Insulin effects on apolipoprotein B production by normal, diabetic and treated-diabetic rat liver and cultured rat hepatocytes

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1. The effect of insulin on apolipoprotein (apo B) secretion was studied in 24 h recirculating liver perfusions of isolated normal, diabetic and insulin-treated diabetic rats. In single perfusions from each group apo B accumulated in the media in a linear fashion. 2. In perfusions of normal rat livers, when the medium contained insulin plus cortisol, apo B production was significantly inhibited (by 35.8 %), demonstrating a hormone effect on apo B secretion. 3. In perfusions of diabetic-rat livers, apo B production was decreased to 11.8 % of normal when the medium contained no hormones, and was not significantly changed by the addition of insulin plus cortisol to the medium, suggesting that the hormone effect on apo B secretion is missing in long-term hypoinsulinaemic states. 4. Treatment of diabetic rats with daily insulin injection restored apo B production and restored the effect of insulin plus cortisol in the medium to inhibit apo B secretion during perfusion. 5. Parallel studies of apo B secretion with insulin alone, cortisol alone and insulin plus cortisol in the medium were performed in primary cultures of hepatocytes to compare results from liver perfusions. 6. Apo B secretion by hepatocytes from normal, diabetic and treated-diabetic rats was inhibited (by 36.8 %, 57.1 % and 57.9 % respectively) when insulin alone was added to the medium. 7. Insulin plus cortisol inhibited apo B secretion by hepatocytes from normal and treated diabetic rats (by 30.2 % and 47.2 % respectively), but failed to inhibit apo B secretion by hepatocytes from diabetic rats.

INTRODUCTION

The influence of hormones, especially insulin, on the long- and short-term regulation of hepatic VLDL synthesis and secretion in primary cultures of hepatocytes has been studied by several investigators, who have measured secretion of lipoprotein lipid components [1–7] as well as secretion of apolipoproteins [2,6–8]. Although there are clear advantages in the study of hormonal effects on hepatic lipoprotein pathways in primary cultures of hepatocytes, there are some disadvantages in that the system potentially may not be representative of the physiological system, owing to the presence of other cell types in the liver and to the presence of counter-regulatory hormones *in vivo*. In the present experiments, we used a recirculating liver-perfusion system [9] to study effects of insulin plus cortisol on hepatic lipoprotein apolipoprotein B (apo B) secretion. Liver perfusion has clear advantages in that not only is it a more physiological system than primary cultures of hepatocytes, but also it constitutes a standard of comparison for other techniques [10]. Because apo B is an essential secretory component of VLDL [11,12] and may be secreted into fractions of lipoproteins denser than VLDL [6,8,13–15], we measured the total apo B secreted into the perfusion medium. The effect of insulin on apo B production was assessed in the presence of cortisol, since together they have been shown to optimize the conditions for plasma protein synthesis and secretion *in vitro* [9]. Parallel studies using primary cultures of rat hepatocytes from normal, diabetic and treated-diabetic rats were performed in order to compare

the individual effects of cortisol and insulin on hepatocyte apo B production.

The objective of the present study was to evaluate the effect of insulin on hepatic apo B production. In rats, injection of insulin *in vivo* has been shown to decrease the net hepatic release of triacylglycerols [16,17]. In humans, decreased hepatic VLDL triacylglycerol production in response to high portal insulin levels has also been demonstrated [18]. Moreover, recent studies using continuous subcutaneous insulin infusions in human subjects have suggested that elevated insulin levels suppress hepatic triacylglycerol output [19]. Consistent with these reports are results of studies using primary cultures of hepatocytes, where insulin has been shown to inhibit VLDL triacylglycerol secretion [1–6] and apo B secretion in a dose-dependent fashion [2,6,7]. In apparent contradiction to these reports are studies of perfused rat livers, where insulin was shown to increase triacylglycerol secretion [20]. Hyperinsulinaemia has also been associated with increases in hepatic VLDL triacylglycerol production [21–23]. In view of the potential importance of hepatically synthesized VLDL apo B as a precursor to LDL apo B and the strong association of plasma LDL levels with atherosclerosis in humans [24], we wished to document the effects of insulin on apo B production by liver cells. The effects of insulin plus cortisol on apo B production in perfused liver of isolated normal, diabetic and treated-diabetic rats have not been previously reported. Furthermore, the individual effects of insulin and cortisol on hepatocyte apo B secretion have not been defined.

Abbreviations used: VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; apo B, apolipoprotein B; apo B_H, apo B of higher *M_r*; apo B_L, apo B of lower *M_r*.

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EXPERIMENTAL

Maintenance of animals

Liver donors were normal male fed Sprague-Dawley rats. The animals were housed in a temperature-controlled (22 °C) windowless room, which was artificially lit for 12 h each day. Rats were fed on a commercial pelleted diet (Diet 5001 from Ralston Purina Co., St. Louis, MO, U.S.A.). The diet contained (by wt.) 50.8 % carbohydrate (mainly starch), 23.4 % crude protein, 4.5 % fat and 5.2 % crude fibre. Food and water were available *ad libitum*. Rats were rendered diabetic by intraperitoneal injection of streptozotocin (60 mg/kg), supplied by Sigma Chemical Co. (St. Louis, MO, U.S.A.). For treatment of diabetes, rats were injected daily for 6–8 months with 2 units of insulin (protamine zinc insulin), kindly supplied by Eli Lilly Co. (Indianapolis, IN, U.S.A.). Rats were used 6–8 months after induction of diabetes and were non-ketotic at the time of perfusion. Diabetic rats gained weight less rapidly than did normal or treated-diabetic rats, while consuming as much as 50 % more food than normals. When diabetic rats were treated with insulin, growth was normalized. Differences in growth were reflected in differences in body weights at the time of perfusion, which were (means $\pm$ S.D., for the numbers of rats in parentheses): normal rats, 529 $\pm$ 41 g (18); treated diabetic rats, 596 $\pm$ 31 g (14); diabetic rats, 396 $\pm$ 34 g (9). Abnormal growth of diabetic rats has been documented elsewhere [25,26]. Apo B results were expressed as mg of apo B secreted/g of liver protein, where protein was measured by the method of Lowry *et al.* [27] or a modification thereof [28]. Calculations of apo B production in 24 h and in timed samples were corrected for the samples removed, for bile production and for infusion volumes and haematocrit.

Rats used for preparation of primary cultures of hepatocytes were normal male fed Sprague-Dawley rats weighing 200–250 g. Diabetic-rat liver donors for hepatocyte preparation were used 10 days to 3 weeks after streptozotocin injection and were non-ketotic at the time of perfusion. Treated diabetic-rat liver donors for hepatocyte preparation were injected with insulin as described above for 5–10 days before the time of perfusion.

Perfusion technique

Livers were perfused for 24 h in a recirculating system as previously described [9]. The common bile duct was routinely cannulated and bile flow monitored. To wash out preformed rat plasma proteins, livers were first perfused with 10 ml of medium which was collected separately and discarded. Livers were then continuously perfused with medium, whose composition was as follows: bovine red cells, 38 ml; Krebs-Ringer bicarbonate containing 3.0 % (w/v) bovine serum albumin (Armour, Fraction V; adjusted to pH 7.4 with 1.0 M-NaOH), 50 ml; glucose, 200 mg; heparin, 10000 units; penicillin, 3000 units; gentamicin, 10 mg; and enough Ringer's solution to bring the volume to 100 ml. The recirculating perfusate was supplemented by a continuous 12 h infusion (1.5 ml/h) totalling 18 ml of Ringer's solution, containing: glucose, 500 mg; penicillin, 3000 units; and a nutritionally complete amino acid mixture of L-amino acid plus glycine, 320 mg. For perfusions supplemented with hormones the medium at the outset contained a priming dose of insulin (51 munits/ml;

366 nm) and of cortisol (50 μ g/ml; 138 μ M); the continuous infusion contained insulin (6.8 units) and cortisol (5.0 mg). Insulin was glucagon-free pig crystallized zinc insulin from Eli Lilly Co., and cortisol was the sodium succinate derivative (Solu-Cortef) from the Upjohn Co. The pH of the medium was continuously monitored and adjusted to pH 7.1 by the intermittent automatic addition of 0.5 M-NaHCO₃ controlled by a Radiometer Titrator (London Co., Yellow Springs, OH, U.S.A.).

Preparation of hepatocyte cultures and incubation procedures

Hepatocytes were prepared for primary culture in serum-free media [6,8] and placed on 60 mm-diam. sterile culture dishes coated with rat tail collagen. Hepatocytes were incubated for 4 h at 37 °C in Waymouth's medium MB 752/1 (1.0 $\times$ 10⁶ cells/ml) containing glutamine, penicillin (200 units/ml) and gentamicin (5 mg/ml) in an atmosphere of air/CO₂ (19:1), after which the medium and non-adherent cells were discarded and adherent cells were washed with 3 $\times$ 2.0 ml of 10 mM-sodium phosphate, pH 7.4, 139 mM-NaCl and 0.1 % bovine serum albumin. Fresh medium (2–3 ml/60 mm dish) containing 0.2 % bovine serum albumin was then added, and cells were incubated for 12–14 h. Where indicated, insulin and cortisol were added to give initial concentrations of 10 nM and 100 nM respectively. At the conclusion of the experiments and after the removal of media, cellular protein was determined by a modification of the Lowry procedure [28].

Quantification of rat apo B in perfusion media

A competitive solid-phase monoclonal-antibody radioimmunoassay with VLDL apo B as standard [29] was used to measure apo B secreted into the medium by the perfused rat liver and by primary cultures of rat hepatocytes. The antibody used was a ¹²⁵I-labelled purified mouse monoclonal, equally reactive against the high- and low-*M_r* forms of rat apo B of VLDL and LDL, designated apo B_H and apo B_L respectively [30]. Briefly, the procedure involves precoating polystyrene wells (Immulon-2 Removawell strips; Dynatech Laboratories, Alexandria, VA, U.S.A.) with rat LDL (15 μ g/ml diluted in 0.015 M-Na₂CO₃/0.035 M-NaHCO₃, pH 9.6, overnight at 4 °C. After adsorption of LDL to the well, unoccupied binding sites are blocked by incubation with 1 % bovine serum albumin in 0.01 M-phosphate buffer (pH 7.4)/0.15 M-NaCl (BSA/PBS) for 4 h at room temperature. After removal of the blocking solution, samples of perfusion medium or hepatocyte culture medium (0.1 ml/well) and ¹²⁵I-labelled purified mouse monoclonal antibody (0.1 ml/well) both diluted in 1 % BSA/PBS, are added. Maximum-binding controls (B₀) are wells incubated with 1 % BSA/PBS and labelled antibody. Apo B in the medium samples (assayed immediately after collection or stored at –20 °C until assay) and the solid-phase apo B of the well compete for the labelled monoclonal antibody overnight at 4 °C. After incubation, the wells are washed three times in 1 % BSA/PBS, residual fluid is aspirated and labelled antibody associated with the well is measured. To prepare standard curves, dilutions of rat VLDL (1.0–100 ng of apo B/0.1 ml) are prepared in 1 % BSA/PBS and added to wells. The apo B content of rat VLDL (*d* < 1.006) was calculated by using Coomassie-Blue-stained SDS/polyacrylamide gels of delipidated VLDL apolipoproteins as described pre-

Table 1. Apo B concentrations of media after 24 h liver perfusion

Livers from normal, streptozotocin-diabetic and treated-diabetic rats were perfused in the presence (+) or in the absence (–) of hormones for 24 h in a recirculating system, after which the media were centrifuged to remove cells. Apo B concentration of each medium was assayed by a monoclonal-antibody immunoassay. Percentage inhibition is the percentage decrease in apo B concentration in perfusions without and with hormones. Values marked * and ** indicate that apo B results of liver perfusions without added hormones are significantly different from those with hormones at $P < 0.025$ and $P < 0.005$ respectively.

Parameter	Hormones ...	Normal rats		Treated diabetic rats		Diabetic rats	
		(–)	(+)	(–)	(+)	(–)	(+)
No. of perfusions		7	11	5	9	4	5
Protein/liver (g)		2.23±0.15	2.56±0.16	3.82±0.30	4.32±0.32	3.86±0.64	2.78±0.08
Apo B/liver protein (mg/g)		1.87±0.24	1.20±0.15*	1.05±0.09	0.59±0.08**	0.22±0.01	0.27±0.02
Inhibition (%)			35.8		43.8		–

viously [6,29]. The apo B content of control samples of perfusion medium or hepatocyte culture medium was unaffected by storage of the samples at -20°C .

Statistical treatment of results

All values in Tables are presented as means $\pm$ S.E.M. for several independent observations. The significance of any observed difference was tested by Student's *t* test.

RESULTS AND DISCUSSION

Apo B measurements

Apo B secretion was measured in studies of the effects of insulin on hepatic apo B production and secretion by normal, diabetic and treated-diabetic rat livers. Two model systems were used for these studies: the 24 h recirculating rat liver perfusion system [9] and primary cultures of rat hepatocytes [6,8]. Lipid secretion was not assessed, as heparin is required to perform the long-term liver perfusions and because heparin releases lipolytic enzymes into the perfusion medium which may have significant effects on secreted lipid over the course of 24 h of perfusion. Measurement of apo B secretion, on the other hand, assesses lipoprotein-particle secretion, as apo B is required for lipoprotein assembly and secretion [11,12] and because there is a finite mass of apo B per lipoprotein particle. In addition, apo B is non-exchangeable, unlike other apolipoproteins.

Effect of diabetes and treatment on apo B production in rat liver perfusions

Isolated liver perfusions without hormones were carried out on seven normal rat livers, and apo B content of the 24 h media was assayed (Table 1, column 1). Because differences in liver weights owing to changes in glycogen and fat in the liver are known to occur in animals in different metabolic states [31,32], the apo B content in the medium was calculated per g of liver protein instead of per g of liver. In normal rat livers, an average of 1.87 ± 0.24 mg of apo B accumulated in medium/g of liver protein in 24 h of perfusion. Assuming that the protein content of normal liver is approx. 22% of the wet weight, the apo B secretory rates ($\mu\text{g/h}$ per g wet wt. of liver) averaged 17.1 ± 2.2 . This value is within reported literature values of 14–22 μg of apo B/h per g of liver as reported by Marsh & Sparks [14], using non-recirculating perfusions.

Isolated liver perfusions without hormones were car-

ried out on four untreated diabetic rats, and apo B content of the 24 h media was assayed (Table 1, column 5). In single perfusions of normal and diabetic rat livers apo B content of samples taken at timed intervals was measured, and it was found to accumulate in the media in a linear fashion (Fig. 1). In diabetic rat livers apo B production was 0.22 ± 0.01 mg/g of liver protein. This represents only 11.8% of normal apo B production (Table 1, column 1 versus column 5; $P < 0.005$). The decrease in apo B secretion is consistent with previous studies using primary cultures of hepatocytes from diabetic rats, demonstrating that apo B secretion was markedly decreased [8]. Because apo B is an essential component of VLDL and LDL, this result is in accord with the observed decrease in VLDL and LDL output by perfused livers of diabetic rats previously reported [33–36]; it also demonstrates the marked effect of long-

Fig. 1. Time course of apo B secretion in (□) normal and (△) diabetic rat liver perfusions

Rat livers were perfused for 24 h with medium supplemented with glucose and amino acids without hormone additions. Samples of perfusion media were taken after 5 min, 2 h, 4 h, 6 h, 8 h, 12 h and 24 h of perfusion. The apo B content of samples was assayed by monoclonal-antibody immunoassay. Values at each time point represent the average of six determinations of each sample, and results are expressed as the mean mg of apo B secreted per g of liver protein. Approximation of secretion to a linear rate was assessed by simple linear regression, yielding a correlation coefficient (*r*) for perfusions of normal rat liver of 0.99 and for perfusions of diabetic liver of 0.94.

Fig. 2. Effect of hormone supplementation of the perfusion media on the time course of apo B secretion by treated-diabetic and diabetic-rat livers

Rat livers were perfused for 24 h with medium supplemented with glucose and amino acids with and without insulin plus cortisol. Samples of perfusion media were taken after 5 min, 2 h, 4 h, 6 h, 8 h, 12 h and 24 h of perfusion. The apo B content of samples was assayed by monoclonal-antibody immunoassay, and the effect of hormone supplementation on the time course of apo B production by treated diabetic rat livers and diabetic rat livers is presented. Values at each time point represent the average of six determinations, and results are expressed as the mean mg of apo B secreted per g of liver protein. Approximation of secretion to a linear rate was assessed by simple linear regression. Correlation coefficients for each liver perfusion were as follows: diabetic-rat liver without hormones, $r = 0.94$; diabetic-rat liver with hormones, $r = 0.98$; treated diabetic-rat liver without hormones, $r = 0.99$; treated diabetic-rat liver with hormones, $r = 0.97$. Liver perfusions: $\diamond$, treated diabetic rat; $\blacklozenge$, treated diabetic rat, with hormone supplementation; $\triangle$, diabetic rat; $\blacktriangle$, diabetic rat, with hormone supplementation.

term hypoinsulinaemia on hepatic apo B production. In view of the increases in plasma apo B observed in the streptozotocin-diabetic rat [8,37,38], our results support the idea that the hyperlipoproteinaemia may relate primarily to intestinally derived lipoproteins, as suggested by earlier studies using diabetic rats fed on a fat-free diet [39]. This increase in plasma apo B is coupled with a defect in the clearance of apo B-containing lipoproteins [8].

Isolated liver perfusions without hormones were carried out on five treated diabetic rats, and apo B content of 24 h media was assayed (Table 1, column 3). Treatment of diabetic rats by daily injection of insulin normalized growth and resulted in a 4.8-fold increase in apo B production, to 1.05 ± 0.09 mg/g of liver protein. Apo B production by treated diabetic-rat livers was significantly less than normal rat liver on a g-of-liver-protein basis (Table 1, column 1 versus column 3; $P < 0.05$). When the total apo B produced by normal and treated diabetic rat liver was compared, they were not significantly different (4.17 and 4.01 mg respectively). Thus apo B production per liver was restored to normal by injection of insulin *in vivo*, supporting the idea that the impairment of apo B secretion by the liver in the diabetic state is due to long-term insulin deficiency rather than a hepatotoxic effect of streptozotocin.

Effects of insulin plus cortisol on hepatic apo B secretion in liver perfusions of isolated normal, diabetic and treated-diabetic rat liver

The effect of insulin on apo B secretion was assessed in liver perfusions of normal, diabetic and treated-diabetic rats by using medium containing cortisol. Insulin and cortisol in perfusion medium optimize plasma protein synthesis and secretion *in vitro* [9], producing a positive nitrogen balance and maximum net biosynthesis of rat serum albumin, fibrinogen, α_1 -acid glycoprotein, haptoglobin and α_2 -acute-phase globulin, as well as maximum incorporation of [14 C]lysine into plasma total protein. Apo B secretion into perfusion medium was linear in single liver perfusions of treated diabetic rats in medium with and without added hormones (Fig. 2). Insulin plus cortisol in medium was not able to restore

apo B production by diabetic-rat liver to normal in 24 h perfusions. These results suggest that more than 24 h of insulin treatment is required for restoration of normal apo B production, or that critical factors exist *in vivo* which together with insulin treatment are able to normalize hepatic apo B production. Comparing liver perfusions with and without insulin, there was a 35.6% and 43.8% decrease in apo B output in the normal and

Table 2. Effect of insulin on hepatic apo B secretion in normal rats

Apo B secretion rates in liver-perfusion studies were calculated on the basis of accumulated apo B in perfusates in 24 h. Results were adjusted to g of protein and are expressed as μ g of apo B secreted/h per g of liver protein. At 4 h after plating of hepatocytes, the medium was removed and cells were washed three times in phosphate-buffered saline containing 0.1% bovine serum albumin. Then 2.0–3.0 ml of Waymouth's medium was added, followed by addition of insulin and cortisol to give initial concentrations of 10 nM and 100 nM respectively. The cells were incubated for 12–14 h and media were collected and apo B was assayed by monoclonal-antibody immunoassay. Apo B secretion rates, calculated on the basis of accumulated apo B in 12–14 h cultures, were adjusted to g of cell protein per dish and are expressed as μ g of apo B secreted per g of liver protein. Values marked * and ** indicate that apo B secretion rates without insulin are significantly different from those with insulin at $P < 0.05$ and $P < 0.025$ respectively.

	Apo B secreted (μ g/h per g of liver protein)		
	Liver perfusion	Primary hepatocytes	
	Cortisol ... (+)	(–)	(+)
No insulin	77.8 ± 10.0 ($n = 7$)	108.7 ± 9.7 ($n = 6$)	120.9 ± 2.2 ($n = 3$)
+ insulin	$50.1 \pm 6.1^{**}$ ($n = 11$)	$68.7 \pm 6.5^{**}$ ($n = 6$)	$84.4 \pm 7.7^*$ ($n = 3$)
Inhibition ...	35.6 %	36.8 %	30.2 %

Table 3. Effect of insulin on hepatic apo B secretion in diabetic rats

Apo B secretion rates in liver-perfusion studies were calculated on the basis of accumulated apo B perfusates in 24 h. Results were adjusted to g of protein and are expressed as μg of apo B secreted/h per g of liver protein. At 4 h after plating of hepatocytes, the medium was removed and cells were washed three times in phosphate-buffered saline containing 0.1% bovine serum albumin. Then 2.0–3.0 ml of Waymouth's medium was added, followed by addition of insulin and cortisol to give initial concentrations of 10 nM and 100 nM respectively. The cells were incubated for 12–14 h, and media were collected and apo B was assayed by monoclonal-antibody immunoassay. Apo B secretion rates, calculated on the basis of accumulated apo B in 12–14 h cultures, were adjusted to g of cell protein per dish and are expressed as μg of apo B secreted per g of liver protein. Values marked * indicate that apo B secretion rates without insulin are significantly different from those with insulin at $P < 0.05$.

Cortisol ...	Apo B secreted ($\mu\text{g}/\text{h}$ per g of liver protein)		
	Liver perfusion	Primary hepatocytes	
	(+)	(–)	(+)
No insulin	9.2 ± 0.5 ($n = 4$)	30.8 ± 4.7 ($n = 5$)	24.9 ± 3.4 ($n = 5$)
+ insulin	11.2 ± 0.5 ($n = 5$)	$13.2 \pm 4.4^*$ ($n = 5$)	27.4 ± 4.8 ($n = 5$)
Inhibition ...	–	57.1 %	–

treated-diabetic rat liver and no apparent effect on apo B secretion by diabetic rat livers (Tables 2, 3 and 4). Although insulin alone in the medium inhibits apo B production by hepatocytes from diabetic rats, when cortisol is present simultaneously in the medium the inhibitory effect is lost. Further studies are required to determine the mechanisms involved.

Effect of insulin on the secretion of apo B by primary cultures of rat hepatocytes

The individual effect of insulin on apo B secretion was examined in parallel experiments using primary cultures of hepatocytes from normal, diabetic and treated-diabetic rats. Apo B secretion rates in liver perfusion and in primary cultures of rat hepatocytes were calculated and are compared in Tables 2, 3 and 4. Apo B secretion rates by hepatocytes were higher than those of perfused livers. This may relate, in part, to the presence in whole livers of non-apo B-secreting cells (Kupffer cells etc.), which would be included in the protein measurement. Apo B secretion by primary cultures of hepatocytes from normal, diabetic and treated-diabetic rats was decreased by 36.8%, 57.1% and 57.9% respectively when medium contained insulin alone.

Cortisol effects on apo B secretion

The individual effect of cortisol on apo B secretion was studied in parallel experiments using primary cultures of hepatocytes from normal, diabetic and treated-diabetic rats. In the absence of insulin, cortisol had little effect on apo B secretion by hepatocytes in any group. In the presence of insulin in hepatocytes and in liver perfusions

Table 4. Effect of insulin on hepatic apo B secretion in treated diabetic rats

Apo B secretion rates in liver-perfusion studies were calculated on the basis of accumulated apo B in perfusates in 24 h. Results were adjusted to g of protein and are expressed as μg of apo B secreted/h per g of liver protein. At 4 h after plating of hepatocytes, the medium was removed and cells were washed three times in phosphate-buffered saline containing 0.1% bovine serum albumin. Then 2.0–3.0 ml of Waymouth's medium was added, followed by addition of insulin and cortisol to give initial concentrations of 10 nM and 100 nM respectively. The cells were incubated for 12–14 h, and media were collected and apo B was assayed by monoclonal-antibody immunoassay. Apo B secretion rates, calculated on the basis of accumulated apo B in 12–14 h cultures, were adjusted to g of cell protein per dish and are expressed as μg of apo B secreted per g of liver protein. Values marked * indicate that apo B secretion rates without insulin are significantly different from those with insulin at $P < 0.005$.

Cortisol ...	Apo B secreted ($\mu\text{g}/\text{h}$ per g of liver protein)		
	Liver perfusion	Primary hepatocytes	
	(+)	(–)	(+)
No insulin	43.8 ± 3.9 ($n = 5$)	74.6 ± 7.9 ($n = 6$)	60.0 ± 3.6 ($n = 3$)
+ insulin	$24.6 \pm 3.5^*$ ($n = 9$)	$31.4 \pm 3.2^*$ ($n = 6$)	$31.7 \pm 3.3^*$ ($n = 3$)
Inhibition ...	43.8 %	57.9 %	47.2 %

of control and of treated-diabetic rats, cortisol did not appear to influence the inhibitory effect of insulin on apo B secretion. In liver perfusions or hepatocytes from diabetic rats, apo B secretion rates were unchanged in the presence of insulin plus cortisol. In contrast, apo B secretion was inhibited in hepatocytes from diabetic rats when cultured with insulin alone. The reason for this 'insulin resistance' to apo B secretion in the diabetic state in the presence of cortisol requires further study.

Roles of insulin in apo B metabolism

We have recently proposed more than one role for insulin in apo B metabolism [8]. The first role is to maintain normal levels of apo B synthesis and secretion. An additional role is to inhibit hepatic apo B production at postprandial insulin concentrations [6]. Considering that the increased lipid synthesis stimulated by insulin occurs at a time when there is a decrease in apo B, there would be more efficient packaging of the lipoprotein particle. To the extent that lipid composition of the lipoprotein affects its ultimate catabolism, the changes in particle composition could have potential consequences related to catabolic pathways. As insulin decreases between meals, hepatic VLDL-apo B secretion increases, promoting lipid delivery to peripheral tissues during the inter-prandial period. When insulin-mediated suppression of hepatic apo B production is decreased, as in long-term hypoinsulinaemic states and possibly insulin-resistant states, apo B production by the liver is released from suppression and is controlled by other factors such as changes in non-esterified fatty acid flux. A similar hypothesis of insulin-mediated suppression of

hepatic triacylglycerol release has been suggested by Gibbons [40] to explain the increased hepatic triacylglycerol secretion observed in some insulin-resistant states.

We have shown previously in rat hepatocyte cultures [6] the dose-dependent effects of insulin on the stimulation of labelled acetate incorporation into triacylglycerol concomitantly with inhibition of apo B synthesis. At low insulin concentrations the effect was primarily on lipid, resulting in triacylglycerol label accumulation in the medium. At higher concentrations, which significantly inhibited apo B, insulin in the medium resulted in lipid accumulation within cells and inhibition of triacylglycerol secretion into the medium. We have hypothesized that under these circumstances apo B may be rate-limiting with respect to VLDL assembly and secretion. Thus, if one measures VLDL-triacylglycerol secretion into the medium, insulin may appear to have a biphasic effect, whereas in fact total triacylglycerol synthesis is increased and apo B secretion is decreased at post-prandial insulin concentrations. We have chosen apo B as an end-point for these reasons, but for these considerations future studies will need to be done which focus on insulin effects on triacylglycerol/apo B ratio as a reflection of particle composition, and studies will need to include both media and cellular measurements. Unregulated hepatic apo B and triacylglycerol production could result in increases in plasma apo B-containing lipoproteins, which could constitute an increased atherogenesis risk factor, as has been observed in certain forms of diabetes [41]. The exact mechanism of the insulin-mediated suppression of apo B production remains to be determined. In this context, it is noteworthy that we have recently demonstrated that apo B_H and apo B_L are secreted by primary cultures of hepatocytes as phosphoproteins containing phosphoserine and phosphotyrosine residues [42]. An appealing hypothesis is that hormonal regulation of hepatic apo B production relates to the phosphorylation state of apo B.

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