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Review

Insulin: The master regulator of glucose metabolism

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ABSTRACT

Insulin is the master regulator of glucose, lipid, and protein metabolism. Following ingestion of an oral glucose load or mixed meal, the plasma glucose concentration rises, insulin secretion by the beta cells is stimulated and the hyperinsulinemia, working in concert with hyperglycemia, causes: (i) suppression of endogenous (primarily reflects hepatic) glucose production, (ii) stimulation of glucose uptake by muscle, liver, and adipocytes, (iii) inhibition of lipolysis leading to a decline in plasma FFA concentration which contributes to the suppression of hepatic glucose production and augmentation of muscle glucose uptake, and (iv) vasodilation in muscle, which contributes to enhanced muscle glucose disposal. Herein, the integrated physiologic impact of insulin to maintain normal glucose homeostasis is reviewed and the molecular basis of insulin's diverse actions in muscle, liver, adipocytes, and vasculature are discussed.

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1. Overview of insulin's effect on glucose metabolism

Following glucose ingestion, insulin secretion is stimulated and the resultant hyperinsulinemia initiates a series of closely integrated metabolic responses that, in normal glucose tolerant individuals, returns the plasma glucose concentration to normal within 2 h [1,2] (Fig. 1). The rise in plasma insulin, in concert with the increase in plasma glucose concentration, results in the inhibition of glucagon secretion [3]. Under postabsorptive conditions, i.e. following an overnight fast, the basal rate of endogenous glucose production (80–90% derived from liver and 10–20% from kidney) is ~2.0 mg/kg·min and is suppressed by 50–60% secondary to the combined actions of increased plasma insulin and glucose and decreased plasma glucagon concentrations [4–6]. More complete suppression of hepatic glucose production is observed when insulin and glucose are administered intravenously [1,7,8]. Under euglycemic conditions intravenous insulin infusion to create pharmacologic hyperinsulinemia (>1000 μU/ml) has no effect to stimulate hepatic glucose uptake [6–8]. In contrast, following glucose ingestion, ~30–40% of the oral load is taken up and disposed of by the liver [5,6,9–11]; this effect is mediated by an increase in the positive portal vein/hepatic artery glucose gradient and mass action effect of hyperglycemia [6,9,12], and requires the presence of insulin [13,14].

Following glucose ingestion, the increase in plasma insulin concentration binds to and activates the insulin receptor on myocytes [15–18], augmenting muscle glucose uptake [1,2,7,10,19,20]. Quantitatively, ~60–70% of an ingested 100 gram glucose load escapes hepatic glucose uptake and is disposed of by muscle and other obligate glucose utilizers (i.e., CNS, formed blood elements, smooth muscle) [1,2,5,10] under the combined effects of hyperinsulinemia plus hyperglycemia, with approximately two-thirds of the glucose entering the glycogen synthetic pathway and one-third entering the oxidative pathway [21,22]. Insulin also stimulates adipose tissue glucose uptake [23], but quantitatively this accounts for the disposal of only about 5–10% of the ingested glucose load [1]. Under basal postabsorptive conditions tissue glucose uptake by non-insulin sensitive tissues (CNS, formed blood elements, smooth muscle) is ~1.2 mg/kg·min [1]. For a 70 kg person, this amounts to ~20 g over 4 h. Thus, of the 60–70 g of glucose that escape splanchnic extraction, 30–40 g are taken up by muscle with 5–10 g being disposed of by fat and ~20 g by other obligate tissues. Thus, hepatic and muscle glucose uptake contribute approximately equally to the disposal of an ingested glucose load. However, it should be noted that adipose tissue also contributes to glucose homeostasis via insulin's effect to inhibit lipolysis, leading a reduction in the plasma free fatty acid (FFA) concentration [24]. FFA inhibit muscle glucose uptake [25,26] and augment hepatic glucose production [27]. Thus, the insulin-mediated decline in plasma FFA concentration simultaneously serves to facilitate muscle glucose uptake and retard hepatic glucose output [28–31].

Insulin also exerts a potent effect on arterial smooth muscle to promote vasodilation [32–35]. This results in enhanced delivery of insulin with glucose to under perfused myocytes, resulting in enhanced disposal of the ingested glucose. Activation of the insulin signaling system in vascular smooth muscle cells activates nitric oxide synthase, leading to enhanced generation of nitric oxide, a potent vasodilator and

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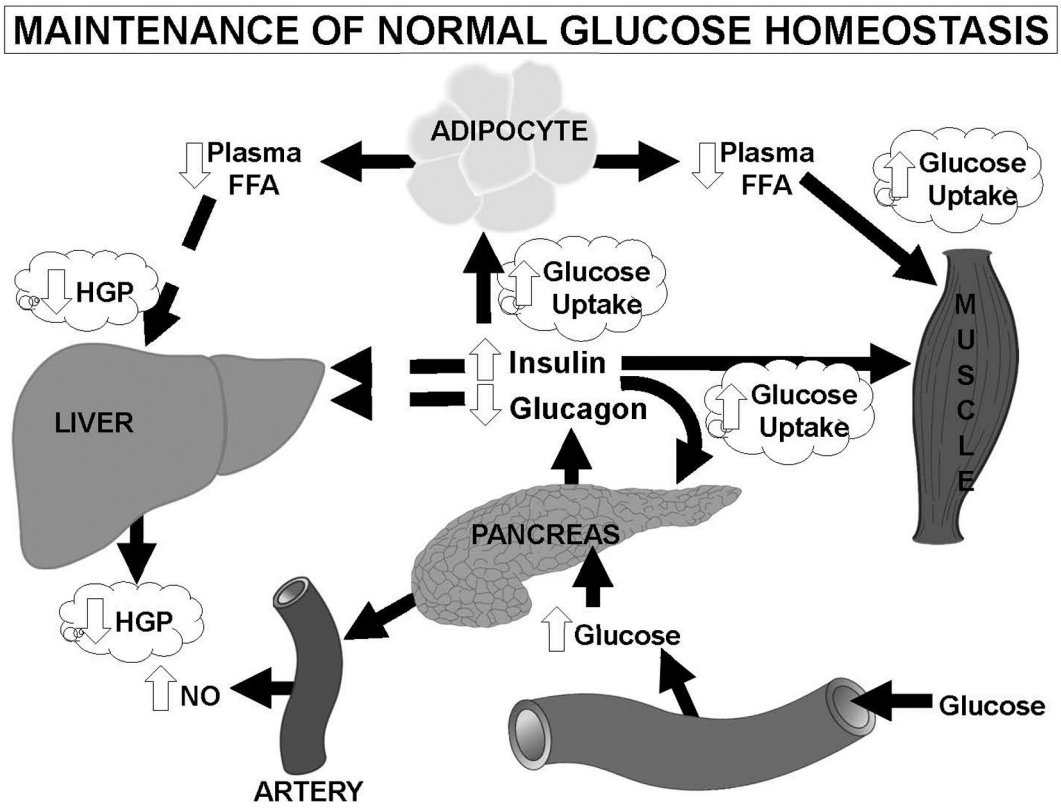

Fig. 1. Overview of insulin's effect on glucose metabolism.

antiatherogenic molecule [36,37]. Thus, insulin provides a cardioprotective effect, while insulin resistance impairs nitric oxide synthesis leading to accelerated coronary artery disease and hypertension [38]. Further, the hyperinsulinemic response to insulin resistance causes stimulation of the mitogen-activated protein kinase (MAPK) pathway leading to enhanced vascular smooth cell growth and proliferation, inflammation, and atherogenesis [38].

Of interest congenital, as well as postnatal, beta cell specific knockout of the insulin receptor in mice leads to glucose intolerance [39] suggesting an important role of the insulin receptor in the regulation of insulin secretion. However, in humans little is known about the role of the beta cell insulin receptor in the regulation of insulin secretion.

2. Physiology of insulin actions in skeletal muscle

The anabolic actions of insulin are epitomized by its regulation of nutrient storage in skeletal muscle. As the body's largest reservoir of both carbohydrate and protein, skeletal muscle function is heavily reliant on the insulin-mediated uptake of glucose and amino acids to sustain these intracellular nutrient pools. In healthy individuals, skeletal muscle glucose uptake increases 10-fold in response to insulin stimulation, making this tissue the overwhelming contributor to total body glucose disposal under hyperinsulinemic euglycemic conditions [1,2,5,40–42]. Following a meal, 60–70% of the ingested carbohydrate is disposed by skeletal muscle [1,2,5,41,42], highlighting the paramount role of insulin-mediated muscle glucose uptake in postprandial glycemic control. A substantial fraction of this glucose disposal, approximately 15–20%, can be attributed to the vasodilatory actions of insulin to increase muscle perfusion [32–35]. More importantly, insulin increases the transport of glucose into the myocytes via the recruitment of glucose transporter 4 (GLUT4) to the plasma membrane from intracellular organelles [43–45], an effect mediated via the insulin signal transduction cascade [15–17,46] (Fig. 2). The GLUT4 transporter is present in all insulin sensitive tissues including skeletal and cardiac muscle and adipose tissue. In skeletal muscle and adipocytes (as well as in RBCs and the brain), GLUT1 is the basal glucose transporter and is insulin insensitive. GLUT2, which has a high Km (~17 mM), is present in the liver, beta cells, and gastrointestinal epithelium and facilitates the rapid equilibration of glucose between the extracellular space and cell cytosol. At the myocellular membrane, insulin binding to the extracellular domain (α subunits) of its cognate receptor leads to transphosphorylation of the intracellular β subunits and subsequent tyrosine phosphorylation of insulin receptor substrate (IRS) proteins [15–17,47,48]. Phosphorylated IRS-1 binds to and activates phosphoinositide 3-kinase (PI3K), resulting in the generation of [3,4,5]-triphosphate (PIP_3) and downstream phosphorylation/activation of the crucial metabolic transducer Akt (protein kinase B) [49]. In skeletal muscle, Akt phosphorylation stimulates the trafficking of intracellular GLUT4 vesicles from their intracellular domain to the plasma membrane [43–47]. Remarkably, and despite extensive progress in delineating the key events involved in the insulin signaling cascade (outlined above and reviewed in ref. [13–15,46]), significant controversy remains as to the absolute dependence of insulin-mediated GLUT4 translocation on the major components of this pathway in muscle [47–49].

Sustained facilitative transport of glucose into the muscle cell requires the maintenance of a glucose concentration gradient, achieved by the rapid intracellular phosphorylation of glucose to glucose-6-phosphate (G6P) by hexokinase II [50–53]. Insulin supports the regulation of glucose phosphorylation in human muscle by upregulating the activity and transcription of hexokinase II [50–53]. Insulin also promotes the downstream metabolism of glucose-6-phosphate through both oxidative and non-oxidative pathways [54,55]. Skeletal muscle glucose oxidation is predominantly driven by the rate of mitochondrial acetyl-CoA generation from pyruvate, a step regulated by the insulin-mediated activation of pyruvate dehydrogenase [56]. As such, insulin orchestrates a switch from fatty acid to glucose oxidation in skeletal

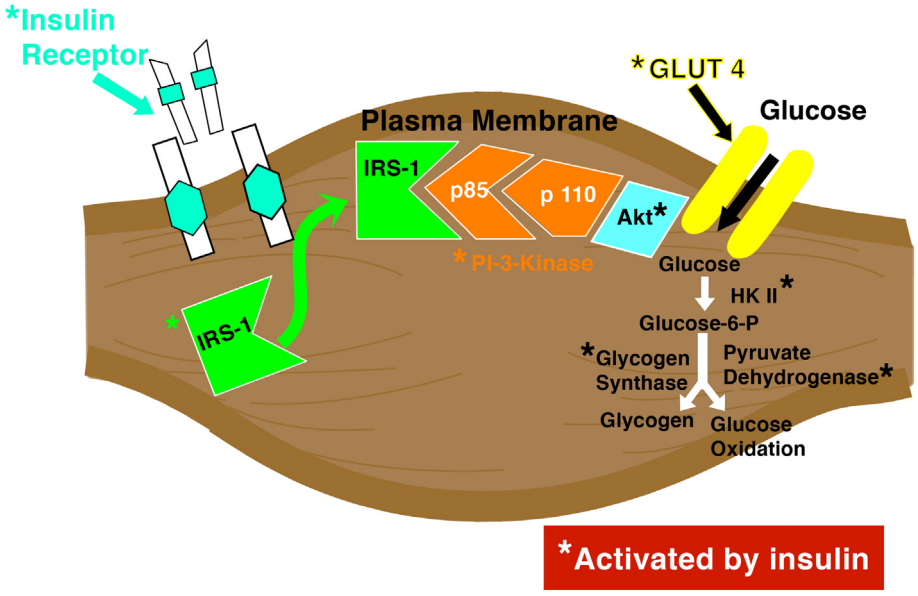

Fig. 2. Schematic representation of the effect of insulin on muscle glucose disposal.

muscle in the fasting to fed transition [1,2,41]. In healthy individuals, insulin infusion also has been shown to increase the capacity for mitochondrial ATP production in skeletal muscle, as well as to upregulate the transcription of multiple components of the mitochondrial electron transport chain [57].

Under conditions of sustained muscle glucose uptake, such as during a euglycemic-hyperinsulinemic clamp, glucose oxidation saturates oxidative energy turnover and the fraction of G6P instead diverted towards muscle glycogen can exceed 70% [58]. Distal components of the insulin signaling cascade, primarily GSK3 phosphorylation (causing its inhibition) by Akt [53], foster glycogen synthesis by dephosphorylating and activating glycogen synthase [56,59–61]. Moreover, net glycogen accretion is further supported by the reciprocal suppression of glycogen breakdown, ensured via insulin's inhibitory actions towards glycogen phosphorylase [62]. However, superseding these mechanisms is the metabolic action of insulin to increase the intramyocellular concentration of G6P, which acts as a major allosteric activator of glycogen synthesis via its reciprocal regulation of both glycogen synthase (activating) and glycogen phosphorylase (inhibitory) [63,64]. The blunted ability of insulin to promote non-oxidative glucose storage represents the fundamental pathophysiology of insulin resistant skeletal muscle in type 2 diabetes [58].

Insulin also plays a pivotal role in the regulation of muscle protein metabolism [65,66]. Physiological concentrations of insulin contribute to the maintenance of skeletal muscle mass by suppressing intramuscular rates of proteolysis [65,66] via the Akt-mediated inactivation of autophagic signaling, including phosphorylation of the forkhead box O transcription factors [67]. Stimulation of the PI3K-Akt axis by insulin also activates mTORC1 (the mechanistic target of rapamycin complex 1), a key effector of cellular protein synthesis [66]. However, the net stimulation of muscle protein synthesis requires a rise in plasma insulin concentration in concert with a rise in plasma amino acid concentration which most typically occurs following ingestion of a meal [65,66,68,69]. Another major physiological role of insulin is the stimulation of sodium-potassium ATPase, which is highly abundant in skeletal muscle where it serves to buffer postprandial potassium levels [70,71]. This increase in Na/K^+-ATPase activity also supports the insulin-mediated, sodium-dependent transport of other compounds critical for skeletal muscle function, such as creatine [72] and carnitine [73].

It should be noted that the regulation of muscle nutrient uptake by insulin may be subject to sex-dependent differences [74]. Insulin-stimulated whole-body [75] and leg [76] glucose disposal has been shown to be higher in women than men, despite a similar activation of muscle insulin signaling [76]. One factor that could explain the sexual dimorphism in muscle insulin action is the relative muscle fiber composition between males and females. For example, the relative area occupied by type I fibers, which have a higher capacity for insulin-stimulated glucose metabolism [77], has been reported to be greater in female versus male skeletal muscle [78]. The advent of single cell and single nuclei sequencing technologies promises to reveal further complexities in the heterogeneity of insulin action within the various cell types of skeletal muscle.

3. Physiology of insulin action in adipocytes

The main action of insulin in the adipocytes is the stimulation of adipogenesis, by promoting TG synthesis and suppression of lipolysis [41,79,80]. Adipose tissue (AT) is the main storage site of fatty acids (FA) that are released in the circulation during lipolysis (i.e., hydrolysis of triglycerides, TG) as non-esterified fatty acids (NEFA) to be used as an energy source during fasting conditions. Insulin plays a key role in adipose tissue metabolism both by inhibiting lipolysis and stimulating glucose uptake and TG synthesis [41,79,80]. NEFA are transported into adipocytes by CD36 while glucose is transported into the adipocytes by GLUT1 (insulin-independent) and GLUT4 (insulin-dependent), the latter being responsible for the majority of glucose transport under insulin-stimulated conditions [81]. This glucose is used for the synthesis glycerol-3-phosphate, since adipocytes do not contain the enzyme glycerol kinase, which is necessary for TG synthesis (Fig. 3). The expression and number of GLUT4 transporters is reduced in obese insulin resistant subjects and even more so in patients with diabetes [82]. Glucose uptake in visceral and subcutaneous fat, measured by positron emission tomography (PET) with ^{18}F-deoxyglucose, is decreased in insulin resistant subjects similarly to muscle glucose uptake [83]. However, given that obese subjects have an increased fat mass, fractional glucose uptake is similar in different fat depots (i.e. subcutaneous, femoral, and visceral fat) in insulin-resistant and insulin-sensitive subjects. In contrast, fractional muscle glucose uptake is significantly decreased in insulin resistant subjects with or without diabetes [83].

Insulin acts through its receptor by activating phosphoinositide 3-kinase (PI3K) for which insulin receptor substrates (IRS-1 and IRS-2) are the main docking proteins [15–17,42,46]. PI3K plays a major role

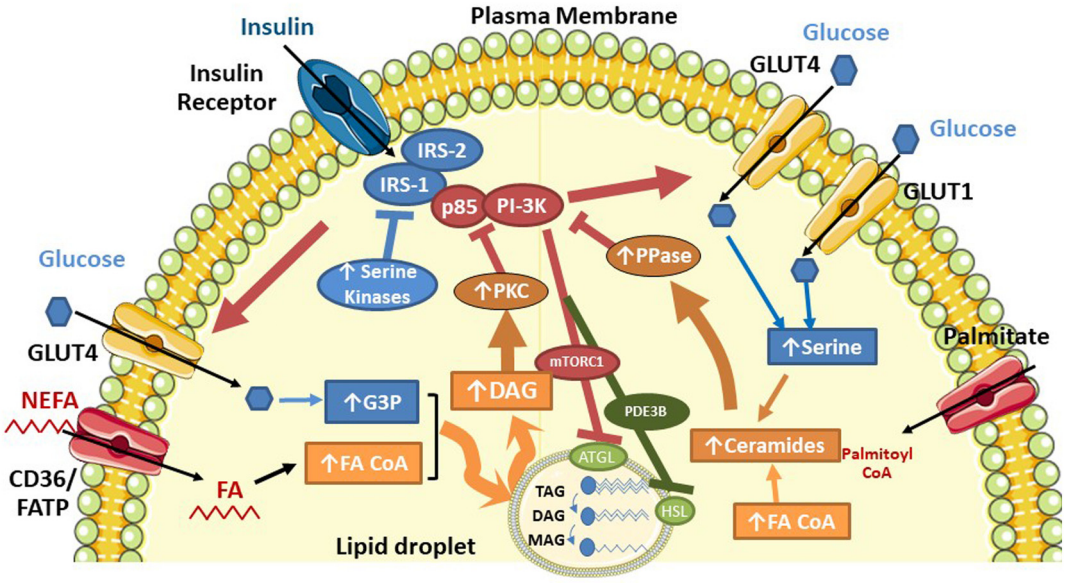

Fig. 3. Effect of insulin on adipocyte metabolism. Adipogenesis starts with the synthesis of diacylglycerols (DAG) from the conversion of glucose to glycerol-3-phosphate (G3P) and the binding of 2 fatty acid (FA) acyl coA (CoA). Adipose tissue triglyceride lipase (ATGL) promotes the hydrolysis of TG to DAG with release of 1 FA; then hormone sensitive lipase (HSL) hydrolyzes DAG to monoacylglycerol (MAG) with the release of 1 FA and then MAG are hydrolyzed by monoglyceride lipase to 1 glycerol and 1 FA. During de-novo synthesis of ceramides a FA-CoA is added to a backbone formed by palmitoyl-CoA and serine. Ceramides impair insulin signal by inhibiting PI-3K. Insulin binds to its specific cell-surface receptor and produces tyrosine phosphorylation and activation of the insulin receptor, which leads to the interaction with the insulin receptor substrates (IRS-1 and IRS-2), in turn activating the phosphatidyl inositol 3-kinase (PI3K) complex. Insulin powerfully inhibits lipolysis mainly through inhibition of ATGL (by activating the mammalian target of rapamycin, mTORC1) and by reducing the activity of HSL (via a PKB/Akt-dependent action) and activation of phosphodiesterase-3B (PDE-3B). The phosphodiesterase catalyzes the breakdown of cAMP to its inactive form, thereby decreasing cAMP levels, which in turn reduces PKA activation and, therefore, also translates into preventing HSL stimulation. The anti-lipolytic effect of insulin is observed already minutes upon binding of the hormone to its receptors.

in adipogenesis and its pharmacological inhibition (with CNIO-PI3Ki) decreases adiposity in obese rhesus monkeys after 3 months of treatment [84].

Resistance in the adipose tissue to the antilipolytic effect of insulin results in increased plasma NEFA concentrations both during fasting and during hyperinsulinemic states (e.g. after a meal, oral glucose tolerance test or during a hyperinsulinemic clamp) [24,80,91]. Insulin decreases lipolysis in a dose response manner [24,80] and is effective at low doses (i.e. EC50 of 68 pmol/l) in subcutaneous adipose tissue of healthy individuals [24,86]. Insulin has an action on the lipases which are responsible of TG hydrolysis. Activation of protein kinase B (PKB) by insulin stimulates the mammalian target of rapamycin (mTORC1) leading to inhibition of adipose tissue triglyceride lipase (ATGL) [87], the first step of TG hydrolysis that results in the formation of diacylglycerol (DAG) and the release of one FA (Fig. 3). Insulin also inhibits hormone-sensitive lipase (HSL) that converts DAG into glycerol and FA and hydrolyzes TAG and MAG [88]; monoacylglycerol (MAG) also can be hydrolyzed by monoglyceride lipase (Fig. 3).

Studies in Pnpla2−/− mice, using both the global and adipose tissue knock out of ATGL, have shown impaired adipose tissue lipolysis, increased TG accumulation and improved systemic insulin sensitivity, with a reduction in circulating plasma NEFA and TG [89–91]. In insulin resistant subjects, expression of ATGL and HSL protein in adipose tissue is decreased and correlates negatively with the fasting plasma insulin concentration and degree of insulin resistance, independent of age, gender, fat cell size, and body composition [92]. This might be a mechanism to counteract excess lipolysis and increased NEFA release in the presence of resistance to the antilipolytic effect of insulin. Surprisingly, in a recent study insulin infusion [93] was shown to activate adipose tissue genes involved in de novo fatty acid (ACC, ACLY, FASN), glycerolipid (GPAM, LPIN1) and cholesterol (HMGCS, HMGCR, MVD) synthesis similarly in obese and lean subjects and in obese subjects who lost weight after bariatric surgery.

Adipocyte dysfunction and inflammation can result from the accumulation of lipotoxic compounds, including diacylglycerols and ceramides [94,95], that are associated with insulin resistance although via different mechanisms (Fig. 3). Ceramides are synthesized either from sphingolipids or de novo when a fatty acid acylCoA (FA-CoA) is added to a backbone formed by palmitoyl-CoA and serine, a reaction catalyzed by ceramide synthase (CERS). White AT ceramide synthase 6 (CERS6) mRNA expression and concentration of CER(C16:0) are increased in obese subjects and CERS6 expression correlates with adiposity and insulin resistance [96]. Thus, excess palmitic acid and serine (that is synthesized from glucose) drive ceramide synthesis. It is well established that saturated fatty acids, in particular palmitic acid, promote lipotoxicity [97]. The composition of the released NEFAs strongly depends on the composition of the TG in the adipose tissue, which in turn depends on the average intake of fatty acids and from de novo lipogenesis [98]. Overfeeding with saturated fatty acids stimulates adipose tissue lipolysis and NEFA uptake in the liver and increases visceral fat accumulation [99,100], while a diet rich in PUFA suppresses peripheral lipolysis [101]. However, hepatic de novo lipogenesis also contributes to FA composition of adipose tissue TG, since newly synthesized FA (mainly palmitate) contribute up to 20% of VLDL-TG in insulin resistant subjects [102].

Different fat depots, i.e., intra-abdominal (perivisceral, omental, cardiac) versus subcutaneous (abdominal or buttock), have different lipid composition with more saturated and less monounsaturated (MUFA) fat in perivisceral adipose tissue compared to the other fat depots [98,103] and this can influence the action of insulin on adipocytes. Further, different fat depots (e.g., cardiac, visceral vs subcutaneous abdominal AT) produce and secrete distinct lipotoxic compounds [104,105]. Since NEFA released into the circulation are taken up by peripheral tissues (mainly liver), the composition of different fat depots can have an important impact on peripheral FA metabolism. Moreover, the impact of visceral fat is often underestimated, since it is highly lipolytic and releases NEFA directly into the portal vein and the great majority of FA are taken up by the liver without appearing in the peripheral circulation. In obese versus lean subjects FA uptake in visceral and subcutaneous AT is increased [106] and displays resistance to the antilipolytic

effect of insulin, leading to a chronic increase in circulating levels of NEFA despite high circulating insulin levels [85,107–109].

4. Insulin action in the liver

4.1. Overview

The liver sits at the end of the portal venous circulation and, therefore, is profoundly influenced by insulin. Because of its strategic location, the liver serves as a buffer to the peripheral circulation by removing 50% of secreted insulin [110], thereby preventing peripheral hyperinsulinemia which can cause insulin resistance in muscle and adipose tissue [111]. During the postabsorptive state, the liver produces glucose at the rate of ~2 mg/kg·min, approximately half via glycogenolysis and half via gluconeogenesis [1–4,112–114]. This supply of glucose by the liver matches the supply of whole body glucose utilization [1–4]. Following glucose ingestion, the maintenance of normal glucose homeostasis requires four closely coordinated effects of insulin and hyperglycemia [1,2,41]: (i) suppression of endogenous (primarily reflects hepatic) glucose production; (ii) stimulation of glucose uptake by the liver; (iii) stimulation of glucose uptake by peripheral tissues, primarily muscle; (iv) inhibition of lipolysis, with resultant decline in plasma FFA and glycerol concentrations. The decrease in plasma FFA concentration plays a key role in augmenting the suppression of hepatic glucose production (HGP) and stimulating muscle glucose uptake [28,29].

4.2. Regulation of hepatic glucose metabolism by insulin

Glucose production by the liver is exquisitely regulated by insulin in coordination with counterregulatory hormones, such as glucagon, neural mechanisms, and substrate supply. Following glucose ingestion, insulin is secreted from the pancreatic β-cells into the portal circulation, where it rapidly and potently inhibits hepatic glucose production (HGP) [1–3,5–8,115] with a half maximal effect of ~30–40 μU/ml, which corresponds to an increment in portal insulin concentration of 10–15 μU/ml [1,80] (Fig. 4). This suppressive effect of insulin on HGP is a primary event [3,115] mediated through the direct suppression of glycogenolysis via the phosphoinositol-3-kinase/Akt signaling cascade, leading to the inhibition of glycogen phosphorylase. Insulin also both directly and indirectly inhibits hepatic gluconeogenesis [1,3,28,29] [see subsequent discussion]. Suppression of glycogenolysis is more sensitive to insulin than is inhibition of gluconeogenesis [1,3].

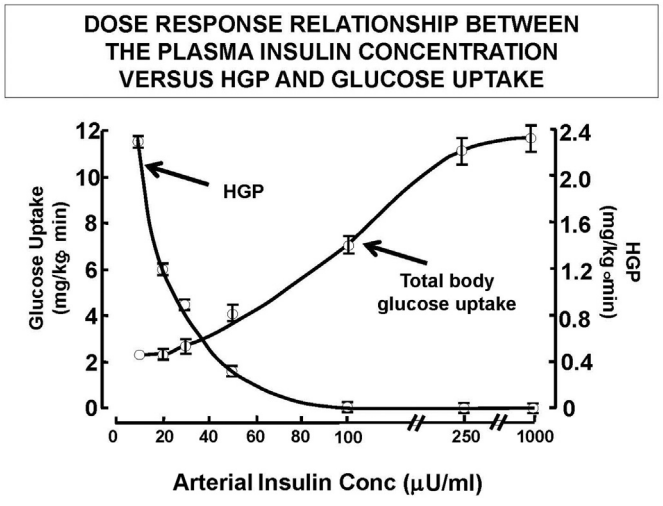

Fig. 4. Dose response relationship between plasma insulin concentration and (i) suppression of hepatic glucose production and (ii) total body (primarily reflects muscle) glucose disposal.

During the fed state, insulin promotes hepatic glucose uptake and storage as glycogen [3,116] via activation of glycogen synthase phosphatase (protein phosphatase 1 or PP1), which dephosphorylates and activates glycogen synthase. Glycogen synthesis and glycogenolysis are reciprocal pathways regulated at the molecular level by the balance between circulating insulin and glucagon concentrations under fed and fasted conditions, respectively. However, glycogenolysis and glycogen synthesis can occur simultaneously, and a significant proportion of G6P derived from glycogenolysis can be recycled back into glycogen [117].

Insulin directly and indirectly inhibits hepatic gluconeogenesis, which contributes ~50% of all HGP following an overnight fast [113]. This pathway relies on the synthesis of glucose from three carbon precursors, including pyruvate, lactate, alanine and glycerol. Gluconeogenesis begins with the conversion of pyruvate to oxaloacetate and then to phosphoenolpyruvate by pyruvate carboxylase (PC) and phosphoenolpyruvate by phosphoenolpyruvate carboxykinase (PEPCK), respectively. The cytosolic variant, PEPCK-C, is thought to be the most important for gluconeogenesis [118]. Several enzymatic steps of glycolysis are reversed to generate fructose-1,6-bisphosphate, which requires a unique enzyme fructose-1,6-bisphosphatase (FBPase). The final step is common to both glycogenolysis and gluconeogenesis and involves the hydrolysis of glucose-6-phosphate (G6P) by G6Pase and the release of free glucose into the circulation via GLUT2.

Insulin is a powerful negative regulator of several gluconeogenic enzymes in hepatocytes. By inhibiting the nuclear localization of the transcription factor forkhead box protein O1 (FOXO1), insulin suppresses the mRNA expression of the genes encoding for G6Pase and PEPCK [119–121]. Additional transcriptional factors and co-activators – including CCAAT enhancer-binding proteins (C/EBPα and C/EBPβ) and PPARG coactivator 1 alpha – also contribute to the regulation of gluconeogenic enzymes by insulin following the activation of the insulin signaling cascade through PI3K and Akt [122–123]. However, multiple studies have demonstrated in humans that the expression of gluconeogenic enzymes is not correlated with fasting glycemia [124], suggesting that alternative mechanisms also mediate the acute effect of insulin on gluconeogenesis. Consistent with this, the allosteric regulation of enzymes involved in gluconeogenesis have been shown to play an important role. The fate of pyruvate is controlled by flux through PC and pyruvate dehydrogenase (PDH), which direct pyruvate to oxaloacetate for gluconeogenesis or acetyl-CoA for oxidation, respectively. The relative activity of these two pathways is influenced by nutritional status and allosteric/covalent modification of PC and PDH. The activity of PDH is inhibited by pyruvate dehydrogenase 4 (PDK4), the expression of which is suppressed by insulin in the fed state; this favors the oxidation of glucose in the liver [125,126]. While there is little evidence that insulin directly regulates the expression or activity of PC, the activity of PC is allosterically activated by acetyl-CoA [127] and, thus, may be indirectly regulated by the effect of insulin on lipid oxidation and PDH activity. The conversion of phosphoenolpyruvate to pyruvate is another important step in glycolysis that is controlled by the pyruvate kinase enzyme (PK). Glucagon and insulin exert reciprocal control over the activity of PK in the liver [128]. Under fasting conditions glucagon inhibits PK activity, favoring the utilization of phosphoenolpyruvate for gluconeogenesis. Insulin opposes this process in the fed state, promoting the uptake and oxidation of glucose in liver.

Insulin indirectly controls gluconeogenesis through primary effects on adipose tissue lipolysis and free fatty acid (FFA) availability. Selectively increasing arterial, but not portal, insulin suppresses lipolysis and inhibits gluconeogenesis in dogs [129]. This indirect effect of insulin on gluconeogenesis is linked to reduced hepatic acetyl-CoA levels [130]. This indirect pathway is relevant when insulin is administered peripherally, disrupting the normal 3:1 ratio of hepatic to peripheral insulin levels. Under normal physiological conditions endogenous secretion of insulin into the portal circulation and direct inhibition of HGP is the most prominent [131].

In contrast to HGP, under euglycemic conditions the stimulatory effect of insulin on hepatic glucose uptake is limited [6–8,10,13,14,132–134], while in the presence of hyperglycemia insulin has a small effect to augment hepatic glucose uptake [75]. Hyperglycemia per se (in the presence of basal insulin levels) enhances hepatic glucose uptake by a mass action effect such that the hepatic clearance of glucose does not change [6–9]. In contrast, oral administration of glucose increases hepatic glucose uptake 4–5 fold despite plasma insulin and glucose concentrations that are much lower than produced by intravenous insulin and glucose infusion [5,6,11]. This "gut factor" effect is reduced by >50% in type 2 diabetic subjects [6,9]. In dogs with chronically implanted catheters in the portal and hepatic veins and artery Cherrington and colleagues [132] demonstrated that creation of combined portal hyperglycemia plus hyperinsulinemia by portal glucose/insulin infusion mimicked the effected of glucose ingestion to augment hepatic glucose uptake, while hepatic denervation obliterated the effect of portal hyperglycemia to augment hepatic glucose uptake in the dog. When acetylcholine was administered and glucose was infused peripherally, hepatic glucose uptake increased markedly, implicating the parasympathetic nervous system in generation of the portal signal [132]. Withdrawal of adrenergic input [135] and increased nitric oxide [136] also have been shown to augment of hepatic glucose uptake.

4.3. The role of insulin in hepatic lipid metabolism

The liver is of central importance in the regulation of hepatic and systemic lipid metabolism. The liver synthesizes large quantities of phospholipids and cholesterol, which are packaged into lipoproteins and made available for the rest of the body. The liver also oxidizes triglycerides and FFA and is the primary site of de novo lipogenesis (DNL). Therefore, alterations in hepatic lipid metabolism have a significant impact on the development of T2D and NAFLD. The main function of insulin in hepatic lipid metabolism is to regulate lipid storage by exerting control over DNL, FFA oxidation and very low-density lipoprotein (VLDL) export. Following meal ingestion the increase in portal insulin augments FFA synthesis from excess acetyl-CoA. The TCA cycle intermediate citrate is exported to the cytosol, where is converted back to acetyl-CoA by the enzyme ATP-citrate lyase (ACLY). Cytosolic acetyl-CoA is then converted to malonyl-CoA by acetyl-CoA carboxylase (ACC), which is followed by the synthesis of fatty acids (primarily palmitate) by fatty acid synthetase (FAS). This process is partly controlled transcriptionally by sterol regulatory element binding protein 1c (SREBP1c) and carbohydrate response element binding protein (ChREBP), which are activated by insulin signaling though the PI3K-Akt pathway and high glucose concentrations during feeding, respectively [137–139]. However, like the regulation of hepatic glucose metabolism, many DNL enzymes are regulated post-transcriptionally via mechanisms that are modified by insulin. For example, ACC undergoes dimerization to enhance its activity, a post-translational modification that is augmented by insulin [140]. Further, ACC is allosterically activated by citrate and glutamate, and negatively regulated by its product malonyl-CoA [140]. The importance of insulin signaling to hepatic lipid storage is highlighted by studies carried out in LIRKO mice which lack the insulin receptor specifically in liver. These mice are protected from dietary induced hepatic lipid accumulation and do not develop hepatic steatosis characteristic of NAFLD [141]. Experiments conducted in these mice also highlight the importance of insulin signaling to cholesterol and lipoprotein homeostasis. Baseline hepatic cholesterol levels are increased two-fold in LIRKO mice and, when challenged with an atherogenic diet, LIRKO mice develop severe hypercholesterolemia and atherosclerosis [141].

5. Insulin and the vasculature

Insulin receptors are present on vascular smooth muscle cells and pharmacologic, as well as physiologic, increases in the plasma insulin concentration augment leg and forearm blood flow [30,33,142–144]. However, not all studies have demonstrated that insulin augments blood flow in peripheral tissues (muscle) in vivo [145,146]. Several factors can explain these varying results [146], of which the level of hyperinsulinemia and duration of insulin infusion are of paramount importance. With plasma insulin concentrations in excess of 100 μU/ml, a dose response effect of hyperinsulinemia is clearly demonstrable [147,148], while at lower, more physiologic plasma insulin concentrations, insulin's vasodilatory effect is more variable [149]. Within the physiologic range of hyperinsulinemia prolongation of the insulin infusion to >2 h produces a more consistent vasodilatory response [142,147]. In insulin resistant individuals with type 2 diabetes [150] and obesity [151], insulin's vasodilatory action is impaired. In both type 2 diabetic and obese individuals the blunted vasodilatory response to insulin has been linked to reduced insulin-stimulated muscle glucose uptake [150,151], but this has not been a consistent observation [142,147].

Insulin initiates its vasodilatory action by binding to the insulin receptor on endothelial cells, leading to activation of the PI-3 kinase/Akt pathway and generation of nitric oxide [151–154]. Consistent with this, *N*-monomethyl-L-arginine (L-NMMA), a specific inhibitor of endothelium-derived NO, inhibits the insulin-mediated increase in forearm blood flow and muscle glucose uptake [155].

Insulin also affects the muscle microvasculature [33,156]. Muscle has a highly organized vasculature in which arterioles branch off from primary arteries and give rise to terminal arterioles that are perpendicular to the muscle fibers. Each terminal arteriole feeds 15–20 capillaries which deliver nutrients to the myocytes [33,156]. Under resting conditions only 30–50% of muscle capillaries are perfused and the proportion increases 2- to 3-fold in response to exercise and insulin [33,147,148]. Using contrast enhanced ultrasound, insulin at physiologic concentrations has been shown to augment microvascular perfusion volume without any change in total limb blood flow and increase muscle glucose uptake in animals and humans [157–160]. The increase in microvascular blood flow precedes and correlates with the increase in forearm blood flow [157] and is blunted in obese subjects [161]. Elevated plasma FFA levels inhibit the insulin-stimulated microvascular response to insulin in healthy subjects [162,163].

Activation of the insulin signal transduction system (PI3K/Akt) plays a critical role in the production of nitric oxide [164,165]. Nitric oxide is a potent vasodilatory and antiatherogenic agent [154,166] and nitric oxide deficiency promotes hypertension and atherosclerosis [165,167]. This explains why, in T2DM and obese individuals in whom insulin signaling is impaired [36,168–170], insulin resistance is closely linked with accelerated atherogenesis [36,171–173]. Insulin also is a potent growth factor [174,175] and its growth promoting effects are mediated via the mitogen-activated protein (MAP) kinase pathway [176]. MAPK can be activated either by IRS-1 or SHC. In insulin resistant states, IRS-1 tyrosine phosphorylation is impaired but activation of SHC is intact [177]. The hyperinsulinemia that occurs secondary to impaired PI3K activation and resultant insulin resistance leads to enhanced MAPK pathway activation [36,177]. Following its activation [18,178], extracellular regulated kinase (ERK) translocates into the nucleus where it catalyzes phosphorylation of transcription factors that promote vascular smooth muscle growth and proliferation, inflammation, and atherogenesis [36,178]. Inhibition of the MAPK pathway blocks the growth promoting effects of insulin without affecting its metabolic effects [179]. Insulin resistance in the PI3K pathway with intact MAPK signaling activates multiple inflammatory pathways, including inhibitor κβ/nuclear factor κβ [180] and c-Jun N-terminal kinase [181]. The preceding scenario explains why in NGT insulin sensitive individuals insulin promotes a healthy vascular milieu, whereas in insulin resistant hyperinsulinemic individuals insulin promotes atherogenesis.

6. Summary

Insulin plays a pivotal role during both fasting and postprandial states in regulating the flux of nutrients – glucose, free fatty acids,

amino acids – between muscle, liver, and adipose tissue to ensure an adequate substrate supply to meet the energy needs of the cell. Less well appreciated is the potent effect of insulin on vascular smooth muscle cells to generate nitric oxide and inhibit atherogenesis. Although beyond the scope of this review, insulin resistance is associated with a constellation of cardiometabolic disturbances (diabetes, obesity, atherosclerotic cardiovascular disease) collectively known as the metabolic (insulin resistance) syndrome which takes its toll in terms of morbidity and mortality.

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Credit authorship contribution statement

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Declaration of competing interest

Ralph A. DeFronzo is a member of the advisory boards of AstraZeneca, Janssen, Lexicon, Boehringer-Ingelheim-Lilly Alliance, Novo Nordisk. Ralph A. DeFronzo is a member of the speakers' bureau of Novo Nordisk and Astra-Zeneca. Ralph DeFronzo has grant support from Astra-Zeneca and Janssen. Amalia Gastaldelli has received honorarium from Novo Nordisk and is consultant for Boehringer Ingelheim, Eli Lilly, Gilead, Inventiva, and Pfizer. Luke Norton and Chris Shannon have no declarations of interests.

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