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OBESE ZUCKER (*fa/fa*) RATS ARE RESISTANT TO INSULIN'S INHIBITORY EFFECT ON HEPATIC APO B SECRETION

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Hepatocytes derived from lean Zucker rats have reduced secretion of apo B and lowered cellular apo B in response to a physiologic range of insulin (0.1 nM - 10 nM). Effects are attenuated in hepatocytes derived from Zucker obese rats and seen only at higher insulin concentrations (>100 nM) with a significant shifting of the dose-response curve. Decreased sensitivity and responsiveness of hepatocytes derived from obese rats suggests insulin resistance and dose-response curves are consistent with coexistent binding and post-binding defects. Inability to inhibit hepatic apo B secretion in the presence of short-term high levels of insulin may have important implications to the balance of intestinal and hepatic triglyceride-rich lipoprotein secretion post-prandially.

Insulin inhibits the secretion of apolipoprotein B (apo B) in primary cultures of rat hepatocytes (1-4). Since there is a single molecule of apo B present per lipoprotein particle (5), insulin also decreases the number of lipoprotein particles secreted. Inhibition of apo B secretion by rat hepatocytes is dose-dependent (1-3), receptor-mediated (6) and is the consequence of decreased apo B synthesis and stimulation of intracellular degradation of newly synthesized apo B (3). In rat hepatocyte cultures treated with insulin for longer than 24 h the insulin effect is attenuated (6) and after 48 and 72 h treatment the effect of insulin is lost (4) suggesting that hepatocytes become resistant to insulin after long-term exposure. To examine the effect of *in vivo* long-term hyperinsulinemia we investigated the dose-response effects of insulin on apo B secretion by hepatocytes derived from the genetically obese (*fa/fa*) Zucker rat. The Zucker rat is a well-characterized model of chronic hyperinsulinemia exhibiting insulin resistance (7), glucose intolerance (8) and marked hypertriglyceridemia (9-11). We report here that hepatocytes derived from obese rats are resistant to insulin's inhibitory action on apo B secretion and results suggest that resistance in this pathway may be

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contributory to development of the hypertriglyceridemic state characteristic of the Zucker obese rat.

MATERIALS AND METHODS

Animals

Male, Zucker (obese, homozygous *fa/fa* rats and lean Fa/? rats) were obtained from Charles River Laboratories, Wilmington, MA and from Genetic Models, Inc., Indianapolis, IN. All animals were studied in the fed state with free access to water and rat chow. Perfusions were performed to prepare hepatocytes between 0800 and 1000 h. Obese animals were on average 37 % heavier than lean control animals. Obese animals had elevated insulin levels (3 to 4 times control) and were hypertriglyceridemic (5 to 20 times control) while serum glucose levels were similar.

Hepatocyte preparation

Hepatocytes were isolated by liver perfusion with collagenase, purified using Percoll (12) and were cultured as described previously (13). Briefly, after seeding cells onto collagen-coated 60 mm culture dishes, hepatocytes were incubated for 14 h in Waymouth's medium MB752/1 containing 0.2%, w/v, bovine serum albumin (BSA) and various concentrations of insulin (0, 0.1, 1, 10, 100 and 1000 nM) (4-5 dishes per condition). After incubation, media were collected and cell lysates were prepared using Triton X-100 solubilization as previously described (3).

Apolipoprotein B assay

Apo B concentrations of cell lysates and media were determined by competitive radioimmunoassay using rat VLDL apo B as a standard and with a monoclonal antibody equally reactive to higher and lower molecular weight forms (14). Cellular protein was measured by a modification of the Lowry method (15). Apo B concentrations of cells and media were assayed in triplicate and results were calculated per mg cell protein for each culture dish. The average apo B concentration (cells, media and total) for each initial insulin condition (4-5 dishes per condition) was then calculated for each liver preparation. The average apo B concentration for each condition from each liver was then averaged with corresponding results from other liver preparations and results are expressed as the average of averages ± standard error of the mean (S.E.M). Differences were determined using Student's *t*-test.

RESULTS

Hepatocytes derived from 10-20 week old Zucker lean control and obese rats were incubated in media with various initial concentrations of insulin (0-1000 nM) for 14 h and apo B concentrations in cells and media were analyzed (Tables 1 and 2). In both groups apo B secretion rates as well as cellular apo B content at 10 weeks of age were similar to those at 20 weeks of age. Hepatocytes derived from obese rats secreted slightly more apo B during 14 h incubations and cellular apo B content was higher ($P < 0.05$) compared with leans over the range of insulin concentrations tested. As shown in Table 1, insulin added to culture media inhibited secretion of apo B by hepatocytes derived from lean rats in a dose-dependent manner while reducing cellular apo B content. These results are similar to those described using hepatocytes

Table 1

Apo B Concentration in Cells and Media of Primary Cultures of Hepatocytes Derived From Lean Control Rats

		APO B (ng/mg CELL PROTEIN)		
[INSULIN]	N	MEDIA	CELL	TOTAL
0 nM	9	868 ± 27	230 ± 23	1098 ± 12
0.1 nM	5	869 ± 32	195 ± 22	1064 ± 21
1 nM	5	749 ± 50	173 ± 13	922 ± 45
10 nM	9	595 ± 20	181 ± 20	776 ± 31
100 nM	9	564 ± 23	166 ± 20	730 ± 33
1000 nM	9	574 ± 32	176 ± 17	750 ± 30

Hepatocytes derived from 10-20 week lean control rats were incubated for 14 h with insulin at various initial concentrations (4 to 5 dishes per condition). At the end of the incubation period, media were collected and apo B concentration of cells and media from each dish were radioimmunoassayed and results were normalized per mg cell protein. Results are expressed as the average of averages for each insulin condition ± S.E.M. from 5-9 rats.

derived from male Sprague-Dawley rats (3). A significant reduction in apo B secretion occurred in control cells at 1 nM insulin and at 100 nM insulin in hepatocytes derived from obese rats both compared to no insulin. A maximal effect on apo B secretion occurred in hepatocytes derived from lean rats at 10 nM insulin [mean reduction of 36.5 % ± 8.8 (S.D.); $P < 0.05$]. As shown in Table 2, apo B secretion by hepatocytes derived from obese rats was similar over insulin concentrations ranging from 0-100 nM. A significant reduction in apo B secretion by hepatocytes derived from obese rats was observed at insulin concentrations ≥ 100 nM insulin [mean reduction of 14.6 % ± 4.8 (S.D.); $P < 0.05$]. In hepatocytes derived from lean rats, cellular apo B content was

Table 2

Apo B Concentration in Cells and Media of Primary Cultures of Hepatocytes Derived From Zucker (*fa/fa*) Rats

		APO B (ng/mg CELL PROTEIN)		
[INSULIN]	N	MEDIA	CELL	TOTAL
0 nM	6	986 ± 36	319 ± 33	1305 ± 8
0.1 nM	5	957 ± 30	335 ± 33	1292 ± 10
1 nM	6	968 ± 46	314 ± 25	1282 ± 34
10 nM	6	916 ± 47	332 ± 30	1248 ± 41
100 nM	6	843 ± 39	335 ± 31	1178 ± 37
1000 nM	6	806 ± 32	307 ± 32	1113 ± 18

Hepatocytes derived from 10-20 week old Zucker obese rats were incubated for 14 h with insulin at various initial concentrations (4 to 5 dishes per condition). Results were calculated as described in Table 1.

reduced by 20.7% ($P < 0.05$) by 10 nM insulin whereas no reduction in cellular apo B in hepatocytes derived from obese rats was observed at any insulin concentration tested.

In Fig. 1A the average percent reduction in apo B secretion by hepatocytes derived from lean and obese rats was plotted against insulin concentration. Hepatocytes derived from obese rats demonstrated a rightward shift in the dose-response curve (decreased insulin sensitivity) plus a significant decrease in maximum percent apo B inhibition compared with lean rats (decreased responsiveness). To eliminate the influence of differences in post-receptor effects the absolute values for apo B inhibition were converted to percentage terms (16-18). The maximum inhibition of apo B secretion for lean and obese rats was taken as 100 % and the inhibition of apo B at each sub maximal insulin level was then plotted as a percentage of the maximum value (Fig. 1B). As seen in Fig. 1B, the dose response curve was shifted to the right in hepatocytes derived from obese rats compared with lean controls. The half-maximally effective insulin concentration for hepatocytes derived from lean rats was 1 nM and for hepatocytes derived from obese rats was 40 nM. Results suggest that resistance to insulin's inhibitory effect on hepatocyte apo B secretion in obese rats is due to both a decrease in cellular insulin receptors and a post-receptor binding defect (17,18).

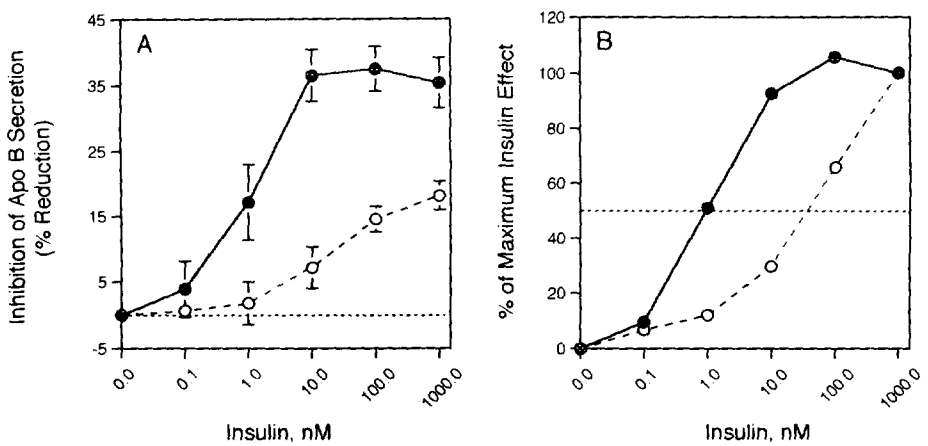

Figure 1. Insulin dose-response curves for inhibition of apo B secretion by hepatocytes derived from lean control rats (●-●-●) and from Zucker obese rats (O-O-O) in 14 h cultures. Panel A: Inhibition of apo B secretion (percent reduction) is plotted against initial insulin concentration in the media in hepatocytes derived from 5 lean control and 5 Zucker obese rats for which complete dose-response curves were generated. Results are the average percent reduction ± S.E.M. The dotted line indicates 0% reduction for reference. **Panel B:** Dose-response curves plotted as a percent of the maximal effect of insulin on apo B secretion. The dotted line for reference indicates 50% of the maximal insulin effect. Comparing lean to obese and measuring insulin concentration in media (Coat-a-Count , Diagnostic Products, Los Angeles, CA) at an initial concentration of 10 nM, final insulin concentrations were within 30% of each other after 14 h incubations.

DISCUSSION

In this study the dose-response effects of insulin on inhibition of hepatic apo B secretion and on reduction of cellular apo B was investigated in hepatocytes derived from Zucker obese (*fa/fa*) rats to determine the effect of chronic hyperinsulinemia *in vivo* on hepatocyte response to insulin. The data indicate that insulin-stimulated inhibition of apo B secretion is attenuated in hepatocytes derived from obese rats and high levels of insulin fail to reduce cellular apo B levels compared with lean controls. Results suggest that, in addition to the well-documented insulin resistance in liver of Zucker rats related to hepatic glucose production (7,19), obese rats are resistant to insulin's inhibitory action on hepatic apo B production.

Analysis of insulin dose-response curves of the inhibition of apo B secretion demonstrates both a rightward shift in the curve (decreased insulin sensitivity) and a decrease in maximal insulin action (decreased insulin responsiveness) in obese rats suggesting the coexistence of both receptor and post-receptor defects. Mild-to-moderate reductions in insulin receptor concentrations of hepatocytes of obese rats have been reported (20-22) although reduced insulin binding is not a consistent finding (23-25). The decrease in maximal insulin action on hepatocyte apo B observed here in obese rats is therefore unlikely to be due to a massive reduction in insulin receptor number (18).

Short-term peak levels of insulin as occur in the post-prandial state are hypothesized to balance hepatic and intestinal triglyceride-rich (TRL) lipoprotein metabolism (26). Resistance in this pathway would have important consequences. First, the inability to reduce hepatic secretion of TRL during the fed state could overload the common triglyceride removal mechanism for chylomicrons and VLDL (27). Saturation of the lipolytic pathway would lead to prolonged plasma residence time of TRL and persistent hypertriglyceridemia (11,28). Second, the secretion of VLDL by liver would no longer be limited by availability of freshly synthesized apo B and may become dependent on other factors such as the size of the hepatic neutral lipid storage pool (29,30). In controls, insulin-inhibited VLDL triglyceride secretion parallels the reduction in apo B secretion in spite of increases in cellular triglyceride synthesis (1,2,4). Over-secretion of VLDL triglyceride is the suggested mechanism for development of hypertriglyceridemia in the obese rat (9-11,31). A third consequence of resistance in this pathway is that increased numbers of VLDL particles may be secreted over the post-prandial period.

As recently suggested by McGarry (32), insulin resistance in pathways involving lipid metabolism may have important consequences in diabetes and insulin resistance syndromes. Consistent with our results are studies by Lewis et al. who demonstrated that chronic hyperinsulinemia in obese women attenuated the ability of insulin to decrease hepatic B100 production using hyperinsulinemic euglycemic clamps (33). The current results demonstrate hepatic insulin resistance in pathways involved in

VLDL production and suggest that the resistance involves both the insulin receptor and postreceptor events.

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