Inhibition of apolipoprotein B100 secretion by lipid-induced hepatic endoplasmic reticulum stress.

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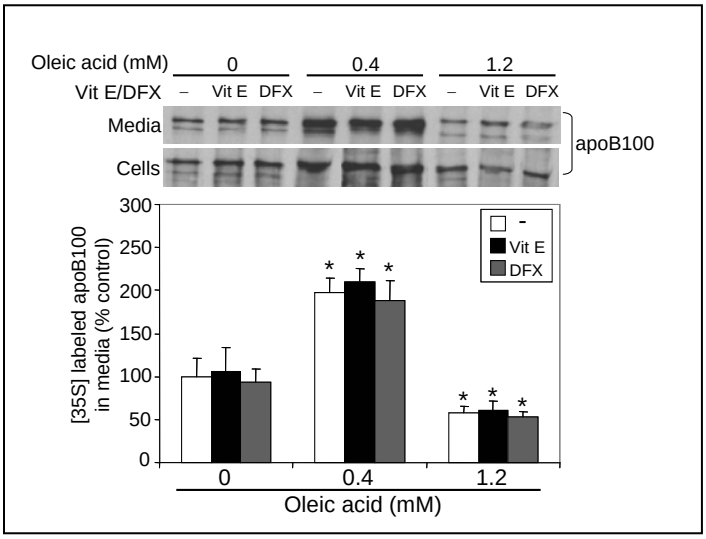
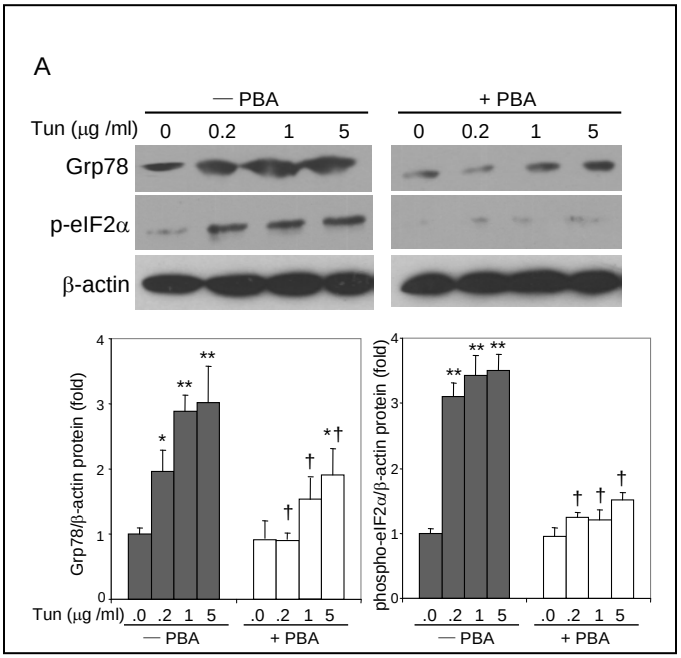
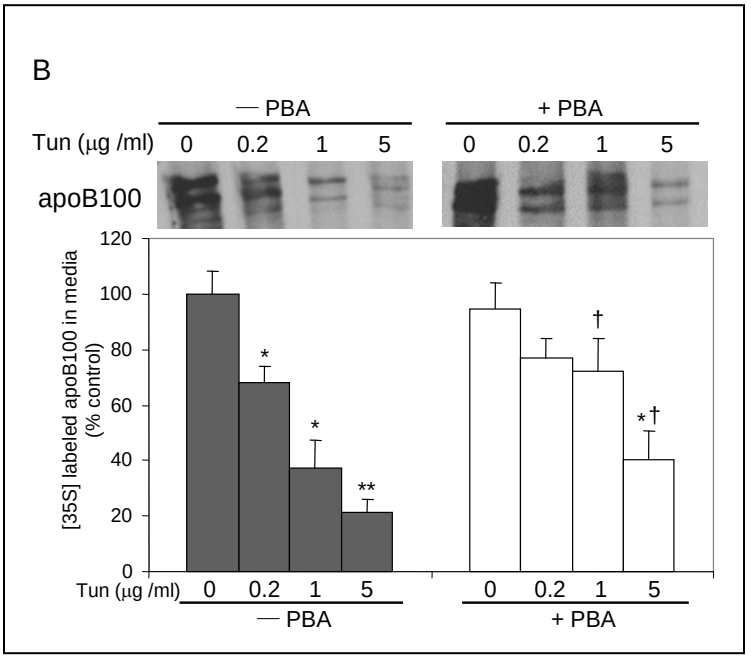
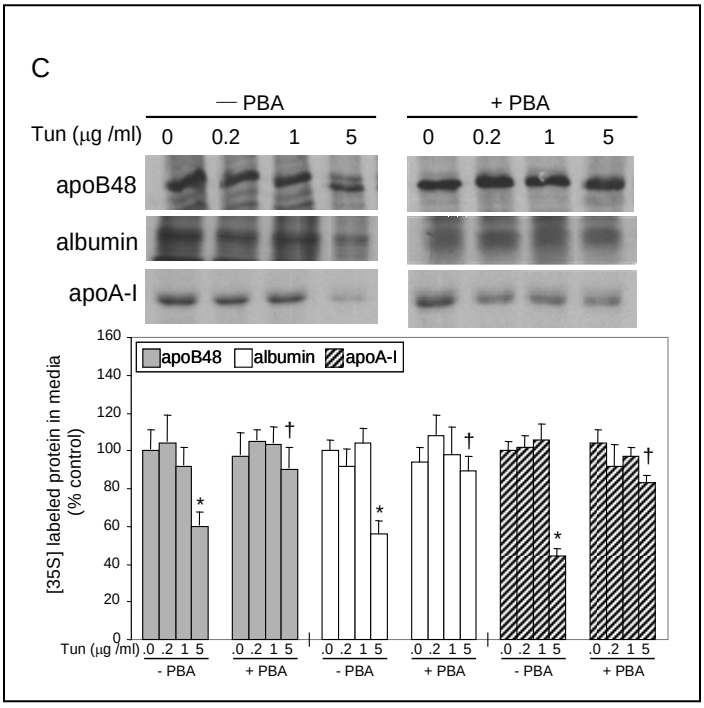
Supplemental Figure S1

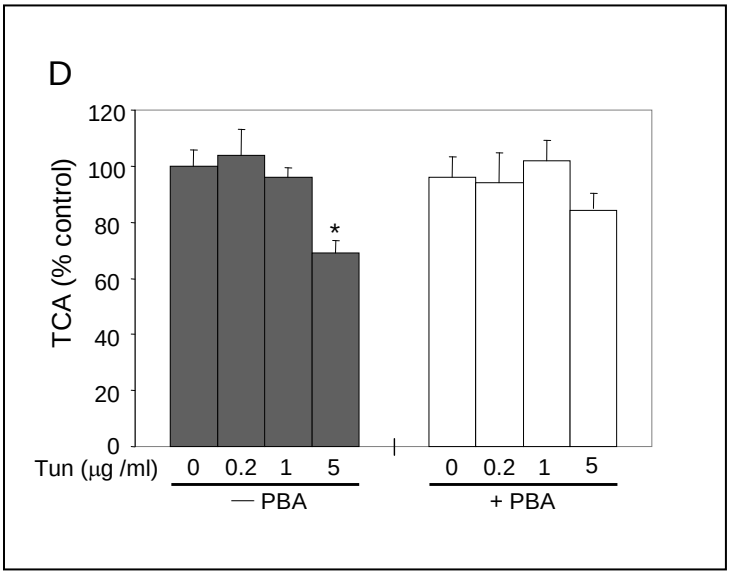


Supplemental Figure S2

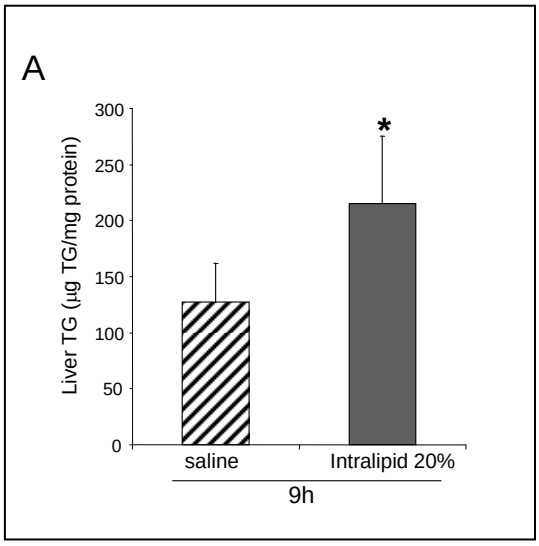


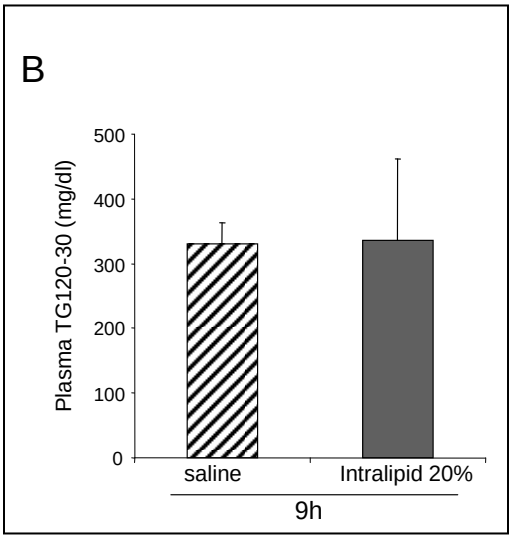


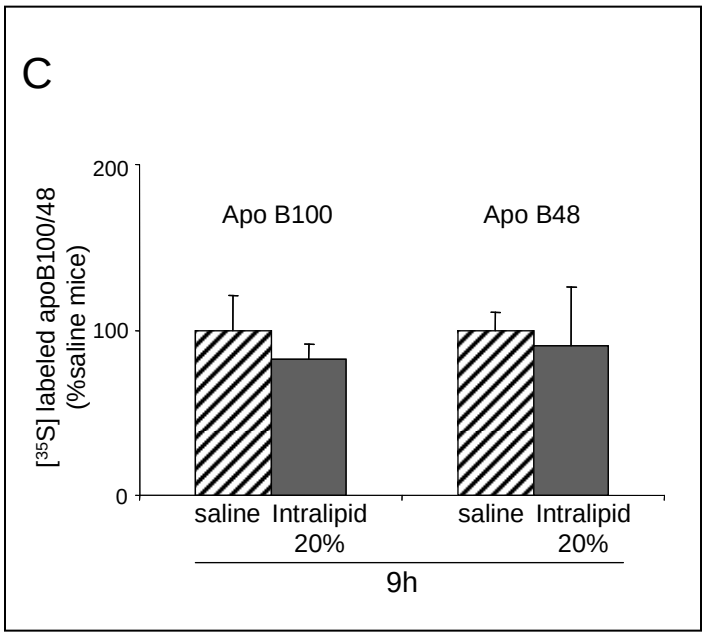




Supplemental Figure S3






C
200
100
0
[35S] labeled apoB100/48
(%saline mice)
Apo B100
Apo B48
saline
Intralipid 20%
saline
Intralipid 20%
9h

Supplemental Figure S1. Neither vitamin E nor desferrioxamine prevents ER stress-associated inhibition of apoB100 secretion. To determine if lipid peroxidation was involved in the non-proteasomal degradation of apoB associated with ER stress, McA cells were treated with OA for 16 hrs, in the presence or absence of either vitamin E (Vit E, 120 μM) or desferrioxamine (DFX, 100 μM) (32). The cells were then incubated in methionine/cysteine-free DMEM for 2 hrs and then [35S]-methionine for 2 hrs. Both of these additional incubations were under the same conditions as the previous 16 hrs. Neither vitamin E nor DFX altered the parabolic effect of increasing concentrations of OA on apoB100 secretion. The data shown are representative of three experiments.

Supplemental Figure S2. Tunicamycin elicits ER stress in a dose dependent manner and this is associated with inhibition of apoB100 secretion that is reversible with PBA. McA cells were incubated with/without PBA (1 mM) for 16 hrs and then with/without PBA plus 0–5 μg/ml tunicamycin (Tun) for an additional 6 hrs. **(A)** Incubations with tunicamycin increased Grp78 protein levels and the levels of phosphorylated eIF2α, as analyzed by immunoblot; activation of these markers of ER stress was significantly blocked by PBA. **(B)** McA cells were incubated with/without PBA (1 mM) for 16 hrs and then with/without PBA plus 0–5 μg/ml tunicamycin for an additional 6 hrs followed by methionine/cysteine-free DMEM for 2 hrs, and then [35S]-methionine for 2 hrs. Tunicamycin decreased apoB100 secretion in a dose dependent manner (left side); PBA treatment restored the secretion of apoB100 at all doses of tunicamycin except 5 μg/ml, where the effect of PBA was only partial (right side). **(C)** Under these same conditions, tunicamycin did not affect apoB48, albumin or apoA-I secretion except at highest concentration (5 μg/ml) (left side); PBA treatment restored the secretion of these proteins at the 5 μg/ml dose of tunicamycin (right side). **(D)** TCA precipitable radioactivity was unaffected except at highest concentration (5 μg/ml) of tunicamycin. However, trypan blue staining was unaffected by all doses of tunicamycin for 6 hrs (data not shown). All data are mean ± SD; n=3 per group.

*P<0.05, **P<0.01 vs incubations in the absence of tunicamycin; †P<0.05 vs incubations with the same concentration of tunicamycin but without PBA.

Supplemental Figure S3. Intravenous infusion of 20% Intralipid for 9 hrs results in steatosis and loss of Intralipid-stimulated apoB and TG secretion. C57BL/6J mice were infused intravenously with saline or Intralipid 20% for 9 hrs (n=4 and n=7, respectively). **(A)** At the end of the 9-hr infusions, mice infused with Intralipid and saline were sacrified and the livers were collected for the measurement of liver TG content. The mean values of liver TG content are expressed as μg of TG/ mg of liver total protein. Infusion of Intralipid for 9 hrs significantly increased liver TG levels compared with the saline. Data are mean ± SD, *p<0.05 versus saline. **(B)** At the end of 9-hr Intralipid infusions, Triton WR1339 was injected intravenously. Blood samples were collected every 30 minutes between the end of the infusion (time 0) and during 2 hours (time 120) to measure plasma TG concentration. The *bar graph* represents the absolute increase in plasma TG levels between 30 and 120 min after Triton injection. 9-hr infusions of Intralipid did not stimulate TG secretion compared to saline. **(C)** [^{35}S]-methionine was injected in the mice at the end of the 9-hr Intralipid infusions to measure the secretion of newly synthesized apoB100 and apoB48. Infusion of Intralipid for 9 hrs did not stimulate either apoB100 or apoB48 secretion compared with saline.