Biochemical and Biophysical Research Communications 430 (2013) 66–71

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Insulin suppression of apolipoprotein B in McArdle RH7777 cells involves increased sortilin 1 interaction and lysosomal targeting

Jeffrey M. Chamberlain, Colleen O'Dell, Charles E. Sparks, Janet D. Sparks *

Department of Pathology & Laboratory Medicine, University of Rochester School of Medicine & Dentistry, Box 626, 601 Elmwood Avenue, Rochester, NY 14642, USA

ARTICLE INFO

Article history:
Received 16 October 2012
Available online 15 November 2012

Keywords:
Insulin
Lipoprotein secretion
Apo B degradation
Bafilomycin A1
Phosphatidylinositide 3-kinase
Sortilin 1

ABSTRACT

Insulin suppresses secretion of very low density lipoprotein (VLDL) apolipoprotein (apo) B in primary rodent hepatocytes (RH) by favoring the degradation of B100, the larger form of apo B, through post-endoplasmic reticulum proteolysis. Sortilin 1 (sort1), a multi-ligand sorting receptor, has been proposed as a mediator of lysosomal B100 degradation by directing B100 in pre-VLDL to lysosomes rather than allowing maturation to VLDL and secretion. The purpose of our studies was to investigate the role of sort1 in insulin-dependent degradation of apo B. Using liver derived McArdle RH7777 (McA) cells, we demonstrate that insulin suppresses VLDL B100 secretion via a phosphatidylinositide 3-kinase (PI3K) dependent process that is inhibitable by wortmannin in a fashion similar to RH. Using McA cells and *in situ* cross-linking, we demonstrate that insulin acutely (30 min) stimulates the interaction of B100 with sort1. The insulin-induced interaction of sort1–B100 is markedly enhanced when lysosomal degradation is inhibited by Bafilomycin A1 (BafA1), an inhibitor of lysosomal acidification. As BafA1 also prevents insulin suppressive effects on apo B secretion, our results suggest that sort1–B100 interaction stimulated by insulin transiently accumulates with BafA1 and favors B100 secretion by default.

1. Introduction

Hypersecretion of apo B-containing very low density lipoprotein (VLDL) by the liver is a hallmark of metabolic syndrome, and a major contributor to the development of hypertriglyceridemia. Insulin negatively regulates VLDL secretion acutely [1], in part, by targeting apo B for post-translational degradation in a post-endoplasmic reticulum (ER) compartment [2]. The process requires insulin activation of phosphatidylinositide 3-kinase (PI3K) [2,3] which translocates to intracellular membranes [4]. Insulin favors the degradation of B100 over B48 [1] which are required proteins for triglyceride-rich lipoprotein (TRL) assembly and are translated from unedited and edited apo B mRNA, respectively [5]. Inhibition of VLDL secretion by insulin minimizes competition between TRL of hepatic and intestinal origin for clearance during the postprandial period [6]. Loss of insulin-mediated regulation of apo B secretion precedes dysregulation of glucose metabolism in obese males [7], and thus may play an early role in dyslipidemia associated with insulin resistance [6].

Degradation of hepatic apo B can occur through a number of processes including ER associated degradation (ERAD), post-ER presecretory proteolysis (PERPP), and receptor-mediated re-uptake [8]. ERAD typically occurs following inadequate lipidation during translation causing B100 to be ubiquitinated and degraded by the proteasome, which is particularly prominent in HepG2 cells [9]. PERPP occurs following incubation of hepatocytes with ω-3 fatty acids [8]. Re-uptake of freshly secreted apo B mainly occurs via binding to the LDL receptor (LDLR), resulting in endosomal trafficking and lysosomal degradation [10]. Insulin stimulated apo B degradation does not occur through ERAD as movement out of the ER is necessary [2], consistent with results of others [11]. Furthermore, insulin-dependent degradation of apo B is maintained in the absence of the LDLR [12], suggesting that the LDLR is not involved. Less is known about the mechanism by which apo B is degraded via PERPP, although recent studies have implicated sortilin 1 (sort1) [13–16]. Sort1 is a multi-ligand sorting receptor that binds B100 during transit through the Golgi apparatus, and also extracellularly at the plasma membrane, resulting in trafficking of B100 to lysosomes for degradation [16]. Over-expression of hepatic sort1 in wild-type mice and restoration of hepatic sort1 expression in *ob/ob* mice results in reduced apo B secretion [15,16]. Binding of sort1 is specific for B100 [14] and B100 degradation is favored with insulin [1], suggesting the possibility

Abbreviations: Apo B, apolipoprotein B; B100, apo B derived from unedited mRNA; VLDL, very low density lipoprotein; PI3K, phosphatidylinositide 3-kinase; ERAD, endoplasmic reticulum associated degradation; PERPP, post ER presecretory proteolysis; BafA1, Bafilomycin A1.

* Corresponding author. Address: Department of Pathology & Laboratory Medicine, University of Rochester Medical Center, Box 626, 601 Elmwood Avenue, Rochester, New York 14642, United States of America. Fax: +1 585 756 5337.
E-mail address: Janet_Sparks@urmc.rochester.edu (J.D. Sparks).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.11.022

that sort1 trafficking may be responsible for insulin-dependent B100 degradation. Data from the current study examining this hypothesis demonstrate that insulin enhances interaction of B100 with sort1 which correlates with suppressed B100 secretion. When insulin suppression of B100 is blocked by inhibiting lysosomal degradation, B100-sort1 interaction is more apparent suggesting interaction as a mechanism for lysosomal B100 targeting.

2. Materials and methods

2.1. Materials

McA cells were obtained from the ATCC (Manassas, VA). Waymouth's 752/1 medium, Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum, horse serum, and most other chemicals including Bafilomycin A1 were from Sigma–Aldrich (St. Louis, MO). Purified bovine serum albumin (BSA) was purchased from Serologicals Proteins Bayer Corp. (Kankakee, IL). Aprotinin solution was purchased from MP Biomedicals (Solon, OH). Sodium orthovanadate was from Fisher Scientific (Fair Lawn, NJ). FuGENE 6 was from Promega Corp. (Madison, WI). Protein A-Sepharose and dithiobis [succinimidyl propionate] (DSP) were obtained from Pierce (Rockland, IL). Protease inhibitor cocktail sets I and III, phosphatase inhibitor cocktail set II, LY 294002, and wortmannin were purchased from Calbiochem (San Diego, CA). PROTEAN®TGX™ SDS polyacrylamide gels (4–15% (w/v) acrylamide), nitrocellulose and PVDF membranes, ECL reagents, and non-fat dry milk were obtained from Bio-Rad Laboratories (Hercules, CA). Rabbit anti-sortilin was purchased from Abcam (Cambridge, MA). Rabbit anti-pAKT (S473) and anti-AKT were purchased from Cell Signaling (Danvers, MA). Mouse anti-glyceraldehyde phosphate dehydrogenase (GAPDH) antibody was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-mouse horseradish peroxidase-linked IgG and Hyperfilm™ were purchased from GE Healthcare (Buckinghamshire, UK). Light chain-specific anti-rabbit horseradish peroxidase-linked IgG was from Jackson ImmunoResearch Labs (West Grove, PA). The plasmid p110* containing constitutively activated mutant PI3K [17] was generously supplied by Dr. Wendy Fantyl, Chiron Corp. (Emeryville, CA). pTracer™-EF/V5-His/lacZ used to assess transfection efficiency was from Invitrogen, Corp. (Carlsbad, CA).

2.2. Cell culture

Primary RH were isolated from Sprague–Dawley rat livers using recirculating collagenase perfusions and cells were cultured on collagen coated dishes as previously described [18]. Wild-type McA cells were seeded onto 100 mm dishes and cultured in complete DMEM (cDMEM) containing 10% (v/v) FBS and 10% (v/v) horse serum, penicillin (100 U/mL) and streptomycin (100 μg/mL), as previously described [19]. After reaching 70–80% confluence, McA cells were washed three times in HBSS containing 0.2% BSA followed by incubation in DMEM containing 1% (w/v) BSA (1% BSA/DMEM) overnight (14–18 h). Experiments were terminated by washing cells three times in ice-cold (4 °C) HBSS containing 0.1 mM sodium orthovanadate and immediately freezing the dishes in liquid nitrogen. McA cells were scraped/thawed on ice into ice-cold lysis buffer containing 1% (v/v) NP40, 25 mM Tris–HCl (pH 7.4), 10% (v/v) glycerol, 150 mM NaCl, 2 mM EDTA, 10 mM tetrasodium pyrophosphate, 2 mM sodium orthovanadate, 1% (v/v) aprotinin, 1% (v/v) phosphatase inhibitor cocktail II, 1% (v/v) protease inhibitor cocktail III, and 1 mM 4-(2-aminoethyl) benzene sulfonyl fluoride (AEBSF). Cellular debris was removed by centrifugation at 17,000*g* for 15 min at 4 °C, and protein concentrations of clarified supernatants were determined using the bicinchoninic acid (BCA) assay (Pierce, Rockland, IL). Lysate proteins were then solubilized in SDS by addition of 1 part 4X Laemmli's gel loading buffer [20] with freshly added DTT (final concentration, 10 mM) per 3 parts lysate followed by heating to 95 °C for 10 min. Samples were stored frozen at −80 °C until further analysis.

2.3. Immunoprecipitation

Apo B was immunoprecipitated from media or clarified cell lysates by addition of polyclonal rabbit anti-rat apo B antibody developed by our laboratory and incubation at 4 °C with end-over-end mixing. Afterwards, Protein A-Sepharose beads were added, and samples were re-incubated to collect immunoprecipitates for 3–4 h at 4 °C. Beads were washed extensively as described [3], and proteins were eluted by addition of 2X Laemmli's buffer with heating at 95 °C for 10 min [20]. Samples were centrifuged briefly to collect eluted proteins, transferred to clean tubes, and stored at −80 °C until further analysis.

2.4. Western blotting

Proteins were separated by SDS–PAGE and electrophoretically transferred to PVDF membranes in Towbin buffer [21]. Non-specific binding was blocked by incubating membranes 2–3 h at 37 °C in TBST buffer (20 mM Tris, pH 7.5, 500 mM NaCl and 0.1% (v/v) Tween 20) containing 5% (w/v) milk. Membranes were incubated with primary antibody overnight at 4 °C with agitation, and after washing membranes 3 times in TBST, were re-incubated for 1 h at room temperature with the appropriate horseradish peroxidase-linked secondary antibody. Membranes were washed twice with TBST followed by several washes with TBS. PhosphoAKT (S473) and AKT mass were evaluated by similar methods except using nitrocellulose membranes and blocking non-specific binding by incubation in 5% (w/v) BSA/TBST. Antibody binding was evaluated by chemiluminescence detection using Hyperfilm™ or imaged directly using the BioRad Laboratories ChemiDocXRS+ system (Hercules, CA). Exposures were digitized and band intensities were quantified using Image Lab 3.0.1 software (Bio-Rad).

2.5. Lipoprotein fractionation

Media lipoproteins secreted by McA cells were separated by sucrose density gradient ultracentrifugation as described [22]. Gradients were centrifuged in a Beckman SW41 Ti rotor at 29,000 rpm for 65 h at 12 °C. Fractions (1 mL) were collected from the top, and apo B in each fraction was immunoprecipitated followed by separation of B100 and B48 by SDS–PAGE and analysis by Western immunoblotting using rabbit anti-rat apo B antibody.

2.6. McArdle RH7777 cell transfections

Transient transfections of McA cells were carried out in 4-well dishes using a 4:1 mixture of plasmid p110* and pTracer™-EF/V5-His/lacZ DNA in 5% (v/v) fetal bovine serum/DMEM and a concentration of FuGENE 6 to DNA of 6:2 according to manufacturer's protocols. Six hours after transfection, medium was removed, McA cells were rinsed in 0.2% (w/v) BSA/HBSS and reincubated in cDMEM containing 1 μM wortmannin for 12 h. McA cells were rinsed three times and half of the cells were incubated in cDMEM containing either 1 μM wortmannin or equivalent volume of DMSO. Cells were terminated; media were collected, and apo B concentrations were measured by RIA. To assess transfection efficiency, in parallel incubations McA cells were terminated by washing in Ca^{2+} and Mg^{2+}-free PBS and following lysis were assayed for β-galactosidase activity using a kit assay (E2000, Promega, Corp., Madison, WI).

2.7. Radioimmunoassay

Media samples were adjusted to contain 1% (v/v) protease inhibitor cocktail I, and apo B concentrations were measured in triplicate using a competitive monoclonal RIA developed against rat apo B and rat VLDL apo B as standard as described previously [23]. Apo B accumulation in the medium was normalized to total cellular protein, and results were calculated per mg cell protein on a dish-by-dish basis.

2.8. Intracellular cross-linking

For cross-linking studies, McA cells were washed twice in HBSS followed by incubation with 2 mM DSP in HBSS or HBSS alone (control) for 1 h at 4 °C. Cross-linking was terminated by addition of Tris–HCl to a final concentration of 25 mM before washing and freezing cells in liquid nitrogen. Cells were scraped into lysis buffer containing 25 mM Tris, pH 7.4, 0.5X PBS, 1% (v/v) NP-40, 0.5% (w/v) Na deoxycholate, 0.1% (w/v) SDS, 0.1% (v/v) aprotinin, 5 mM benzamidine, 2 mM EGTA, 2 mM vanadate, 1 mM AEBSF, 20 nM okadaic acid, and 1% protease inhibitor cocktail III. Lysates were adjusted to 3.5% (w/v) with BSA and cellular apo B was immunoprecipitated as described above. Immunoprecipitated B100 was quantified by immunoblotting using a mouse anti-rat B100 specific monoclonal antibody developed by our laboratory [23].

2.9. Statistics

RIA results are expressed as means ± S.E.M. and are average of averages of 3–5 individual plates for each condition in n replicate experiments. Results for cross-linking studies are expressed as means ± S.D., where n = the number of independent experiments performed. Significant differences were determined using Student's t-test with P-values < 0.05 being considered significant.

3. Results and discussion

We have previously established that insulin suppresses hepatic VLDL apo B secretion [1,24–26] which correlates with activation of PI3K and translocation of activated PI3K to the ER where lipoprotein assembly is initiated [3,4]. The mechanism responsible for reduced secretion involves both reduced synthesis and increased apo B degradation, both of which favor B100 over B48 [1]. This finding is important as we seek to understand mechanisms responsible for development of hypertriglyceridemia in human insulin resistance states. The recent discovery of sort1 as a factor for regulated secretion of hepatic VLDL [13–16] suggests sort1 as a potential mediator of insulin action. In the current study, we utilized serum-deprived McA cells as a hepatic model system to study insulin action on VLDL apo B secretion. Incubation of McA cells with insulin reduced the accumulation of apo B in the medium as measured by RIA by 33 ± 6% ($P < 0.005$, $n = 6$) indicating that McA cells respond to insulin similarly to RH. McA cells synthesize both B100 and B48-containing lipoproteins, and analysis of media anti-apo B immunoprecipitates indicates that insulin favors decreased secretion of B100 compared with B48 containing lipoproteins (Fig. 1A). Suppressive effects are also observed in incubations with vanadate which reduced apo B secretion by 56 ± 5% ($P < 0.001$, $n = 4$) with a substantial reduction of B100 (Fig. 1A). Vandate is insulin mimetic in RH [27] most likely due to its ability to inhibit protein tyrosine phosphatases [28]. Insulin reduced primarily VLDL-B100 containing particles as shown following sucrose density fractionation of media lipoproteins (Fig. 1B), consistent with finding that insulin inhibits the maturation of pre-VLDL and formation of mature VLDL particles [11]. Transient expression of constitutively activated class I PI3K is shown to inhibit apo B secretion by McA cells and this effect was prevented by wortmannin (Fig. 1C), consonant with the observation that the activity of PI3K is involved in suppressive effects on apo B secretion.

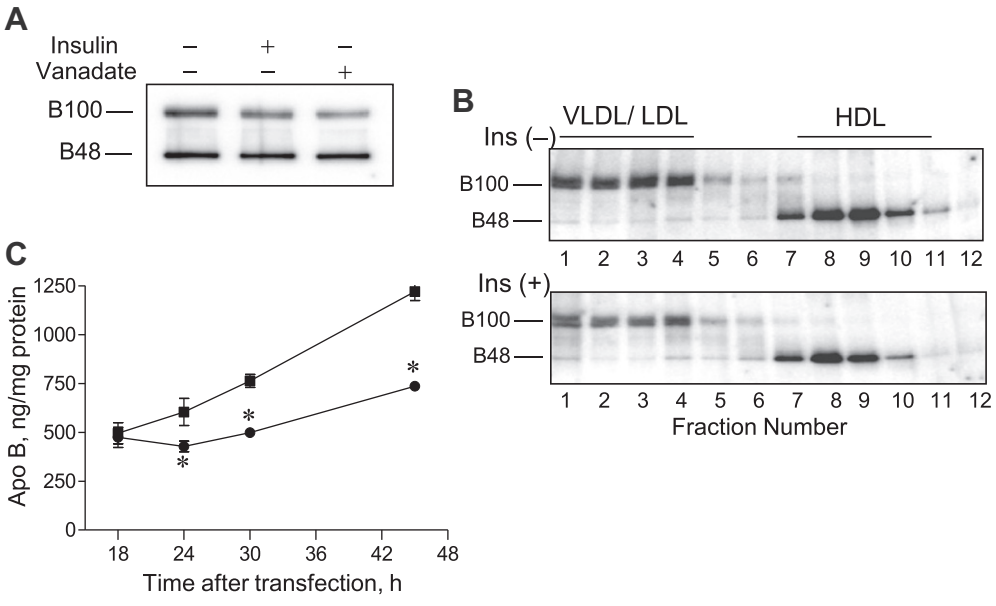

Fig. 1. Insulin suppresses VLDL B100 secretion in insulin sensitive McA cells in a PI3K-dependent manner. (A) McA cells were serum-starved followed by incubation with insulin (500 nM) or vanadate (100 μM) for 5 h. Media apo B was immunoprecipitated and analyzed by Western immunoblotting using rabbit anti-rat polyclonal antibody. (B) Media lipoproteins from McA cells incubated with or without insulin were fractionated by sucrose density gradients, and each fraction was immunoprecipitated and analyzed by Western immunoblotting. Fractions containing VLDL/LDL and HDL are indicated. (C) McA cells were transiently transfected with a plasmid containing a constitutively active p110*, and following transfection, were maintained for 18 h in cDMEM containing wortmannin (1 μM). Half of the cells were reincubated in medium containing wortmannin (■–■), while the other half received medium containing an equivalent volume of DMSO (●–●), and incubation was continued. Media apo B concentrations were measured by RIA at various time points thereafter and results normalized to cell protein. Results are the average ng apo B/mg cell protein ± S.D. from quadruplicate wells at each time point. Cellular β-galactosidase activity levels were equivalent at all time points for wortmannin and DMSO treated McA cells indicating comparable transfection efficiencies. *$P < 0.05$ *versus* McA cells treated with wortmannin continuously.

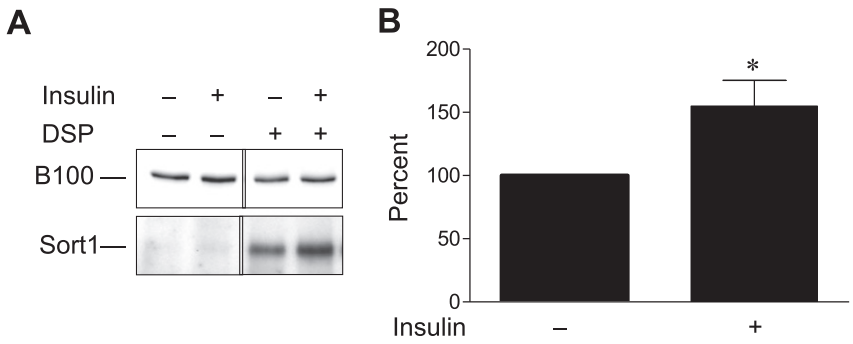

Fig. 2. Acute insulin treatment increases cross-linking of sort1 to B100. McA cells were treated with or without insulin (1 μM) for 30 min and cells were immediately cross-linked by DSP addition (2 mM). Apo B was immunoprecipitated from cell lysates and cross-links were reversed by incubation in SDS gel loading buffer. Following protein separation by SDS–PAGE, coimmunoprecipitated sort1 was analyzed by immunoblotting with anti-sortilin antibodies. (A) Immunoblots of anti-apo B immunoprecipitates. (B) The relative increase of sort1 bound to apo B with insulin treatment was expressed as a percentage of the no insulin condition. Results are expressed as the mean ± S.D., $n = 4$. $^*P < 0.05$ *versus* basal conditions. B100 in immunoprecipitates was determined by immunoblotting with a mouse anti-rat B100 specific monoclonal antibody.

Recent studies demonstrate that sort1 selectively binds B100 [14], and is suggested to be responsible for trafficking B100 to lysosomes for degradation [16]. Previous studies indicate that insulin-dependent apo B degradation takes place in a post-ER compartment [2] which might involve lysosomes. Considering that sort1, like insulin, shows preference for B100, we hypothesized that sort1 binding to B100 might be involved in insulin-dependent apo B degradation. Due to the transient nature of the interaction of B100 and sort1, we utilized the cell permeable cross-linking agent DSP to identify this interaction. McA cells were incubated with insulin followed by cross-linking; cell lysates were prepared, and apo B was immunoprecipitated. Sort1 present in immunoprecipitates was evaluated by immunoblotting (Fig. 2A). Insulin significantly increased sort1 present in apo B immunoprecipitates by 60 ± 19% ($P < 0.05$, $n = 4$) (Fig. 2B). Without the use of DSP, little interaction between sort1 and B100 is detectable.

We have previously shown that movement of apo B out of the ER is necessary for insulin-dependent degradation [2]. This underscores the complexity of the process responsible for targeting B100, considering that insulin mediates PI3K translocation to the ER where apo B is translated [4]. Recent studies indicate that sort1-dependent degradation of B100 occurs in lysosomes and may involve intracellular vesicles or endolysosomal fusion. To examine the role of lysosomes in apo B degradation, we utilized BafA1 whose main effect in hepatocytes is prevention of lysosomal acidification through inhibiting the vacuolar H^+-ATPase [29]. In both RH and McA cells, BafA1 prevented insulin suppressive effects on apo B secretion (Fig. 3A). Importantly, BafA1 did not block the ability of insulin to stimulate S473 phosphorylation of AKT which showed a 2.8-fold increase in the absence of BafA1, and 3.2-fold increase in the presence of BafA1 in control incubations (Fig. 3B).

We next addressed whether BafA1 had any effect on insulin-stimulated sort1–B100 interaction. Using a strategy similar to that described in Fig. 2, McA cells were preincubated with or without BafA1 for 1 h followed by addition of insulin for 30 min prior to cross-linking with DSP. The interaction of sort1 with B100 was significantly increased by an average of 4-fold (Fig. 4A and B). Because BafA1 prevents lysosomal degradation, results suggest that with insulin treatment, sort1–B100 complexes build-up in a prelysosomal compartment. The transient increase in B100-bound to sort1, combined with the fact that BafA1 prevents insulin suppression of apo B secretion, suggests that sort1–B100 interaction is responsible for insulin mediated B100 degradation.

The presented studies implicate sort1 binding in insulin-mediated targeting of B100 to lysosomes for degradation. Sort1 has been shown to be involved in regulation of LDL cholesterol through genome wide association studies [13,14]. Moreover, a role for sort1 has been demonstrated in regulated VLDL secretion and LDL up-

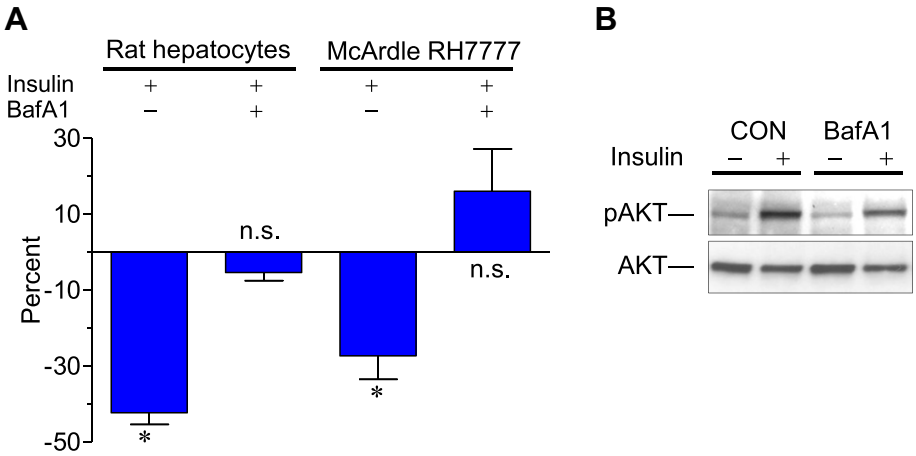

Fig. 3. Pretreatment of RH and McA cells with BafA1 blocks insulin suppression of apo B secretion. (A) RH and McA cells were preincubated with BafA1 (100 nM) or vehicle (DMSO) for 1 h followed by incubation with or without insulin (500 nM) for 6 h. Media apo B concentrations were analyzed by RIA and corrected for cell protein. Results were calculated as a percentage of the no insulin condition ($n = 3$). $^*P < 0.05$ compared to the no insulin condition; n.s., not significant. (B) Cell lysates derived from McA cells with or without BafA1 (100 nM) and subsequently treated with or without insulin (500 nM) were analyzed for insulin-dependent phosphorylation of AKT at S473 relative to total AKT mass by Western immunoblotting.

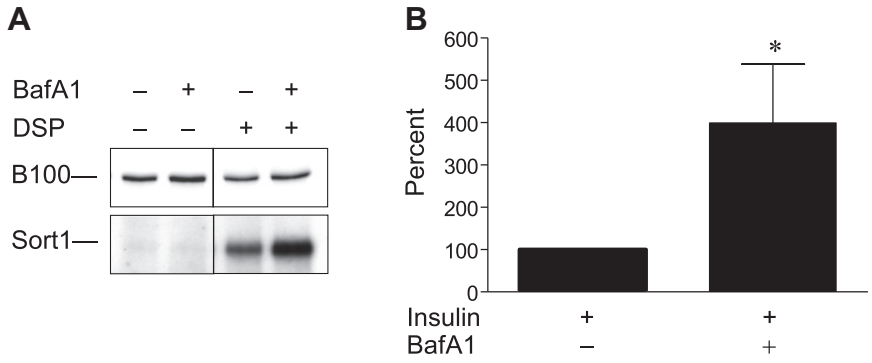

Fig. 4. BafA1 treatment increases insulin stimulated interaction of sort1 with B100. McA cells were pre-incubated for 1 h with BafA1 (100 nM) or with vehicle (DMSO) followed by incubation with insulin (500 nM) for 30 min. Immediately thereafter, cells were cross-linked using DSP (2 mM). Apo B was immunoprecipitated from cell lysates and cross-links were reversed by incubation in SDS gel loading buffer. Following protein separation by SDS–PAGE, coimmunoprecipitated sort1 was analyzed by immunoblotting with anti-sort1 antibodies. The relative increase of sort1 bound to apo B with BafA1 treatment in anti-apo B immunoprecipitates was calculated as a percentage of the no BafA1 condition. Results are expressed as the mean ± S.D., $n = 3$. $^{*}P < 0.05$ *versus* no BafA1 condition. B100 in immunoprecipitates was determined by immunoblotting with a mouse anti-rat B100 specific monoclonal antibody.

take [16]. The role of suppressed hepatic sort1 in ER stress of obese mice has also been shown [15]. The current study suggests an additional role for sort1 in insulin-dependent apo B degradation and VLDL secretion establishing a potential novel mechanism for insulin action. Insulin mediated changes occur in VLDL secretion during the postprandial transition, and loss of this pathway may be the earliest event in insulin resistance resulting in increased VLDL output in obesity and associated metabolic syndrome [6]. Future studies will be necessary to determine the precise targeting event that favors increased sort1–B100 interaction and the relative role of PERPP *versus* endosomal degradation in insulin-dependent B100 degradation.

Acknowledgments

This study was supported by R01 DK078131 through the National Institutes of Health (J.D.S.). We gratefully acknowledge the helpful discussions related to sortilin with Morten S. Neilsen, University of Aarhus, Denmark.

References

[1] J.D. Sparks, C.E. Sparks, Insulin modulation of hepatic synthesis and secretion of apolipoprotein B by rat hepatocytes, J. Biol. Chem. 265 (1990) 8854–8862.
[2] J.D. Sparks, T.L. Phung, M. Bolognino, C.E. Sparks, Insulin-mediated inhibition of apolipoprotein B secretion requires an intracellular trafficking event and phosphatidylinositol 3-kinase activation: studies with brefeldin A and wortmannin in primary cultures of rat hepatocytes, Biochem. J. 313 (Pt 2) (1996) 567–574.
[3] D.V. Chirieac, N.O. Davidson, C.E. Sparks, J.D. Sparks, PI3-kinase activity modulates apo B available for hepatic VLDL production in apobec-1−/− mice, Am. J. Physiol. Gastrointest. Liver Physiol. 291 (2006) G382–G388.
[4] T.L. Phung, A. Roncone, K.L. Jensen, C.E. Sparks, J.D. Sparks, Phosphoinositide 3-kinase activity is necessary for insulin-dependent inhibition of apolipoprotein B secretion by rat hepatocytes and localizes to the endoplasmic reticulum, J. Biol. Chem. 272 (1997) 30693–30702.
[5] N.O. Davidson, G.S. Shelness, APOLIPOPROTEIN B: mRNA editing, lipoprotein assembly, and presecretory degradation, Annu. Rev. Nutr. 20 (2000) 169–193.
[6] J.D. Sparks, C.E. Sparks, K. Adeli, Selective hepatic insulin resistance, VLDL overproduction, and hypertriglyceridemia, Arterioscler. Thromb. Vasc. Biol. 32 (2012) 2104–2112.
[7] L.P. Sorensen, E. Sondergaard, B. Nellemann, J.S. Christiansen, L.C. Gormsen, S. Nielsen, Increased VLDL-triglyceride secretion precedes impaired control of endogenous glucose production in obese, normoglycemic men, Diabetes 60 (2011) 2257–2264.
[8] E.A. Fisher, M. Pan, X. Chen, X. Wu, H. Wang, H. Jamil, J.D. Sparks, K.J. Williams, The triple threat to nascent apolipoprotein B. Evidence for multiple, distinct degradative pathways, J. Biol. Chem. 276 (2001) 27855–27863.
[9] H.N. Ginsberg, E.A. Fisher, The ever-expanding role of degradation in the regulation of apolipoprotein B metabolism, J. Lipid Res. 50 (Suppl.) (2009) S162–S166.
[10] K.J. Williams, R.W. Brocia, E.A. Fisher, The unstirred water layer as a site of control of apolipoprotein B secretion, J. Biol. Chem. 265 (1990) 16741–16744.
[11] A.M. Brown, G.F. Gibbons, Insulin inhibits the maturation phase of VLDL assembly via a phosphoinositide 3-kinase-mediated event, Arterioscler. Thromb. Vasc. Biol. 21 (2001) 1656–1661.
[12] D.V. Chirieac, J. Cianci, H.L. Collins, J.D. Sparks, C.E. Sparks, Insulin suppression of VLDL apo B secretion is not mediated by the LDL receptor, Biochem. Biophys. Res. Commun. 297 (2002) 134–137.
[13] K. Musunuru, A. Strong, M. Frank-Kamenetsky, N.E. Lee, T. Ahfeldt, K.V. Sachs, X. Li, H. Li, N. Kuperwasser, V.M. Ruda, J.P. Pirruccello, B. Muchmore, L. Prokunina-Olsson, J.L. Hall, E.E. Schadt, C.R. Morales, S. Lund-Katz, M.C. Phillips, J. Wong, W. Cantley, T. Racie, K.G. Ejebe, M. Orho-Melander, O. Melander, V. Koteliansky, K. Fitzgerald, R.M. Krauss, C.A. Cowan, S. Kathiresan, D.J. Rader, From noncoding variant to phenotype via SORT1 at the 1p13 cholesterol locus, Nature 466 (2010) 714–719.
[14] M. Kjolby, O.M. Andersen, T. Breiderhoff, A.W. Fjorback, K.M. Pedersen, P. Madsen, P. Jansen, J. Heeren, T.E. Willnow, A. Nykjaer, Sort1, encoded by the cardiovascular risk locus 1p13.3, is a regulator of hepatic lipoprotein export, Cell Metab. 12 (2010) 213–223.
[15] D. Ai, J.M. Baez, H. Jiang, D.M. Conlon, A. Hernandez-Ono, M. Frank-Kamenetsky, S. Milstein, K. Fitzgerald, A.J. Murphy, C.W. Woo, A. Strong, H.N. Ginsberg, I. Tabas, D.J. Rader, A.R. Tall, Activation of ER stress and mTORC1 suppresses hepatic sortilin-1 levels in obese mice, J. Clin. Invest. 122 (2012) 1677–1687.
[16] A. Strong, Q. Ding, A.C. Edmondson, J.S. Millar, K.V. Sachs, X. Li, A. Kumaravel, M.Y. Wang, D. Ai, L. Guo, E.T. Alexander, D. Nguyen, S. Lund-Katz, M.C. Phillips, C.R. Morales, A.R. Tall, S. Kathiresan, E.A. Fisher, K. Musunuru, D.J. Rader, Hepatic sortilin regulates both apolipoprotein B secretion and LDL catabolism, J. Clin. Invest. 122 (2012) 2807–2816.
[17] Q. Hu, A. Klippel, A.J. Muslin, W.J. Fantl, L.T. Williams, Ras-dependent induction of cellular responses by constitutively active phosphatidylinositol-3 kinase, Science 268 (1995) 100–102.
[18] J.D. Sparks, J. Cianci, J. Jokinen, L.S. Chen, C.E. Sparks, Interleukin-6 mediates hepatic hypersecretion of apolipoprotein B, Am. J. Physiol. Gastrointest. Liver Physiol. 299 (2010) G980–G989.
[19] J.D. Sparks, H.L. Collins, I. Sabio, M.P. Sowden, H.C. Smith, J. Cianci, C.E. Sparks, Effects of fatty acids on apolipoprotein B secretion by McArdle RH-7777 rat hepatoma cells, Biochim. Biophys. Acta 1347 (1997) 51–61.
[20] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685.
[21] H. Towbin, T. Staehelin, J. Gordon, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications, Proc. Natl. Acad. Sci. USA 76 (1979) 4350–4354.
[22] J. Boren, S. Rustaeus, S.O. Olofsson, Studies on the assembly of apolipoprotein B-100- and B-48-containing very low density lipoproteins in McA-RH7777 cells, J. Biol. Chem. 269 (1994) 25879–25888.
[23] J.D. Sparks, M. Bolognino, P.A. Trax, C.E. Sparks, The production and utility of monoclonal antibodies to rat apolipoprotein B lipoproteins, Atherosclerosis 61 (1986) 205–211.
[24] J.D. Sparks, C.E. Sparks, L.L. Miller, Insulin effects on apolipoprotein B production by normal, diabetic and treated-diabetic rat liver and cultured rat hepatocytes, Biochem. J. 261 (1989) 83–88.
[25] O.G. Bjornsson, J.M. Duerden, S.M. Bartlett, J.D. Sparks, C.E. Sparks, G.F. Gibbons, The role of pancreatic hormones in the regulation of lipid storage, oxidation and secretion in primary cultures of rat hepatocytes. Short- and long-term effects, Biochem. J. 281 (Pt 2) (1992) 381–386.
[26] J.D. Sparks, C.E. Sparks, Obese Zucker (fa/fa) rats are resistant to insulin's inhibitory effect on hepatic apo B secretion, Biochem. Biophys. Res. Commun. 205 (1994) 417–422.

[27] T.K. Jackson, A.I. Salhanick, J.D. Sparks, C.E. Sparks, M. Bolognino, J.M. Amatruda, Insulin-mimetic effects of vanadate in primary cultures of rat hepatocytes, Diabetes 37 (1988) 1234–1240.
[28] W. Qiu, R.K. Avramoglu, N. Dube, T.M. Chong, M. Naples, C. Au, K.G. Sidiropoulos, G.F. Lewis, J.S. Cohn, M.L. Tremblay, K. Adeli, Hepatic PTP-1B expression regulates the assembly and secretion of apolipoprotein B-containing lipoproteins: evidence from protein tyrosine phosphatase-1B overexpression, knockout, and RNAi studies, Diabetes 53 (2004) 3057–3066.
[29] D.J. Klionsky, Z. Elazar, P.O. Seglen, D.C. Rubinsztein, Does bafilomycin A1 block the fusion of autophagosomes with lysosomes?, Autophagy 4 (2008) 849–950