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Apolipoprotein A-1 (apoA-1) deposition in, and release from, the enterocyte brush border: A possible role in transintestinal cholesterol efflux (TICE)?

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ABSTRACT

Transintestinal cholesterol efflux (TICE) has been proposed to represent a non-hepatobiliary route of cholesterol secretion directly "from blood to gut" and to play a physiologically significant role in excretion of neutral sterols, but so far little is known about the proteins involved in the process. We have previously observed that apolipoprotein A-1 (apoA-1) synthesized by enterocytes of the small intestine is mainly secreted apically into the gut lumen during fasting where its assembly into chylomicrons and basolateral discharge is at a minimal level. In the present work we showed, both by immunomicroscopy and subcellular fractionation, that a fraction of the apically secreted apoA-1 in porcine small intestine was not released from the cell surface but instead deposited in the brush border. Cholesterol was detected in immunoisolated microvillar apoA-1, and it was partially associated with detergent resistant membranes (DRMs), indicative of localization in lipid raft microdomains. The apolipoprotein was not readily released from microvillar vesicles by high salt or by incubation with phosphatidylcholine-specific phospholipase C or trypsin, indicating a relatively firm attachment to the membrane bilayer. However, whole bile or taurocholate efficiently released apoA-1 at low concentrations that did not solubilize the transmembrane microvillar protein aminopeptidase N. Based on these findings and the well known role played by apoA-1 in extrahepatic cellular cholesterol removal and reverse cholesterol transport (RCT), we propose that brush border-deposited apoA-1 in the small intestine acts in TICE by mediating cholesterol efflux into the gut lumen.

1. Introduction

Apolipoprotein A-1 (apoA-1) is the major apolipoprotein of high density lipoproteins (HDL) and belongs to the class of exchangeable apolipoproteins capable of moving between different lipoproteins [5,48]. The common structural motif of these proteins is the amphipathic α-helix, enabling them to bind reversibly to lipid surfaces as well as to self-assemble when in solution [31,36,38]. Functionally, apoA-1 plays an essential role in cellular cholesterol efflux and reverse cholesterol transport (RCT) and is thus a key player in the overall cholesterol homeostasis [21]. At the cell surface, apoA-1's interactive partner in removal of free cholesterol is mainly thought to be the ATP-binding cassette transporter A1 (ABCA1), an integral membrane protein exporting cholesterol and phospholipids [7]. In the process, lipid-free/poor apoA-1 becomes lipidated, forming discoidal HDL particles that are subsequently remodeled in a stepwise manner to the other types of HDL circulating in the plasma [21].

In addition to the liver, the small intestine is a major site for synthesis and export of lipoproteins [29,30,35]. During absorption of dietary fat apoA-1, together with other apolipoproteins, including apolipoprotein B-48 (apoB-48) is assembled into chylomicrons in the endoplasmic reticulum of the enterocytes. From here, they are transferred along the secretory pathway to the Golgi complex and finally discharged from the basolateral side of the cells toward the lymph system. Enterocytic synthesis of apoA-1 and apoB-48 is not acutely regulated neither by dietary triglyceride nor biliary lipid [18–20]. Thus, a substantial small intestinal synthesis also occurs in the fasting state despite the decreased amounts of fat transported, possibly owing to the fact that fat feeding increases the size, but not the number, of chylomicrons produced in the small intestine [28]. In the fasting state, a major fraction of the apolipoproteins synthesized by enterocytes therefore remains non-lipoprotein bound and is secreted in a free state [1,34,37]. Under these conditions, we previously observed that a high proportion of lipid free/poor apoA-1 and apoB-48 is not secreted basolaterally but apically through the brush border to the gut lumen [16].

The brush border of the jejunum is primarily known to be the major site for absorption of dietary/biliary cholesterol, but interestingly the proximal part of the small intestine has also been proposed to act as a site for cholesterol excretion by a mechanism termed transintestinal cholesterol efflux (TICE) [9,42,46]. Unlike RCT, TICE thereby represents a non-hepatobiliary, direct route of cholesterol secretion "from blood to gut". Historically, evidence supporting such an alternative excretion

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mechanism has accumulated from both animal and human studies, where biliary obstruction/diversion did not prevent the appearance of fecal neutral sterols. More recently, TICE has been directly demonstrated in experiments using perfusion of isolated intestinal segment, and it has been estimated to contribute about one-third of the overall neutral sterol secretion in humans, whereas in mice it may play an even more prominent role in cholesterol secretion than the hepatobiliary route [43,44]. In addition, non-biliary excretion of neutral sterols has been revealed by targeted depletion of hepatic cholesterol esterification [8]. A connection to the general fat metabolism was implied by the observation that activation of the peroxisome proliferator-activated receptor δ (PPARδ) increased TICE more than two-fold [47]. However, key molecular players involved in TICE so far remain elusive in that no apparent involvement could be established with candidate cholesterol transporting proteins such as scavenger receptor class B type I (SR-BI), Niemann–Pick C1-like 1 protein (NPC1L1), or members of the ATP-binding cassette transporters ABCA1, ABCG1, or ABCG5/8 [42,46].

The aim of the present work was to study in closer detail the fate of apically secreted apoA-1 in small intestinal enterocytes. We observed that substantial amounts of the apolipoprotein are not directly released to the gut lumen but instead deposited in the brush border. The brush border-associated apolipoprotein was partially associated with detergent-resistant membranes (DRMs), indicating a localization in lipid rafts, and cholesterol was detected in immunoisolated microvillar apoA-1. ApoA-1 was not readily released from microvillar vesicles by trypsin, phosphatidylcholine-specific phospholipase C, or high salt, implying a relatively strong association with the membrane, but it could be solubilized by low concentrations of whole bile or taurocholate. Since these agents have previously also been shown to promote TICE, we propose that apoA-1, synthesized by the enterocytes in the fasting state and deposited in the brush border, may take part in this important physiological process.

2. Materials and methods

2.1. Materials

Rabbit antibodies to human apolipoprotein A-1 (apoA-1), human apolipoprotein B (apoB), and secondary antibodies for immunoblotting and immunogold electron microscopy were from DAKO (www.dako.dk), a rabbit antibody to intestinal alkaline phosphatase (IAP) was from AbD Serotec (www.biogenesis.co.uk/), and Alexa-coupled anti-rabbit antibodies and ProLong Gold antifade reagent with DAPI were from Invitrogen (www.invitrogen.com). Rabbit antibodies to human apoA-1 and aminopeptidase N were prepared as previously described [11,26]. ApoA-1 from human plasma (>85% pure), bovine bile, and sodium taurocholate were from Sigma-Aldrich (www.sigmaaldrich.com), and Protein A Sepharose CL-4B was from GE Healthcare (wwwgehealthcare.com). Pig small intestines were surgically removed by licensed staff from anesthetized animals that were fasted overnight at the Department of Experimental Medicine, the Panum Institute, University of Copenhagen.

2.2. Immunofluorescence microscopy

10–20 cm pieces of the small intestine were taken 1–2 m from the Pyloris and rinsed in ice cold RPMI medium. Mucosal segments of about 0.1 g were then rapidly excised and fixed in 4% paraformaldehyde in 0.1 M sodium phosphate, pH 7.2 (buffer A), for 2 h at 4 °C. Following a rinse in buffer A they were frozen in precooled 2-methylbutane and sectioned in a Leica CM1850 cryostat at −20 °C. For immunolabeling of apoA-1 or apoB, the sections were incubated for 1 h at room temperature with the respective primary antibodies (1:200–1000 dilution) in 50 mM Tris–HCl, 150 mM NaCl, 0.5% ovalbumin, 0.1% gelatin, 0.05% Tween 20, 0.2% telostan gelatin, pH 7.2 (buffer B), followed by a second incubation for 1 h with Alexa-coupled antibodies to rabbit IgG (1:200 dilution in buffer B). Controls with omission of primary antibodies were routinely included in all experiments. The sections were finally mounted in antifade medium and examined in a Leica DM 4000B microscope fitted with a Leica DC 300FX digital camera.

2.3. Immunogold electron microscopy

For ultracryosectioning, mucosal sections were fixed in 4% paraformaldehyde as described above. After a rinse in buffer A they were immersed overnight in 2.3 M sucrose, containing 1% paraformaldehyde. After a rinse in buffer A they were mounted on a metal pin and frozen in liquid nitrogen. Sections were cut in a RMC MT 6000-XL ultramicrotome, collected with a sucrose droplet, and attached to formvar-coated nickel grids. Labeling was performed with anti-apoA-1 antibodies (1:100 dilution) for 1 h followed by labeling with secondary gold-coupled antibodies (1:100 dilution) for 1 h, essentially as previously described [24]. Controls with omission of primary antibodies were routinely included in all experiments. The sections were examined in a Zeiss EM 900 electron microscope fitted with a Mega View II digital camera.

2.4. Subcellular fractionation of intestinal mucosa and immunoisolation of apoA-1

Mucosa was scraped from 5 to 10 cm pieces of pig jejunum and fractionated into soluble, Mg^{2+}-precipitated membranes (basolateral and intracellular membranes) and microvillar vesicles by the divalent cation precipitation method [6]. Briefly, the mucosa was homogenized in 10 volumes of 2 mM Tris–HCl, 50 mM mannitol, pH 7.1, containing 10 μg/ml aprotinin and leupeptin. After centrifugation at 500 *g*, 5 min, $MgCl_2$ was added to the supernatant to yield a final concentration of 10 mM. After 10 min on ice, the preparation was centrifuged at 1500 *g*, 10 min, to pellet basolateral- and intracellular membranes. The supernatant was subsequently centrifuged at 48 000 *g*, 30 min, to yield a pellet of microvillar vesicles and a supernatant of soluble proteins.

For immunoisolation, protein A-Sepharose was swelled in 50 mM HEPES-HCl, 150 mM NaCl, pH 7.1, (HB) and incubated with anti-apoA-1 antibodies for 1 h at room temperature under rotation. Before use, the protein A Sepharose-coupled antibodies were washed three times in HB. For immunoisolation, Mg^{2+}-precipitated- and microvillar membranes were resuspended in 0.5 ml HB and solubilized by addition of 1% Triton X-100 at room temperature. After 10 min, the extracts were cleared by centrifugation at 20 000 *g*, 10 min. ApoA-1 was immunoisolated from the cleared membrane extracts and the soluble fraction by incubation with ~50 μl protein A Sepharose-coupled apoA-1 antibody for 1 h at room temperature under rotation. After incubation, the protein A Sepharose was washed three times in HB.

2.5. Velocity sedimentation by density gradient centrifugation and detergent resistant membrane (DRM) analysis

For velocity sedimentation, microvillar vesicles were resuspended in 0.9 ml HB and solubilized by addition of 0.1 ml 10% Triton X-100. After 10 min incubation at room temperature the preparation was centrifuged at 20 000 *g*, 10 min, and the resulting supernatant layered on top of a linear 10–30% sucrose gradient made up in the same buffer and centrifuged in a SW40Ti rotor (Beckman Instruments, Palo Alto, USA) at 28 000 rpm (g_{av} = 97 400) for about 20 h at 4 °C, essentially as previously described [12].

For DRM analysis, microvillar vesicles were resuspended and solubilized with Triton X-100 as described above except that the preparation was kept on ice throughout the procedure. Flotation of DRMs by

sucrose gradient ultracentrifugation was then performed as described earlier [13].

After centrifugation, the sucrose gradients were fractionated, and protein was precipitated from the fractions by addition of an equal volume of acetone. After 15 min on ice, protein was pelleted by centrifugation at 20 000 *g*, 10 min.

2.6. Release of apoA-1 from microvillar membrane vesicles

Microvillar membrane vesicles, freshly prepared as described in Section 2.4, were briefly washed in HB, pelleted by centrifugation at 20 000 *g*, 40 min, and carefully resuspended in HB. Samples of 50 µl were immediately used for membrane release experiments with the following agents: phosphatidylcholine-specific phospholipase C, trypsin, high salt (1 M NaCl), bovine whole bile, or sodium taurocholate. After incubation at 37 °C, the samples were centrifuged as described above, the pellets and supernatants collected and analyzed by SDS/PAGE followed by immunoblotting.

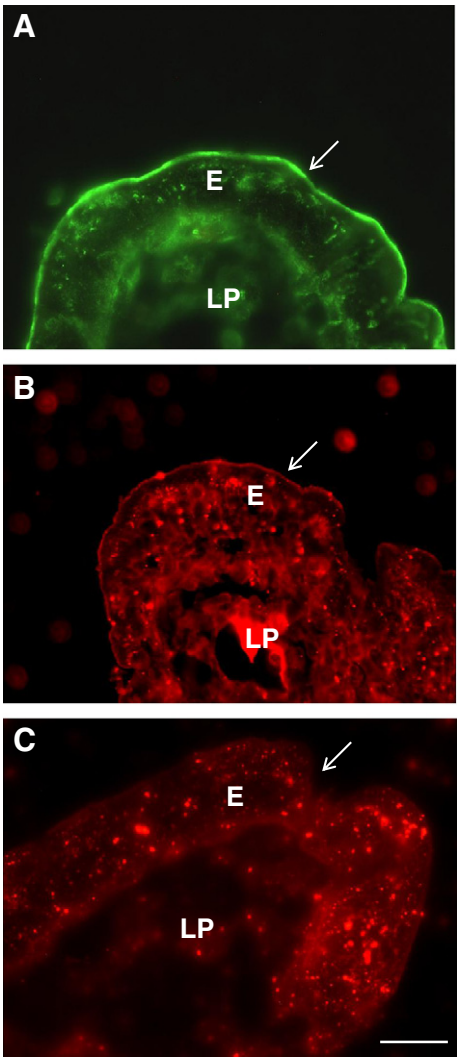

Fig. 1. Localization of apoA-1 and apoB by immunofluorescence microscopy. A, B: Frozen sections of paraformaldehyde-fixed-porcine jejunum from an animal fasted overnight were labeled for apoA-1, using either an antibody previously described [11] (A), or a commercial antibody (B). In both cases the brush border (arrows) was labeled as well as bright punctae scattered in the cytoplasm, probably representing lipoproteins awaiting discharge from the cells. In addition, some diffuse labeling of the lamina propria (LP) was seen. C: A section labeled for apoB showed a similar labeling pattern in the cytoplasm of the enterocytes as in A and B, but no staining of the brush border. Bar: 20 µm.

2.7. SDS/PAGE and immunoblotting

Samples were denatured by boiling for 3 min in the presence of 1% SDS and 10 mM dithiothreitol and subjected to SDS/PAGE in 15% gels as described [32]. After electrophoresis and electrotransfer of proteins onto Immobilon PVDF membranes, immunoblotting was performed with primary antibodies to apoA-1 (1:400 dilution) or IAP (1:1000 dilution), followed by horseradish peroxidase-coupled secondary antibodies (1:2000 dilution). An electrochemiluminescence (ecl) reagent was used according to the manufacturer's (GE Healthcare, www.gehealthcare.com) protocol to develop the blots. After immunoblotting, total protein was stained with Coomassie brilliant blue R250 (0.2%) dissolved in an ethanol/H_2O/acetic acid mixture (50:43:7).

2.8. Cholesterol determination

Cholesterol associated with apoA-1, immunoisolated from a microvillar fraction as described above, was determined spectrophotometrically by a cholesterol oxidase/peroxidase assay [22,25].

3. Results

3.1. ApoA-1 deposition in the enterocyte brush border of overnight fasted pigs

Fig. 1 shows the localization of apoA-1 in enterocytes of overnight fasted animals, using either an antibody previously prepared in our laboratory [11] (Fig. 1A), or a commercially obtained antibody (Fig. 1B). In both cases, a distinct labeling of the apical brush border was visible, and in addition, both antibodies brightly labeled punctae of various sizes scattered in the cytoplasm, most likely representing lipoproteins awaiting discharge from the cells. No labeling was detectable along the basolateral surfaces, but a diffuse labeling of the lamina propria, in particular below the basal membrane of the enterocytes, was seen. An antibody to apoB showed much the same labeling as that observed for apoA-1, except that the brush border labeling was absent (Fig. 1C). Together, these results imply that both apolipoproteins are assembled into lipoproteins but that only apoA-1 is deposited in the brush border during its apical secretion from the enterocytes in the fasting state. The apical deposition of apoA-1 was also studied by immunogold electron microscopy, and Fig. 2 shows prominent apoA-1 labeling of the microvilli of an enterocyte. The apolipoprotein was evenly distributed along the length of the microvilli and was also seen in small clusters below the cell surface, possibly representing exocytic vesicles.

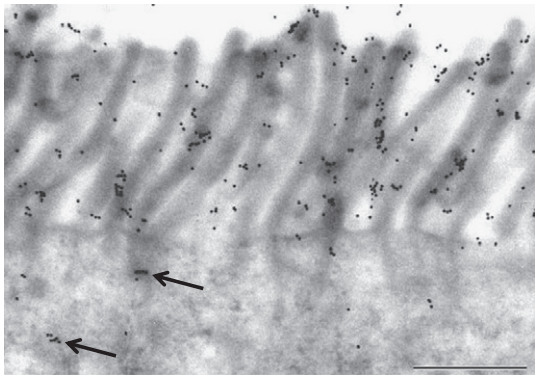

Fig. 2. Localization of apoA-1 by immunogold electron microscopy. Electron micrograph of an ultracryosection showing labeling of apoA-1 in the brush border region of an enterocyte. The apolipoprotein was seen equally distributed from the bottom to the tip of the microvilli. In addition clusters of immunogold particles were present in the apical cytoplasm (arrows), possibly representing exocytic vesicles carrying apoA-1 to the cell surface. Bar: 0.5 µm.

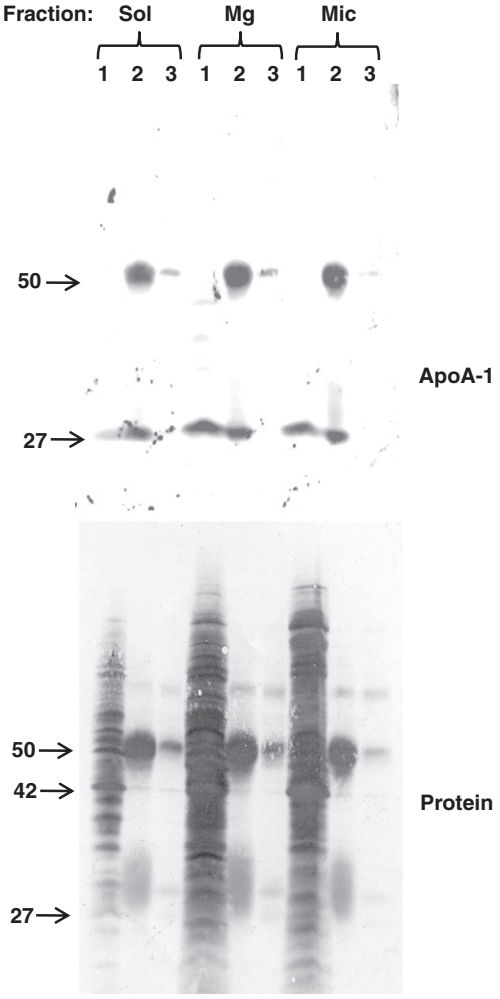

Fig. 3. Immunoisolation of apoA-1 from mucosal subcellular fractions. SDS/PAGE of apoA-1 immunoisolated from the soluble (Sol), Mg^{2+}-precipitated (Mg)- and microvillar (Mic) fractions of porcine jejunum, as described in Materials and methods. 1: Total fraction, 2: protein A-Sepharose immunoisolates, and 3: control protein A-Sepharose without conjugated antibodies. After electrophoresis, apoA-1 was visualized by immunoblotting and total protein by staining with Coomassie brilliant blue. Arrows indicate the position of apoA-1 (27 kDa), immunoglobulin heavy chains (50 kDa) and actin (42 kDa).

As shown in Fig. 3, the 27 kDa polypeptide of apoA-1 could be specifically immunoisolated not only from the soluble- and Mg^2-precipitated fractions, but also from detergent solubilized microvillar vesicles. Finally, as shown in Table 1, cholesterol was detected in the immunoisolated microvillar apoA-1, suggesting a close contact between the two molecules.

We therefore conclude that the brush border labeling for apoA-1 observed both by immunofluorescence- and immunogold microscopy genuinely represents a deposition of the apolipoprotein at the luminal surface of the small intestine.

Table 1
Detection of cholesterol associated with microvillar apoA-1. ApoA-1 was immunoisolated from microvillar membranes and subjected to a spectrophotometric cholesterol assay as described in Materials and methods. The absorbance (expressed in mAU) of the immunoisolated microvillar apoA-1 (apoA-1 i.p.), a control (antibody-protein A-Sepharose) (antibody-PAS control), and a cholesterol standard was determined in parallel. The results listed in the table are the mean values of two separate experiments.

Sample	ApoA-1 i.p.	Antibody-PAS control	Cholesterol standard (2.3 μg)
Absorbance (mAU)	14.3	1.8	99.8

3.2. ApoA-1 in the brush border resides partially in lipid rafts

The enterocyte brush border harbors a large number of digestive enzymes [40], and many of them, including aminopeptidase N and sucrose-isomaltase, reside partially in DRMs, probably owing to cross-linking to the glycolipids by galectins 2 and 4 [14,15]. As shown in Fig. 4, some of the brush border-deposited apoA-1 was solubilized by ice-cold Triton X-100 and remained in the two bottom fractions of the density gradient, whereas some partitioned with DRMs in the floating fractions like IAP, a glycosylphosphatidylinositol-anchored marker of lipid rafts. Contrary to IAP however, the DRM-associated apoA-1 was mainly seen in fractions of relative low density, suggesting that it binds to DRMs having a relative high lipid/protein ratio. Altogether, the result of the DRM analysis implies that apoA-1 may interact with both lipid raft- and non-raft domains of the brush border.

Fig. 5 shows a velocity sedimentation experiment performed with microvillar vesicles extracted by Triton X-100 at room temperature, a temperature at which also lipid raft microdomains are solubilized. Here, apoA-1 essentially remained in the two upper fractions of the gradient, indicating that it does not form stable higher molecular weight complexes with any resident brush border proteins. Previously, velocity sedimentation was used to reveal the existence of complexes formed between microvillar enzymes, such as aminopeptidase N and sucrase-isomaltase, with galectin-4 [17].

3.3. Brush border apoA-1 is released by bile and taurocholate

ApoA-1 has previously been solubilized from the surface of fibroblasts by phosphatidylcholine-specific phospholipase C [27], but as shown in Fig. 6A, apoA-1 was not readily released from microvillar vesicles by incubation with this lipase. Similarly, neither incubation with trypsin nor a wash with 1 M NaCl were effective in releasing the apolipoprotein, since the small amounts released by all three agents were similar to that of an integral transmembrane protein, aminopeptidase N.

TICE is known to be greatly stimulated by using bile salts as cholesterol acceptors [44,45], and as shown in Fig. 6B, whole bile solubilized apoA-1 at low concentrations (0.02–0.04%) at which aminopeptidase N remained associated with the microvillar membrane. The latter protein required 0.3–0.6% bile for solubilization, a concentration range that can thus be taken as the one at which the whole membrane becomes solubilized. Like whole bile, taurocholate was capable of a selective release of apoA-1 at low concentrations (0.02–0.04%, corresponding to ~0.4–0.8 mM) (Fig. 6C). Together, these experiments show that apoA-1 is firmly associated with the brush border membrane but is readily released by exposure to the physiological detergents of the digestive tract also known to promote TICE. Furthermore, the low concentrations of bile/taurocholate needed for apoA-1 release in these in vitro experiments imply that the apolipoprotein

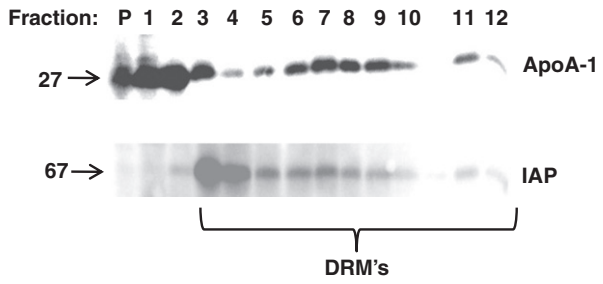

Fig. 4. DRM analysis of microvillar apoA-1. Microvillar vesicles were extracted with 1% Triton X-100 on ice and layered at the bottom of a sucrose gradient for centrifugation as described in Materials and methods. After centrifugation the gradient was divided into 12 fractions and a pellet (P) of sedimented protein. ApoA-1 and the 67-kDa DRM marker IAP (both marked by arrows) were developed by immunoblotting.

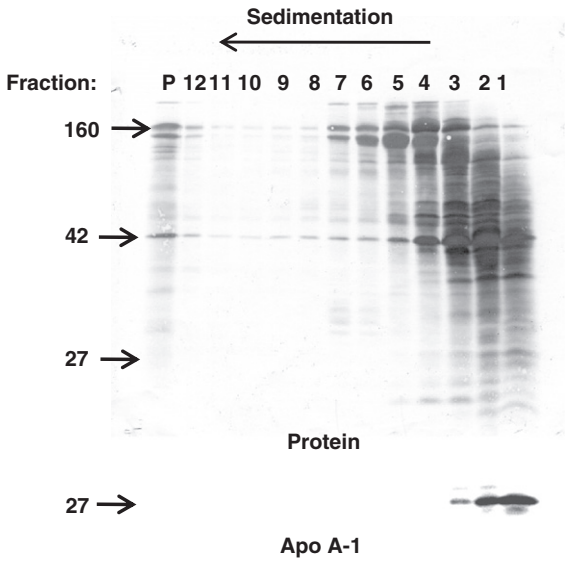

Fig. 5. Velocity sedimentation analysis of microvillar apoA-1. Microvillar vesicles were extracted with 1% Triton X-100 at room temperature and layered on top of a sucrose gradient for centrifugation as described in Materials and methods. After centrifugation the gradient was divided into 12 fractions and a pellet (P) of sedimented protein. ApoA-1 was visualized by immunoblotting and total protein by staining with Coomassie brilliant blue. Arrows indicate the position of apoA-1, actin, and aminopeptidase N (160 kDa).

might be able to contribute to TICE with only a low luminal content of bile constituents, i.e. in the early fasting state.

4. Discussion

Studies on apoA-1 of hepatic origin and as a constituent of HDL have long established a close relationship between its amphipathic α-helical structure, enabling it to interact reversibly with lipid surfaces [5,48], and its pivotal role in cellular cholesterol efflux and RCT [21]. Regarding the small intestinal apoA-1, its synthesis by enterocytes was previously shown to be independent of both the quantity and quality of dietary triglycerides [20], suggesting other functional roles for the apolipoprotein in addition to that defined by its participation in dietary fat absorption [30]. Furthermore, we observed several years ago that much of the newly synthesized apoA-1 under fasting conditions, is secreted apically toward the gut lumen [16], showing that the small intestine does not contribute all its apoA-1 to the systemic circulation. The main finding of the present work was that a fraction of apically secreted lipid free/poor apoA-1 is not readily released from the cell but instead deposited in the brush border. A likely scenario for this would be that either during the intracellular transport or exocytosis of a transport vesicle, some but not all of the apoA-1 molecules within the vesicle become embedded into the exoplasmic leaflet of the membrane. The amphipathic helical structure of apoA-1 makes the molecule well suited for such a dynamic/transient association with the membrane, much the same way in which it operates in RCT [21]. Its resistance to a high-salt wash and treatment with phospholipase C or trypsin implied that the deposition is not a mere entrapment in the glycocalyx between the microvilli. In addition, the release of apoA-1 by low concentrations of whole bile or taurocholate is suggestive of a selective and functional interaction with sterol constituents of the bile. Combining these observations, we therefore propose that apoA-1 in the brush border may take part in the last step of TICE, the efflux of free cholesterol into the gut lumen prior to the fecal excretion. That cholesterol was detected in immunoisolated microvillar apoA-1 supports our hypothesis, but admittedly it needs to be confirmed by more in vivo-like experiments. Nevertheless, the following factors also argue in favor of

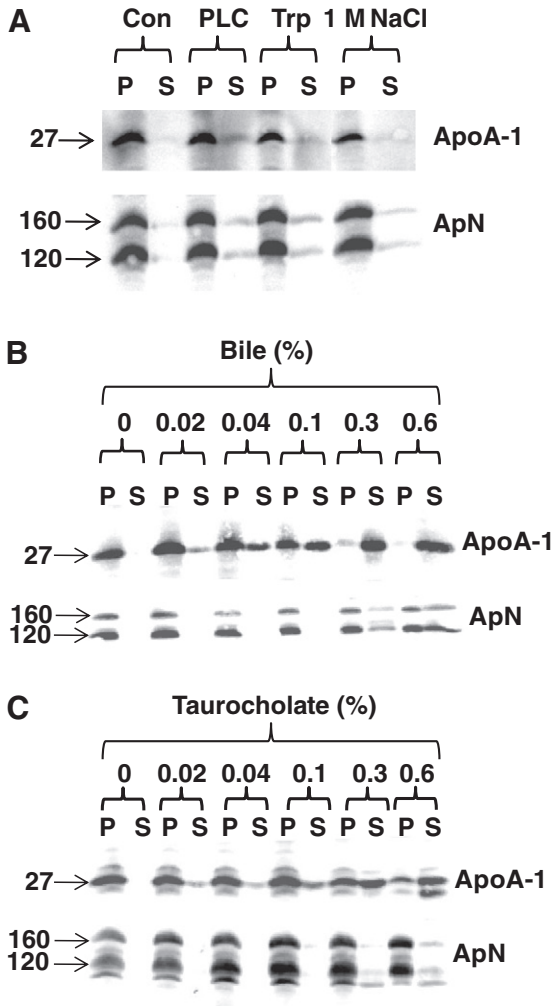

Fig. 6. Release of microvillar apoA-1 by bile and taurocholate. A: Microvillar vesicles prepared as described in Materials and methods, washed once and resuspended in 50 μl HB in the absence (Con) or presence of 10 U/ml phosphatidylcholine-specific phospholipase C (PLC), 50 μg/ml trypsin (Trp) or 1 M NaCl. After incubation for 1 h at 37 °C, the samples were centrifuged at 20 000 *g*, 40 min and the pellet (P) and supernatants (S) subjected to SDS/PAGE. ApoA-1 and the 160 kDa- and 120 kDa bands of aminopeptidase N (positions marked by arrows) were visualized by immunoblotting. B, C: Experimental setup as in A except that the microvillar vesicles were incubated for 10 min at 37 °C with the indicated concentrations of whole bile (B) or sodium taurocholate (C).

our proposal: 1) ApoA-1 biosynthesis by the enterocytes constitutes about 1–2% of the cell's total protein synthesis also in the absence of triglycerides, with the highest rate of expression in the jejunum [20]. TICE reportedly occurs throughout the small intestine, but with the highest rate in the proximal part [43]. The longitudinal expression profile of apoA-1 thus matches that of TICE, and the ample amounts of apolipoprotein present have a quantitative potential for mediating removal of physiologically relevant amounts of cholesterol. Furthermore, apoA-1 will mainly be available for TICE during fasting, where its apical secretion is at the highest level [16]. 2) During apical exocytosis apoA-1 gets in a favorable, close contact with the exoplasmic leaflet of the nascent brush border membrane, thus avoiding having to penetrate the "unstirred" layer of mucin that faces proteins approaching the cell surface from the gut lumen. This may be important since in a previous work using cultured dog gallbladder cells, exogenously added apoA-1 failed to promote cellular cholesterol efflux [45]. 3) ApoA-1, either free or as part of HDL, has been shown to inhibit cholesterol uptake into isolated microvillar vesicles [4], possibly via a direct interaction with SR-BI [39]. Although intestinal apoA-1 during fat absorption in vivo is not apically secreted, but mainly

assembled into basolaterally secreted chylomicrons, this observation nevertheless supports the notion that the apolipoprotein is involved in cholesterol traffic across the brush border. 4) The partial association of brush border apoA-1 with DRMs may enhance the lipid efflux. Thus, in studies with model membranes, it was observed that lipid-free apoA-1 binds tightly to homogeneous phospholipid bilayers, but is insufficient in lipid removal from them [38]. In contrast, efficient lipid solubilization by apoA-1 occurs from bilayers with a high degree of interfacial packing defects, i.e. from membranes made of mixed lipids rich in boundaries between raft- and non-raft microdomains. 5) Only about 10% of the total membrane lipids of porcine microvillar vesicles are made up of cholesterol [10]. This is less than half the amounts expected of a "normal" cell membrane, suggesting that cholesterol levels are actively controlled in the brush border. An apoA-1-mediated cholesterol efflux would contribute by exerting a cholesterol-lowering effect on the brush border lipid composition although a high proportion of glycosphingolipids in the brush border may also partly account for this [10].

Considering the many potential candidate proteins for participation in TICE, none has so far convincingly been shown to play a crucial role in the process. In the small intestine, the ABCG5/8 heterodimer is known to be the main transporter in the brush border responsible for sterol efflux [3,33], but double knockouts only showed moderately reduced levels of fecal neutral sterols [49]. To our knowledge TICE has not been studied in apoA-1 knockout mice, but in a recent work it was observed that apoA-1 deficiency in mice does not affect biliary secretion of lipids or formation of gallstones [2]. However, when fed a lithogenic diet, apoA-1 knockout mice exhibited an impaired hypercholesterolemic response, suggesting that the apolipoprotein does play a role but is not a rate-limiting step under normal dietary conditions. We have previously localized transporters implicated in absorption of dietary/biliary cholesterol, such as SR-BI and NPClL1, in the porcine brush border and observed them to be responsive to dietary conditions [23,41]. However, in the present work we were not able to show any convincing interaction between apoA-1 and these transporters neither by velocity sedimentation nor by coimmunoprecipitation- or overlay experiments (results not shown). Since apoA-1 is also capable of mediating a passive diffusional lipid removal [38], it may conceivably perform in such a solitary mode at the brush border, as suggested by the velocity sedimentation experiment shown in Fig. 5.

5. Conclusions

Despite its well known ability to remove extrahepatic cellular cholesterol and its key role in RCT, apoA-1 has not previously been considered a likely candidate protein for TICE. The data presented here show that cholesterol is associated with immunoisolated microvillar apoA-1, which by its transient, bile-dependent deposition in the brush border, is both well placed and well suited to participate functionally in the last step of TICE, i.e. the efflux of cholesterol into the gut lumen in the fasting state. Further studies may help to define in greater detail how apoA-1 may achieve this.

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