941

Thornalley, P. J. (1990). The glyoxalase system: New developments towards functional characterization of a metabolic pathway fundamental to biological life. *Biochemical Journal*, *269*(1), 1–11. https://doi.org/10.1042/bj2690001

Thornalley, P.J. (2003). Glyoxalase I – structure, function and a critical role in the enzymatic defence against glycation. *Biochemical Society Transactions*, *31*(6), 1343–1348. https://doi.org/10.1042/bst0311343

Thornalley, P.J., Hooper, N. I., Jennings, P. E., Florkowski, C. M., Jones, A. F., Lunec, J., & Barnett, A. H. (1989). The human red blood cell glyoxalase system in diabetes mellitus. *Diabetes Research and Clinical Practice*, *7*(2), 115–120. https://doi.org/10.1016/0168-8227(89)90101-0

Thornalley, P J. (1988). Modification of the glyoxalase system in human red blood cells by glucose in vitro. *Biochemical Journal*, *254*(3), 751–755. https://doi.org/10.1042/bj2540751

Thornalley, Paul J. (1991). Population genetics of human glyoxalases. *Heredity*, *67*(2), 139–142. https://doi.org/10.1038/hdy.1991.73

Thornalley, Paul J. (1993). The glyoxalase system in health and disease. *Molecular Aspects of Medicine*, *14*(4), 287–371. https://doi.org/10.1016/0098-2997(93)90002-U

Thornalley, Paul J. (1996). Pharmacology of methylglyoxal: Formation, modification of proteins and nucleic acids, and enzymatic detoxification - A role in pathogenesis and antiproliferative chemotherapy. *General Pharmacology*, *27*(4), 565–573. https://doi.org/10.1016/0306-3623(95)02054-3

Thornalley, Paul J., Battah, S., Ahmed, N., Nikolaos, K., Agalou, S., Babaei-Jadidi, R., & Dawnay, A. (2003). Quantitative screening of advanced glycation endproducts in cellular and extracellular proteins by tandem mass spectrometry. *Biochemical Journal*, *375*(3), 581–592. https://doi.org/10.1042/bj20030763

Thornalley, Paul J., & Rabbani, N. (2011). Glyoxalase in tumourigenesis and multidrug resistance. *Seminars in Cell and Developmental Biology*, *22*(3), 318–325. https://doi.org/10.1016/j.semcdb.2011.02.006

Thornalley, Paul J. (1998). Glutathione-dependent detoxification of α-oxoaldehydes by the glyoxalase system: involvement in disease mechanisms and antiproliferative activity of glyoxalase I inhibitors. *Chemico-Biological Interactions*, *111–112*, 137–151. https://doi.org/10.1016/S0009-2797(97)00157-9

Tillil, H., & Köbberling, J. (1987). Age-Corrected Empirical Genetic Risk Estimates for First-Degree Relatives of IDDM Patients. *Diabetes*, *36*(1), 93–99. https://doi.org/10.2337/diab.36.1.93

Tramunt, B., Smati, S., Grandgeorge, N., Lenfant, F., Arnal, J.-F., Montagner, A., & Gourdy, P. (2020). Sex differences in metabolic regulation and diabetes susceptibility. *Diabetologia*, *63*(3), 453–461. https://doi.org/10.1007/s00125-019-05040-3

Tsutsui, H., Ide, T., Hayashidani, S., Suematsu, N., Shiomi, T., Wen, J., Nakamura, K., Ichikawa, K., Utsumi, H., & Takeshita, A. (2001). Enhanced Generation of Reactive Oxygen Species in the Limb Skeletal Muscles From a Murine Infarct Model of Heart Failure. *Circulation*, *104*(2), 134–136. https://doi.org/10.1161/01.CIR.104.2.134

Ulgen, E., Ozisik, O., & Sezerman, O. U. (2019). pathfindR: An R Package for Comprehensive Identification of Enriched Pathways in Omics Data Through Active Subnetworks. *Frontiers in Genetics*, *10*. https://doi.org/10.3389/fgene.2019.00858

Uotila, L. (1989). Glutathione thiol esterases. In: Glutathione: Chemical, Biochemical and Medical Aspects, Coenzymes and Cofactors. *Wiley-Interscience, New York*, *3*, 767–804.

Vander Jagt, D. L., & Hunsaker, L. A. (2003). Methylglyoxal metabolism and diabetic complications: roles of aldose reductase, glyoxalase-I, betaine aldehyde dehydrogenase and 2-oxoaldehyde dehydrogenase. *Chemico-Biological Interactions*, *143–144*, 341–351. https://doi.org/10.1016/S0009-2797(02)00212-0

Vander Jagt, D. L., Robinson, B., Taylor, K. K., & Hunsaker, L. A. (1992). Reduction of trioses by NADPH-dependent aldo-keto reductases. Aldose reductase, methylglyoxal, and diabetic complications. *The Journal of Biological Chemistry*, *267*(7), 4364–4369. http://www.ncbi.nlm.nih.gov/pubmed/1537826

Vassileva, G., Hu, W., Hoos, L., Tetzloff, G., Yang, S., Liu, L., Kang, L., Davis, H. R., Hedrick, J. A., Lan, H., Kowalski, T., & Gustafson, E. L. (2010). Gender-dependent effect of Gpbar1 genetic deletion on the metabolic profiles of diet-induced obese mice. *Journal of Endocrinology*, *205*(3), 225–232. https://doi.org/10.1677/JOE-10-0009

Vijan, S. (2019). Type 2 Diabetes. *Annals of Internal Medicine*, *171*(9), ITC65–ITC80. https://doi.org/10.7326/AITC201911050

Vomhof-DeKrey, E. E., Lee, J., Lansing, J., Brown, C., Darland, D., & Basson, M. D. (2019). Schlafen 3 knockout mice display gender-specific differences in weight gain, food efficiency, and expression of markers of intestinal epithelial differentiation, metabolism, and immune cell function. *PLOS ONE*, *14*(7), e0219267. https://doi.org/10.1371/journal.pone.0219267

Voss, M., Paterson, J., Kelsall, I. R., Martín-Granados, C., Hastie, C. J., Peggie, M. W., & Cohen, P. T. W. (2011). Ppm1E is an in cellulo AMP-activated protein kinase phosphatase. *Cellular Signalling*, *23*(1), 114–124. https://doi.org/10.1016/j.cellsig.2010.08.010

Vozarova, B., Weyer, C., Lindsay, R. S., Pratley, R. E., Bogardus, C., & Tataranni, P. A. (2002). High White Blood Cell Count Is Associated With a Worsening of Insulin Sensitivity and Predicts the Development of Type 2 Diabetes. *Diabetes*, *51*(2), 455–461. https://doi.org/10.2337/diabetes.51.2.455

Wang, C., Wang, C., Huang, H., & Wei, Y. (2013). Mitochondrial dysfunction leads to impairment of insulin sensitivity and adiponectin secretion in adipocytes. *The FEBS Journal*, *280*(4), 1039–1050. https://doi.org/10.1111/febs.12096

Wang, Q., Zhang, M., Xu, M., Gu, W., Xi, Y., Qi, L., & Li, B. (2015). *Brown Adipose Tissue Activation Is Inversely Related to Central Obesity and Metabolic Parameters in Adult Human*. 1–13. https://doi.org/10.1371/journal.pone.0123795

Wang, T., Kartika, R., & Spiegel, D. A. (2012). Exploring Post-translational Arginine Modification Using Chemically Synthesized Methylglyoxal Hydroimidazolones. *Journal of the American Chemical Society*, *134*(21), 8958–8967. https://doi.org/10.1021/ja301994d

Wang, W., & Lo, A. (2018). Diabetic Retinopathy: Pathophysiology and Treatments. *International Journal of Molecular Sciences*, *19*(6), 1816. https://doi.org/10.3390/ijms19061816

Wang, Y. C., McPherson, K., Marsh, T., Gortmaker, S. L., & Brown, M. (2011). Health and economic burden of the projected obesity trends in the USA and the UK. *The Lancet*, *378*(9793), 815–825. https://doi.org/10.1016/S0140-6736(11)60814-3

Westwood, M. E., & Thornalley, P. J. (1995). Molecular characteristics of methylglyoxal-modified bovine and human serum albumins. Comparison with glucose-derived advanced glycation endproduct-modified serum albumins. *Journal of Protein Chemistry*, *14*(5), 359–372. https://doi.org/10.1007/BF01886793

Wortmann, M., Hakimi, M., Fleming, T., Peters, A. S., Sijmonsma, T. P., Herzig, S., Nawroth, P. P., Böckler, D., & Dihlmann, S. (2016). A Glyoxalase-1 Knockdown Does Not Have Major Short Term Effects on Energy Expenditure and Atherosclerosis in Mice. *Journal of Diabetes Research*, *2016*, 1–8. https://doi.org/10.1155/2016/2981639

Xue, M., Weickert, M. O., Qureshi, S., Kandala, N.-B., Anwar, A., Waldron, M., Shafie, A., Messenger, D., Fowler, M., Jenkins, G., Rabbani, N., & Thornalley, P. J. (2016). Improved Glycemic Control and Vascular Function in Overweight and Obese Subjects by Glyoxalase 1 Inducer Formulation. *Diabetes*, *65*(8), 2282–2294. https://doi.org/10.2337/db16-0153

Yamauchi, T., Kamon, J., Minokoshi, Y., Ito, Y., Waki, H., Uchida, S., Yamashita, S., Noda, M., Kita, S., Ueki, K., Eto, K., Akanuma, Y., Froguel, P., Foufelle, F., Ferre, P., Carling, D., Kimura, S., Nagai, R., Kahn, B. B., & Kadowaki, T. (2002). Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nature Medicine*, *8*(11), 1288–1295. https://doi.org/10.1038/nm788

Yaney, G. C., & Corkey, B. E. (2003). Fatty acid metabolism and insulin secretion in pancreatic beta cells. *Diabetologia*, *46*(10), 1297–1312. https://doi.org/10.1007/s00125-003-1207-4

Yuan, M., Konstantopoulos, N., Lee, J., Hansen, L., Li, Z.-W., Karin, M., & Shoelson, S. E. (2001). Reversal of Obesity- and Diet-Induced Insulin Resistance with Salicylates or Targeted Disruption of Ikkβ. *Science*, *293*(5535), 1673–1677. https://doi.org/10.1126/science.1061620

Zaharia, O. P., Strassburger, K., Strom, A., Bönhof, G. J., Karusheva, Y., Antoniou, S., Bódis, K., Markgraf, D. F., Burkart, V., Müssig, K., Hwang, J.-H., Asplund, O., Groop, L., Ahlqvist, E., Seissler, J., Nawroth, P., Kopf, S., Schmid, S. M., Stumvoll, M., … Ziegler, D. (2019). Risk of diabetes-associated diseases in subgroups of patients with recent-onset diabetes: a 5-year follow-up study. *The Lancet Diabetes & Endocrinology*, *7*(9), 684–694. https://doi.org/10.1016/S2213-8587(19)30187-1

Zhang, H., Ni, J., Yu, C., Wu, Y., Li, J., Liu, J., Tu, J., Ning, X., He, Q., & Wang, J. (2019). Sex-Based Differences in Diabetes Prevalence and Risk Factors: A Population-Based Cross-Sectional Study Among Low-Income Adults in China. *Frontiers in Endocrinology*, *10*. https://doi.org/10.3389/fendo.2019.00658

Zheng, D., MacLean, P. S., Pohnert, S. C., Knight, J. B., Olson, A. L., Winder, W. W., & Dohm, G. L. (2001). Regulation of muscle GLUT-4 transcription by AMP-activated protein kinase. *Journal of Applied Physiology*, *91*(3), 1073–1083. https://doi.org/10.1152/jappl.2001.91.3.1073

Zhou, B., Lu, Y., Hajifathalian, K., Bentham, J., Di Cesare, M., Danaei, G., Bixby, H., Cowan, M., Ali, M., Taddei, C., Lo, W., Reis-Santos, B., Stevens, G., Riley, L., Miranda, J., Bjerregaard, P., Rivera, J., Fouad, H., Ma, G., … Zuñiga Cisneros, J. (2016). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4·4 million participants. *The Lancet*, *387*(10027), 1513–1530. https://doi.org/10.1016/S0140-6736(16)00618-8

Zhou, Y., Lee, J., Reno, C. M., Sun, C., Park, S. W., Chung, J., Lee, J., Fisher, S. J., White, M. F., Biddinger, S. B., & Ozcan, U. (2011). Regulation of glucose homeostasis through a XBP-1–FoxO1 interaction. *Nature Medicine*, *17*(3), 356–365. https://doi.org/10.1038/nm.2293

Zou, X., Zhou, X., Zhu, Z., & Ji, L. (2019). Novel subgroups of patients with adult-onset diabetes in Chinese and US populations. *The Lancet Diabetes & Endocrinology*, *7*(1), 9–11. https://doi.org/10.1016/S2213-8587(18)30316-4

8. Personal Publications

Publications

Morgenstern, J., Katz, S., Krebs-Haupenthal, J., Chen, J., Saadatmand, A., Cortizo, F. G., Moraru, A., Zemva, J., **Campos, M. C**., Teleman, A., Backs, J., Nawroth, P., & Fleming, T. (2020). Phosphorylation of T107 by CamKIIδ Regulates the Detoxification Efficiency and Proteomic Integrity of Glyoxalase 1. *Cell Reports*, *32*(12), 108160. https://doi.org/10.1016/j.celrep.2020.108160

Morgenstern, J., Kliemank, E., **Campos, M. C**., Nawroth, P., & Fleming, T. (2020). Michaelis-Menten Kinetics Measurements of Aldo-Keto Reductases for Various Substrates in Murine Tissue. *STAR Protocols*, *1*(3), 100206. https://doi.org/10.1016/j.xpro.2020.100206

Morgenstern, J., **Campos Campos, M**., Nawroth, P., & Fleming, T. (2020). The Glyoxalase System—New Insights into an Ancient Metabolism. *Antioxidants*, *9*(10), 939. https://doi.org/10.3390/antiox9100939

Fleming, T., von Nettelbladt, B., Morgenstern, J., **Campos, M**., Le Marois, M., Bartosova, M., Hausser, I., Schwab, C., Fischer, A., Nawroth, P. P., Szendroedi, J., & Herzig, S. (2021). Methylglyoxal Induces Endothelial Dysfunction via Stunning-like Phenotype. *BioRxiv*, 2021.11.18.469085. https://doi.org/10.1101/2021.11.18.469085

Manuscript in preparation

Campos, M., Morgenstern, J., Kliemank, E., Poth, T., Volk, N., Szendroedi, J., Nawroth, P. & Fleming, T. (2022-2023). Gender specific alterations in glucose homeostasis driven by Glyoxalase 1.

Conference proceedings

M. Campos, T. Fleming, J. Morgenstern & P. Nawroth – *Glucose homeostasis is affected by the loss of Glyoxalase 1* – **Young Investigators Research Day** – Heidelberg, Germany – Poster Presentation – September 2019

M. Campos, T. Fleming, J. Morgenstern & P. Nawroth – *Glucose homeostasis is affected by the loss of Glyoxalase 1* - **5th Heidelberg International Symposium on Diabetic Complications (HiDC)** – Heidelberg, Germany – Poster Presentation – November 2019

M. Campos, J. Morgenstern, T. Poth, N. Volk, J. Szendroedi, P. Nawroth & T. Fleming – *Gender specific alterations in glucose homeostasis driven by glyoxalase 1: New road towards type 2 diabetes?* – **EASD 2021** – Online Congress – Short Oral Presentation – September 2021

M. Campos, J. Morgenstern, T. Poth, N. Volk, J. Szendrödi, P. Nawroth & T. Fleming – *Regulation and impact of Glyoxalase 1 in diabetes – experimental data* – **Diabetes Kongress DDG 2022** – Berlin, Germany – Oral presentation – May 2022

M. Campos, J. Morgenstern, T. Poth, N. Volk, J. Szendrödi, P. Nawroth & T. Fleming – *Gender specific alterations in glucose homeostasis driven by Glyoxalase 1* – **50. ÖDG Jahrestagung 2022** – Salzburg, Austria – Poster Presentation – November 2022

Currvoiculum Vitae

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Education

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Eidesstattliche Verischerung

1. Bei der eingereichten Dissertation zu dem Thema *„The consequences of the loss of Glyoxalase 1 on cellular metabolism"* handelt es sich um meine eigenständig erbrachte Leistung.

2. Ich habe nur die angegebenen Quellen und Hilfsmittel benutzt und mich keiner unzulässigen Hilf Dritter bedient. Insbesondere habe ich wörtlich oder sinngemäß aus anderen Werken übernommene Inhalte als solche kenntlich gemacht.

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