The roles of insulin and fatty acids in the regulation of hepatic very-low-density lipoprotein assembly

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INTRODUCTION

Abnormal insulin production and insulin action are associated with disturbed lipoprotein metabolism, and this linkage might provide an explanation for the relationships between metabolic diseases, such as diabetes and obesity, and cardiovascular disease. As the liver is the exclusive source of endogenous lipoproteins secreted as very-low-density lipoprotein (VLDL), the role of insulin on hepatic VLDL assembly is important. The processes involved in insulin action and lipoprotein assembly are very complex and incompletely understood. Unambiguous definition of their interactions is even more difficult and, as a result, many investigators have taken a reductionist approach by studying these interactions *in vitro*. However, this approach is not an end in itself. It is not sufficient to know how, for instance, insulin modulates apolipoprotein B (apoB) and lipid interactions within a single hepatocyte in a culture dish or an isolated perfused liver. The full picture will emerge only when we know whether such modulations, if they occur, are affected and modified by other insulin-dependent metabolic processes occurring simultaneously in the whole body. One of the most important of these simultaneous events is the suppression of fatty acid (FA) release from adipose tissue, thus limiting the supply of an important VLDL precursor.

The difference between what insulin is capable of doing in a strictly defined chemical environment *in vitro* and what insulin actually does in the real world of whole body physiology *in vivo* strikes at the very core of the controversy surrounding its precise role in hepatic VLDL assembly. In many cases, the consequences of nutritional manipulations and pathophysiological changes which result, amongst other things, in changes in plasma insulin have been attributed to insulin itself rather than to other major associated metabolic perturbations. This paper will deal, first of all, with a review of experiments carried out over a number of years which have sought to define the direct effects of insulin in carefully controlled chemical environments in various types of liver preparations *in vitro*. These experiments have been carried out mainly with animal models and the effects of insulin resistance in the donor animals will also be considered.

This review will also discuss the extent to which these *in vitro* observations can be reproduced *in vivo*, as seen following the administration of exogenous insulin to human subjects and animal models. Of greater physiological relevance, however, are the effects of increased endogenous insulin production following oral glucose. Finally, we will review some recent developments in the understanding of the molecular and cell biology of VLDL assembly, and also the pathway by which FA are incorporated into VLDL, which have made it possible to design experiments to test the effects of insulin and FA on potential regulatory targets.

WHAT ARE THE DIRECT EFFECTS OF INSULIN ON VLDL PRODUCTION IN ISOLATED LIVER PREPARATIONS?

In general, studies with isolated perfused livers have not yielded consistent results. In the earliest work[1], insulin had no effect on VLDL output, possibly because of the relatively low insulin concentration, which would have undergone rapid hepatic degradation. Other studies showed an inhibitory effect on the secretion of VLDL triacylglycerol (TAG)[2] and apoB[3]. In some cases, insulin either stimulated or inhibited VLDL TAG output according to either the perfusion flow rate[4] or the nutritional state of the donor animals[5]. In contrast, since the original report by Durrington and colleagues[6], all studies with hepatocyte cultures have shown that exposure to insulin for periods of up to 24 h inhibits the secretion of every constituent of VLDL, including TAG[6,7], cholesteryl ester[7], apoB[8,9] and apolipoprotein E (apoE)[8]. This effect has been shown in primary hepatocytes from a wide range of species, including humans[10], rats (see above), hamsters[11], and in the human hepatoma cell line HepG2[12]. The effective concentrations of insulin used in the above experiments (0.3–100 nM) were generally higher than the maximum postprandial concentration of insulin (~1 nM) in the portal blood[13], possibly reflecting the rapid degradation of insulin by hepatocytes.

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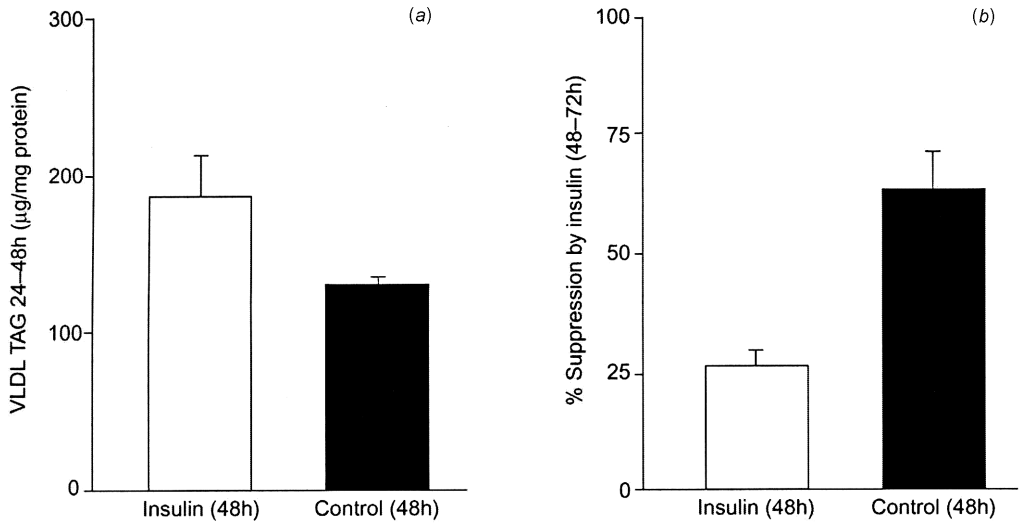

Figure 1 **Long-term exposure of hepatocytes to insulin.** VLDL=very-low-density lipoprotein; TAG=triacylglycerol

The time dependence of the inhibitory effects of insulin on the secretion of VLDL apoB and TAG has been studied and shows that at an initial concentration of 78 nM, these effects persist for up to 24 h[14]. An inhibitory effect on the secretion of newly synthesized apoB was observed after as little as 2 h exposure to insulin[15]. Exposure periods longer than 24 h, however, did not show any inhibitory effects. For instance, in the experiment shown in Figure 1*a*, hepatocytes were cultured for 48 h in the presence or absence of insulin, and VLDL TAG output was measured over the final 24 h (i.e. between 24 and 48 h of culture). During this period, insulin stimulated VLDL TAG output compared to the controls. Furthermore, when the control cells were cultured for a further 24 h (48–72 h) either in the presence or absence of insulin, the cells remained sensitive to insulin inhibition and VLDL TAG output was suppressed by 60–65% (Figure 1*b*). However, when the cells that had been exposed previously to insulin for 48 h were treated in the same way, the inhibitory effect of insulin was attenuated and VLDL output was suppressed by only 25%. In other words, chronic exposure of hepatocytes to a high concentration of insulin desensitized the cells to the subsequent acute inhibitory effect of insulin on the secretion of VLDL[16], possibly as a result of insulin receptor downregulation[8].

Returning to the acute effect of insulin, the question remained as to the fate of the cellular TAG and apoB that were not secreted when insulin was present in the medium. Table 1 shows that insulin had no significant effect on the total amount of TAG present (i.e. cellular plus secreted). However, the inhibitory effect on secretion was accompanied by an accumulation of cellular TAG, most of which accumulated within the cytosolic storage pool in the form of lipid droplets. It did not remain within the secretory apparatus[17]. Therefore, insulin prevented the recruitment of stored hepatocellular TAG for VLDL assembly in the secretory apparatus of the cell. From a physiological viewpoint, it might be argued that the effect of insulin at the hepatic level is indistinguishable from the well-established insulin-mediated promotion of TAG storage within adipose tissue caused by the inhibition of hormone sensitive lipase[18]. The suppression of VLDL apoB output by insulin was not accompanied by a cellular accumulation of apoB (Table 1). The decreased quantity of cellular plus VLDL apoB recovered in the presence of insulin was probably not a result of transcriptional regulation since insulin had no effect on apoB mRNA[19]. By contrast, the most important regulatory target for apoB appears to be post-translational at the level of degradation[20]. In this respect, Sparks and Sparks[9] have

Table 1 **Effects of insulin on the distribution of TAG and apoB between hepatocytes and medium**

	TAG (μg/mg cell protein)[16]			***ApoB (ng/mg cell protein)[9]***		
	Cell	***Medium***	***Total***	***Cell***	***Medium***	***Total***
Insulin–	180±38	111±32	292±29	282	780	1062
Insulin+	257±23	60±9	318±25	226	380	606

shown clearly that insulin enhances apoB degradation, an effect that is probably secondary to a decreased transfer of intracellular lipid into the secretory pathway[15].

DOES INSULIN RESISTANCE AFFECT THE VLDL RESPONSE TO INSULIN?

If the acute inhibitory effect of insulin described above is an accurate reflection of the insulin response at the whole-body level, then it might be predicted that this inhibitory effect on VLDL output would be lost or attenuated if the liver became insensitive to insulin. The result would be an increased output of VLDL TAG and apoB compared with the normal, insulin-sensitive liver. This relatively high output in insulin-resistant states might be particularly pronounced postprandially when the liver is exposed to a high insulin concentration. In contrast to other groups[21], we have previously proposed that in insulin-resistant states, such as obesity and non-insulin-dependent diabetes, such a desensitization of the liver may be a contributory factor to the high hepatic output of VLDL under these conditions[22]. To test this hypothesis, we selected several animal models of insulin resistance: the obese Zucker rat[23], the chronically fructose-fed rat[24] and the lactating rat[25]. Hepatocytes were prepared from these donor animals, and the concentration-dependent effects of insulin on VLDL TAG and apoB output were compared with those in hepatocytes from corresponding insulin-sensitive donor controls. Figure 2 shows the differences in the insulin-induced responses of hepatocytes prepared from fatty Zucker rats and from their lean littermates. In the absence of insulin, the 'obese' hepatocytes secreted about twice as much VLDL TAG as the 'lean' hepatocytes. More importantly, however, whereas insulin was able to suppress TAG output in the 'lean hepatocytes,

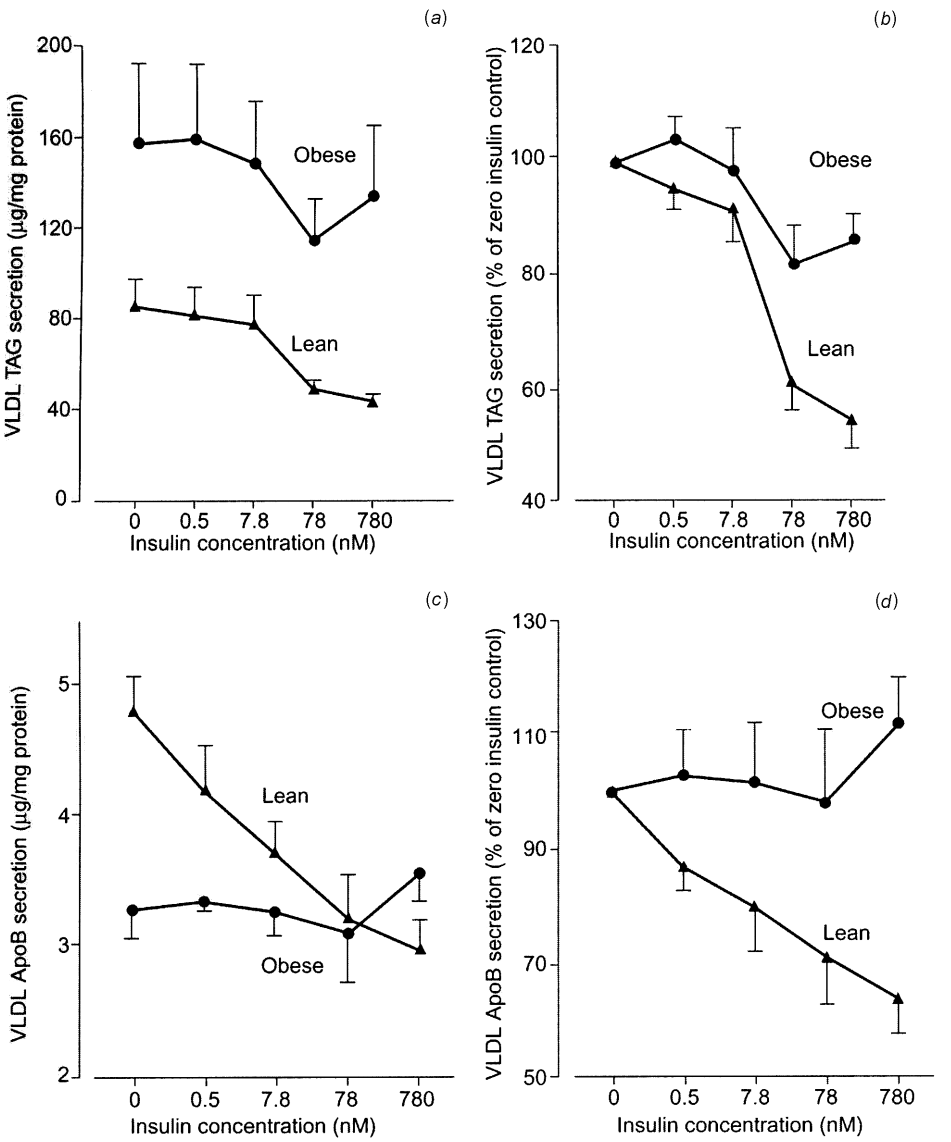

Figure 2 **Effects of increasing concentrations of insulin on (*a*, *b*) very-low-density lipoprotein (VLDL) triacylglycerol (TAG) and (*c*, *d*) VLDL apolipoprotein-B (apoB) release from 'lean' and 'obese' hepatocytes**

there was no significant suppression in the 'obese' hepatocytes, when expressed as either absolute quantities (Figure 2*a*) or fractional inhibition (Figure 2*b*). A similar lack of insulin suppression of VLDL apoB was also observed in the hepatocytes from the obese animals but not in those from the lean animals (Figure 2*c* and *d*). Similar results were also obtained in the hepatocytes cultured from lactating rats (compared with virgin and post-weaning mothers), and in hepatocytes cultured from 7-day fructose-fed rats (compared with chow-fed controls). Therefore, there appear to be two components to the high rates of VLDL TAG secretion in the livers of insulin-resistant rats: a high basal rate and a decreased sensitivity to the inhibitory effects of insulin. Interestingly, when the hepatocytes from the obese Zucker rats were cultured for a further 24 h (i.e. 24–48 h after seeding) and challenged with insulin alone, the dose response of VLDL secretion was identical to that in hepatocytes from the lean littermates cultured at the same time[23]. It would appear, therefore, that the 'obese' hepatocytes are not inherently insulin resistant: their insensitivity derives exclusively from the environment to which the liver was exposed *in vivo*. Normal insulin sensitivity may be restored following exposure to a suitable environment *in vitro*. In all of the animal models, the hepatic TAG content *in vivo* was increased compared with their respective control group. A high hepatic TAG content alone is not sufficient to induce insulin resistance, since increasing the TAG content of normal hepatocytes *in vitro* does not affect the insulin dose–response curve for VLDL output. However, an increased hepatic TAG content increases the basal level of VLDL secretion (see below).

The liver of the fatty Zucker rat is exposed to a chronically hyperinsulinaemic environment (plasma insulin 65±7 μU/mL versus 12±1 μU/mL in the lean littermates)[23]. Since chronic exposure of normal hepatocytes to a high insulin concentration *in vitro* desensitized the cells to the subsequent inhibitory effect of acute insulin challenge on VLDL output (see above), this raised the question of whether a similar mechanism *in vivo* would explain the lack of insulin responsiveness of VLDL secretion in hepatocytes derived from the donor fatty Zucker rats. To test this hypothesis, normal Wistar rats were made chronically hyperinsulinaemic by subscapular implantation of osmotic minipumps that delivered insulin at a constant rate for 7 days[14]. Control animals were fitted with minipumps that delivered vehicle alone. After 7 days, the plasma insulin concentrations were 201±64 μU/mL and 31±4 μU/mL for the hyperinsulinaemic and control groups, respectively. Hepatocytes were prepared from each group of animals and the cells challenged *in vitro* with increasing concentrations of insulin. Figure 3 shows that the basal output of VLDL TAG in the absence of insulin was significantly higher in the hepatocytes derived from the hyperinsulinaemic animals compared with the normoinsulinaemic controls. This pattern resembled that in the insulin-resistant 'obese' Zucker hepatocytes. However, the cells from the chronically hyperinsulinaemic animals remained sensitive to the inhibitory effects of insulin on VLDL secretion *in vitro*. In fact, when calculated as fractional suppression, the insulin dose–response curves of the 'hyperinsulinaemic' and the 'control' hepatocytes were virtually superimposable (Figure 3*b*). It would appear, therefore, that although chronic hyperinsulinaemia *in vivo* is associated with a higher output of VLDL, it is not in itself sufficient to induce resistance to the normal inhibitory effect of insulin. In the obese Zucker rat, therefore, some other factor in

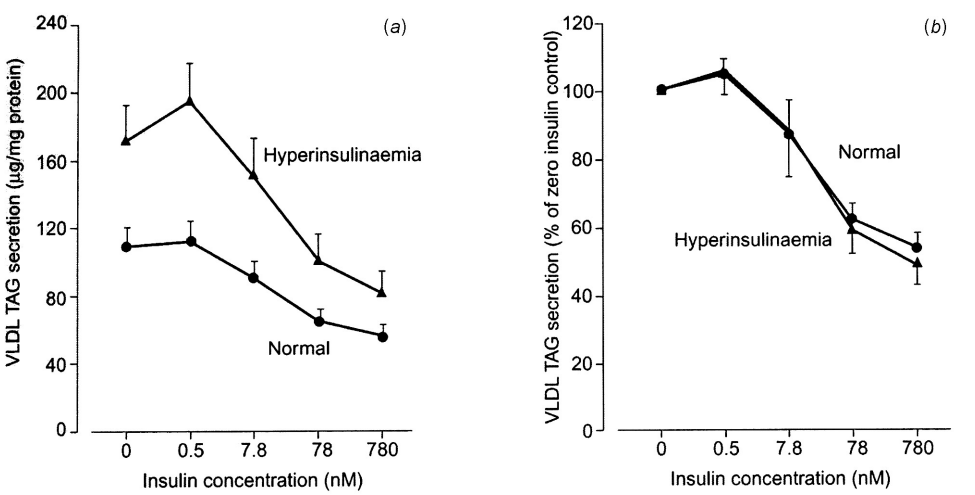

Figure 3 **Chronic *in vivo* hyperinsulinaemia does not blunt the hepatic response to insulin *in vitro*.** VLDL=very-low-density lipoprotein; TAG=triacylglycerol

addition to chronic hyperinsulinaemia is necessary for hepatic resistance to insulin with respect to the control of VLDL output. Interestingly, in the chronically hyperinsulinaemic but otherwise normal rats, there was a decreased level of plasma TAG despite the increased VLDL output. This was associated with a significant increase in epididymal fat pad size, implying that adipose tissue TAG clearance from the plasma was increased under these conditions[14].

WHY ARE CHRONIC CHANGES IN PLASMA INSULIN CORRELATED POSITIVELY WITH CHANGES IN HEPATIC VLDL OUTPUT?

The above experiment illustrates the paradox that lies at the heart of the controversy surrounding the role of insulin in the regulation of hepatic VLDL output, i.e. in the absence of insulin resistance, chronic exposure of the liver to high insulin *in vivo* elevates VLDL output despite the fact that the derived hepatocytes secrete considerably less VLDL during acute exposure to insulin *in vitro*. It has been argued previously that because chronic hyperinsulinaemia and hypoinsulinaemia are associated, respectively, with high and low outputs of hepatic VLDL, there must be a direct causal relationship between plasma insulin levels and VLDL secretion. Furthermore, ambient plasma insulin levels are correlated positively with the sensitivity of the liver to FA-induced changes in VLDL output[26]. To understand the mechanisms involved here, it is necessary to discriminate between the long-term and indirect role of insulin in creating a metabolic environment that is conducive to a high VLDL output and the direct, acute inhibitory effect on VLDL secretion. For instance, in the experiment described above, in which chronic hyperinsulinaemia was maintained by osmotic minipump implantation, there was evidence for an increased hepatic uptake of glucose[14], which would itself result in an increased VLDL output[6]. It is also likely that *de novo* FA synthesis was also increased, which would tend to channel exogenous FA into the esterification/secretion pathway via a malonyl-coenzyme A(CoA)-mediated inhibition of FA oxidation[27]. Conversely, livers from insulin-deficient animals have low rates of FA synthesis and low levels of malonyl-CoA, so it is likely that exogenous FA are directed primarily into the oxidative rather than the esterification/secretion pathway. Thus, changes in the rate of endogenous FA synthesis, rather than insulin per se, would predetermine the fate of exogenous FA and would explain the apparent changes in the sensitivity of livers from sucrose-fed, starved and diabetic animals to FA-mediated changes in esterification/secretion. This does not alter the fact that hepatocytes from these livers decrease their VLDL output when exposed acutely to insulin.

In defining the overall effects of insulin at the physiological level, therefore, it is necessary to distinguish between the effects of hyperinsulinaemia alone, which indirectly elevate VLDL output, and the effects of insulin resistance, which reduce the sensitivity of the liver to the direct inhibitory effect of insulin on VLDL secretion.

DOES INSULIN ACUTELY SUPPRESS HEPATIC VLDL RELEASE *IN VIVO* VIA A DIRECT EFFECT ON THE LIVER?

Several groups have shown conclusively that in human subjects, hepatic VLDL apoB and TAG outputs decrease during a hyperinsulinaemic, euglycaemic clamp. Similar results were obtained irrespective of whether VLDL output was measured indirectly by isotope dilution techniques[28–30] or directly by determination of veno-arterial differences for VLDL across the splanchnic bed[13]. In these experiments, it was impossible to discriminate between a direct inhibitory effect of insulin and a decreased hepatic FA flux as contributory factors to the decreased output of VLDL. Three independent observations, however, provide good evidence for a direct effect of insulin. First, even when hepatic FA supply was not limited, VLDL TAG output became suppressed during the clamp, although not to the same extent[31]. Second, direct administration of portal insulin produced a decreased VLDL TAG output into the hepatic vein[32]. Finally, a tolbutamide-mediated increase in portal insulin also suppressed hepatic VLDL output[33]. When insulin-resistant subjects were examined during a hyperinsulinaemic, euglycaemic clamp, the suppression of hepatic VLDL output was attenuated compared with normal subjects[28,29], confirming the previous hypothesis (see above)[22]. The above experiments provided strong circumstantial, but not conclusive, evidence for a direct regulatory role of increased portal insulin produced as part of the postprandial response. Such an endogenous insulin surge forms part of the framework of the complex metabolic and hormonal changes that occur in the postprandial state, and it has proved difficult to design an experiment in which a change in portal insulin is the only variable postprandially. This is obviously an important problem to resolve, since any abnormality of insulin action on lipoprotein metabolism will be most apparent in the postprandial state.

To address this question, Bulow and colleagues[13] measured splanchnic VLDL output in the fasting state and then at 30-min intervals up to a total of 5 h following oral consumption of 75 g of glucose. Splanchnic glucose output peaked at 2.83 mmol/min, 30 min after the glucose load and hepatic venous plasma insulin concentration peaked at 250 pmol/L 30 min later. Although splanchnic VLDL TAG output changed significantly after the glucose load, there was no immediate decrease corresponding to the peak of portal insulin concentration, despite a decreased splanchnic uptake of nonesterified fatty acids (NEFA) (Figure 4). Thus, in both the clamp experiments and the experiments with

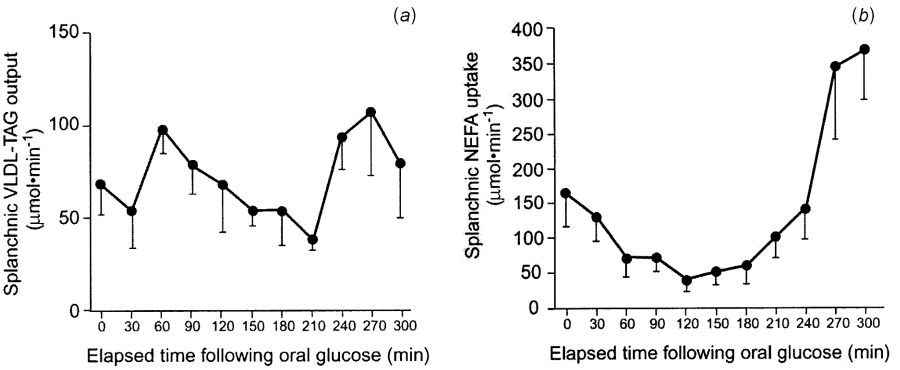

Figure 4 **Splanchnic non-esterified fatty acid (NEFA) uptake (*a*) and splanchnic very low-density lipoprotein (VLDL) output (*b*) following a glucose load**

oral glucose, insulin levels increased and FA decreased. However, only the former experiments showed a decreased splanchnic VLDL output. The major physiological difference affecting the environment of the liver that resulted from the different experimental protocols was an increase in portal glucose concentration following an oral glucose load. In this respect, recent studies have shown that acute exogenous hyperglycaemia increased the output of VLDL TAG even in the face of a decreased splanchnic NEFA flux[34]. These conditions have been reproduced under a more strictly defined chemical environment *in vitro*, in which it was shown that glucose resulted in a concentration-dependent increase in VLDL apoB output in cultured hepatocytes, which was dependent upon an increased mobilization of hepatocellular TAG stores[35]. It is noteworthy that in the experiment with oral glucose described above, splanchnic uptake of NEFA alone was insufficient to account for all of the VLDL ouput over the course of the experiment, suggesting that the increased splanchnic glucose was associated with an increased mobilization of stored hepatic TAG.

Although further experiments are required, the results from *in vivo* and *in vitro* studies provide strong evidence that the acute increase in portal insulin postprandially, in the absence of any other change, is directly responsible for a decreased output of hepatic VLDL. This direct effect may be modified by other metabolic and hormonal changes occurring in the environment of the liver postprandially, and it is suggested that a major role of insulin is to attenuate any glucose-mediated increase in VLDL output in the immediate postprandial state. If this is the case, then it is proposed that insulin serves to prevent VLDL output and temporarily promote the hepatic storage of TAG postprandially in three ways: (1) by directly inhibiting VLDL secretion; (2) by decreasing hepatic NEFA flux; and (3) by potentiating the rapid removal of glucose, which itself stimulates VLDL output. It follows that resistance to any of these effects of insulin would lead to an abnormally high output of hepatic VLDL.

WHAT ARE THE MOLECULAR MECHANISMS INVOLVED IN THE REGULATION OF VLDL ASSEMBLY BY FA AND INSULIN?

Some recent developments in our understanding of the molecular and cellular basis of VLDL assembly have highlighted potential regulatory targets for insulin and FA action. Figure 5 summarizes our current, although as yet incomplete, understanding of mechanisms by which VLDL is assembled within the hepatocyte. This description is based upon the so-called two-step model of VLDL synthesis[36], in which a small, dense, apoB-containing precursor particle (formed from newly translocated apoB in Step 1) fuses with a large apoB-free particle of TAG to give a mature VLDL particle (Step 2). Much attention is currently being focused on factors that regulate, or limit, the quantity of TAG available for the maturation phase, since this will determine the ultimate size of the secreted VLDL particle and, hence, whether it is metabolized by an 'atherogenic' or 'non-atherogenic' pathway in the plasma[37].

One of the factors that may influence VLDL particle size is the metabolic route by which extracellular FA, arising mainly from adipose tissue TAG lipolysis, are made available within the hepatocyte for the assembly of VLDL. Thus, plasma NEFA entering the hepatocyte are esterified to TAG by a process involving microsomal diacylglycerol acyl transferase (DGAT 1). However, a body of evidence suggests that this newly synthesized TAG is not immediately available for VLDL assembly[18]. Instead, it is transferred to a cytosolic storage pool, where it is mobilized by a process that involves lipolysis followed by re-esterification of the lipolytic products at a different site in the endoplasmic reticulum (ER). The re-esterification probably requires a different isoform of DGAT (DGAT 2)[38]. The lipolysis/re-esterification process is closely linked, structurally, with the intracellular site at which bulk lipid is transferred to the small VLDL precursor to give mature VLDL (Step 2 in Figure 5). However, only a part of this newly mobilized TAG is utilized to complete the VLDL assembly process

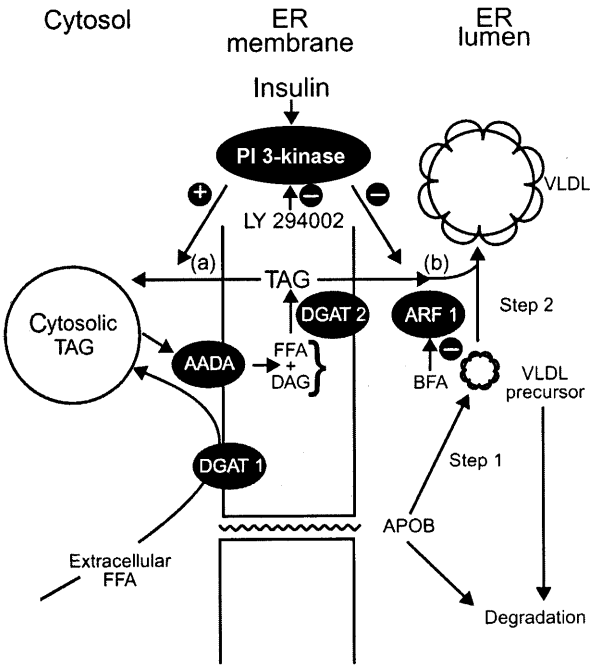

Figure 5 **Mobilization of triacylglycerol (TAG) for very-low-density lipoprotein (VLDL) assembly.** AADA=Arylacetamide deacetylase; ARF 1=adenosine diphosphate (ADP) ribosylation factor 1; BFA=Brefeldin A; DGAT 1=diacylglycerol acyl transferase 1; DGAT 2=diacylglycerol acyl transferase 2; ER=endoplasmic reticulum; FFA=free fatty acid; PI 3-kinase=phosphatidylinositol 3-kinase; ApoB=apolipoprotein B [Source: Trickett *et al.*, 2001—from Ref 9]

(Pathway (*b*) in Figure 5). The remainder is recycled back to the cytosolic storage pool (Pathway (*a*) in Figure 5). The molecular mechanisms that regulate the recycling/secretion options for mobilized TAG are unknown, but they are sensitive to insulin signalling (see below).

We have also identified a gene that we believe is involved in the lipolysis of cytosolic TAG. It is identical to a gene previously assigned a role in carcinogen activation called arylacetamide deacetylase (AADA). AADA has significant homology with hormone-sensitive lipase (HSL). However, unlike HSL, it is targeted to, and retained within, the ER of the cell by virtue of an unusual amino terminal signal anchor sequence. *In vitro* translocation has also shown that the protein is translocated into ER membranes, the site at which lipolytic activity might be expected to be located[18]. Stable transfection of the AADA gene into HepG2 cells increases TAG secretion by two- to threefold[18] and significantly increases the secretion of newly synthesized apoB (unpublished data). In many cases, transcription of the AADA gene (as shown by nuclear run-on assays) parallels closely the rate of hepatic lipid secretion[39]. Insulin, however, has no direct inhibitory effect on AADA transcription following acute (0–24 h) exposure of hepatocytes to insulin. Instead, insulin redirects less of the newly recruited TAG from lipolysis and esterification into the secretory pathway (Pathway (*b*) in Figure 5), so that a greater proportion is recycled back to the cytosolic storage pool (Pathway (*a*) in Figure 5).

With respect to the molecular mechanisms involved in the insulin-mediated rechannelling of intracellular lipid, there are two possible alternatives: insulin may either inhibit some factor involved directly in the bulk lipid transfer step (Step 2 in Figure 5), or it may inhibit the production of the VLDL precursor (Step 1 in Figure 5), so that the mobilized TAG returns to the cytosol by default. Experimental discrimination between these two possible alternatives requires a means of uncoupling Step 1 from Step 2 in the VLDL assembly process. This is possible using the elegant experimental design, published by Olofsson and colleagues[36], that uses low concentrations of the fungal metabolite Brefeldin A (BFA) to inhibit the maturation step of VLDL assembly in rat hepatoma cells without affecting the formation of the VLDL precursor. BFA inhibits nucleotide exchange on adenosine diphosphate (ADP)-ribosylation factor 1 (ARF 1), a small guanosine triphosphate (GTP) binding protein belonging to the Ras superfamily, the activity of which is crucial for TAG transfer during the maturation phase. BFA-mediated inhibition of ARF 1 activity (and thus, of TAG transfer) is rapidly reversible, and the maturation phase of VLDL assembly may be restored following removal of BFA from the medium. Using BFA, we designed pulse-chase experiments in which insulin signalling was intact at only one of the two steps of the overall VLDL assembly process. Transmission of the insulin signal to the site of VLDL synthesis in the ER involves phosphatidylinositol 3-kinase (PI 3-kinase), and insulin signal transduction may be abolished at any time by addition of the PI 3-kinase inhibitor LY 294002[40]. Thus, using BFA to prevent further metabolism of the VLDL precursor-associated apoB during a pulse-chase experiment, it was possible to study specifically the kinetics of VLDL maturation from the precursor when BFA was removed from the medium.

Rat hepatocytes secrete VLDL associated with both apoB-48 and apoB-100, and when insulin was present in the culture medium during both steps of VLDL assembly (precursor formation and maturation), as expected, there was a decreased secretion of labelled apoB-48 VLDL and apoB-100 VLDL (Figure 6). However, when insulin was removed from the medium during the maturation phase only (i.e. following removal of BFA), there was no inhibition of VLDL apoB secretion[15]. Similarly, when insulin remained in the medium during the maturation step (second step), but signal transduction via PI 3-kinase was abolished by addition of LY 294002, VLDL apoB output was not inhibited (Figure 6)[15]. These results suggest that insulin did not affect the formation of the VLDL precursor when VLDL maturation was prevented by BFA. A decreased availability of the precursor under these conditions would not have

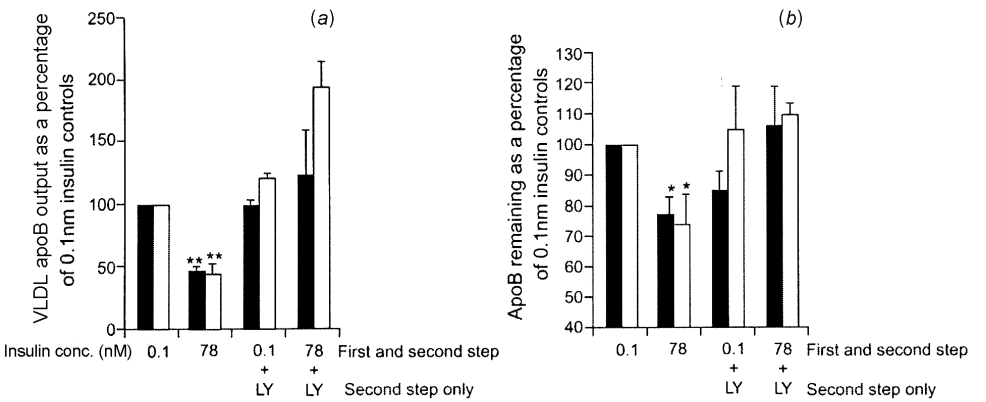

Figure 6 **Abolition of insulin signalling during the second step of VLDL assembly decreases apoB degradation and restores VLDL apoB output.** (*a*) Very low-density lipoprotein (VLDL) output; (*b*) apolipoprotein B (apoB). ■ ApoB-100; □ apoB-48. **Significantly lower than 0.1 nm insulin controls ($P < 0.01$); *Significantly lower than 0.1 nm insulin controls ($P < 0.05$)

permitted the full restoration of VLDL assembly when insulin signalling was abolished (either by removal of insulin or inhibition of PI 3-kinase) during the maturation phase only (following removal of BFA). Therefore, if the formation of the VLDL precursor was not insulin sensitive, then the normal, overall inhibition of VLDL assembly must have derived exclusively from direct insulin interference with some factor responsible for the bulk TAG transfer step (Step 2 in Figure 5). Whether this involved ARF 1 or one of its downstream consequences such as phospholipase D-dependent phosphatidic acid formation[36] remains to be determined. In this respect, it has been shown previously that a substantial proportion of VLDL TAG is derived from cellular phospholipids[41,42]. A clearer understanding of the molecular mechanisms operating here is essential for identification of a regulatory mechanism determining nascent VLDL particle size.

The insulin-mediated increase in apoB degradation has been referred to previously. In the experiment described above, abolition of insulin signalling during Step 2 only also abolished the overall stimulation of apoB degradation observed when insulin was present during both steps of VLDL assembly. This result again implied that insulin acted directly during the maturation phase, and that a decreased availability of TAG for this step directed the VLDL precursor into a degradation pathway[15] (*see* Figure 6).

HOW DO FAs INCREASE VLDL OUTPUT?

Changes in FA flux to the liver gave rise to corresponding changes in the size of the cytosolic pool of TAG, serving as the immediate substrate pool for VLDL assembly (Figure 7). As described above, extracellular FA are stored temporarily in this pool and secreted as VLDL when physiological conditions permit. Although the size of the intrahepatic pool of TAG is ultimately dependent on (amongst many other factors) the delivery of extrahepatic FA, it is the size of the TAG pool rather than the immediate FA flux that is responsible for the acute regulation of VLDL secretion[18]. A good example of this is the lack of correlation between splanchnic FA flux and VLDL output immediately following an oral glucose load (Figure 4). All the animal models of insulin resistance described above had a high hepatic TAG content, and in every case derived hepatocytes showed a high basal rate of VLDL secretion in the absence of insulin. It is likely, therefore, that a high hepatic TAG content in pathophysiological states such as type 2 diabetes and obesity is responsible in part for the high rates of VLDL output observed under these conditions. The other contributory factor, of course, is resistance to the inhibitory effect of insulin, as described above.

WHY DOES THE LIVER SECRETE VLDL?

It has been proposed that the liver acts as a buffer to prevent the potentially toxic effects of excess FA on peripheral tissues. This is achieved by detoxification of FA via esterification and temporarily storing the product as TAG[18]. In this respect, the capacity of mammalian, including human,

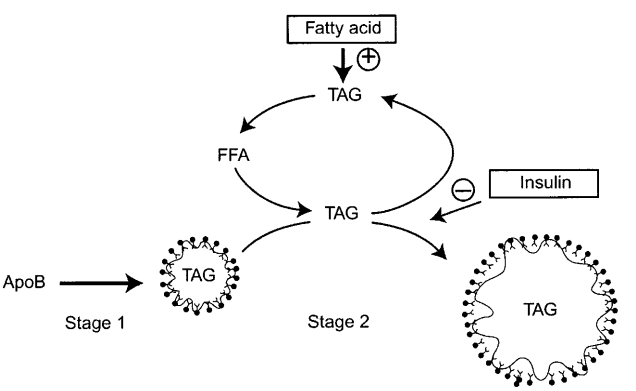

Figure 7 **Insulin and fatty acid regulation of triacylglycerol (TAG) transfer to very low-density lipoprotein (VLDL).** FFA=Free fatty acid; ApoB=apolipoprotein B

liver is quite large but not limitless. It is likely that under normal circumstances, the liver releases stored TAG as VLDL under physiological circumstances in which peripheral tissues are able to deal with it efficiently. These would include periods during which chylomicron entry into the plasma is low, e.g. in the post-absorptive state when plasma insulin concentrations are low. This buffering effect of the liver would result in a diurnal periodicity of VLDL secretion that arises from the deliberate asynchrony of hepatic TAG synthesis (FA esterification) and TAG release as VLDL, and would be regulated, at least in part, by diurnal fluctuations in portal insulin concentration (see above). Thus, the hepatic processing of FA and their ultimate secretion as a benign derivative, VLDL, may simply represent yet another facet of the liver's general role as a frontline defence to safeguard body tissues from the potentially dangerous, but nevertheless inevitable, consequences of major and abrupt physiological transitions.

CONCLUSIONS

A large body of evidence from experiments carried out both *in vitro* and *in vivo* suggests that an acute physiological increase in endogenous insulin production rapidly suppresses hepatic VLDL output. In the postprandial state, this response may be modified by other changes in the metabolic and hormonal environment of the liver. Nevertheless, insulin would suppress any tendency towards a postprandial increase in VLDL output. This regulatory role of insulin is attenuated severely under conditions of insulin resistance, resulting in an abnormally high hepatic VLDL secretion. Chronic hyperinsulinaemia (but not necessarily insulin resistance) results indirectly in a high rate of exogenous FA esterification and, ultimately, a high rate of VLDL output. Exogenous FA, however, are not utilized immediately for VLDL TAG synthesis but enter an intermediate storage pool, which is mobilized by lipolysis and re-esterification. Exogenous FA increase VLDL output by increasing the size of the TAG storage pool. Insulin directly inhibits VLDL output by decreasing the proportion of newly mobilized TAG entering the secretory pathway (*see* Figure 7). This effect is achieved by limiting the availability or activity of some critical factor required for the maturation phase of VLDL assembly.

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