REVIEW

Hyperinsulinaemia: does it tip the balance toward intrahepatic fat accumulation?

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Abstract

In health, the liver is metabolically flexible over the course of the day, as it undertakes a multitude of physiological processes including the regulation of intrahepatic and systemic glucose and lipid levels. The liver is the first organ to receive insulin and through a cascade of complex metabolic processes, insulin not only plays a key role in the intrahepatic regulation of glucose and lipid metabolism, but also in the regulation of systemic glucose and lipid concentrations. Thus, when intrahepatic insulin signalling becomes aberrant then this may lead to perturbations in intrahepatic metabolic processes that have the potential to impact on metabolic health. For example, obesity is associated with intrahepatic fat accumulation (known as nonalcoholic liver disease (NAFLD)) and hyperinsulinaemia, the latter as a result of insulin hypersecretion or impaired hepatic insulin extraction. Although insulin signalling directly alters intra- and extrahepatic metabolism, the regulation of hepatic glucose and fatty acid metabolism is also indirectly driven by substrate availability. Here we discuss the direct and indirect effects of insulin on intrahepatic processes such as the synthesis of fatty acids and peripherally regulating the flux of fatty acids to the liver; processes that may play a role in the development of insulin resistance and/or intrahepatocellular triacylglycerol (IHTAG) accumulation in humans.

Key Words

- insulin
- liver
- *de novo* lipogenesis
- adipose tissue

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Introduction

The liver is a key metabolic organ that performs a multitude of physiological processes, including the regulation of systemic lipid and glucose metabolism. It provides metabolic flexibility where, over the course of a day, hepatocytes rapidly transition back and forth between the metabolic tasks of energy storage and supply (1). The liver serves as an intermediary organ between exogenous (dietary) and endogenous energy supply to extrahepatic organs; thus, perturbations in hepatic metabolism can impact widely on metabolic disease risk. An example of this is the accumulation of intra-hepatocellular fat, which is likely to be due to an imbalance between fatty acid delivery to the liver, fatty acid synthesis within the liver and fatty acid removal (via oxidation or export as triacylglycerol (TAG)) from the liver. Pathological accumulation of intra-hepatocellular TAG (IHTAG) is denoted as nonalcoholic liver disease (NAFLD), which encompasses a spectrum of conditions from hepatic steatosis to cirrhosis (2); adiposity is almost invariably an underpinning factor. NAFLD is associated with other metabolic diseases including type 2 diabetes and cardiovascular disease, through multiple pathophysiological mechanisms including impaired insulin-dependent regulation of glucose and hepatic lipid metabolism (3). Hyperinsulinaemia is a common finding in obesity, and may be the result of insulin hypersecretion and/or impaired hepatic insulin extraction; individuals with hyperinsulinaemia are often said to have 'insulin resistance' (4, 5). Here we discuss the direct and indirect effects of insulin on intrahepatic processes (e.g. fatty acid synthesis) and systemic processes (e.g. the regulation of fatty acid flux from adipose tissue) and how dysregulation of these processes may play a

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role in the development of insulin resistance and/or IHTAG accumulation in humans. Although there are new therapies in the pipeline for the treatment of NAFLD/insulin resistance, there are many challenges associated with development (as reviewed in 6) and although of potential interest, they are outside the scope of our review.

Regulation of hepatic insulin extraction

The liver plays a key role in the regulation of systemic insulin concentrations. Insulin is secreted directly into the portal vein and a decline in hepatic insulin extraction may lead to profound changes in peripheral insulin concentrations. Data from *in vivo* canine and human studies have estimated that hepatic insulin extraction in the fasting state is up to 80% (7, 8, 9); after consumption of a mixed meal, portal vein insulin concentrations are approx. three times that in the systemic circulation (1). Peiris *et al.* (9) demonstrated that obese compared to non-obese women had significantly higher insulin production and calculated portal vein insulin concentrations in the fasted state and after intravenous and oral glucose administration. They determined that the mean estimated hepatic insulin extraction fraction was significantly lower in women with upper-body obesity compared to non-obese subjects (9). On the basis of this observation, the authors suggested that the pronounced hyperinsulinaemia observed with upper-body obesity was, in part, due to a defect in hepatic insulin extraction (9). Recently, Utzschneider *et al.* (10) investigated the effect of IHTAG accumulation on hepatic insulin extraction by undertaking two-step hyperinsulinaemic-euglycaemic clamps and oral glucose tolerance tests in non-diabetic individuals with and without NAFLD. After modelling the data they found hepatic insulin extraction was not directly associated with IHTAG accumulation rather, it was associated with skeletal muscle and adipose tissue insulin resistance (10). These findings suggest that factors from other organs, such as adipose tissue, may play a role in modulating hepatic insulin extraction.

Hepatic insulin clearance: the influence of fatty acids

The concept that adipose tissue-derived factors may influence hepatic insulin extraction has been around for about 30 years (11). It has been suggested that non-esterified fatty acids (NEFAs) (also call free fatty acids) derived from adipose tissue TAG hydrolysis may be important metabolic regulators of hepatic insulin extraction (5, 12). *In vitro* work using primary rat hepatocytes demonstrated that specific fatty acids (oleic, palmitic, stearic, palmitoleic, and eicosapentaenoic acids) were equally effective, in a concentration-dependent manner, at reducing the number of binding sites for insulin and its uptake and degradation by the hepatocyte (11). Svedberg *et al.* (13) then utilised an *in situ* perfused rat liver model to demonstrate that the addition of a fatty acids (usually oleic acid complexed to albumin) in concentrations between 240 and 350 µmol/L, decreased hepatic insulin clearance in older, heavier rats compared to younger, lean rats. Moreover, perfusing livers with very high concentrations (1000 and 2250 µmol/L) of NEFA inhibited hepatic insulin clearance compared to perfusing with a NEFA concentration of 350 µmol/L (13). By altering the perfusate glucose concentrations from 5 mmol/L to 20 mmol/L, when NEFA concentrations were between 240 and 350 µmol/L, no effect of the glucose on hepatic insulin clearance was observed (13), demonstrating glucose concentrations do not have an effect when NEFA concentrations are in a physiological range. Using a canine model, Wiesenthal *et al.* (5) investigated the effect of fatty acids on hepatic insulin extraction by controlling portal insulin levels (via direct intra-portal infusion of insulin) and experimentally elevated NEFA concentrations (to supraphysiological levels) through infusion of intralipid and heparin. They found fractional hepatic insulin extraction and peripheral insulin clearance to be significantly lower with the raised NEFA concentrations compared to a saline infusion (5). In line with the findings from the rodent perfusion models, the authors concluded that fatty acids impaired hepatic insulin extraction and suggested this could be a contributing factor to obesity-related peripheral hyperinsulinaemia (5).

Evidence from a limited number of *in vivo* human studies are compelling. By using an intralipid and heparin infusion model to experimentally elevate NEFA concentrations to ~1000 µmol/L, the effect of two levels of glycaemia (7 and 11 mmol/L) were compared in eight lean, pre-menopausal women (14). It was found that high concentrations of NEFA had insulinotropic effects when glucose infusion concentrations were at 7 mmol/L but not at 11 mmol/L and that the raised NEFA had a suppressive effect on endogenous insulin clearance independent of the glucose concentration, although the two substrates appear to be additive (14). Taking the findings from experimental studies together, they suggest that the concentration of NEFA, rather than glucose, to which the liver is exposed is a regulator of hepatic

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insulin clearance. However, although it appears elevated levels of NEFA may decrease hepatic insulin extraction, and this is more evident with ageing and obesity, it is challenging to determine how much of a role they may play as these studies have experimentally elevated levels of systemic NEFA to unphysiological concentrations (15), which would not be seen in a postprandial state, when insulin excursions were maximal. Therefore, it would be of interest to determine the effect that physiological concentrations of NEFA, under different nutritional states, have on hepatic insulin clearance *in vivo* in humans across of spectrum of phenotypes (e.g. lean, obese, younger, older adults, males and females etc).

Hepatic insulin clearance: the influence of phenotype

The liver is the major organ responsible for insulin turnover and there are a cascade of steps involved, including binding to a specific membrane receptor, internalisation and intracellular compartmentalisation of the receptor complex, and proteolytic degradation by specific enzymes (14). For the initial step of hepatic insulin extraction to occur, insulin receptors must be present and it is plausible, the total number of receptors available may influence extraction. By measuring insulin receptors on hepatic membranes Arner *et al.* (16) observed that obese individuals had a lower number of receptors compared with lean individuals. In further work, they reported that specific insulin binding was two-fold greater in individuals with type 2 diabetes mellitus (at that time called NIDDM) due to a large increase in the number of insulin receptors compared to control, non–type 2 diabetes subjects (17). Overall, the findings suggest that hepatic insulin receptors are upregulated in type 2 diabetes and downregulated in obesity, despite the observation that both type 2 diabetes and obese subjects have hepatic insulin resistance (18, 19). It has been suggested that the prevailing insulin concentration may modulate insulin binding, with an overall inverse relationship between insulin receptor number and plasma insulin level with a high number of insulin receptors being associated with a lower plasma insulin level (18, 19). However, this is a simplistic view given the complex nature of hepatic insulin turnover. Thus, it is likely that the underlying cause for hepatic insulin resistance in different phenotypes exists at a site beyond the initial interaction between the hormone and liver cell receptor (17).

Studying the regulation and metabolism of insulin at the post-receptor level under physiological conditions, in humans is challenging. Findings from *in vitro* cellular work using rodent primary hepatocytes noted that culturing cells in 500 µmol/L of palmitate or 400 µmol/L oleate had profound effects on all steps in the intra-hepatocellular cascade of insulin metabolism (11, 20). However, hepatocytes *in vivo* are exposed to a flux of different macronutrients (fats, sugars and proteins), and never just a single fatty acid. Thus, it remains unclear what effects fatty acids, in physiological ratios and concentrations, alone and in combination with other macronutrients, have on intra-hepatocellular insulin metabolism in humans.

Hepatocyte insulin signalling

Insulin action has been suggested to regulate hepatic glucose and lipid metabolism by direct and indirect mechanisms (1). For example portal insulin acts on the liver to directly activate glycogen synthesis and *de novo* lipogenesis (DNL) and inhibit gluconeogenesis, whilst peripherally insulin may indirectly regulate hepatic glucose and lipid metabolism by inhibiting adipose tissue lipolysis and promote glucose uptake into skeletal muscle (1). The hepatic insulin signalling cascade is complex and involves multiple steps. Briefly, the after consumption of a mixed meal, insulin binds to, and activates the insulin receptor which then recruits and activates insulin receptor substrate (IRS) which activates phosphoinositide-3-phosphate kinase (PI3K). From the activation of PI3K, phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) is generated and activates 3-phosphoinositide-dependent protein kinase 1 (PDK1), leading to partial phosphorylation of Akt, with mTORC2 fully phosphorylating Akt. Once Akt is fully phosphorylated then there is a divergence in the pathways regulating glucose and lipid metabolism (21). At this point, there are two key actions at the gene transcription level: (i) insulin stimulates the phosphorylation of Forkhead box protein O1 (FoxO1), a transcription factor regulating gluconeogenesis, which results in a downregulation of genes required for gluconeogenesis and results in a decrease in hepatic glucose output; and (ii) insulin activates the transcription factor sterol regulatory element-binding protein 1c (SREBP1c) which enhances the transcription of genes required for fatty acid and TAG synthesis (22). It has been postulated that a mismatch in these signals gives rise to selective hepatic insulin resistance (22), which is characterised by excess glucose production in the face of accelerated hepatic lipogenesis (21). In this scenario,

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insulin signalling fails to appropriately phosphorylate FoxO1 and the mRNAs for genes required for gluconeogenesis (i.e. phosphoenolpyruvate carboxylase (*PEPCK*) and glucose 6-phosphatase (*G6Pase*)) are not downregulated. Thus, hepatic glucose production is not decreased whilst the hepatocyte maintains insulin sensitivity for the SREBP1c pathway, leading to accelerated DNL (22). Overall, the regulation of hepatic glucose and lipid metabolism is the result of multiple inputs to activate Akt; the pattern of insulin delivery may have an impact on this, with pulsatile portal delivery being more physiological than fixed insulin delivery (23).

Regulation of hepatic glucose metabolism

The factors regulating hepatic glucose output have long been debated. The traditional view is after consumption of a meal containing carbohydrate, hepatic insulin reduces the transcription of gluconeogenic enzymes, leading to direct suppression of hepatic glucose output (12). However, Samuel and Shulman (23) commented that an insulin-mediated reduction in the transcription of gluconeogenic enzymes does not explain the rapid suppression of hepatic glucose production after meal consumption in healthy subjects. Since the mid-1950s it has been suggested that the regulation of hepatic glucose output by insulin is mediated indirectly by extrahepatic factors (24). In support of this concept, studies undertaken in canine and rodent models have demonstrated: (i) that the suppression of endogenous glucose production closely matched the time course of peripheral rather than portal insulin concentrations (12); and (ii) the insulin-mediated suppression of adipose TAG hydrolysis resulted in a decreased intrahepatic acetyl-CoA content, reduced pyruvate carboxylase activity and flux, along with a decreased glycerol flux (from adipose tissue TAG) to the liver (23). On the basis of these observations, Samuel and Shulman (23) have suggested that although hepatic insulin signalling may establish the transcriptional tone of gluconeogenic enzymes and determine hepatic gluconeogenic capacity, the ability of insulin to acutely regulate hepatic gluconeogenesis occurs predominantly via the indirect mechanism of inhibition of white adipose tissue lipolysis.

Regulation of hepatic lipid metabolism

Hepatic insulin action also regulates lipid homeostasis by reducing very-low-density lipoprotein (VLDL)-TAG secretion into systemic circulation. Secretion of VLDL-TAG is often enhanced in individuals classified as insulin resistant or who have NAFLD (21); it has been suggested that raised plasma or VLDL-TAG concentrations are proportional to the amount of hepatic DNL occurring (25). However, this could be questioned as only a small fraction of VLDL-TAG originates from DNL-derived fatty acids (26, 27, 28, 29, 30, 31). Moreover, it is often speculated that the induction of intrahepatic DNL may contribute to (hepatic) insulin resistance (32, 33) and (ultimately) the development of NAFLD. However, it remains unclear whether an increase in DNL may precede, and thus contribute to the development of IHTAG accumulation and insulin resistance or vice versa. There a number of in-depth reviews in recent years that extensively cover the transcriptional control of glucose and lipid metabolism within the liver by insulin (1, 21, 34, 35, 36). As the intrahepatic regulation of lipid and glucose metabolism is complex and interrelated, it is plausible that these pathways are regulated through a combination of direct and indirect mechanisms, which are challenging to investigate in humans, *in vivo*. It is likely that the contribution of the direct and indirect mechanisms, within the respective pathways, will vary between individuals and be influenced by the phenotype and genotype of the individual.

Hepatic DNL

In humans the process of synthesising non-lipid precursors (e.g. sugars and protein) in to fatty acids (known as DNL) occurs primarily in the cytoplasm of hepatocytes (37). The synthesis of sugars to fatty acids involves the acetyl-CoA produced during glycolysis being utilised as a substrate for acetyl-CoA carboxylase (ACC1), which is the enzyme that catalyses the irreversible carboxylation of acetyl-CoA to produce malonyl-CoA, an intermediate in the DNL pathway. Malonyl-CoA is further elongated by fatty acid synthase (FAS) (38) until the major quantitative end product of the DNL pathway, palmitoyl-CoA is formed (37, 39). Along with the contribution of newly synthesised fatty acids to the intrahepatic pool there are other indirect effects of increased hepatic DNL which may contribute to IHTAG accumulation, including (i) the suppression of mitochondrial fatty acid oxidation; malonyl-CoA inhibits carnitine palmitoyl transferase-1b (CPT1B) activity (40, 41); (ii) palmitoyl-CoA is a precursor for ceramide synthesis (42, 43); and (iii) the accumulation of DNL-derived fatty acids may lead to endoplasmic reticulum (ER) stress, leading to cellular dysfunction and up-regulation of pro-inflammatory pathways (44, 45, 46).

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Insulin resistance and hepatic DNL

The relative contribution of DNL-derived fatty acids to IHTAG in individuals with NAFLD has been reported to be 26% in the fasted state (29). As measuring the contribution of different fatty acid sources directly in IHTAG is impractical, the valid assumption has been made that VLDL-TAG reflects hepatic TAG composition (29). The relative contribution of DNL-derived fatty acids to VLDL-TAG is reported to be around 10% or less, in individuals without NAFLD (26, 27, 28, 47, 48, 49, 50, 51, 52) and markedly higher, between 14 and 25%, in individuals with NAFLD and/or defined as insulin resistant (28, 29, 47, 48, 49, 51, 52). We have previously reported higher hepatic DNL in individuals defined as insulin-resistant (based on HOMA-IR) compared to insulin-sensitive individuals, despite no difference in BMI or IHTAG content (51). These findings suggest IHTAG accumulation is not always associated with insulin resistance and vice versa; thus it remains unclear whether insulin resistance is a cause or a consequence of IHTAG accumulation. As TAG is not a signalling lipid, it has been proposed that the bioactive lipids, diacylglcyerols (DAGs) and ceramides mediate lipid-induced hepatic insulin resistance (53). Observational data from a limited number of human studies suggest intrahepatic DAG content is associated with hepatic insulin resistance, whilst the intrahepatic ceramide content is not (53). Moreover, findings comparing 'metabolic NAFLD' with 'genetic (*PNPLA3*) NAFLD' demonstrate that individuals defined as having 'metabolic NAFLD' (i.e. individuals with a high HOMA-IR and IHTAG content) have increased intrahepatic DAG and ceramide content compared to those without 'metabolic NAFLD'. For 'genetic NAFLD' (i.e. carriers of the *PNPLA3* I148 gene variant who have higher IHTAG but a similar HOMA-IR to non-variant *PNPLA3* carriers), there is no difference in the intrahepatic content of DAGs and ceramides (54). These data suggest that hepatic insulin resistance, rather than IHTAG content, is an important factor in the intrahepatic DAG and ceramide content, although remains unclear what comes first, an increase in DAG and ceramides or insulin resistance. A possible explanation for the increased intrahepatic ceramide content noted with insulin resistance may be due to increased hepatic DNL, as there would be increased availability of palmitoyl-CoA for ceramide synthesis (42, 43). In support of this concept, evidence from animal work has suggested that a reduction in hepatic DNL leads to a reduced incorporation of DNL-derived fatty acids into hepatic ceramides (55).

As hepatic DNL has been associated with hyperinsulinaemia, even after matching for BMI and IHTAG content (51, 56, 57), this suggests a direct effect of impaired insulin signalling. Higher DNL in individuals with NAFLD has been associated with increased expression of, Liver X Receptor (LXR), SREBP1c and carbohydrate-responsive element-binding protein (ChREBP), which are the master regulators of hepatic DNL (58, 59, 60). Using murine models, Shimomura *et al.* (61) demonstrated that although chronic hyperinsulinaemia downregulated IRS-2, which is an essential component of the insulin signalling pathway in the liver, insulin continued to upregulate SREBP1-c, which activated lipogenic pathways. To examine the direct effect of loss of insulin action in the liver, Michael *et al.* (62) developed liver insulin receptor knockout (LIRKO) mice and found they exhibited: (i) severe glucose intolerance, (ii) failed to suppress hepatic glucose production, and (iii) had impaired hepatic glycogen storage, in conjunction with hyperinsulinaemia due to a combination of increased insulin secretion and decreased insulin clearance. Biddinger *et al.* (63) reported that despite hyperglycaemia and hyperinsulinaemia, the LIRKO mice had low plasma TAG and no elevation in IHTAG, suggesting SREBP1c and lipogenic enzymes were not upregulated; Brown and Goldstein (22) termed this 'pure insulin resistance'.

Taking these observations together it has been proposed that in selective hepatic insulin resistance, insulin fails to suppress gluconeogenesis but continues to stimulate fatty acid and TAG synthesis (22). Semple *et al.* (4) examined the hypothesis of selective post-receptor insulin resistance in humans, by characterising lipid metabolism in patients with primary defects in the insulin receptor or downstream protein kinase AKT2 and comparing to patients with familial partial lipodystrophy, who were insulin signalling competent but hugely insulin resistant. By studying the combination of subjects with severe insulin resistance due to signalling defects at the level of the insulin receptor and those with severe insulin resistance associated with lipodystrophy or severe insulin resistance of undefined molecular aetiology, Semple *et al.* (4) reported that patients with lipodystrophy had exaggerated metabolic dyslipidaemia with high plasma TAG and low HDL cholesterol concentrations whilst patients with either genetic or acquired insulin receptoropathy had strikingly normal plasma lipid

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profiles, despite their extreme hyperinsulinaemia. They then measured fasting hepatic DNL and IHTAG content in healthy controls ($n=6$), patients with loss-of-function insulin receptor ($n=4$), patients with *AKT2* mutations ($n=2$) and patients with familial partial lipodystrophy ($n=3$) and found that compared to controls the loss-of-function insulin receptor patients has similar hepatic DNL and IHTAG content whilst the patients with *AKT2* mutations and partial lipodystrophy had significantly higher hepatic DNL and IHTAG (4). This work provides evidence that in humans, metabolic dyslipidaemia and IHTAG accumulation are the result of selective post-receptor hepatic insulin resistance (4).

Nutritional regulation of hepatic DNL

The amount of dietary carbohydrate and/or fat consumed may influence hepatic DNL. For example, by comparing the effects of a short-term (3 day) higher-fat (~37% total energy (TE), carbohydrate 48% TE) diet to a lower-fat (~23% TE, carbohydrate 59% TE) diet on hepatic DNL in obese individuals with and without type 2 diabetes, Wilke *et al.* (39) found hepatic DNL was notably higher on the low-fat diet with >70% of the palmitate in VLDL-TAG being newly synthesised. In contrast, on the higher-fat diet the *de novo* synthesis of fatty acids was notably reduced (39). Studies on the LIRKO mice demonstrated that feeding a high-carbohydrate diet induced mammalian target of rapamycin complex 1 (CASTOR1), which is an upstream activator of the SREBP1c via insulin-independent signalling pathways (64). Moreover, feeding a high-fructose (60%) diet resulted in the activation of ChREBP and lipogenic gene expression, independent of hepatic insulin signalling (64). Therefore in humans consuming a diet high in free sugars (glucose and fructose), it is plausible hepatic lipogenic gene activity is activated via both insulin signalling-dependent and -independent pathways. In support of this, co-ingestion of glucose and fructose has been found to exacerbate hepatic DNL to a greater extent than consumption of the same amount of either glucose or fructose alone (65, 66, 67, 68). Metabolic studies, utilising stable-isotope labelled fructose, have demonstrated that the contribution of ingested fructose to DNL-derived fatty acids is very low (69), thus supporting an indirect effect of upregulating the DNL pathway as previously discussed (66). A crucial factor regulating DNL is the availability of substrate for the pathway, which typically comes from excess dietary carbohydrate, although there is some evidence from human genetic studies that this can vary between people. Santoro *et al.* (70) demonstrated that obese adolescents with the rs1260326 SNP in the glucokinase regulatory protein (*GCKR*) gene had higher rates of hepatic DNL, which was associated with greater glycolysis, than obese adolescents without the variant. This demonstrates the importance of the glycolytic carbon flux in hepatic DNL, which if continued overtime may play a role in IHTAG accumulation. In support of this, findings from genome-wide association analysis have found that variants in or near *GCKR* are associated with IHTAG accumulation (measured by computer tomography) and histologically defined NAFLD (71).

Although it is often suggested that hepatic DNL is one of the primary mechanisms for the development of NAFLD, this is yet to be clearly demonstrated *in vivo*, in humans. It is clear that hepatic DNL occurs in to a greater extent in the postprandial, compared to the fasting state and this is exacerbated in individuals with NAFLD and/or those defined as being insulin resistant. It is likely that chronically elevated hepatic DNL plays a role, but is not the sole cause, for the development of NAFLD. Increased DNL would impact on a number of pathways, including an increased contribution of fatty acids within the intrahepatic pools, suppression of hepatic fatty acid oxidation, an increased production of bioactive lipids, such as ceramides (Fig. 1A and B) and ER stress, although all of these mechanisms remain to be demonstrated as casual in humans.

Peripheral effects of insulin on adipose tissue derived fatty acids

Insulin is a key regulator of the acute changes in the flux of adipose tissue-derived fatty acids to the liver. Plasma NEFA concentrations (representing predominantly fatty acids derived from the hydrolysis of subcutaneous adipose tissue TAG) are highest in the fasting state and decrease after the consumption of a mixed meal due to insulin-mediated suppression of adipose tissue TAG hydrolysis (72, 73, 74, 75). In the fasting state, the relative contribution of systemic NEFA to VLDL-TAG has been reported to be between 75 and 84% in lean, insulin-sensitive individuals (26, 28, 30, 31, 76, 77) whilst for obese/insulin-resistant individuals and those with NAFLD, the relative contribution is between 42 and 72% (28, 29, 30, 31, 76, 77).

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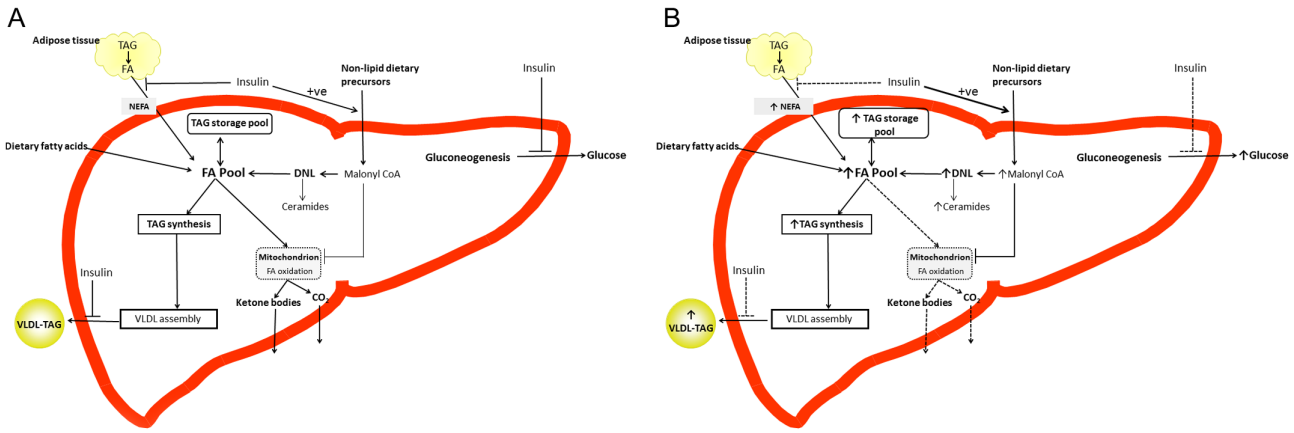

Figure 1

Overview of insulin regulation on intrahepatic pathways in the postprandial state in healthy (A) and individuals with NAFLD or defined as insulin resistant (B). In the fasting state non-esterified fatty acids (NEFA) from the lipolysis of subcutaneous and visceral adipose tissue enter the liver and mix with fatty acids (FAs) from the cytosolic triacylglycerol (TAG) storage pool and those from *de novo* lipogenesis (DNL). FAs are then preferentially partitioned toward the oxidation pathway where the acetyl Co-A produced can enter the tricarboxylic acid cycle to produce CO_2 or ketogenic pathway, where the ketone bodies 3-hydroxybutyrate and acetoacetate are produced. FAs are also esterified to TAG and utilised in the production of very low-density lipoprotein (VLDL) particles. In the transition to the postprandial state, and after consumption of a mixed meal, FAs from the diet also enter the liver and mix with endogenous sources. The postprandial increase in plasma insulin concentrations suppresses adipose tissue lipolysis, decreasing the flux of NEFA to the liver. Within the liver, insulin upregulates the DNL pathway, leading to suppression in FA oxidation, VLDL production and secretion, and gluconeogenesis. Thus, during the postprandial period, cellular metabolism rapidly shifts away from energy supply to energy storage (A) and back again. In individuals with an 'unhealthy' phenotype (e.g. NAFLD, insulin resistance) the postprandial increase in plasma insulin concentrations will not suppress adipose tissue lipolysis to the same extent as in a 'healthy' individual, leading to a higher flux of NEFA to the intrahepatic FA pool. Within the liver, insulin will further upregulate the DNL pathway, leading to a greater contribution of FA to the FA pool, FA oxidation will be suppressed, and ceramide production may be increased, whilst VLDL production and secretion and gluconeogenesis are not attenuated. During the postprandial period in these individuals FAs will partitioned toward esterification pathways and utilised in the production of VLDL particles or stored in the cytosolic TAG storage pool rather than entering oxidation pathways (B).

Plasma NEFA concentrations: the influence of nutritional state

In the postprandial period, chylomicron-derived dietary TAG are hydrolysed by adipose tissue lipoprotein lipase (LPL), which is upregulated by insulin. Although the majority of liberated fatty acids are taken up by adipose tissue, some escape uptake and appear in the systemic plasma NEFA pool. These fatty acids are often referred to as spillover NEFA and may constitute 40–50% of the total plasma NEFA pool, during the nadir in postprandial NEFA concentrations (73, 78, 79). It is often suggested that the contribution of chylomicron-derived spillover NEFA to the systemic NEFA pool is higher in insulin-resistant compared to insulin-sensitive individuals; however, we have previously found an inverse relationship between HOMA-IR and chylomicron-derived spillover NEFA (79). These data suggest a reduction in adipose tissue LPL activity with insulin resistance, which is in line with the observation that adipose tissue LPL expression is significantly reduced in obese compared to lean individuals (73, 80). Although it is likely the hepatic uptake of chylomicron remnant TAG can channel substantial amounts of dietary fat directly to the liver, the NEFA pathway still represents the largest contribution of fatty acids to the liver for VLDL-TAG production and IHTAG (26, 29, 30, 31). The relative contribution of systemic NEFA to VLDL-TAG in the postprandial state has been estimated to be between 28 and 57% for lean/insulin-sensitive individuals (26, 28, 30, 31), and 28 and 30% for obese/insulin-resistant individuals and those with NAFLD (28, 29, 30, 31). In our single meal feeding studies, we utilised stable-isotope tracer methodology to determine the relative contribution of endogenous systemic NEFA to VLDL-TAG in the postprandial state and found the relative contribution to be substantially lower in insulin-resistant compared to insulin-sensitive individuals, however when expressed as an absolute concentration, the contribution was similar between groups (30). In our sequential meal feeding studies, we did not find a difference in either the relative or absolute contribution of endogenous systemic NEFA to VLDL-TAG between lean insulin-sensitive and abdominally obese insulin-resistant males (73). Thus, the insulinaemic state of an individual in the postabsorptive and postprandial state may have an impact on the flux of fatty acids from adipose tissue to the liver (Fig. 1A and B).

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Plasma NEFA concentrations: the influence of phenotype

Plasma NEFA concentrations are reported by some (81), but not all (73) to be elevated with obesity, NAFLD and type 2 diabetes; however, it is clear that plasma NEFA concentrations are not simply a consequence of increased adipose tissue mass. This was clearly demonstrated by Karpe *et al.* (15) when they plotted the release of NEFA from subcutaneous abdominal adipose tissue (per 100 g tissue) against total fat mass (kg) and found a strong inverse association, demonstrating that as fat mass increased NEFA release per unit weight of adipose tissue was decreased. Moreover, they found a similar inverse association when they plotted NEFA release from subcutaneous abdominal adipose tissue (per 100 g tissue) against fasting plasma insulin concentrations (15). On the basis of these observations, the authors suggested that hyperinsulinaemia, or long-term adaptation to hyperinsulinaemia, are means by which fatty acid release is downregulated (15). The situation is likely to be more complicated in individuals with type 2 diabetes, which is normally described as a state of relative insulin deficiency. It is possible that insulin insufficiency will negatively impact on both adipose tissue fat storage and antilipolysis, with a net effect of greater delivery of fatty acids to the liver compared with the non-diabetic state.

Adipose tissue-derived NEFA flux and hepatic fatty acid uptake

Using a 4-day stable-isotope labelling procedure, it was demonstrated that the contribution of plasma NEFA to fasting IHTAG was between 45 and 74% of the liver TAG (29), suggesting an increased flux of fatty acids from subcutaneous adipose tissue may be an underlying cause for some, but not all, individuals that develop NAFLD. Work by Iozzo *et al.* (82) investigating hepatic fatty acid uptake using position emission tomography/computed tomography in combination with labelled palmitate (^{11}C) or a palmitate analogue fluoro-6-thia-heptadecanoic acid (^{18}F-FTHA), found hepatic fatty acid uptake tended to be higher in obese compared to overweight non-diabetic individuals. Using the same methodology, Immonen *et al.* (83) reported that morbidly obese individuals had significantly higher hepatic fatty acid uptake before and 6 months after bariatric surgery, compared to lean individuals, despite IHTAG content being normalised. It was suggested that the persistence of high hepatic fatty acid uptake was coupled with diverting fatty acids towards oxidation, rather than esterification pathways as reduction in IHTAG was maintained after weight loss (83).

There is a strong association between IHTAG content and visceral adiposity (27, 84), however determining the contribution of fatty acids from visceral adipose tissue to IHTAG, in humans, *in vivo*, is challenging. As visceral adipocytes are more lipolytically active than subcutaneous adipocytes *in vitro* (85, 86) it is plausible that an increased visceral fat mass leads to an increased release of NEFA into the portal vein, exposing the liver to a greater fatty acid flux than would be predicted from systemic NEFA concentrations. By utilising isotope dilution/hepatic vein catheterisation techniques in lean and obese men and women, Nielsen *et al.* (87) provided support for this concept as they demonstrated that obese individuals had a significantly higher splanchnic fatty acid uptake and release than lean individuals in the fasting state. As adipose tissue lipolysis is under the control of insulin, Meek and colleagues (88) investigated whether there was a differential response to insulin between upper-body and splanchnic adipose tissue, compared to lower body (leg) adipose tissue. By using isotope dilution/hepatic vein catheterisation techniques in healthy men and women with a BMI between 18 and 27 kg/m^2, they demonstrated that moderate hyperinsulinaemia appeared to increase the proportions of fatty acids reaching the liver from visceral fat compared with systemic sources. They concluded that visceral adipose tissue lipolysis is more resistant to insulin suppression than leg lipolysis (88). Thus, in situations of obesity-induced insulin resistance or in individuals with visceral obesity, it is likely that the insulin-mediated suppression of systemic NEFA is not a good surrogate marker of NEFA delivery to the liver. Moreover, after consumption of a mixed meal, when insulin has an antilipolytic effect on adipose tissue TAG, it is likely that the NEFA flux to the liver would be further exaggerated in individuals with visceral obesity compared to those without.

The flux of fatty acids from adipose tissue lipolysis is under the regulation of insulin and as an individual expands their fat mass depots, it is clear the metabolic state of the subcutaneous adipose tissue changes such that it becomes more metabolically quiescent. Despite a lower flux of fatty acids from subcutaneous adipose tissue (per unit mass) in obesity, it is still the major contributor of fatty acids to the liver and it is likely the contribution from visceral fat is exacerbated in obesity.

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Conclusion

Insulin signalling is an important factor that links intra- and extrahepatic metabolism. Within the liver, insulin signalling regulates a number of pathways including glucose and fatty acid uptake, storage, synthesise and output. Although insulin resistance is often suggested to be synonymous with hyperinsulinaemia in the context of normal blood glucose concentrations (4), there has been the suggestion that hepatic insulin resistance manifests a 'selective' profile, where both DNL and gluconeogenesis remain upregulated. However, in 'total' insulin resistance, DNL is downregulated whilst gluconeogenesis is upregulated (22); the nuances of hepatic insulin resistance remain to be determined. Whether insulin resistance is the cause or effect of IHTAG remains to be elucidated. It is clear however that intrahepatic DNL and the flux of fatty acids from adipose tissue and dietary fat is dependent on a number of metabolic factors; not least ambient and postprandial insulin concentrations, which in combination with the phenotype of an individual (i.e. BMI, plasma insulin concentrations) and lifestyle factors (e.g. habitual diet) may play a role in tipping the balance of IHTAG toward accumulation, rather than away from it.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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