Restoration *in vitro* of normal rates of very-low-density lipoprotein triacylglycerol and apoprotein B secretion in hepatocyte cultures from diabetic rats

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Hepatocytes derived from diabetic rats were cultured in serum-free Waymouth's medium containing various supplements, after an initial 4 h period during which the cells were allowed to attach to the culture dish in the presence of foetal-bovine serum (10%). After removal of serum, these cells secreted much less very-low-density lipoprotein (VLDL) apoprotein B (apoB) and triacylglycerol than those derived from normal rats when cultured for 24 h in the basal medium. Inclusion of oleate (0.75 mM) in the medium initially increased the output of apoB and triacylglycerol, but the rates remained lower than those observed in normal hepatocytes and declined to zero after 72 h. This time-dependent decline in VLDL output was prevented by addition of dexamethasone to the oleate-containing medium. Levels of apoB and triacylglycerol output characteristic of normal hepatocytes could only be completely restored, however, by further addition of a

mixture of lipogenic substrates (lactate plus pyruvate) to the medium. Restoration of normal levels of VLDL secretion in diabetic hepatocytes *in vitro* by this means was accompanied by a normal inhibitory response of apoB and triacylglycerol output to short-term (24 h) treatment with insulin or glucagon. Exposure of the cells to insulin for 72 h enhanced the secretion of VLDL, whereas treatment with glucagon for the same period potentiated the original inhibitory effect. The defective secretion of VLDL apoB observed when diabetic hepatocytes were cultured in the basal medium for 24 h could also be rectified by inclusion of a mixture of oleate (0.75 mM), lactate (10 mM), pyruvate (1 mM), dexamethasone (1 μ M) and insulin (78 nM) in the medium during the 4 h attachment period in the presence of serum. Under these conditions, the increase in the secretory response of triacylglycerol was not so pronounced.

INTRODUCTION

Isolated liver preparations derived from insulin-deficient rats secrete less very-low-density-lipoprotein (VLDL)-associated triacylglycerol [1–4] and apoprotein B (apoB) [5–7] than do those from normal animals. This results, at least in part, from a decreased rate of translation of apoB mRNA [8]. Attempts to restore normal VLDL secretion rates by direct treatment of such preparations with insulin *in vitro* have not been successful [1, 4, 5]. By contrast, however, VLDL secretion was restored to normal in isolated liver preparations derived from diabetic animals previously treated with insulin *in vivo* [1, 5]. These discrepant effects *in vivo* and *in vitro* suggest that rectification by insulin of defective VLDL assembly and/or secretion in diabetic animals occurs by an indirect rather than by a direct mechanism. In other words, *in vivo* insulin mediates the production of some factor(s) which is directly responsible for the stimulation of hepatic VLDL production. The aim of the present work is to provide information about the mechanism of the indirect effect by simulating conditions *in vitro* in which the rate of VLDL apoB and triacylglycerol secretion in hepatocytes derived from diabetic animals is restored to a normal level.

MATERIALS AND METHODS

Maintenance of animals

Male Wistar rats were fed and housed as described previously [9]. Non-ketotic insulin deficiency was induced by streptozotocin injection via the tail vein (50 mg/kg body wt.) in 0.01 M citrate

buffer (pH 4.5) under light diethyl ether anaesthesia. Since hypoglycaemia occurs 7 h after injection of the drug, glucose was added to the drinking water between 7 and 20 h after streptozotocin treatment [10]. The mean plasma glucose concentration of the experimental diabetic animals was 21.30 ± 1.6 mM. Animals were used 5 days after streptozotocin treatment.

Preparation of hepatocyte cultures and incubation procedures

Hepatocytes were prepared under sterile conditions and were plated out (3.0 ml/dish) after suspension $[(0.5–0.75) \times 10^6$ cells/ml] in Waymouth's medium MB752/1 containing added amino acids, antibiotics and heat-inactivated foetal-bovine serum [11]. At the time of plating some of the dishes were supplemented with oleate (0.75 mM), dexamethasone (1 μ M), lactate (10 mM), pyruvate (1 mM) and insulin (78 nM). After the initial 4 h period, the cells became attached to the dish as a monolayer. The medium was removed and the cells washed twice with 3.0 ml of Dulbecco's PBS [12]. Basal medium was then added, followed by supplements added singly or in combination where necessary. When present, these supplements were added to give initial concentrations of dexamethasone (1 μ M), lactate (10 mM), pyruvate (1 mM) and oleate (0.75 mM). A combination of all these supplements is referred to as the fully supplemented medium ('FSM'). In some cases insulin or glucagon were added to give initial concentrations of 78 nM and 0.1 μ M respectively. Hepatocytes were cultured for periods of 24 h (Day 1), 48 h (Day 2) or 72 h (Day 3). At the end of each 24 h period, the experiment was either terminated by removal of the medium and harvesting the cells (see below) or continued after removal of the medium,

followed by addition of medium of composition identical with that used previously. In each case the amount of VLDL secreted into the medium during the previous 24 h period was determined.

Determination of VLDL triacylglycerol and cellular triacylglycerol

VLDL was isolated from the cell medium as described previously [9]. The cell monolayer was washed with PBS and the cells were removed from the dish [12]. Portions of the cell pellet and of the VLDL-containing fraction were used for the extraction of total lipids [11], and portions of these extracts were used for the enzymic determination of triacylglycerol [13]. This assay utilized a kit from Boehringer-Mannheim (Triglycerides GPO-PAP). Where required, the triacylglycerol was purified from the total lipid extract by t.l.c. [14]. In all cases, manipulative losses of triacylglycerol were accounted for by addition of glycerol [^{14}C]trioleate as internal standard.

Determination of VLDL apoB by an e.l.i.s.a. assay

Rat plasma VLDL apoB was used to prepare standard curves for these assays. This was obtained by treatment of rats with Triton WR1339 injected via the tail-vein [15]. VLDL-enriched plasma was obtained 3.5 h later and the VLDL fraction obtained by ultracentrifugation. SDS/PAGE of a portion of the VLDL fraction on a 15%-acrylamide gel showed that the major apoproteins present were apoB₁₀₀ and apoB₄₈, with the latter present in the greatest amount (results not shown). Neither apoE nor apoC was present in this preparation, in accordance with a previous observation [16] that Triton removes peripheral apoproteins from VLDL. Thus the protein content of the VLDL is exclusively apoB, and this was determined by Coomassie Blue dye binding [17]. An aliquot of the VLDL standard was diluted with PBS (10 mM Na_2HPO_4 , 3.7 mM NaH_2PO_4 , 0.15 M NaCl, 1.5 mM NaN_3) containing Tween 20 (0.05%) to give a protein concentration of approx. 5 $\mu\text{g}/\text{ml}$. A range of dilutions was prepared, and 0.1 ml of each dilution (containing approx. 20–200 ng of apoB) was transferred in triplicate to the wells of an e.l.i.s.a. plate (Nunc-Immuno, Inter-Med, Denmark). PBS containing Tween 20 was added to 0.3 ml of the hepatocyte-secreted VLDL to give a concentration identical with that in the standards. Secreted VLDL samples (0.1 ml of each) were then pipetted, in duplicate, into the wells of the plate. The samples were kept at 4 °C overnight to allow binding of the apoB to the plate. Excess unbound material was removed by sharply inverting the plate, and the coated wells were washed three times with 0.05% Tween/PBS. Anti-(human apoB) antiserum (raised in sheep) was diluted 100-fold with 0.05% Tween/PBS. A 0.1 ml portion of this solution was added to each well and the plates were kept at room temperature for 2 h. Excess unbound antibody was removed, and the wells were washed three times as described above. Anti-sheep IgG (raised in donkey) linked to alkaline phosphatase was diluted 1000-fold with 0.05% Tween/PBS, 0.1 ml of this solution was added to each well and the plate was kept at room temperature for 2 h. Excess antibody was removed, and the plates were washed three times with PBS only. Finally, 200 μl of substrate solution (1 mg/ml *p*-nitrophenyl phosphate in 0.04 M Na_2CO_3 /0.06 M NaHCO_3 buffer, pH 9.6, containing 0.5 mM MgCl_2) was added to each well. After 30 min at room temperature, the A_{405} of each well was measured (reference filter at 620 nm) by using an Anthos Reader 2001 (Anthos Labtec Instruments). A typical standard curve is shown in Figure 1.

Figure 1 Standard curve for e.l.i.s.a. assay of apoB

For details see the Materials and methods section.

Other methods

Cellular protein was measured by the method of Lowry et al. [18]. Oleate bound to BSA (essentially fatty-acid-free) was prepared by the method of Van Harken et al. [19]. The BSA concentration in the culture medium was 0.5%.

Statistical methods

All values are expressed as means $\pm$ S.E.M. of several independent observations. The degree of significance of observed differences was tested by either a paired or unpaired *t* test.

Materials

All radiochemicals were obtained from Amersham International (Little Chalfont, Bucks., U.K.). The anti-(human apoB) antiserum was obtained from Boehringer-Mannheim, and the anti-sheep IgG was obtained from Sigma (Poole, Dorset, U.K.).

RESULTS AND DISCUSSION

Manipulation of the culture medium of hepatocytes derived from diabetic rats revealed synergistic interactions between glucocorticoids, lipogenic substrates and fatty acids, a combination of which was capable of rectifying defects in the assembly and/or the secretion of VLDL particles. For instance, during the first 24 h in serum-free basal medium (Day 1) diabetic-rat hepatocytes secreted 11.0 nmol of VLDL triacylglycerol/mg of protein (Table 1). This was similar to that observed in a previous study [4] and was an order of magnitude lower than that secreted by hepatocytes from normal animals under identical conditions (Table 2). There was a similar decrease in the rate of secretion of VLDL apoB in the hepatocytes derived from the diabetic animals compared with that from the normal (Tables 1 and 2) when cultured in the serum-free basal medium. Decreased rates of VLDL and apoB secretion have also been observed in isolated perfused livers and in cultured hepatocytes derived from diabetic rats [1–8]. In the present work, when the culture period was prolonged for a further 48 h, during which the basal medium was replaced with fresh medium at 24 h intervals (Day 2 and Day 3), the already low rates of apoB and triacylglycerol secretion in the diabetic-rat hepatocytes decreased to zero (Table 1). Addition of dexamethasone to the medium of cultured hepatocytes derived from normal animals has previously been shown to increase the

Table 1 Stimulation of VLDL apoB and triacylglycerol output by hepatocytes from diabetic rats by supplementation of the culture medium *in vitro*

Hepatocytes from diabetic rats were cultured for periods of 24 h, 48 h or 72 h, either in basal Waymouth's medium, or in basal medium plus oleate, mixtures of dexamethasone, lactate and pyruvate, oleate plus dexamethasone or oleate plus dexamethasone plus lactate and pyruvate. This latter is the fully supplemented medium (FSM). For culture periods lasting longer than 24 h, the medium was removed and replaced with fresh medium of identical composition every 24 h. Measurements of secreted VLDL apoB and triacylglycerol were made during the final 24 h of each culture period (i.e. Day 1, Day 2 or Day 3). Units for apoB are $\mu\text{g}/\text{mg}$ of protein. Units for triacylglycerol are nmol/mg of protein. The values represent means $\pm$ S.E.M. of between 3 and 5 individual cell preparations, except in specific instances in which two preparations only were used (number in parentheses). Values marked * and ** are significantly different ($P < 0.05$ and $P < 0.01$ respectively by paired *t* test) from the corresponding values obtained in the basal medium alone. Abbreviations: O, oleate; D, dexamethasone; LP, lactate + pyruvate; TAG, triacylglycerol.

Additions to basal medium	Day 1		Day 2		Day 3	
	apoB	TAG	apoB	TAG	apoB	TAG
—	1.04 $\pm$ 0.64	11.0 $\pm$ 5.6	0.18 $\pm$ 0.18	1.0 $\pm$ 1.0	0 $\pm$ 0	0 $\pm$ 0
D + LP	5.35 $\pm$ 2.09*	74.6 $\pm$ 34.2*	1.59 $\pm$ 0.59	43.4 $\pm$ 20.1	0 (2)	0 (2)
O	2.63 $\pm$ 0.59	36.6 $\pm$ 4.4	1.04 $\pm$ 0.42	12.4 $\pm$ 6.7	0.07 $\pm$ 0.02	0 $\pm$ 0
O + D	3.77 $\pm$ 1.59*	41.8 $\pm$ 8.0*	2.75 $\pm$ 0.12**	84.4 $\pm$ 24.1*	0.89 $\pm$ 0.24*	62.4 $\pm$ 26.6*
O + D + LP (FSM)	6.50 $\pm$ 2.25*	130.7 $\pm$ 43.8*	2.31 $\pm$ 0.54*	119.3 $\pm$ 35.0*	1.05 $\pm$ 0.20*	142.1 $\pm$ 61.8*

Table 2 VLDL triacylglycerol secretion by hepatocytes from normal rats: effects of supplementation of the culture medium

Hepatocytes from normal rats were cultured for 24 h either in basal Waymouth's medium or with supplements (see the legend to Table 1). The values represent means $\pm$ S.E.M. of several individual cell preparations (numbers in parentheses). The value marked * is significantly different from the control (no additions). Abbreviations: O, oleate; D, dexamethasone; LP, lactate + pyruvate.

Addition(s) to basal medium	VLDL triacylglycerol output (nmol/mg of protein)	VLDL apoB output ($\mu\text{g}/\text{mg}$ of protein)
—	148 $\pm$ 24 (4)	7.00 $\pm$ 2.10 (3)
D + LP	215 $\pm$ 32* (4)	4.48 $\pm$ 0.82 (3)
O	178 $\pm$ 33 (4)	5.11 $\pm$ 1.46 (3)
O + LP	178 $\pm$ 33 (4)	4.89 (1)
O + D + LP (FSM)	177 $\pm$ 31 (7)	5.16 $\pm$ 0.89 (6)

Figure 2 Cellular triacylglycerol levels in hepatocytes from diabetic rats

For details of culture conditions, see legend to Table 1: $\triangle$, basal medium alone; $\bullet$, basal medium + oleate; $\circ$, basal medium + oleate + dexamethasone; $\blacksquare$, basal medium + oleate + dexamethasone + lactate + pyruvate; $\blacktriangle$, basal medium + oleate + dexamethasone + lactate + pyruvate.

rate of secretion of VLDL [20,21]. This effect was enhanced by the simultaneous presence of the lipogenic precursors, lactate and pyruvate [11]. However, in hepatocytes from diabetic rats, although addition of dexamethasone alone enhanced VLDL triacylglycerol secretion [4], this increased rate remained well below that observed in normal hepatocytes. In the present work, supplementation of the basal medium with a mixture of dexamethasone and lipogenic substrates (lactate plus pyruvate) produced a 5–7-fold increase in the amounts of apoB and triacylglycerol which were secreted during the first 24 h in culture. This may have been due to a potentiation by dexamethasone of lipogenesis *de novo* from lactate and pyruvate [22]. A similar combination led to only a 50% increase in VLDL triacylglycerol secretion in normal hepatocytes and there was no increase in apoB secretion (Table 2). Addition of oleate to the medium containing dexamethasone and lipogenic substrates led to a further increase ($P < 0.01$) in the rate of apoB secretion (Table 1). This may have resulted from a maximization of the rate of cellular triacylglycerol synthesis (Fig. 2). Under these conditions, the diabetic-rat hepatocytes secreted as much apoB as did hepatocytes from normal animals cultured under identical conditions (Table 2).

When oleate alone was present in the culture medium of hepatocytes from diabetic rats, the initial rates (i.e. on Day 1) of apoB and triacylglycerol secretion could not be maintained during longer culture periods (i.e. Days 2 and 3; Table 1). Addition of dexamethasone to the oleate-containing medium prevented the time-dependent decline in the secretion of triacylglycerol and, to a lesser extent, that of apoB. Although cells cultured for 72 h in the presence of either oleate or a mixture of dexamethasone plus lipogenic substrates were unable to secrete significant amounts of apoB and triacylglycerol, a combination of these conditions forming the fully supplemented medium produced a large increase in the secretion of these components of VLDL (Table 1). The decrease in the secretion of VLDL with time in the absence of the full complement of supplements was not due to a general decline in the metabolic performance or viability of the cells. This was evidenced by examination under the light microscope, in which the cells remained flattened and a brightly reflective monolayer was maintained. Neither was there any decline in the rate of triacylglycerol synthesis as measured by the increase in the accumulated totals of VLDL-triacylglycerol and cellular triacylglycerol over the 3-day period. For instance, in the presence of oleate alone, when the secretion of VLDL

Table 3 Effects of initial pre-treatment of hepatocytes from diabetic rats on their subsequent rates of VLDL apoB and triacylglycerol secretion

Hepatocytes were suspended in basal Waymouth's medium containing 10% foetal-bovine serum and, in addition, insulin (73 nM), dexamethasone (1 μ M), oleate (0.75 mM), lactate (10.0 mM) and pyruvate (1 mM), and transferred to culture dishes. After 4 h, the medium was removed, the monolayer was washed twice with PBS and basal Waymouth's medium was added. A mixture of dexamethasone and lipogenic substrates or a mixture of dexamethasone, lipogenic substrates and oleate were added where appropriate. The amounts of VLDL triacylglycerol and apoB secreted into the medium during the next 24 h were determined. The values represent means $\pm$ S.E.M. of between 3 and 5 independent hepatocyte preparations. Abbreviations: O, oleate; D, dexamethasone; LP, lactate + pyruvate.

Additions to basal medium	Secretion of VLDL	
	apoB (μ g/mg of protein)	Triacylglycerol (nmol/mg of protein)
—	5.73 $\pm$ 0.59	47.0 $\pm$ 7.0
D + LP	6.75 $\pm$ 1.33	74.0 $\pm$ 13.5
D + LP + O (FSM)	4.59 $\pm$ 1.33	105.7 $\pm$ 26.4

decreased to negligible levels (Table 1), these values were (in μ g/mg of cell protein): Day 1, 109 $\pm$ 9; Day 2, 244 $\pm$ 48; Day 3, 364 $\pm$ 90.

In general, the ability of the diabetic-rat cells to secrete apoB and triacylglycerol was related to the intracellular triacylglycerol content (Table 1 and Figure 2). This relationship has been noted previously for normal hepatocytes [23]. However, this factor alone was incapable of sustaining a significant output of VLDL in the diabetic cells, since, after 72 h of culture, although there was no significant difference in the intracellular content of triacylglycerol in cells cultured with dexamethasone plus oleate compared with those containing oleate alone (Figure 2), under the latter conditions apoB and triacylglycerol outputs were negligible (Table 1).

As described above, immediately after preparation the cells were allowed to attach to the dish for 4 h in the presence of foetal-bovine serum. Other supplements were normally absent at this stage. If, however, all the supplements plus insulin were present during this 4 h period, their subsequent removal and replacement with basal medium resulted in a greatly enhanced secretion of apoB during the next 24 h period ($P < 0.01$; Table 3), compared with that observed without this pre-treatment (Table 1). This enhanced rate equalled that observed in normal hepatocytes which had not received this pre-treatment (Table 2) and could not be further stimulated by the inclusion of further

supplements (e.g. lipogenic substrates etc.) to the basal medium during the 24 h culture period (Table 3). Thus a short treatment *in vitro* of hepatocytes from diabetic rats in this way was sufficient to rectify the defect in apoB secretion caused by insulin deficiency *in vivo* and to sustain a normal rate of secretion in an unsupplemented medium.

Previous studies using hepatocytes from diabetic rats have shown that treatment with insulin alone *in vitro* was incapable of restoring normal rates of lipogenesis *de novo*, whereas insulin administered to the intact diabetic rats markedly enhanced lipogenesis in subsequently cultured hepatocytes [24]. It was suggested [24] that *in vivo* insulin action was indirect and mediated by some factor(s), which then directly stimulate(s) hepatic lipogenesis. Similarly disparate results between the effects of insulin *in vivo* and in tissue culture also arise at the level of gene expression, and are particularly evident as regards the expression of genes for *c-fos*, fatty acid synthetase, malic enzyme and albumin (for a review see [25]). As mentioned above, a similar discrepancy arises between the effects of insulin *in vitro* and *in vivo* on hepatic VLDL assembly and/or secretion in diabetic rats [1, 5] and it is possible that, at least for lipogenesis *de novo* and VLDL production, this putative mediator(s) of insulin action is the same. However, the presence of lipogenic substrates alone had little effect on lipogenesis *de novo* [24] and VLDL triacylglycerol secretion [4] in hepatocyte cultures from diabetic rats. Neither was the glucocorticoid dexamethasone alone capable of substantially stimulating VLDL triacylglycerol output [4]. The present work suggests, however, that a combination of dexamethasone and lipogenic substrates may have a synergistic effect on the output of VLDL triacylglycerol and apoB, and that this interaction is maximized when oleate is simultaneously present (D + LP versus FSM; Table 1; $P < 0.01$).

Effects of insulin and glucagon

Having restored normal rates of VLDL secretion in the diabetic-rat hepatocytes, we decided to determine whether these cells responded normally to the effects of insulin and glucagon. To check this, hepatocytes cultured in the fully supplemented medium (FSM) were treated with either insulin or glucagon for periods of time ranging from 24 to 72 h. Where appropriate, the medium was changed every 24 h and the amounts of VLDL triacylglycerol and apoB secreted into the medium were determined during the final 24 h of each culture period (Table 4). As observed previously with cells from normal animals [23], the inhibitory effect of glucagon increased with increasing time of culture. In the present work, this was also generally the case (46.5 $\pm$ 9.1%, 11.5 $\pm$ 5.0% and 19.3 $\pm$ 9.4% of control for triacyl-

Table 4 Effects of insulin and glucagon

Hepatocytes from diabetic rats were cultured for periods of 24 h (Day 1), 48 h (Day 2) or 72 h (Day 3) as described in the Materials and methods section. At the end of each 24 h period, the medium was removed and replaced with fresh medium of identical composition. Measurements of VLDL apoB and triacylglycerol were made during the final 24 h of each culture period. Abbreviations: FSM, fully supplemented medium; I, insulin (73 nM); G, glucagon (0.1 μ M); TAG, triacylglycerol. Units for apoB are μ g/mg of protein. Units for triacylglycerol are nmol/mg of protein. The values represent means $\pm$ S.E.M. of between 3 and 5 cell preparations. Values marked * and ** are significantly different ($P < 0.05$ and $P < 0.01$) from those observed in the supplemented medium (paired *t* test).

Conditions	Day 1		Day 2		Day 3	
	apoB	TAG	apoB	TAG	apoB	TAG
FSM	5.60 $\pm$ 1.96	130.5 $\pm$ 43.8	2.31 $\pm$ 0.54	119.2 $\pm$ 35.0	1.05 $\pm$ 0.26	141.9 $\pm$ 61.9
FSM + I	2.46 $\pm$ 0.41*	39.9 $\pm$ 19.0**	1.68 $\pm$ 0.72	35.5 $\pm$ 22.3**	1.73 $\pm$ 0.48	74.1 $\pm$ 51.5**
FSM + G	2.62 $\pm$ 0.81*	55.9 $\pm$ 19.1*	0.62 $\pm$ 0.23*	21.8 $\pm$ 16.4*	0.12 $\pm$ 0.12**	40.0 $\pm$ 21.3*

glycerol and $48.7 \pm 9.1\%$, $24.9 \pm 2.4\%$ and $4.9 \pm 4.9\%$ of control for apoB on Day 1, Day 2 and Day 3 respectively). With insulin, although as with normal cells there was an inhibitory response of triacylglycerol secretion for up to 48 h, unlike normal hepatocytes [9] there was no reversal of this inhibition during the final 24 h of a 72 h period of exposure. By contrast, the insulin response of apoB was identical with that observed in normal animals in which the initial inhibition of apoB secretion was reversed after 72 h exposure to the hormone. This discrepancy between the effects of insulin on the secretion of VLDL apoB and triacylglycerol between 48 and 72 h led to a decrease in the amount of triacylglycerol per VLDL particle (as measured by the change in the triacylglycerol/apoB ratio; medium alone, ratio = 115.7 ± 30.2 ; with insulin, ratio = 28.4 ± 14.0 ; $P < 0.01$ by paired *t* test). In other words, the effect of insulin on day 3 of culture was to promote the secretion of a larger number of smaller VLDL particles.

Finally, the absolute amounts of apoB measured with the anti-(human apoB) antiserum were considerably higher than those measured previously with a monoclonal antibody against rat apoB [23,26]. The reasons for this are uncertain, but may be related to differences in the immunoreactivity of apoB₄₈ and apoB₁₀₀. It is also possible that degradation fragments of apoB are secreted with the VLDL fraction. The monoclonal antibody does not appear to cross-react significantly with these fragments (J. D. Sparks and C. E. Sparks, personal communication), but they may be immunoreactive towards the polyclonal antibody.

Concluding remarks

In conclusion, the present work has shown that it is possible to reverse the inhibitory effects of diabetes on the assembly and secretion of hepatic VLDL by manipulation of a chemically defined culture medium *in vitro*. This reversal of the inhibitory effect did not involve exposure of the cells to insulin, which in itself further inhibited the secretion of VLDL [4]. Whether manipulations described in the present work *in vitro* reflect the mechanism by which insulin administered to diabetic rats *in vivo* restores hepatic VLDL secretion is not known. However, the plasma concentration of lipogenic precursors such as lactate and pyruvate, an essential element of the 'cocktail' used *in vitro*, might be expected to increase as a result of insulin administration. The treated cells responded normally to the inhibitory effect of

glucagon, but, although their initial response to insulin was similar to that of normal cells, longer-term treatment with insulin did not reverse the initial inhibitory effect on the secretion of VLDL triacylglycerol, in contrast with its longer-term effect on apoB.

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