Universitat de Barcelona

Facultat de Farmàcia i Ciències de l'Alimentació

Treball Final de Grau

# Pathogenesis of insulin resistance and hyperinsulinemia associated to obesity and timeline of appearance of these conditions in Type 2 Diabetes

Marta Puig Figueras

Revisió bibliogràfica

Departament de Bioquimica I

Fisiologia

22 de Juny , 2021

*Review*

# Pathogenesis of insulin resistance and hyperinsulinemia associated to obesity and timeline of appearance of these conditions in Type 2 Diabetes: A bibliographic review

**Marta Puig Figueras***

**Abstract:** Type 2 diabetes is an heterogenous condition characterized by fasting hyperglycemia , insulin resistance and impaired insulin secretion. For years insulin resistance has been proposed as the primary effect of overnutrition, ultimately leading to fasting hyperglycemia and Type 2 diabetes when beta cells become dysfunctional and are no longer able to sustain sufficient insulin secretion to compensate for decreased insulin sensitivity. However recently a novel hypothesis has emerged suggesting beta cell overstimulation as the primary effect of nutritional excess ultimately causing impaired insulin sensitivity and obesity. This bibliographic review describes the physiology of insulin action as well as the pathophysiology of insulin resistance and hyperinsulinemia associated to obesity. Furthermore it provides evidence from different studies regarding the timing of appearance of these conditions in Type 2 diabetes. While it is clear that nutritional excess associated to obesity is the major forerunner of Type 2 diabetes conflicting evidence still exists regarding the time of appearance of hyperinsulinemia and insulin resistance during the progression of the disease. Therefore further research needs to be done to have an improved understanding of the mechanisms underlying the pathogene-sis of Type 2 diabetes to find novel and improved therapeutic interventions for these pa-tients.

**Resum:** La diabetis tipus 2 és una malaltia heterogènia caracteritzada per hiperglucèmies recurrents inclús en dejú i també alteracions en la sensibilitat i la secreció d' insulina. Des de fa anys s'ha proposat la resistència a la insulina com a principal conseqüència de la sobre ingesta que finalment comporta a l'aparició de diabetis tipus 2 quan les cèl·lules beta es tornen disfuncionals i ja no són capaces de mantenir una secreció d'insulina suficient per compensar la baixa sensibilitat de la insulina. Tanmateix, recentment ha sorgit una nova hipòtesi que proposa la sobre estimulació de les cèl·lules beta com l'efecte principal de l'excés nutricional que finalment comporta l'aparició de la resistència a la insulina i l'obesitat. Aquesta revisió bibliogràfica descriu la fisiologia de l'acció de la insulina, així com la fisiopatologia de la resistència a la insulina i la hiperinsulinèmia associada a l'obesitat. A més, proporciona proves de diferents estudis sobre el moment d'aparició d'aquestes afeccions en la diabetis tipus 2. Tot i que està clar que l'excés nutricional associat a l'obesitat és el principal precursor de la diabetis tipus 2 encara existeixen proves contradictòries sobre el moment d'aparició de la hiperinsulinèmia i la resistència a la insulina durant la progressió de la malaltia. Per tant, cal fer més investigacions per comprendre millor els mecanismes subjacents a la patogènesi de la diabetis tipus 2 per trobar intervencions terapèutiques noves i millorades per a aquests pacients.

**Citation:** Puig Figueras, Marta. Pathogenesis of Insulin Resistance and Hiperinsulinemia associated to obesity and timeline of these conditions in Type 2 diabetes: A bibliographic review .


**Keywords:** Type 2 Diabetes, Hyperinsulinemia, Insulin resistance, Obesity

## 1. Introduction

Obesity is defined by World Health Organization (WHO) as a Body Mass Index (BMI) (that is the ratio between the weight in grams *versus* the square of the height in meters) equal or greater to 30 in adults, and it's prevalence worldwide has nearly tripled since 1975 (1). At least one in thirteen annual deaths in the EU can be attributed to excess weight meaning that almost 279.000 people die every year due to obesity only in the European region (2). Obesity was declared as a global epidemic by WHO in 1997 and currently affects over 650 million people worldwide (1). Equally alarming is the data regarding childhood obesity, in Spain over 17 % of children aged 6 to 9 were obese in 2019 according to the ALDINO study (3). In addition, obesity is a major contributor to the global burden of non-transmittable diseases, in a meta-analysis obesity showed statistical significant associations to different types of cancers (such as colorectal and breast cancer), cardiovascular diseases, asthma, gallbladder disease, osteoarthritis, chronic back pain and Type 2 diabetes (T2D) (4). Even though BMI and waist circumference have been consistently correlated with T2D in multiple studies and obe- sity is a well-recognized modifiable risk factor for the onset of this disease (5) the mechanisms underlying this association are still highly debated. Concordantly with this data, in a retrospect study conducted in the US, approximately 78.2% of patients with T2D were obese or overweight (6). Diabetes is a common metabolic disorder characterized by fasting hyperglycemia due to impaired insulin secretion by pancreatic islets (Type 1 diabetes,T1D) and/or insulin resistance (IR), that is the failure to response to insulin by target tissues (T2D) as well as, disturbed carbohydrate, fat and protein metabolism (7). According to WHO statistics, in 2014 there were 422 million people suffering from diabetes worldwide and the number today is four times what it was in 1980 (8). Furthermore the results from the International Diabetes Federation atlas showed that a total of 4.2 million deaths among 20-79 years old people were attributable to Diabetes in 2019 (9). T2D is the most common type of diabetes accounting for 90% of all cases, and in these patients can lead to both macrovascular complications such as cardiovascular disease, stroke and peripheral vascular disease and microvascular complications which include nephropathy, neuropathy, retinopathy (10). Worldwide an overall of 32,3% of patients with T2D present cardiovascular disease being the major cause mortality among them (11). For years researchers have put efforts into finding the mechanisms linking obesity with IR and hyperinsulinemia (HI), two traits that are present early in T2D with the aim to find novel targets for disease treatment. However, there are still many controversies regarding the pathological mechanisms involved in the development of HI and IR in obese patients as well as their time of appearance during the onset of T2D (12). Current drug treatments in T2D patients are based on insulin secretion because traditionally it was accepted that IR is the primary abnormality in T2D and that β cell insulin hypersecretion (HI) is a physiological compensatory mechanism to protect the organism from hyperglycemia (13). However recent studies demonstrating that insulin treatment in patients with T1D further exacerbates IR have given rise to a new opposing hypothesis (14). The validation of this alternative hypothesis where HI is the primary trait of overnutrition inducing obesity and IR would give rise to potentially novel drug treatment for T2D. To summarize, there is still a lot of controversy regarding the sequence of events during the progression of T2D in obesity as well as the molecular mechanisms underlying this process. Therefore, this review aims to provide an insight into the molecular mechanisms involved in the pathogenesis of HI and IR in obesity and to contrast the current data available regarding this topic to provide more evidence on the time of appearance of these conditions during onset of T2D.

## 2. Methods

To conduct this bibliographic review on IR and HI linked to obesity, online databases and official web pages were used. Three documents were provided initially by the author's tutor to get a general overview on the topic: a perspective on *"Insulin action and resistance in obesity and type 2 diabetes*", a review on "*The role of the c-Jun N-terminal kinase (JNK) pathway in insulin resistance* " as well as a pre-clinical experiment reported in "*In Vivo JNK Activation in Pancreatic b-Cells Leads to Glucose Intolerance Caused by Insulin Resistance in Pancreas"*. The online databases used for the bibliographic research were mainly PubMed but also, Cercabib and Scopus, all of which were accessed through the "Centre de recursos per a l'Aprenentatges I la investigació"(CRAI) from the Universitat de Barcelona (UB). Finally websites of professional organizations such as WHO, Agencia Española de Seguridad Alimentaria y Nutrición (AESAN) and The International Diabetes Federation were used to search for specific guidelines and statistical data. In addition other web pages such as The Medical Biochemistry Page was also used to search for additional information on the topic. The following keywords were used initially to find the most relevant articles and studies: "incidence" AND ("obesity" OR "Overweight"), "Diabetes" AND ("waist circumference" OR "Body mass index"), "insulin" AND ("secretion" OR "trafficking"),“ Insulin

action ” AND “insulin resistance”, "insulin resistance" AND ("Hyperinsulinemia" OR "insulin hypersecretion") AND Obesity, ("B cell dysfunction" OR " B cell death") AND (lipotoxicity OR glucolipotoxicity). Inclusion criteria consisted of all published articles including reviews, journals, books, meta-analysis, clinical trials, systemic reviews and randomized control trials published in English between 2000 and 2020. The articles were selected based on the relevance for the topic prioritizing the most recent ones as well as the ones with higher scientific evidence such as human randomized trials and systematic and meta-analysis, although other articles were also considered. Furthermore, additional studies used were found through the references from the main articles selected. Finally, all the Figures and Tables present in this review have been created by the author.

## 3. Insulin secretion, Insulin receptor (InR) signaling pathway and metabolic effects

### *3.1 Insulin synthesis and secretion from pancreatic islet β cells*

Insulin is a well-known hormone that exerts multiple regulatory functions in the organism mainly controlling metabolic pathways but also exerting other functions as a growth factor (15). Insulin is a peptide synthesized and secreted by the β-cells of Langerhans islets in the pancreas (15). Insulin mRNA is initially translated as a preproinsulin by the ribosomes attached to the membrane of the rough endoplasmic reticulum (rER) which is cleavage to proinsulin during its insertion into the lumen of rRE through the removal of its N-terminal peptide and a subsequent folding occurs which involves sulfur bound formation. Then folded proinsulin is transported from the RE to the Golgi through immature secretory vesicles and undergoes a final cleavage on the C-peptide to finally become mature insulin (16,17). The majority of mature insulin molecules are stored in a reserved pooled of granules which are released in the sustained phase of insulin secretion also called post-absorptive phase and only a small fraction are thought to be part of the ready releasable pool which are released upon acute insulin stimulation in the postprandial phase (16,17). The mechanisms involved in insulin secretion are complex and involve numerous pathways. Evolutionarily, B cells have adapted to respond to different nutritional factors that go from diet components (monosaccharides, amino acids, fatty acids) to nutrient sensing hormones like incretins and leptin and even neuronal inputs (16). Although all of these stimulus may potentiate glucose-stimulated insulin secretion (GSIS), glucose is still the primary regulator of insulin exocytosis. In the postprandial phase glycemia arise enabling glucose to enter to the β cells through a specific glucose transporter called GLUT-2, which unlike other glucose transporters has low glucose affinity and enables the cell to respond only to postprandial glycemia. Internalized glucose is phosphorylated by glucokinase (GK) into Glucose-6 phosphate (G6P). This reaction is irreversible and is considered a rate-limiting step in glucose metabolism. Additionally GK is considered an important glucose sensor for its relatively low affinity to glucose as well as for its property to not be inhibited by the product of the reaction G6P, enabling a high influx of glucose to enter the cell. Concordantly G6P is converted through glycolysis into pyruvate that is further oxidized by the TCA cycle to produce ATP thus increasing the ATP/ADP ratio, which in turn leads to the closure of the ATP-Sensitive potassium ( K+ATP) channels. This closure leads to a cytoplasmic increase in positive ions, which results in β cell depolarization and opening of the voltage dependent $Ca^{2+}$ channels that eventually activates insulin trafficking and exocytosis (15–17). This **simplified explanation of the GSIS is represented in Figure 1** but it is also important to mention that other signals that result from glucose metabolism such as NADPH, malonyl-CoA, glutamate and glycerol-3-phosphate metabolic coupling factors such as long-chain Acyl-CoA, and diacylglycerol (DAG) may also amplify insulin secretion through an K+ATP channel-dependent manner (16). The mechanisms in which other components from the diet, especially free fatty acids (FAA) may amplify GSIS pathway will be later discussed in this review. Additionally, a study conducted with pancreatic islets from rats demonstrated an alternative pathway in which glucose can stimulate insulin secretion. This study showed that while (GSIS) was abolished when $Ca^{2+}$ influx was inhibited by a combination of diazoxide (opener of KATP cannel), and nifedipine (blocker of voltage-dependent $Ca^{2+}$ channels) an increase in insulin release was seen in the presence foreskin which is an activator of adenylyl cyclase (18). Thus indicating that an alternative pathway involving the generation of cAMP could explain the induction of GSIS. Concordantly with this results, incretins such as GLP-1 have been demonstrated to use the cAMP to stimulate insulin secretion by binding to the adenylyl cyclase coupled receptor (16).

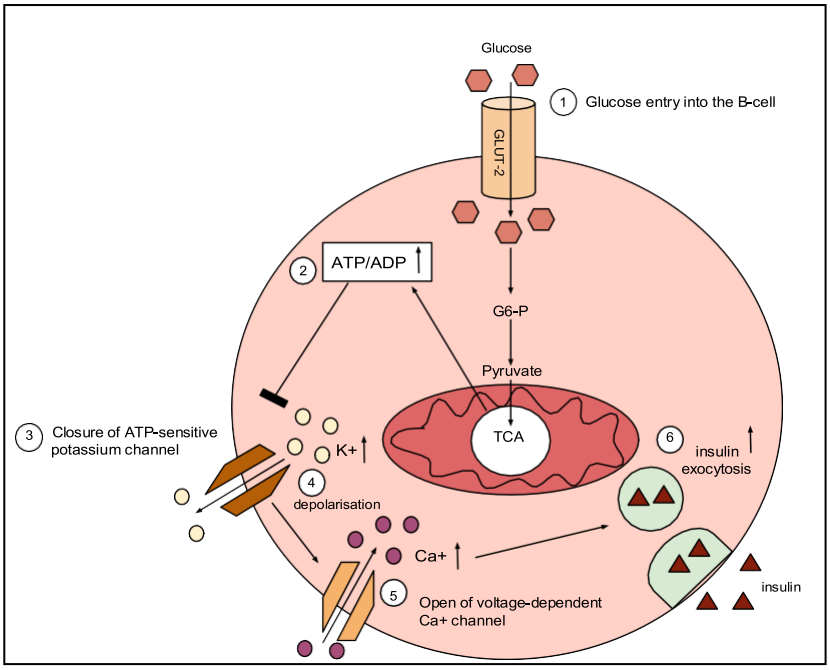



**Figure 1.**Glucose-Stimulated Insulin Secretion in Beta Cells (see text for description)

*3.2 Insulin receptor (InR) signaling pathway and regulatory mechanisms*

Insulin exerts its biological functions in the organism by binding to the InR located in the cell surface of numerous cell types even tough insulin metabolic effects occur mainly in white adipose tissue (WAT), skeletal muscle and liver. InR is a tetrameric protein, composed by two extracellular α subunits and two transmembrane β subunits joined by disulfide bounds, and possesses an intrinsic tyrosine kinase activity (19,20). Once insulin binds to the extracellular subunits a conformational change occurs and the InR undergoes autophosphorylation on specific carboxyl-terminal Tyr residues creating docking sites for specific intracellular molecules with an SH2 domain (19). Importantly, InR signaling activates two different pathways. The first one is known as the Grb2-SOS-Ras-MAPK pathway through which insulin acts as a growth factor exerting mitogenic functions and promoting cell growth and differentiation (15). In contrast to this, the other activated pathway, the IRS-PI3K-AKT cascade mediates insulin metabolic functions. Thus for the purpose of this review only this second signaling pathway will be described. Tyr autophosphorylation of the IR results in the recruitment of different InR substrates (IRS) which in turn result phosphorylated on Tyr residues by the hormone-bound InR and, thereby, recruit the lipid kinase PI3K through its SH2 domains. Activated PI3K kinase phosphorylates membrane lipids to convert inactive phosphatidylinositol 4,5-bisphosphate (PIP2) into activated phosphatidylinositol-3,4,5-trisphosphate (PIP3), which in turn activates the phosphoinositide-dependent kinase (PDK-1). Lastly PDK-1 phosphorylates AKT at the Thr308 residue, a site also targeted by mTORC2 complex. Three different AKT/PKB kinases have been identified but AKT2 seems to be the most important in insulin-mediated glucose homeostasis (19,20). To conclude many effector proteins are phosphorylated on their Ser/Thr residues downstream of AKT and mediate many important metabolic functions of insulin such as inhibiting hepatic glucose production and increasing glucose uptake, and biosynthesis of glycogen, protein and lipid synthesis in different target tissues (12,20). The specific molecular mechanisms required for insulin mediated functions downstream of AKT will be described in detailed latter in this review

*3.3 Inhibitory mechanisms of InR signaling*

InR cascade also has negative modulators that attenuate and suppress insulin action , the main inhibitory mechanisms in which InR **signaling is suppressed are described in Figure 2**. . However, although many of the antagonistic signaling mechanisms that turn off the InR cascade exert a physiological function on modulating insulin response, prolonged activation of those inhibitory mechanisms could eventually lead to IR. An important molecule exerting a negative regulation on the InR pathway is insulin. Insulin decreases InR levels in the membrane through a ligand-induced internalization and lysosomal degradation of the InR. Therefore, while insulin arises after a meal it also paradoxically downregulates its own receptor in the cell surface (20).

Additionally lipid and protein phosphatases are also implicated in InR signaling attenuation. The two phosphatases known to dephosphorylate PI3 and thereby attenuate downstream InR signaling include PTEN and SHIP2. These phosphatases act by converting active PIP3 into an inactive form, PIP2 (19,20). Protein tyrosine phosphatase that negatively regulates InR signaling include Protein Tyrosine Phosphatase (PTP)1B, which acts by dephosphorylating tyrosine residues on InR and IRS, PHLPP and PP2, which constitute a distinct fam-ily of Ser/Thr protein phosphatases that dephosphorylate AKT thereby contributing to a negative feedback on InR signaling (20). Some antagonists like GRB or SOCS act as InR tyrosine kinase pseudosubstrates, bind to the IR subunits and thereby, prevent IRS proteins from binding to InR. Furthermore, GRB and SOCS dock to the phosphor-Tyr residue of the InR through their SH2 domain and in addition, SOCS recruits ubiquitin ligase promoting IRS proteasomal degradation. Intriguingly even though these proteins mostly act as insulin antagonists, they also exert protective effect on the InR Tyr kinase domain by preventing dephosphorylation (19,20). Lastly, other antagonist mechanisms that attenuate InR signaling are caused by IRS Ser/Thr phosphorylation. Different protein kinases are known to use this pathway to downregulate insulin action and the emerging potential role for these kinases in obesity-induced IR has been a focus of interest in the last years. These Ser/Thr kinases include S6 kinase (S6K) induced by the mTORC1 complex, IκB kinase β (IKK), c-Jun N-terminal kinase (JNK) and protein kinase C (PKC). Unrestrained activation of these kinases causes Ser/Tre phosphorylation of IRS, which results in InR-IRS dissociation and signaling downregulation. Interestingly JNK, IKK, S6K and mTORc1 kinases can all be induced by pro-inflammatory cytokines like TNF-α ,IL-1β and IL-6 which could explain the link between the chronic low-grade inflammatory state seen in obese patients with systemic impaired insulin action. Additionally, ectopic fat lipid accumulation can induce PKC activity in those tissues in a DAG dependent manner. PKC also impairs InR autophosphorylation by phosphorylating Thr1160 in the activation loop (12,19,20). Further explanation on how ectopic lipid accumulation and WAT inflammation can lead to systemic IR will be discussed in more detail latter in this review.

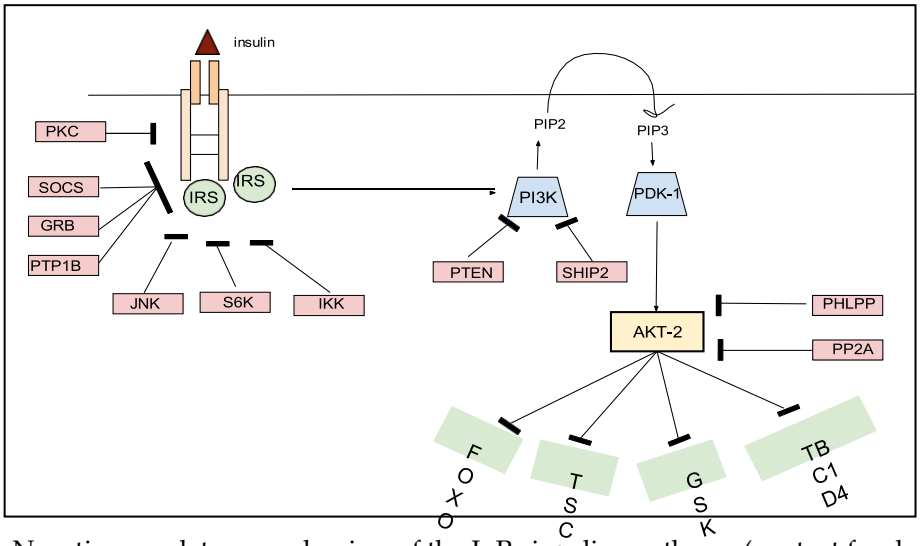



**Figure 2.** Negative regulatory mechanism of the InR signaling pathway (see text for description).

## 4.Metabolic effects of insulin signaling

As discussed earlier systemic metabolic responses to insulin are mostly dependent on AKT activation but insulin also exerts some of its functions independently of AKT (12). PI3K/AKT phosphorylation leads to a number of downstream signaling effects mediated through the phosphorylation of multiple target proteins including: the forehead family box O (FOXO) transcription factors; the protein tuberous sclerosis 2 (TSC2) which in turn activates mTORC1 and its downstream targets ribosomal protein S6 kinase (S6K) and sterol regulatory element binding protein 1c (SREBP1c); glycogen synthase kinase 3β (GSK3β) and the RabGAP TBC1 domain family member 4 (TBC1D4) among other substrates (20). Additionally, even though insulin downstream signaling positively activates numerous substrates implicated in glucose, lipid and protein metabolism, in this section only the main effects of insulin on glucose and lipid metabolism will be discussed **and visually represented in Figure 3** as they are the primary substrates whose metabolism appears to be disrupted in the progression of obesity-associated T2D.

### *4.1 Insulin signaling in the skeletal muscle*

#### *4.1.1 Glucose Uptake*

Skeletal muscle is responsible for about 90% of total-body glucose disposal hence it is important in regulating systemic glucose metabolism and homeostasis (21). Myocytes constitute a highly-energy consuming cells so insulin stimulation of glucose uptake is essential for proper muscle contractility and substrate availability (22). Similarity to the adipose tissue, insulin promotes glucose entry into the cell by stimulating the translocation and latter fusion of GLUT-4 storage vesicles with the plasma membrane. This process requires PI3K/AKT activation followed by downstream inactivation of the TBC1D4 /AS160 substrate, which normally blocks the Rab GTPase proteins that regulate vesicle trafficking. Therefore insulin-mediated AKT activation leads to GLUT-4 storage vesicles (SV) translocation and fusion with the membrane allowing glucose entry into the cell (21–23). Interestingly, exercise can also inactivate AS160 in a AMPK-dependent manner independently on insulin action in the skeletal muscle. Recently a study aimed to show the effects of exercise on obese diabetic, obese non-diabetic and lean non-diabetic subjects demonstrated that exercise had indeed a direct effect on AMPK stimulated glucose-uptake in all subjects. Intriguingly, it also concluded that obese T2D patients needed a higher intensity exercise to stimulate the AMPK-AS160 axis to the same level as lean subjects (24).

#### *4.1.2 Glycogen synthesis*

There is no doubt that insulin exerts a pivotal role as a storage hormone, so it is no surprise the main fate of insulin-stimulated glucose uptake in the skeletal muscle is glycogen synthesis (22). Insulin controls intracellular glycolytic flux by effectively regulating the catabolic and anabolic pathways of glycogen synthesis using both allosteric- and phosphorylation-based mechanisms. Firstly, insulin signaling through AKT2 inhibits GSK3 while simultaneously activating protein phosphatase 1 (PP1). The combination of inactive GSK3 and active PP1 leads to the formation of a dephosphorylated and active form of glycogen synthase (GS). Secondly, insulin positive regulation of hexokinase II allows the conversion of glucose into G6P which is a potent allosteric activator of the GS which in turn results in net glycogen synthesis (22).

While insulin acute activation of GS is essential for glycogenesis to occur, this process is not sufficient for net glycogen synthesis thereby insulin also simultaneously reduces the glycogenolysis pathway by decreasing the activity of glycogen phosphorylase (GP). Similarly to the regulation mechanisms of GS, insulin inactivates phosphorylase kinase (PK) though PP1 activation which eventually promotes the dephosphorylation and inactivation of GP. Conclusively insulin direct and allosteric effects on intracellular glycolytic flux leads to a positive net glycogen synthesis in myocytes (15,20–22).A study conducted in muscle-deficient InR (MIRKO) mice, with selective muscle insulin resistance, demonstrated the importance of proper myocyte InR signaling in maintaining systemic homeostasis. The data reoccluded in that study showed that specifically inducing muscle inactivation of InR signaling in MIRKO mice had systemic consequences on hole-body substrate distribution .Surprisingly these mice showed normal glycemia but instead they displayed an increase in total body fat tissue as well as serum lipids. Together this data suggests that a severe impairment in the insulin-signaling pathway in muscle consequently leads to a compensatory increase in insulin-stimulated glucose uptake in the adipocytes consequently leading to hyperlipidemia, which is associated with the progression of T2D in obese humans demonstrating the effects of muscle resistance in hole-body glucose homeostasis (25).

### *4.2 Insulin signaling in the Liver*

#### *4.2.1 Glycogen synthesis*

In the fed state insulin modulates hepatocyte glycolytic flux using both phosphorylation and allosteric mechanisms that resemble to those discussed previously in the skeletal muscle. However glucose-uptake in the hepatocyte, unlike in the myocyte, is insulin-independent therefore insulin control over of net glycogen synthesis is not as prominent. However insulin still regulates GS allosterically by elevating intracellular GK expression which in turn increases intracellular G6P level enabling GS activation.Additionally, insulin also regulates glycogen synthesis through the InR signaling pathway by covalently phosphorylating and inactivating GSK-3 followed by activation of PP1 which together lead to complete GS activation (21,22). Similarity, insulin also blocks hepatic glycogenolysis through PP1 activation and consequent dephosphorylation and inhibition of PK, which attenuates GP activity. Importantly, in addition to insulin action , hyperglycemia also

exerts an important role in the hepatocyte where it controls net glycolytic flux. Indeed, glucose and its metabolites are both able to simultaneously inactivate liver GP while also enabling the translocation of GK from the nucleus to the cytoplasm increasing glucose-G6P flux and GS activity. Furthermore given that glucose itself is a potent allosteric inactivator of liver GP, this enables the liver-type glycogen targeting subunit of PP1 (GL) to release active PP1. Taken together, this shows that even though glucose metabolites are potent allosteric regulators, the hepatic InR signaling is also required for full control of hepatic glycolytic flux (15,20–22).

*4.2.2 Suppression of hepatic glucose production (HGP)*

One of the hallmarks of T2D is impaired postprandial glycemia hence the importance of insulin to effectively suppress hepatic glucose production in the postprandial state. Normally, hepatic stimulation of glycogenolysis and gluconeogenesis occurs as a physiologic response to compensate for low blood glucose in the fasted state enabling the release of glucose into the bloodstream so other tissues can use it as a fuel source. Meanwhile in the postprandial phase insulin gets released into the bloodstream after pancreatic activation and it acutely suppress hepatic gluconeogenesis to maintain euglycemic levels (15,23). Insulin can suppress hepatic gluconeogenesis through direct and indirect mechanisms. Actually, several enzymes involved in hepatic glucose production are downregulated upon InR signaling which is explained by ability of insulin to directly suppress the activity of two well-characterized transcriptional factors: FOXO and CRTC2. Upon activation FOXO increases the expression of numerous gluconeogenic genes primary by binding to the transcriptional coactivator peroxisome proliferative activated receptor also known as PGC1-α.Therefore, PI3K/AKT activation causes FOXO1 translocation from the nucleus to the cytoplasm where it remains retained and inactive. Concordantly with this, a experimental study showed that complete ablation of hepatic FOXO1 expression on mice led to impaired hepatic glucose production resulting in 50% decrease of fasted glycogenolysis and gluconeogenesis demonstrating the link between FOXO and HGP. In addition to FOXO, a transcriptional complex which involves the CREB-regulated transcription coactivator 2 (CRTC2) also controls gluconeogenic gene expression in an insulin-dependent manner. However, CRTC2 is inhibited by a different kinase known as salt-inducible kinase 2 (SIK2), which is also responsive to InR signaling. Lastly, while FOXO activation is associated with the long lasting fasted states, CRTC2 seems to be crucial in the first few hours of fasting (22). Although the transcriptional mechanisms described above are responsible for the changes observed in gene expression, they cannot explain insulin acute suppression of hepatic gluconeogenesis. Thus an alternative hypothesis has emerged proposing that insulin acute inhibition of hepatic gluconeogenic flux is likely an indirect effect mediated primarily through insulin suppression of adipocyte lipolysis (15,22). Experimentally, selective inhibition of adipocyte lipolysis in dogs has been reported to mediate acute suppression of HGP (26). Equally another study surprisingly showed that in the absence of both AKT and Foxo1, mice adapted appropriately to both the fasted and fed state, and insulin suppressed HGP normally (27). There are mainly two possible mechanisms proposed for FFA mediated stimulation of HGP, the first one involves allosteric activation of the gluconeogenic enzyme, pyruvate Carboxylase (PC) through Acetyl-CoA , which is a metabolite of FFA oxidation. The second one involves glycerol, also a metabolite of FFA oxidation, which is a precursor for hepatic glucose synthesis (22). In conclusion, in the fasted state the adipocyte releases glycerol and FFA into the circulation and within the hepatocyte, FFA are oxidized to Acetyl-CoA, which together with glycerol promote HGP during fasting. However in the postprandial state insulin indirectly suppresses HGP within a few minutes by blocking adipocyte lipolysis, which rapidly reduces intracellular Acetyl-coA and glycerol concentrations in the hepatocytes turning off the gluconeogenic flux (15,22).

*4.2.3 Induction of hepatic De Novo Lipogenesis (DNL)*

Hepatic insulin signaling directly stimulates transcription of lipogenic enzymes to promote intracellular lipid storage and decrease the availability of extracellular FFA for systemic oxidation thereby enabling glucose to be utilized by the tissues as the main fuel during the fed state (22).Insulin signaling through PI3K/AKT phosphorylates and inhibits tuberous sclerosis complex 2 (TSC2) which in turn activates the mammalian target of rapamycin (mTORC1) resulting in the upregulation of a potent hepatic lipogenic transcription factor known as sterol regulatory element binding protein 1 (SREBP1). Active SREBP1 then enables the expression of numerous enzymes involved in hepatic lipogenesis such as: acetyl-CoA carboxylase 1, fatty acid synthase and glycerol-3-phosphate acyltransferase 1 (22,23). Although SREBP1 activation is necessary for hepatic DNL, inhibition of FOXO is also required for complete activation of this pathway. Insulin mediated DNL activation through transcriptional mechanisms is a rather slow process but insulin can also acutely stimulate hepatic DNL by directly phosphorylating lipogenic enzymes like GK, ACC or ATP citrate lyase (15). Furthermore, an

experimental *in vivo* study, conducted in mice defective in hepatic AKT2 expression, demonstrated the requirement of insulin/AKT signaling on hepatic lipogenesis and development of steatosis during insulin-resistance states. Taken together this experiment concluded that loss of hepatic AKT signaling resulted in reduced hepatic triglyceride (TG) levels, as well as lipogenic gene expression. Surprisingly, diet-induced obese mice showed a reduced hepatic TG content but instead displayed no difference on lipogenesis gene expression which remained unhunched raising the hypothesis that insulin is involved in multiple pathways of hepatic lipid metabolism (28). Interestingly ,hepatocyte DNL can also be induced by other mechanisms independently of insulin signaling since the liver also responds to nutrients like glucose and proteins which directly activate CREBP-1 and mTORC1 expression respectively, two potent transcriptional factors that also induce hepatic DNL (22). This has been proposed as an explanation for the known paradox of "selective hepatocyte insulin resistance" thus its relevance in obesity-induced insulin resistance will be discussed latter in this review.

### *4.3 Insulin signaling in the White Adipose Tissue*

#### *4.3.1 Induction of glucose uptake*

Similarity to the skeletal muscle, insulin signaling in the adipocyte also promotes glucose uptake inducing the translocation and fusion of GLUT-4 vesicles with the plasma membrane through TBC1D4 inactivation and regulation of other AKT substrates involved in GSV trafficking (22,23) .Unlike the skeletal muscle, glucose uptake by the adipocytes only accounts for 5-10% of the total-body glucose disposal however impaired adipose glucose uptake has systemic effects on insulin sensitivity. Experimentally, a study conducted using transgenic mice (G4A-/-) with reduced adipocyte GLUT-4 concentrations showed that selectively reducing adipocyte glucose transporter caused systemic insulin resistance in both liver and skeletal muscle demonstrating the role of adipocyte glucose uptake on systemic glucose homeostasis (29). Recently, a new mechanism has emerged proposing the activation of cAMP responsive-element binding protein (CREBP) as the main responsible for the beneficial effects of adipocyte glucose uptake on systemic insulin sensitivity. Several studies have demonstrated that glucose metabolites act by directly activating, carbohydrate (CREBP) and therefore, promoting the transcriptional regulation of lipogenic and glycolytic genes which in turn induce adipocyte lipogenesis and reducing lipolysis thereby decreasing ectopic lipid accumulation and systemic insulin resistance (12,27).

#### *4.3.2 Induction of lipogenesis*

Although there is still a lot of controversy regarding the specific mechanisms in which insulin acutely regulates the net lipogenic transcriptional program insulin action on lipogenesis is believed to be likely mediated through activation of the AKT/TSC2/mTORC1 pathway thereby enabling the upregulation of the lipogenic transcription factor SREBP1. Simultaneously, AKT-mediated inactivation of FOXO also promotes lipogenesis since this transcription factor usually controls the expression of adipose triglyceride lipase (ATGL) which inhibits lipogenesis (21,22). Additionally, insulin-stimulated glucose uptake indirectly promotes lipogenesis by providing glycerol-3-phosphate, an essential substrate for fatty acid esterification, and also activating CREBP, a transcriptional factor that upregulates expression of lipogenic like ACC and FAS. However, fatty acid esterification in the adipocyte is more dependent on substrate availability than on acute insulin stimulation of enzymatic activity (22).

#### *4.3.3. 4.3.3 Inhibition of lipolisis*

Probably the most relevant physiological function of insulin action in the adipocyte is the rapid suppression of triglyceride lipolysis and reduction of plasma FFA level.

Insulin's ability to acutely suppress lipolysis after feeding depends on proper activation of the phosphodiesterase 3B (PDE3B). Complete activation of PDE promotes cAMP degradation and attenuation of PKA lipolytic activity. The adrenergic cAMP/PKA pathway is usually involved in the stimulation of lipolytic enzymes like perilipin (PLIN) and hormone-sensitive lipase (HSL) so acute suppression of this pathway by insulin's activity leads to a decreased adipocyte lipolysis. However, while this action relies on insulin regulation of PDE activity is seems to be independent of AKT activation indicating that other unknown mechanisms are also involved in the acute effects of insulin on lipolysis (12,21,22). Recently, a study was conducted using AKT2 null mice to assess whether under a fed state insulin would still be able to acutely suppress lipolysis. Surprisingly the AKT2

null mice displayed normal plasma FFA concentrations in the fed state suggesting that alternative AKT-independent mechanisms exist in which insulin is able to regulate adipocyte lipolysis under hyperinsulinemic states. (30)

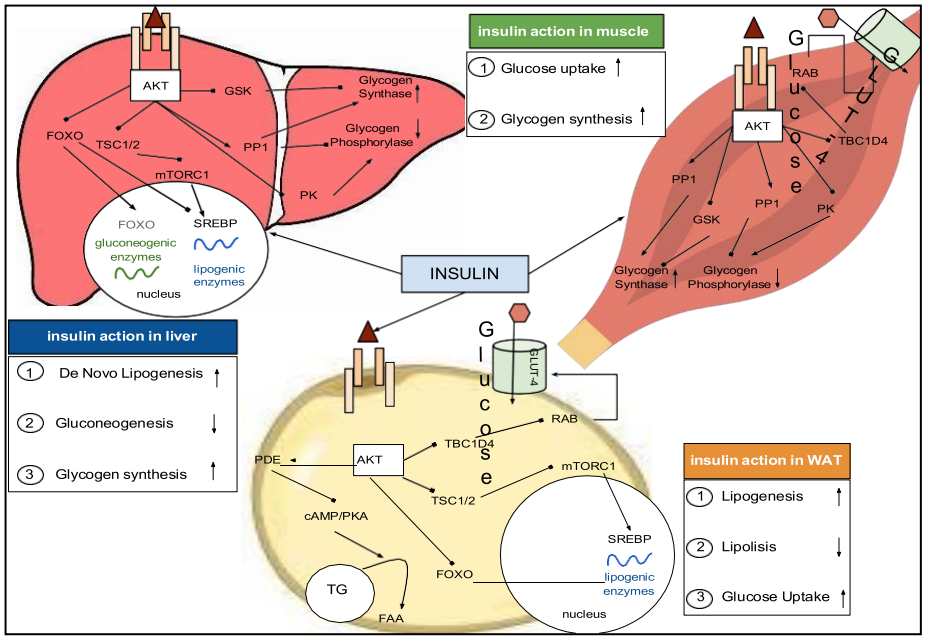



**Figure 3.** Summary of the main metabolic actions of insulin in the peripheral tissues (see text for description).

## 5. Insulin resistance and obesity

The term "insulin resistance" is used to describe an abnormal state whereby target cells are unable to fully respond to insulin signaling thereby decreasing systemic insulin sensitivity (12). Insulin insensitivity results in numerous detrimental effects such as impaired suppression of adipocyte lipolysis and endogenousglucose production as well as decreased plasma glucose uptake which together accelerate the progression of the pathogenesis of T2D. Insulin insensitivity is a characteristic feature associated with obesity however the mechanisms of how overnutrition in prone individuals causes peripheral insulin resistance is still under debate (15,22). In this section the main mechanisms in which obesity causes insulin resistance will be discussed as well as the pathophysiology of impaired insulin sensitivity in the main peripheral target tissues.

### *5.1Potential mechanisms of obesity-induced insulin resistance*

There is no doubt that overnutrition and consequent obesity are responsible for the pathogenies of peripheral IR, however there is still controversy evolving the specific mechanisms underlying this process. Current evidence supports that some of the fundamental mechanisms which are involved in the pathogenesis of obesity-associated IR are ectopic lipid accumulation, nutrient stress and inflammation.

#### *5.1.1 Ectopic Lipid accumulation*

Abnormal deposition of lipids in non-adipose tissues such as liver and skeletal muscle also known as ectopic lipid accumulation occurs as a consequence of adipocyte dysfunction and unrestrained lipolysis which together cause systemic "lipotoxicity" (19). Ectopic lipids originate in both liver and skeletal muscle when there is a mismatch between lipid oxidation and lipid delivery in these tissues and are believed to be responsible for the pathogenesis of systemic IR (22,23). Supporting this hypothesis a study was conducted using transgenic mice with muscle and liver-specific overexpression of lipoprotein lipase to determine the effects of increased fatty acid delivery to these tissues on insulin signaling. The findings clearly demonstrated a strong association between an increased accumulation of intracellular fatty acid-derived metabolites such as long fatty acylCoA, ceramide and DAG with impaired insulin action in these tissues(31). Chronic overnutrition promotes intrahepatic and intramyocellular accumulation of bioactive lipids, however in physiologic conditions intracellular FFA are either stored as triglycerides or oxidized in the mitochondria indicating that TG themselves most likely do not impair cellular insulin signaling. In contrast, excessive delivery of FFA to those tissues increases the rates of fatty acid oxidation (FAO) in the mitochondria in an attempt to compensate for

the chronic lipid oversupply however the rates of lipid utilization is insufficient to prevent the accumulation of lipid metabolites such as DAG and ceramides which are thought to interfere with insulin signaling transduction in these tissues (22,23). A possible explanatory mechanism by which DAG accumulation may induce IR in these tissues involves PKC activation. Thus, as previously discussed, PKC is a potent negative regulatory kinase that acts by phosphorylating IRS proteins on specific Ser residues, thereby suppressing the Tyr phosphorylation by InR resulting in impaired insulin signaling (15,19,22). Consistent with this, experimentally infusing lipid emulsion in rats demonstrated that increased intracellular DAG and fatty acyl-CoA concentrations were associated with increased PKC activation and reduced insulin-stimulated IRS-1 tyrosine phosphorylation and PI3-kinase activation. These data therefore support the role of DAG/PKC axis on muscle and liver induced IR. Surprisingly this study showed no increased TG or ceramide intracellular content during the lipid infusion(32). Similarity, the relationship between hepatocyte DAG, ceramide, acylcarnitine content and impaired insulin action was evaluated in 16 obese subjects and found no direct association between ceramides and hepatocyte IR (33). Contradictory to this, the expression of ceramide synthase enzymes (CerS1–6) and C16:0 ceramide was found elevated in adipocytes of obese humans and strongly correlated with impaired insulin action. Additionally, in this study they found that CerS6-deficient (CerS6D/D) mice exhibited reduced C16:0 ceramides and were protected from diet-induced IR. Interestingly CerS6 deficiency also led to increased lipid oxidation in brown adipose tissue (BAT) and in the liver, proposing Cer62 as a negative regulator for fatty acid β-oxidation (34). Taken together these studies may indicate a potential role for ceramides on the pathogenesis of IR even though the precise mechanisms of ceramide action remains poorly defined, however an accepted mechanism proposes AKT inhibition as the main target of ceramides. Therefore, ceramide may interfere with AKT downstream effectors through activation of both protein phosphatase 2A (PP2A) and PKC activity. Activated PP2A dephosphorylates, and thus inactivates, AKT, simultaneously, activation of PKC phosphorylates AKT on Ser residues suppressing its activity which then results in an impaired insulin downstream signaling (15,19,22,23).

*5.1.2 Nutrient derived oxidative stress*

Obesity is often accompanied by nutrient oversupply which not only causes ectopic accumulation of bioactive lipid metabolites like DAG and ceramides as discussed above but also increases the cell responses to nutrient overload causing ER stress and mitochondrial dysfunction, two conditions implicated in the pathogenesis of IR. ER stress can be caused by elevated ROS production, accumulation of unfolded proteins, increased demand of the synthetic machinery or increased plasma level of FFA, all of which are associated with nutrient oversupply. Consequently, ER responds by activating homeostatic system known as the unfolded protein response (UPR) to protect the organelle for any further damage. Initiation of the UPR response leads to the activation of numerous effectors such as the c-Jun NH2-terminal kinase (JNK). JNK is a well-known Ser/Thr protein kinase that targets IRS proteins upon insulin stimulation thereby, initiating a negative feedback loop to turn off insulin signaling in physiologic conditions (22). However, in obesity there are conditions such as increased ER stress, ROS production and pro-inflammatory cytokines that sustain JNK activation in insulin target tissues thus resulting in systemic attenuation of insulin action (35). Experimentally, *in vivo* JNK activation in pancreatic β cells of transgenic mouse was sufficient to induce pancreatic insulin resistance and resulted in impaired capacity of the pancreas to secrete insulin in response to hyperglycinemia. This data demonstrates that sustained activation of JNK indeed suppresses IRS downstream signaling thereby decreasing insulin autocrine action on pancreatic β cells (36). Interestingly, ER stress also induces de novo lipogenesis by activating SREBP and XBP1 transcriptional program which in turn promote hepatic steatosis and IR through the DAG/PKC axis explained earlier (22,35). Consistent with this, experimental attenuation of the ER stress markers in the liver of *ob*/*ob* mice using the chaperone glucose-regulated protein 78 (GRP78) was associated with attenuated SREBP-1 expression independent of insulin action. These mice also showed reduced hepatic TG and cholesterol content and a consequent increased in insulin sensitivity (37). In the adipose tissue ER stress-mediated JNK activation induces pro-inflammatory gene expression such as TNF-α which in turn sustains JNK activity thereby working as a feed-forward mechanism that maintains chronically activated JNK. In this scenario, this unrestrained activation of JNK in the adipocytes exacerbates the pro-inflammatory state and macrophage infiltration which in turn attenuates insulin signaling (35). Additionally ER stress increases WAT lipolysis probably in a PKA dependent manner and additional perilipin phosphorylation (22). Recently, an experimental study also demonstrated that the ER associated lipogenic DGAT1 enzyme has a crucial role in protecting the ER from lipotoxic stress by promoting re-esterification of intracellular lipids (38). Therefore an increase in fatty acid supply may impair the ability of ER associated DGAT-1 enzyme to properly induce

lipid re-esterification resulting in an increase WAT lipolysis and worsening of the pathogenesis of IR. As mentioned above mitochondrial dysfunction as a consequence of nutrient overload is also implicated in the pathogenesis of IR. Contradictory to what was previously believed, mitochondria respond to cellular ATP demand rather than substrate availability. Therefore, a new model has emerged proposing metabolic inflexibility and excessive FAO as the main cause of obesity-related IR in the skeletal muscle , as opposed to the previous models that suggested that IR was a consequence of reduced FAO and consequent deposition of lipid-derived toxic molecules (19,22). Recently, a new study was conducted to assess whether the lipid-induced IR in the skeletal muscle was indeed a consequence of excessive FAO rather than reduced β-oxidation. This study was done using targeted metabolomics as well as transgenic mice that were deficient in malonyl-CoA decarboxylase (MCD), an enzyme that promotes mitochondrial β-oxidation. Together they found that IR in the myocytes required β-oxidation, which probably explains the protective effect observed in mice lacking MCD enzyme towards diet-induced glucose intolerance. According to the results, insulin disfunction in the myocyte is likely due to an increase rather than diminished activity of the mitochondria (39). Therefore, these studies demonstrate that in a situation of nutrient oversupply, the mitochondria adapt by increasing the rate of FAO, however, the ATP demand does not increase to match the substrate availability. Therefore, the increased rate of FAO is not sufficient to prevent ectopic lipid deposition because intracellular demand of ATP is inflexible. These results in IMCL accumulation and activation of the DAG/PKC axis which consequently drives IR. In parallel, when oxidation rates exceed the ATP demand ROS and other toxic metabolites are produced which together activate JNK, thereby impairing insulin signaling. Eventually, chronically elevated ROS production damages the mitochondria, reducing its ability to efficiently elevate FAO rates thereby increasing IMCL deposition and muscle IR (22). Consistent with this, to examine the role of mitochondria ROS production on adipocyte IR, wild type (WT) mice were given a high-fat diet (HFD) for a week to observe if changes in insulin sensitivity correlated with those in ROS production. As expected after only one week of HFD, the WT mice exhibited an impaired insulin sensitivity that was correlated with an increase in ROS production as well as JNK activation suggesting that oxidative stress is one of the contributing factors of obesity-induced WAT IR (40).

*5.1.3 Inflammation*

Chronic low-grade systemic inflammation is a common trait observed in metabolically obese patients and is usually associated with the progression of IR and T2D. Experimentally using mouse models with genetic and HDF-induced obesity reveled that increased adiposity led to macrophage infiltration and consequent transcriptional up-regulation of pro-inflammatory genes, thereby demonstrating the potential role of inflammation in the pathogenesis of obesity-induced IR (41). However, another study concluded that while chronic inflammation was responsible for the long-term effects of HFD in IR, lipotoxicity was the first effector of the initial disruptions observed in the insulin signaling. This study used both WT mice and immunocompromised mice which were fed a HFD to assess whether inflammation preceded systemic IR. Surprisingly the immunocompromised mouse models all exhibited IR after the short-term HFD indicating that inflammation may not be the primary insult in T2D but rather an exacerbating factor of the disease. To summarize, this data points out that while sustained chronic inflammation may be necessary for long-term HFD induced IR it is not necessary for the short-term effects of HFD (42). Inflammation initially starts in the WAT and the mechanisms underlying this process involve adipose-tissue macrophages (ATM) activation and subsequent secretion of pro-inflammatory cytokines ultimately leading to impaired insulin signaling. However even though chronic adipose inflammation results in impaired insulin signaling, pro-inflammatory signaling in the WAT is actually essential in maintaining metabolically healthy adipocytes (22,43). Suppression of adipose-specific of pro-inflammatory markers in three different mouse models showed that reduced adipocyte inflammatory response upon HFD resulted in an increased ectopic lipid accumulation and metabolic dysregulation, therefore, demonstrating the role of adipocyte inflammation in proper tissue remodeling and lipid storage (43). Long-term exposure to lipid oversupply promotes adipose tissue expansion by increasing both adipocyte cell numbers (hyperplasia) and size (hypertrophy). Hypertrophied adipocytes present elevated stress levels which induce the secretion of signaling chemokines such a IL-8 or macrophage chemotactic protein-1 (MCP-1) that together recruit pro-inflammatory macrophages into the adipose tissue (15,44). Concordantly with this, elevated plasma level of MCP-1 was found in genetically obese diabetic (*db*/*db*) mice and in WT mice with obesity induced by a HFD. Furthermore, IR, hepatic steatosis, and macrophage accumulation in adipose tissue induced by a HFD were reduced in MCP-1 KO mice compared with WT animals, thereby demonstrating the role of MCP-1 in obesity-associated macrophage infiltration (44). Activated macrophages and adipocytes secrete pro-

inflammatory cytokines such as TNF-α, IL1β, or IL-6, which in turn impair intracellular insulin signaling through the activation of Ser/Tre kinases such as JNK and IKK. As mentioned above JNK and IKK both inhibit insulin signaling through IRS phosphorylation, and they further exacerbate the inflammatory response by enabling the transcription of pro-inflammatory genes thereby interfering with the InR signaling in the adipocytes (23,35). Indeed, mice with selective suppression of macrophage JNK-expression preserved systemic insulin sensitivity after HFD feeding in contrast to WT mice which exhibited systemic IR, therefore demonstrating the association between JNK activation with obesity-induce IR and inflammation (15,45). Another mechanism in which lipid oversupply can drive adipose tissue inflammation involves the activation of Toll-like receptors (TLR). TLR belong to the innate immune system and are generally activated upon pathogen infection, however, saturated fatty acids are also able to bind and activate TLR pathway and thereby promote the release of pro-inflammatory cytokines such as IL-1β, IL-6, MCP-1, and TNF-$\alpha$ (15,19). Additionally, inflammation seems to mediate IR in systemic tissues indirectly by inducing unrestrained activation of adipocyte lipolysis which results in increased plasma FFA level and consequent ectopic lipid deposition in the liver and skeletal muscle, thereby, impairing insulin signaling in these tissues. Therefore, it is possible that FFA and ectopic deposition are an important link between chronic adipose inflammation and systemic IR (22). Finally, adipokines secreted by the adipocytes such as leptin and adiponectin, also have a role on systemic insulin sensitivity. In obesity, elevated levels of leptin are correlated with decreased systemic insulin signaling while adiponectin, an insulin sensitizing hormone, seems to be reduced in obese patients, which together exacerbates the pathogenesis of obesity-induced IR (22).

*5.2 An integrated physiological perspective on tissue IR.*

All mechanisms discussed above in which chronic overnutrition causes systemic IR can be described in unique model in which several simultaneous responses to overfeeding (nutrient derived toxic metabolites, cellular stress, inflammation) converge to facilitate ectopic lipid deposition and consequent IR in peripheral tissues. **Figure 4 is a visual representation of an oversimplified model** of the pathogenesis of IR that aims to integrate all of three consequences of chronic overfeeding, however it is important to note that such mechanisms realistically occur simultaneously through complex interactive mechanisms rather that separated to each other.

Overnutrition is the element linking obesity with systemic IR. In the postprandial phase, after nutrient supply, adipose tissue acts as a nutrient sink enabling the deposition and storage of the excess of nutrients. However, as excessive nutrient intake continues the subcutaneous adipose tissue is unable to appropriately expand thereby increasing adipocyte nutrient-derived ER stress and ROS production which are responsible for the early defects in InR signaling (22). Experimentally feeding a HDF in transgenic *ob/ob* mice overexpressing adiponectin demonstrated that PPARγ activation though adiponectin resulted in increased adipocyte cell number and overall expansion of adipose tissue mass. The expansibility of subcutaneous fat tissue was associated with reduced TG level in the liver and skeletal muscle and consequent improvement in overall insulin sensitivity therefore providing evidence for the importance of adequate expansion of subcutaneous adipose tissue in peripheral insulin sensitivity (46). Even the smallest attenuation of WAT insulin signaling can already impair insulin control over adipocyte lipolysis which can have detrimental effects on systemic insulin sensitivity. Experimental attenuation of PTEN activity( an inhibitor of the InR signaling) resulted in enhanced insulin sensitivity in adipocytes and this was sufficient to induce systemic metabolic improvements in insulin sensitivity in both liver and skeletal muscle which indicates that reducing fatty acid delivery to these tissues improves overall insulin sensitivity (47)- Moreover, reduced glucose uptake in IR adipocytes results in decreased ChREBP activation of lipogenic gene transcription as well as proper G3P generation which leads to reduced fatty acid esterification thereby increasing adipocyte delivery of plasma FFA. Furthermore, canonically stressed adipocytes secrete chemokines that recruit and activate ATM. Activated ATM then secrete pro-inflammatory cytokines such as TNF-α that increase adipocyte lipolysis directly by decreasing the expression of lipid droplet proteins, and indirectly by exacerbating the activity of stress kinases (JNK ansd IKK) thereby suppressing insulin signaling. Together, this results in insulin inability to suppress adipose lipolysis which causes increased plasma FFA and consequent systemic lipotoxicity (22,23,31). Therefore as discussed above myocellular lipid deposition impairs muscle insulin signaling through DAG mediated activation of PKC. In humans muscle IR seems to occur before hepatic IR and results in impaired ability of insulin to stimulate GLUT-4 mediated glucose uptake and glycogen synthesis. Impaired glucose uptake in the skeletal muscle leads to an increase of total body glucose disposal thereby increasing glycemia and glucose delivery to the

liver which promotes hepatic DNL and ectopic lipid deposition (IHTG) (15,22). Chronic overnutrition, adipocyte lipolysis and skeletal muscle impaired glucose uptake increase plasma FFA concentrations and subsequent delivery of FFA into the liver. Similarly to the skeletal muscle, intracellular accumulation of ectopic lipids impairs hepatic insulin signaling through the DAG/PKC signaling thereby resulting in sustained hepatic glucose production (HGP) which is responsible for the fasting hyperglycemia present in T2D. Chronically unrestrained hepatic gluconeogenesis may be a direct consequence of insulin inability to inhibit FOXO activity, however initial impaired insulin suppression of HGP may be a consequence of adipocyte IR rather than hepatic IR (22,23). This was confirmed in a study using an *in vivo* metabolomics approach that found that allosteric regulation of hepatic PC activity by acetyl CoA, through insulin suppression of WAT lipolysis, was indeed a critical factor in the regulation of HGP by insulin *in vivo* (48). Intriguingly, under HFD feeding conditions insulin is unable to suppress hepatic FOXO activity while it remains sufficient to induce hepatic expression of SREBP, a lipogenic factor. This selective hepatic IR has been a source of debate in the recent years and two possible explanations for this paradox have emerged (12). Firstly, a possible mechanism for continued insulin activation of SREBP-1c in hepatic IR is that these pathways have different intrinsic sensitivities to insulin, therefore while the initial reduction of insulin signaling may be enough impair insulin ability to suppress FOXO, it may be insufficient to suppress SREBP-1-dependent lipogenesis.

Additionally, chronic overnutrition activates several lipogenic factors such as ChREBP and SREBP-1 independently of insulin action. Indeed this substrate-mediated activation of hepatic lipogenesis may involve both glucose activation of ChREBP and SREBP-1 and amino acid activation of mROC-1. The question of whether insulin control of hepatic glycogen metabolism becomes resistant in diabetes is confounded because of the insulin-independent regulatory mechanisms of glycogen synthesis such as allosteric control of GP by glucose (19,22).

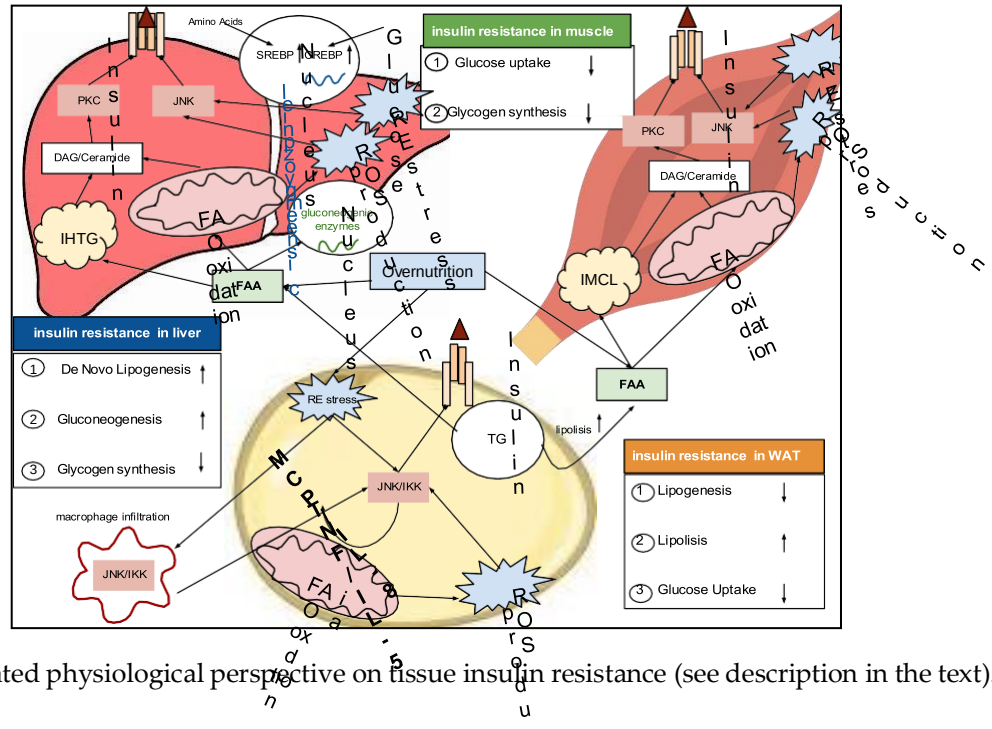



**Figure 4.** An integrated physiological perspective on tissue insulin resistance (see description in the text).

## 6. Hyperinsulinemia (HI) and obesity

Obesity is often accompanied by elevated concentrations of plasma insulin level (HI) under both fasted and postprandial states. HI, IR and glucose intolerance are all initial hallmarks of T2D however sustained pancreatic exposure to excess nutrients can lead to a significant loss of functional β-cells specially in the genetically prone individuals which in turn results in fasted hyperglycemia, the main hallmark of diabetes (12). Traditionally HI was thought to be a mere compensatory mechanism of the pancreas to protect the tissues from systemic IR however recent studies have proved this might be wrong (49). For instance, a 24-year follow-up study conducted using a sample of 515 Healthy Normoglycemic adults found that fasted HI in these people was the most significant predictor for the progression of dysglycemia thereby proposing HI as an independent

risk factor for T2D (50). Many hypotheses have been proposed to explain the HI observed in obesity, as discussed earlier the traditionally accepted mechanism was based on the observation that β cell mass was considerably increased in obese patients which led to thinking that HI was just a mechanism of the pancreas to compensate for the decrease in peripheral insulin sensitivity thereby protecting them from hyperglycemia. However overnutrition can somehow directly stimulate pancreatic insulin secretion before T2D occurs as demonstrated in a 4-day lipid infusion in healthy normal glucose tolerant subjects without a family history of T2D that found potentiated glucose-stimulated insulin secretion in the absence of hyperglycemia Insulinemia was studied in an observational study of 65 normoglycemic obese and non-obese subjects and found that while fasted insulin level was a consequence of pancreatic hypersecretion, impaired hepatic insulin clearance was responsible for the increased plasma insulin levels after oral glucose ingestion (52). Additionally, a variety of studies, point to fasting HI was the most relevant in obesity and as mentioned before, fasting insulinemia mainly depends on pancreatic hypersecretion therefore in this section the mechanism in which different nutrients enhance GSIS will be discussed to give a better understanding on how overnutrition seems to promote initial fasted HI. Evolutionary β cells have adapted to respond to numerous nutrient-derived stimulus, and even tough glucose is the main trigger for insulin secretion in β-cells, other signals such as amino acids, FAA and incretins also enhance GSIS (53). Experimentally, inhibiting the amplifying pathway of GSIS in β-cells resulted in a decrease in insulin secretory response upon high calorie feeding thereby protecting mice from diet-induce obesity which indicates that overnutrition can modulate insulin secretion (54).

*6.1 Amino acids and incretins effect on GSIS*

Individual amino acids at physiological levels do not trigger insulin secretion however certain combination of amino acids such as glutamine and leucine can enhance GSIS by increasing intracellular ATP/ADP ratio and consequent opening of the voltage dependent Ca2+ channels. Another mechanism in which amino acids can increase insulin exocytosis is by stimulating the secretion of gut incretins such as GLP-1 and GIP. Incretins are hormones of the gastrointestinal (GI) tract that are secreted after food ingestion, specially upon glucose and amino acid stimulation. The release of GLP-1 and GIP from the GI cells enables them to bind to specific cell-surface receptors located in pancreatic β cells thereby potentiating GSIS through stimulation of adenyl cyclase (16). Experimentally, using isolated pancreatic β-cells demonstrated that the ability of GLP-1 to potentiate insulin release was mediated in part by stimulating intracellular lipolysis through PKA activation. Remarkably, these experiments suggested that cAMP activation by incretins somehow activated intracellular lipases such as HSL ultimately increasing FFA and long-chain acyl-CoA (LC-CoA), two potent mediators of insulin exocytosis. This hypothesis was supported by the observation that increasing concentrations of lipase-inhibitor orlistat resulted in diminished incretin effect on insulin secretion (55). To summarize, incretins could be one of the contributing factors linking excessive nutrient consumption to HI, in such case, overnutrition would promote sustained release of incretins from the GI tract into the circulation which would consequently sustain chronic stimulation of insulin secretion in pancreatic β cells

*6.2 Fatty acids effect on GSIS:*

Experimentally culturing β-cells in excess nutrients leads to intracellular deposition of lipid derived molecules and consequent increase in insulin hypersecretion at submaximal glucose levels thereby suggesting that lipids are indeed potent modulators of GSIS in pancreatic β-cells (56). Consistent with this, another experiment using rats deprived of food for 18–24 h, found that the ability of β-cells to secrete insulin in response to glucose was dependent on the levels of circulating FFA during the fasted state (57). Therefore FAA are undoubtedly potent stimulators of GSIS, however, neither elevated glucose or FFA supply alone seem to alter the β cell secretory function, because in the absence of glucose, FFA are completely oxidized in the cell and *vice versa*. However chronic pancreatic exposure to both, glucose and FFA, results in an initial pancreatic insulin hypersecretion but as this progresses the excess nutrient intake eventually damage β cell functionality thereby, decreasing their ability to release insulin into the circulation (53,58). Thus initial elevated plasma FFA level, particularly in the presence of glucose, increases pancreatic insulin secretion by accumulating intracellular lipid intermediates such as FFA-derived LC-CoA esters, phospholipids, DAG, ceramides and malonyl-CoA , that are potent enhancer signaling molecules of the GSIS pathway. Plasma FAA can stimulate pancreatic insulin secretion mainly through three important mechanisms **all summarized in Figure 5**. The first one involves malonyl-CoA formation through anaplerotic and cataplerotic processes. Thus after glucose entry into the cell, it gets metabolized into pyruvate which is then converted to citrate by PC enzyme, which then escapes the

mitochondria and gets transformed into malonyl-CoA through two metabolic processes involving citrate liase and ACC enzymes. The resultant malonyl-CoA inhibits FAO by suppressing the activity of carnitine palmitoyl-transferase (CPT)-1 mitochondrial transporter. Reduction of FAO leads to intracellular accumulation of lipids intermediates like LC-CoA that either stimulate insulin secretion directly or promote the formation of other complex lipids such as DAG and other phospholipids (59). Concordantly with this, a study conducted using streptolysin-O-permeabilized clonal insulin-secreting cells (HIT T-15) demonstrated that one of the mechanisms in which FAA seem to acutely stimulate GSIS is through intracellular accumulation of LC-CoA. Interestingly inhibition of protein kinase activity as well as intracellular ATP failed to suppress LC-CoA-stimulated insulin release thereby suggesting that the ability of LC-CoA to promote insulin exocytosis is likely independently of protein kinase-induced phosphorylation and instead may involve LC-CoA protein binding and acetylation (60). The second one involves the glycolipid (GL)/FFA) cycle and the ability of glucose to concomitantly induce FFA esterification while also activating lipolysis at the same time. Thus, glucose derived Glycerol-3P promotes the esterification of LC-CoA into more complex lipid molecules such as DAG and TG, but glucose simultaneously induces lipolysis enabling the release of FFA. Together, these opposing functions of glucose on lipid metabolism results in the accumulation of multiple intracellular lipid molecules such as DAG, ceramides, phospholipids and FFA (58,59). Both the TG/FFA cycling and malonyl-CoA/LC-CoA pathway engage GSIS through intermediates like DAG, which activate PKC either directly thereby inducing insulin exocytosis and it also binds to Munich-13 , a protein involved in the insulin vesicle secretion. Another intermediate that results from these pathways is LC-CoA that is able to acetylate essential proteins such as SNAO-25 and synaptogamin which are proteins that enable vesicle fusion with plasma membrane and consequent insulin release. Lastly, recently it was discovered that β-cells have a G-couple FFA receptor known as FFAR-1 through which FFA can directly stimulate insulin exocytosis by increasing intracellular Ca+ concentrations (59). All the above discussed mechanisms explain the ability of excess FFA and glucose to initially enhance insulin secretion, however chronic pancreatic exposure to excess nutrient supply eventually causes irreparable damage to the pancreatic β cells through a process known as “glucolipotoxicity “ (Gltox) thereby driving β cell apoptosis (53,58). In order to gain insight into the molecular mechanisms involved in Gltox, gene profiling and metabolic analyses were performed in INS832/13 cells that were cultured in glucose and palmitate to promote (Gltox). The results discovered that Gltox was associated with elevated levels of intracellular ROS production, cholesterol, ceramide, and TG levels as well as decreased GSIS and FAO suggesting that elevated glucose and FAA are indeed involved in changes in the expression of genes implicated in lipid partitioning, sterol metabolism, mitochondrial function, and oxidative stress (61). Therefore high rates of both FFA and glucose entry into the cell not only lead to the accumulation of toxic lipid compounds (ceramide, DAG) that interfere with InR signaling, but also causes a tremendous metabolic burden to ER and mitochondria thereby increasing intracellular ROS production and ER stress both of which have very detrimental effects into the cell specially since β cells are not provided by strong antioxidant defensive mechanisms. Therefore, the effects of Gltox may be one of the main contributors leading to β-cell dysfunction and apoptosis (53,58).

To summarize, elevated levels of FAA, amino acids and incretins all seem to potentiate GSIS in the pancreas which results in elevated plasma insulin levels during sustained periods of overfeeding even in the absence of detectable increases in plasma glucose levels or peripheral IR. Equally important to the amount of food consumption are the temporal feeding patterns, experimentally using mice with time restricted feeding and mice that were fed frequently throughout the day demonstrated that disruption of the normal feeding cycle was a better predictor for obesity, HI, hepatic steatosis, and inflammation than HFD itself indicating that sustained stimulation of pancreatic insulin secretion by nutrient consumption can be a primary cause of fasted HI (62).

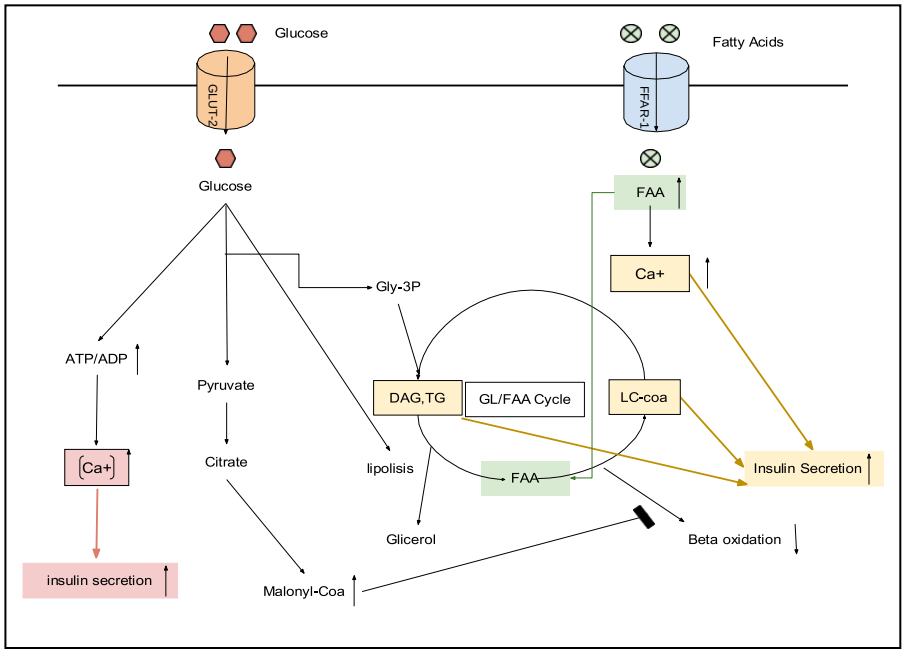



**Figure 5.** An Overview on the cross talk between lipid and glucose metabolism in β cells and insulin secretion.(see text for description)

## 7.Time of appearance of HI and IR during the development of T2D

Nutritional excess, a condition often seen in obesity, is a major forerunner of T2D and it is responsible for the initial metabolic dysregulations that occur early in the progression of the disease such as peripheral IR and insulin hypersecretion. The molecular mechanisms in which chronic overnutrition attenuates insulin sensitivity in peripheral tissues while simultaneously enhancing insulin secretion in the pancreas have been discussed above however conflicting evidence exist regarding the time of appearance of these events during the progression of obesity and T2D.

There are currently two conflicting viewpoints regarding the sequence of events that occur in T2D. The traditional view supports that an early decline of peripheral insulin sensitivity is the primary effect of nutrient excess which in turn causes a progressive increase in insulin secretion. In contrast the novel alternative hypothesis proposes that an increase in pancreatic secretion is the first abnormality in T2D triggering peripheral IR and obesity (13).However up until this date whether pancreatic insulin hypersecretion precedes systemic IR or the other way around is still under debate because they are both conditions that occur almost simultaneously in the early phases of T2D thereby making it difficult to separate one to another. Therefore in this section scientific evidence supporting each one of these opposing hypothesis will be reviewed to have a better understanding on the primary abnormality that occurs early in the progression of the disease.

### *7.1 Model of primary insulin hypersecretion in the pathogenesis of T2D*

This novel model suggesting that primary HI causes IR in metabolic tissues is based on the hypothesis that insulin hypersecretion is the initial effect of nutrient excess which in turn causes reduced insulin sensitivity in target tissues. Ultimately, T2D develops when pancreatic β-cells become “exhausted” and are no longer able to compensate for the increased insulin demand of resistant tissues (12). To test this hypothesis and see whether chronic HI is causative in the development of IR, normoglycemic lean rats were subjected to sustained insulin infusions during a 6 week period to assess the effects of HI on glucose tolerance. Interestingly while moderate insulin infusion was sufficient to cause glucose intolerance in healthy lean rats, a seven day-insulin therapy in obese rats significantly improved insulin signaling in the liver and skeletal muscle indicating that in obesity when IR is already present insulin infusions can effectively improve insulin secretory capacity (63). Additionally, the role of primary HI in the progression of dysglycemia has also been tested in humans. For example an observational trial conducted in a large cohort of healthy normoglycemic subjects was able to identify the metabolic effects of primary insulin hypersecretion. Remarkably, the results demonstrated that

subjects with initial insulin hypersecretion, independent of IR, had a worse metabolic phenotype and lipid profile compared with the rest of the cohort. Furthermore these subjects also exhibited an impaired glucose tolerance and insulin sensitivity and were a more prone to develop T2D after a 3-years follow-up (64). Therefore the above experimental studies demonstrate that HI can somehow reduce insulin sensitivity in target tissues yet the mechanisms underlying this process are not fully understood. However, the observation that patients with insulinomas also develop mild suppression of insulin signaling despite the frequent occurrence of hypoglycemic episodes suggests that primary insulin hypersecretion can directly induce systemic IR even in the absence of obesity or nutrient excess that are usually responsible for the initial suppression of insulin signaling as discussed in the previous sections. Furthermore, even tough weight gain did occur in 40% of the patients there seem to be no correlation between IR and body weight before surgery thereby indicating that other aspects were responsible for the decrease in insulin sensitivity (65). A possible explanation could be that IR in these patients develops as a protective mechanism to maintain euglycemia and protect the tissues from glucose overload. An additional mechanism in which continuously high insulin levels can directly drive IR is through downregulation of its own cell-surface receptor thereby interfering with its own signaling. Either way it seems unlikely that HI is just a compensatory mechanism of primary IR since initial HI in these patients occurs without the increases in blood glucose that would theoretically be required to stimulate β cells. An additional mechanism in which HI impairs systemic insulin sensitivity may involve the activation of inflammatory pathways.

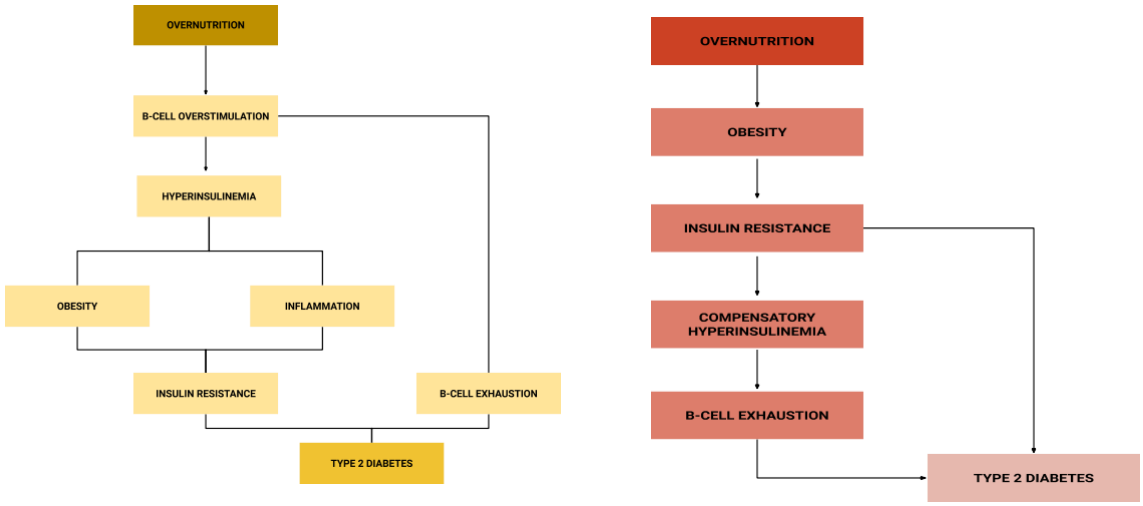
Experimentally reducing circulating insulin levels in obese mice through either diazoxide or streptozotocin treatments attenuated WAT inflammatory markers such as macrophage infiltration and cytokine expression therefore improving whole-body insulin sensitivity. Remarkably, infusion of insulin in lean mice even in euglycemic conditions also increased WAT inflammation. Together this results indicate that lowering circulating insulin levels can attenuate the low-grade chronic inflammatory state often seen in obesity however insulin alone is not the only reason capable of inducing adipocyte inflammation other factors such as chronic overnutrition are also required for this to occur (66). Similarly, T2D patients undergoing insulin treatment for 6 months also experience abdominal weight gain and increase in the levels of pro-inflammatory cytokines such as MCP-1, TNF-$\alpha$ and IL-1β in the adipose tissue. Moreover, in this study the increase in inflammatory markers in diabetic patients was independent of weight gain however the group that experienced a more pronounced weight gain had an increased inflammatory status in the adipose tissue (67). Therefore, while insulin treatment is often a necessity in these patients to achieve proper glycemic control and avoid hypergly- cemia, side effects such as abdominal weight gain and increase low-grade inflammation may actually exacer-bate the progression of the disease. Taken together, these studies demonstrate that obesity-associated HI can promote inflammation in the adipose tissue therefore causing adipocyte IR through the activation of stress kinases such as JNK. Lastly, given that insulin is a well-known lipogenic hormone chronically sustained insu- lin secretion is expected to promote increased adiposity and weight gain. Indeed, an observational study found that fasted plasma HI preceded obesity in children from the Pima Indian population, thereby suggesting in- sulin as an independent risk factor for the development of obesity and consequent progression of T2D (68). Although it seems likely that insulin is associated with obesity to test if HI is causative in the onset of obesityit is necessary to study weather attenuating insulin secretion can also protect towards diet-induced obesity. This was tested in a study comparing the effects of a HDF on female mice that were heterozygous for the ancestral insulin gene Ins2 with the control group with both copies of the Ins2 gene. The results showed that female Ins1−/−:Ins2+/− mice had reduced insulin secretion and they also displayed less weight gain on a HFDthan did the control group. Remarkably, even tough mice lacking both copies of Ins2 gene reached the equiv-alent degree of HI on a HFD than the control group after a year, they remained leaner than the control groupthereby providing evidence that attenuation of insulin secretion in young mice provides long-term protection against diet-induced obesity (69).

However, the consistency of weather insulin has a causative role in the onset of obesity has been challenged by other studies where weight gain was not observed in hyperinsulinemic conditions thereby indicating that there is still a lot of cofounding evidence. Either way, insulin effects on adiposity could provide an additional explanation for the systemic attenuation of insulin signaling upon insulin hypersecretion.

*7.2 Model of primary IR in the pathogenesis of T2D*

The traditional hypothesis supports chronic nutrient excesses as the initial cause of abdominal obesity which then leads to reduced insulin sensitivity in peripheral tissues and consequent HI. Ultimately T2D occurs when β cells are no longer able to sustain sufficient insulin secretion (12,13). Therefore, if decreased insulin sensitivity is the primary abnormality in T2D, progressive β-cell expansion accompanying overnutrition is expected to occur to compensate for the increased insulin demand. Indeed, the ability to increase β-cell mass to overcome IR was demonstrated in a study where female mice were given HDF.The results showed that β-cell volume was indeed increased after 3 months on HDF feeding therefore providing evidence that expanded β-cell mass is an important adaptation towards primary IR to maintain normal glycemia (70). Additionally, chronic treatment with corticosterone in adult mice resulted in rapid induction of severe IR which in turn led to a rapid adaptive increased in insulin secretion. This massive β-cell mass expansion was partially a consequence of increased β-cell neogenesis and proliferation (71).

Similarly, another study also showed that increases in β-cell mass can also take place to overcome low insulin production. Thus, this study using mice carrying a mutation for either Ins1 or Ins2 gene found that knock-out mice were insulin deficient and consequently, developed increased β-cell hyperplasia to compensate for the lack of insulin production thereby indicating that pancreatic islets are able to respond to different stimulus, not just decreased peripheral insulin sensitivity (72). Together this data supports the hypothesis that HI is a necessary response to properly adapt to IR and maintain euglycemia because when this ability of β-cells to compensate for increased insulin demand is impaired diabetes occurs. Observations on certain groups at risk of suffering T2D such as people with Cushing syndrome, gestational diabetes or PCOS have also demonstrated that primary reduced insulin action is what initially drives insulin hypersecretion. For instance, during pregnancy a marked IR occurs as a physiologic response of the body to provide the fetus with a constant flow of glucose. Usually this systemic IR is accompanied by an increased insulin secretion through β-cell hyperplasia and hypertrophy. However an impaired capacity of the pancreas to compensate for the increased demand on insulin may lead to gestational diabetes during pregnancy. Therefore this demonstrates that in these women HI occurs as an adaptive response to primary IR rather as opposed to HI being the initial effect of nutrient excess (73). The exact mechanisms contributing to the compensatory increase of insulin secretion upon IR are still unknown, however, a study using transgenic mice with combined IR and β cell failure indicate that expansion of β-cell mass in response to IR requires FOXO1 nuclear exclusion from β cells since it is a repressor of a critical regulator of cell survival known as Pdx1. Additionally, the results also suggest that β-cell hyperplasia occurs in existing β-cells as a response to locally acting growth factors through a paracrine mechanism (74). Remarkably, some studies have shown that impaired insulin function precedes increased insulin secretion. For example, in a study were mice were given glucocorticoids (GC) both pancreatic insulin content and islet volume were increased to compensate for the initial attenuation of insulin action. The results showed GC-treated mice displayed an increase in ectopic fat accumulation and weight gain accompanied by an increased IR thereby suggesting that the rise in pancreatic islet β-cell secretion was compensation for the increased demand for insulin (75). Lastly, some of the current drug treatments available for diabetic patients such as metformin and thiazolidinediones improve glycemic control of diabetic patients by enhancing peripheral insulin sensitivity. Recently, a randomized prospective trial was conducted on newly diagnosed T2D adults to evaluate the effects of rosiglitazone and metformin treatment during a 4-year period. Together the results showed that treatment with both insulin-sensitizers drugs, metformin and thiazolidinediones, improved peripheral insulin sensitivity and reduced overall β-cell secretory response thereby suggesting an early attenuation on insulin signaling as the principal defect in T2D (76).




**(a)** **(b)**

**Figure 6.** Models for primary insulin hypersecretion (**a**) and IR (**b**) in the pathogenesis of T2D.

## 8.Conclusions

Nutritional excess associated to obesity, is the major forerunner of T2D and it is responsible for the appearance of the initial metabolic defects that characterize the disease such as impaired pancreatic secretion and reduced peripheral insulin sensitivity. Although both of these metabolic defects are present early in the pathogenesis of T2D whether HI precedes IR or *vice versa* is still highly debatable. However, new findings have demonstrated that overnutrition can directly stimulate insulin hypersecretion which in turn leads to decreased peripheral insulin sensitivity and obesity. Increasing insulin levels may help to achieve a better glycemic control once hyperglycemia occurs but, on the light of recent data, in the initial stages when glucose levels are within the normal range elevating insulin levels may only lead to worsened IR. Therefore, novel therapeutic strategies should focus on suppressing insulin secretion to improve peripheral insulin sensitivity rather than searching for drugs that promote insulin hypersecretion, especially in the early stages of the disease.

To conclude further studies are needed in order to have a better understanding on the mechanisms underlying the pathogenesis of T2D to come up with better therapeutic interventions for patients suffering this disease, however, changing people lifestyle and eating habits is always the best preventive mechanism for obesity and its comorbidities.

**Author Contributions:** Literature review of the sections discussed and writing of the manuscript

**Acknowledgments:** None

**Funding:** This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.
**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare that they have no competing interests

**References**

1. Obesity and overweight [Internet]. [cited 2021 Apr 12]. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
2. Banegas JR, López-García E, Gutiérrez-Fisac JL, Guallar-Castillón P, Rodríguez-Artalejo F. A simple estimate of mortality attributable to excess weight in the European Union. European Journal of Clinical Nutrition. 2003;57(2):201–8.
3. Agencia Española de Seguridad Alimentaria y Nutrición. Surveillance Study on Nutrition, Physical Activity, Child Development and Obesity. 2020;(September).
4. Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham CL, Anis AH. The incidence of co-morbidities related to obesity and overweight: A systematic review and meta-analysis. BMC Public Health. 2009;9:1–20.

5. Vazquez G, Duval S, Jacobs DR, Silventoinen K. Comparison of body mass index, waist circumference, and waist/hip ratio in predicting incident diabetes: A meta-analysis. Epidemiologic Reviews. 2007;29(1):115–28.
6. Iglay K, Hannachi H, Howie PJ, Xu J, Li X, Engel SS, et al. Prevalence and co-prevalence of comorbidities among patients with type 2 diabetes mellitus. Current Medical Research and Opinion. 2016;32(7):1243–52.
7. Kazi AA, Blonde L. Classification of diabetes mellitus. Vol. 21, Clinics in Laboratory Medicine. 2001. 1–13.
8. Diabetes [Internet]. [cited 2021 Apr 12]. Available from: https://www.who.int/news-room/fact-sheets/detail/diabetes
9. Saeedi P, Salpea P, Karuranga S, Petersohn I, Malanda B, Gregg EW, et al. Mortality attributable to diabetes in 20–79 years old adults, 2019 estimates: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Research and Clinical Practice [Internet]. 2020;162:108086. Available from: https://doi.org/10.1016/j.diabres.2020.108086
10. Complications [Internet]. [cited 2021 Apr 12]. Available from: https://www.idf.org/aboutdiabetes/complications.html
11. Einarson TR, Acs A, Ludwig C, Panton UH. Prevalence of cardiovascular disease in type 2 diabetes: A systematic literature review of scientific evidence from across the world in 2007-2017. Cardiovascular Diabetology [Internet]. 2018;17(1):1–19. Available from: https://doi.org/10.1186/s12933-018-0728-6
12. Czech MP. Insulin action and resistance in obesity and type 2 diabetes. Vol. 23, Nature Medicine. Nature Publishing Group; 2017. p. 804–14.
13. Esser N, Utzschneider KM, Kahn SE. Early beta cell dysfunction vs insulin hypersecretion as the primary event in the pathogenesis of dysglycaemia. Diabetologia. 2020;63(10):2007–21.
14. Gregory JM, Smith TJ, Slaughter JC, Mason HR, Hughey CC, Smith MS, et al. Iatrogenic hyperinsulinemia, not hyperglycemia, drives insulin resistance in type 1 diabetes as revealed by comparison with GCK-MODY (MODY2). Diabetes. 2019;68(8):1565–76.
15. Insulin Function, Insulin Resistance, and Food Intake Control of Secretion - The Medical Biochemistry Page [Internet]. [cited 2021 Mar 21]. Available from: https://themedicalbiochemistrypage.org/insulin-function-insulin-resistance-and-food-intake- control-of-secretion/
16. Fu Z, R. Gilbert E, Liu D. Regulation of Insulin Synthesis and Secretion and Pancreatic Beta-Cell Dysfunction in Diabetes. Current Diabetes Reviews. 2012;9(1):25–53.
17. Vakilian M, Tahamtani Y, Ghaedi K. A review on insulin trafficking and exocytosis. Gene. 2019;706(April):52–61.
18. Takei M, Dezaki K, Ishii H, Nishio SI, Sato Y, Suzuki S, et al. A new experimental model of ATP-sensitive K+ channelindependent insulinotropic action of glucose: A permissive role of cAMP for triggering of insulin release from rat pancreatic β-cells. Endocrine Journal. 2013;60(5):599–607.
19. Boucher J, Kleinridders A, Kahn CR. Insulin Receptor Signaling in Normal. Cold Spring Harb Perspect Biol 2014. 2014;6:a009191.
20. Haeusler RA, McGraw TE, Accili D. Metabolic Signalling: Biochemical and cellular properties of insulin receptor signalling. Nature Reviews Molecular Cell Biology. 2018;19(1):31–44.
21. Huang X, Liu G, Guo J, Su ZQ. The PI3K/AKT pathway in obesity and type 2 diabetes. International Journal of Biological Sciences. 2018;14(11):1483–96.
22. Petersen MC, Shulman GI. Mechanisms of insulin action and insulin resistance. Vol. 98, Physiological Reviews. American Physiological Society; 2018. p. 2133–223.
23. Samuel VT, Shulman GI. The pathogenesis of insulin resistance: Integrating signaling pathways and substrate flux. Journal of Clinical Investigation. 2016;126(1):12–22.
24. Sriwijitkamol A, Coletta DK, Wajcberg E, Balbontin GB, Reyna SM, Barrientes J, et al. Effect of acute exercise on AMPK signaling in skeletal muscle of subjects with type 2 diabetes: A time-course and dose-response study. Diabetes. 2007;56(3):836–48.
25. Kim JK, Michael MD, Previs SF, Peroni OD, Mauvais-Jarvis F, Neschen S, et al. Redistribution of substrates to adipose tissue promotes obesity in mice with selective insulin resistance in muscle [In Process Citation]\rTissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect simila. J Clin Invest. 2000;105(12):1791–7.

26. Mittelman SD, Bergman RN. Inhibition of lipolysis causes suppression of endogenous glucose production independent of changes in insulin. American Journal of Physiology - Endocrinology and Metabolism. 2000;279(3 42-3):630–7.
27. Lu M, Wan M, Leavens KF, Chu Q, Monks BR, Ahima RS, et al. HHS Public Access. 2012;18(3):388–95.
28. Leavens KF, Easton RM, Shulman GI, Previs SF, Birnbaum MJ. Akt2 Is Required for Hepatic Lipid Accumulation in Models of Insulin Resistance. Cell Metabolism [Internet]. 2009;10(5):405–18. Available from: http://dx.doi.org/10.1016/j.cmet.2009.10.004
29. Herman MA, Peroni OD, Villoria J, Schön MR, Abumrad NA, Blüher M, et al. glucose metabolism. 2012;484(7394):333–8.
30. Koren S, DiPilato LM, Emmett MJ, Shearin AL, Chu Q, Monks B, et al. The role of mouse Akt2 in insulin-dependent suppression of adipocyte lipolysis in vivo. Diabetologia. 2015;58(5):1063–70.
31. Kim JK, Fillmore JJ, Chen Y, Yu C, Moore IK, Pypaert M, et al. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. Proceedings of the National Academy of Sciences of the United States of America. 2001;98(13):752 2–7.
32. Yu C, Chen Y, Cline GW, Zhang D, Zong H, Wang Y, et al. Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. Journal of Biological Chemistry [Internet]. 2002;277(52):50230–6. Available from: http://dx.doi.org/10.1074/jbc.M200958200
33. Magkos F, Su X, Bradley D, Fabbrini E, Conte C, Eagon JC, et al. Intrahepatic diacylglycerol content is associated with hepatic insulin resistance in obese subjects. Gastroenterology. 2012;142(7):1444–6.
34. Turpin SM, Nicholls HT, Willmes DM, Mourier A, Brodesser S, Wunderlich CM, et al. Obesity-induced CerS6-dependent C16:0 ceramide production promotes weight gain and glucose intolerance. Cell Metabolism. 2014;20(4):678–86.
35. Carme Caelles M, Jordi L-M, Isabel AM, Díaz-Delfín; J, Morcillo; M, Bayod C. The role of the c-Jun N-terminal kinase (JNK) pathway in insulin resistance. Research Signpost. 2016;37(2):77–90.
36. Lanuza-Masdeu J, Isabel Arévalo M, Vila C, Barberà A, Gomis R, Caelles C. In vivo jnk activation in pancreatic β-cells leads to glucose intolerance caused by insulin resistance in pancreas. Diabetes. 2013;62(7):2308–17.
37. Kammoun HL, Chabanon H, Hainault I, Luquet S, Magnan C, Koike T, et al. GRP78 expression inhibits insulin and ER stress-induced SREBP-1c activation and reduces hepatic steatosis in mice. Journal of Clinical Investigation. 2009;119(5):1201–15.
38. Chitraju C, Mejhert N, Haas JT, Diaz-Ramirez LG, Grueter CA, Imbriglio JE, et al. Triglyceride Synthesis by DGAT1 Protects Adipocytes from Lipid-Induced ER Stress during Lipolysis. Cell Metabolism [Internet]. 2017;26(2):407-418.e3. Available from: http://dx.doi.org/10.1016/j.cmet.2017.07.012
39. Koves TR, Ussher JR, Noland RC, Slentz D, Mosedale M, Ilkayeva O, et al. Mitochondrial Overload and Incomplete Fatty Acid Oxidation Contribute to Skeletal Muscle Insulin Resistance. Cell Metabolism. 2008;7(1):45–56.
40. Paglialunga S, Ludzki A, Root-McCaig J, Holloway GP. In adipose tissue, increased mitochondrial emission of reactive oxygen species is important for short-term high-fat diet-induced insulin resistance in mice. Diabetologia. 2015;58(5):1071–80.
41. Xu H, Tartaglia LA, Chen H, Xu H, Barnes GT, Yang Q, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance Find the latest version : Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. Journal of Clinical Investigation. 2003;112(12):1821–30.
42. Lee YS, Li P, Huh JY, Hwang IJ, Lu M, Kim JI, et al. Inflammation is necessary for long-term but not short-term high-fat diet-induced insulin resistance. Diabetes. 2011;60(10):2474–83.
43. Wernstedt Asterholm I, Tao C, Morley TS, Wang QA, Delgado-Lopez F, Wang Z v., et al. Adipocyte inflammation is essential for healthy adipose tissue expansion and remodeling. Cell Metabolism [Internet]. 2014;20(1):103–18. Available from: http://dx.doi.org/10.1016/j.cmet.2014.05.005
44. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity1. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, others. MCP-1 contributes to m. The Journal of Clinical Investigation [Internet]. 2006;116(6):1494. Available from: http://www.jci.org/cgi/content/abstract/116/6/1494

45. Sil et al. 2013. 基因的改变NIH Public Access. Bone. 2013;65(6):903–9.
46. Kim JY, van de Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM, et al. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. Journal of Clinical Investigation. 2007;117(9):2621–37.
47. Morley TS, Xia JY, Scherer PE. Selective enhancement of insulin sensitivity in the mature adipocyte is sufficient for systemic metabolic improvements. Nature Communications [Internet]. 2015;6:1–11. Available from: http://dx.doi.org/10.1038/ncomms8906
48. Perry RJ, Camporez JPG, Kursawe R, Titchenell PM, Zhang D, Perry CJ, et al. Hepatic acetyl CoA links adipose tissue inflammation to hepatic insulin resistance and type 2 diabetes. Cell. 2015;160(4):745–58.
49. Page MM, Johnson JD. Mild Suppression of Hyperinsulinemia to Treat Obesity and Insulin Resistance. Vol. 29, Trends in Endocrinology and Metabolism. Elsevier Inc.; 2018. p. 389–99.
50. Basal-State Hyperinsulinemia in Healthy Normoglycemic Adults Is Predictive of Type. 2009;(May):1464–6.
51. Kashyap S, Belfort R, Gastaldelli A, Pratipanawatr T, Berria R, Pratipanawatr W, et al. <A Sustained Increase in Plasma Free Fatty Acids.pdf>. Aderican Diabetes Association. 2003;52:14–8.
52. Bonora E, Zavaroni I, Bruschi F, Alpi O, Pezzarossa A, Guerra L, et al. Peripheral hyperinsulinemia of simple obesity: Pancreatic hypersecretion or impaired insulin metabolism? Journal of Clinical Endocrinology and Metabolism. 1984;59(6):1121–7.
53. Prentki M, Joly E, El-Assaad W, Roduit R. Malonyl-CoA Signaling, Lipid Partitioning, and Glucolipotoxicity. Diabetes [Internet]. 2002;51(suppl 3):S405–13. Available from: http://diabetes.diabetesjournals.org/content/51/suppl_3/S405.abstract
54. Vetterli L, Carobbio S, Frigerio F, Karaca M, Maechler P. The Amplifying Pathway of the ␣ -Cell Contributes to. 2016;291(25):13063–75.
55. Yaney GC, Civelek VN, Richard AM, Dillon JS, Deeney JT, Hamilton JA, et al. Glucagon-like peptide 1 stimulates lipolysis in clonal pancreatic β-cells (HIT). Diabetes. 2001;50(1):56–62.
56. Erion KA, Berdan CA, Burritt NE, Corkey BE, Deeney JT. Chronic exposure to excess nutrients left-shifts the concentration dependence of glucose-stimulated insulin secretion in pancreatic β-cells. Journal of Biological Chemistry [Internet]. 2015;290(26):16191–201. Available from: http://dx.doi.org/10.1074/jbc.M114.620351
57. Stein DT, Esser V, Stevenson BE, Lane KE, Whiteside JH, Daniels MB, et al. Essentiality of circulating fatty acids for glucose-stimulated insulin secretion in the fasted rat. Journal of Clinical Investigation. 1996;97(12):2728–35.
58. Nolan CJ, Prentki M. The islet β-cell: fuel responsive and vulnerable. Trends in Endocrinology and Metabolism. 2008;19(8):285–91.
59. Nolan CJ, Madiraju MSR, Delghingaro-Augusto V, Peyot ML, Prentki M. Fatty acid signaling in the β-cell and insulin secretion. Diabetes. 2006;55(SUPPL. 2).
60. Deeney JT, Gromada J, Høy M, Olsen HL, Rhodes CJ, Prentki M, et al. Acute stimulation with long chain acyl-CoA enhances exocytosis in insulin-secreting cells (HIT T-15 and NMRI β-cells). Journal of Biological Chemistry. 2000;275(13):9363–8.
61. El-Assaad W, Joly E, Barbeau A, Sladek R, Buteau J, Maestre I, et al. Glucolipotoxicity alters lipid partition ing and causes mitochondrial dysfunction, cholesterol, and ceramide deposition and reactive oxygen species production in INS832/13 β-cells. Endocrinology. 2010;151(7):3061–73.
62. Hatori M, Vollmers C, Zarrinpar A, Ditacchio L, Bushong EA, Gill S, et al. Article Time-Restricted Feeding without Reducing Caloric Intake Prevents Metabolic Diseases in Mice Fed a High-Fat Diet. Cell Metabolism [Internet]. 2012;15(6):848–60. Available from: http://dx.doi.org/10.1016/j.cmet.2012.04.019
63. Hamza SM, Sung MM, Gao F, Soltys CM, Smith NP, Macdonald PE, et al. Biochimica et Biophysica Acta Chronic insulin infusion induces reversible glucose intolerance in lean rats yet ameliorates glucose intolerance in obese rats ☆. BBA - General Subjects [Internet]. 2017;1861(2):313–22. Available from: http://dx.doi.org/10.1016/j.bbagen.2016.11.029

64. Tricò D, Natali A, Arslanian S, Mari A, Ferrannini E. Identification, pathophysiology, and clinical implications of primary insulin hypersecretion in nondiabetic adults and adolescents. JCI Insight [Internet]. 2018 Dec 20 [cited 2021 Mar 1];3(24). Available from: https://pubmed-ncbi-nlm-nih-gov.sire.ub.edu/30568042/

65. Furnica RM, Istasse L, Maiter D. A severe but reversible reduction in insulin sensitivity is observed in patients with insulinoma. Annales d'Endocrinologie [Internet]. 2018;79(1):30–6. Available from: http://dx.doi.org/10.1016/j.ando.2017.08.001

66. Pedersen DJ, Guilherme A, Danai L v., Heyda L, Matevossian A, Cohen J, et al. A major role of insulin in promoting obesity-associated adipose tissue inflammation. Molecular Metabolism [Internet]. 2015;4(7):507–18. Available from: http://dx.doi.org/10.1016/j.molmet.2015.04.003

67. Jansen HJ, Stienstra R, van Diepen JA, Hijmans A, van der Laak JA, Vervoort GMM, et al. Start of insulin therapy in patients with type 2 diabetes mellitus promotes the influx of macrophages into subcutaneous adipose tissue. Diabetologia. 2013;56(12):2573–81.

68. Odeleye OE, Courten M de, Pettitt DJ, Ravussin E. Fasting Hyperinsulinemia Is a Predictor of Increased Body Weight Gain and Obesity in Pirna Indian Children. 1997;46(AUGUST).

69. Templeman NM, Clee SM, Johnson JD. Suppression of hyperinsulinaemia in growing female mice provides long-term protection against obesity. 2015;2392–402.

70. Ahrén J, Ahrén B, Wierup N, Ahrén J, Ahrén B, Wierup N. Increased β -cell volume in mice fed a high-fat diet : A dynamic study over 12 months Increased β-cell volume in mice fed a high-fat diet A dynamic study over 12 months. 2022;i:9–13.

71. Courty E, Besseiche A, Thu T, Do H, Liboz A, Aguid FM, et al. Adaptive b -Cell Neogenesis in the Adult Mouse in Response to Glucocorticoid-Induced Insulin Resistance. 2019;68(January):95–108.

72. Leroux L, Desbois P, Lamotte L, Duvillié B, Cordonnier N, Jackerott M, et al. a Null Mutation for Ins1 or Ins2. 2000;150–3.

73. Devlieger R, Casteels K, Assche FA van. Reduced adaptation of the pancreatic B cells during pregnancy is the major causal factorfor gestational diabetes: Current knowledge and metabolic effects on the offspring.

74. Okamoto H, Hribal ML, Lin H v., Bennett WR, Ward A, Accili D. Role of the forkhead protein FoxO1 in β cell compensation to insulin resistance. Journal of Clinical Investigation. 2006;116(3):775–82.

75. Fransson L, Franzén S, Rosengren V, Wolbert P, Sjöholm Å, Ortsäter H. b-cell adaptation in a mouse model of glucocorticoid-induced metabolic syndrome. Journal of Endocrinology [Internet]. 2013;219:231–41. Available from: http://joe.endocrinology-journals.org

76. Le ARTIC, Loves S, Groningen L van, Filius M, Mekking M, Brandon T, et al. Effects of Diazoxide-Mediated Insulin Suppression on Glucose and Lipid Metabolism in Nondiabetic Obese Men. 2018;103(January):2346–53.