The effect of exogenous cholesterol and lipid-modulating agents on enterocytic amyloid-β abundance

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Dietary cholesterol may influence Alzheimer's disease risk, because it regulates the synthesis of amyloid-β. It was recently demonstrated in enterocytes of wild-type mice that intracellular amyloid-β expression is enhanced in response to a high-fat diet made up of SFA and cholesterol. Intestinally derived amyloid-β may be associated with postprandial lipoproteins in response to dietary fats and could be a key regulator in chylomicron metabolism. The present study was designed to investigate the role of cholesterol in modulating amyloid-β abundance in enterocytes. Wild-type mice were fed a low-fat diet supplemented with 2 % (w/w) cholesterol. The effects of cholesterol absorption inhibition and cholesterol biosynthesis inhibition utilising ezetimibe and atorvastatin, respectively, were also studied. Quantitative immunohistochemistry was utilised to determine enterocytic amyloid-β homeostasis. We found that enterocytic amyloid-β concentration was significantly attenuated in mice fed the 2 % (w/w) cholesterol diet. However, blocking cholesterol absorption reversed the cholesterol-feeding effect. Consistent with a suppressive effect of cholesterol on enterocytic amyloid-β abundance, atorvastatin, an inhibitor of cholesterol biosynthesis, enhanced amyloid-β. However, providing exogenous cholesterol abolished the atorvastatin-induced effect. In contrast to the suppression of enterocytic amyloid-β by dietary cholesterol, mice fed a diet enriched in SFA had markedly greater abundance. Collectively, the findings suggest that exogenous and endogenous cholesterol reduce amyloid-β concentration in enterocytes by suppressing production, or enhancing secretion associated with postprandial lipoproteins. Intestinally derived amyloid-β will contribute to the pool of plasma protein and may influence cerebral amyloid homeostasis by altering the bi-directional transfer across the blood–brain barrier.

Amyloid-β: Cholesterol: Chylomicrons: Cholesterol-modulating drugs

Amyloid-β (Aβ) is the main component of proteinaceous deposits found in the brain tissue of subjects with Alzheimer's disease[1]. Aβ is a polypeptide of thirty-nine to forty-three amino acids produced from proteolytic cleavage of the Aβ protein precursor (AβPP)[2,3] by sequential action of β- and γ-secretases[3,4]. Cleavage of AβPP within the Aβ domain at amino acid 17 by the α-secretase pathway will alternatively generate a membrane-bound carboxyl-terminal derivative, which is non-pathogenic[3]. Historically, Aβ generation was thought to occur only at the cell membrane[5,6]. However, cell-culture studies have shown that Aβ is also generated at the endoplasmic reticulum and secreted *via* the Golgi apparatus[7–9].

The origin of cerebrovascular Aβ deposits is controversial. There is little evidence for increased Aβ production in sporadic, late-onset Alzheimer's disease. Rather, decreased Aβ clearance from the brain has been put forward as one alternative hypothesis[10]. A number of studies have also shown *in vivo* transport of circulating Aβ across the blood–brain barrier, thereby contributing to total brain parenchymal Aβ load[11]. Consistent with the concept of a vascular origin for cerebral Aβ was the finding that intravenous injection of anti-Aβ-IgG completely blocked the influx of peripheral Aβ across the blood–brain barrier[10]. Circulatory Aβ could be derived from vascular smooth muscle cells and endothelial cells[6,12], or from blood platelets[13]. However, in recent studies, we also reported that the absorptive epithelial cells of the small intestine have substantial abundance of Aβ[14].

We found that enterocytic Aβ was substantially increased with the ingestion of a diet enriched in saturated fat and cholesterol, but, in contrast, was completely abolished by fasting[14], clearly showing dietary regulation. These findings may provide insight into the mechanisms underlying epidemiological studies and animal feeding studies that have demonstrated a positive relationship between fat intakes and accelerated amyloid pathology in Alzheimer's disease[15–18]. It is our contention that dietary fat-induced elevations in plasma Aβ could

compromise blood–brain barrier integrity, resulting in altered cerebral Aβ homeostasis and inflammatory sequelae. Our hypothesis is supported by studies in transgenic animal models that over-express the AβPP in neurons[19,20]. In these animals, a high-fat diet exacerbates Aβ burden, demonstrating that cerebrovascular deposition is influenced by circulatory effects, irrespective of the actual cellular origin of the Aβ peptide.

Several animal studies suggest that cholesterol is a pro-amyloidogenic dietary lipid. Rabbits fed 2 % (w/w) cholesterol have a dramatic increase in intraneuronal Aβ accumulation positively associated with the duration of feeding[18] and this is reversed when exogenous cholesterol is removed from the diet[21]. Similarly, in young double-transgenic APPsw and PS1$_{M146V}$ mice, dietary cholesterol significantly accelerates Aβ deposition[16]. In neuronal cells, Aβ production was positively associated with cholesterol availability[18] and, conversely, treatment with cholesterol synthesis or cholesterol esterification inhibitors negatively modulated Aβ biogenesis[22,23].

Enterocytes at the proximal region of the small intestine are responsible for the absorption of dietary cholesterol, released into the lymphatics primarily as esters associated with chylomicrons[24,25]. A number of studies have demonstrated that cholesterol regulates chylomicron biosynthesis[26–28]; hence, our previous finding of enhanced Aβ abundance in enterocytes in response to a high-fat dietary regimen could therefore reflect a cholesterol-induced stimulation of Aβ production. Indeed, several lines of evidence suggest that intestinally derived Aβ forms part of the chylomicron structure and thereafter serves as a regulatory apolipoprotein[29,30].

In the present study we compared wild-type mice that were maintained on sterol-free v. cholesterol-supplemented feed. An additional group of cholesterol-fed mice was also provided with ezetimibe, a potent compound of the 2-azitidinone class of drugs[31] that inhibits cholesterol absorption[32]. Control mice, fed a sterol-free diet, given ezetimibe alone, were used to rule out pleiotropic effects of the agent.

The effects of cholesterol biosynthesis inhibition on enterocytic Aβ abundance was also studied in mice that were given atorvastatin, a potent 3-hydroxy-3-methylglutaryl CoA reductase inhibitor (the rate-limiting step of the cholesterol biosynthetic pathway)[33]. Thereafter, we investigated whether the purported effect of statin therapy would be overcome by provision of dietary cholesterol supplementation.

Methods and materials

Animals and diet conditions

The animal housing, handling and experimental procedures described for the present study were approved by the Curtin University Animal Experimentation and Ethics Committee. Female wild-type mice (C57BL/6J), aged 6 weeks, were housed in groups and randomly divided into the diet and drug treatment groups (six mice per group). All mice were maintained in a 12 h light and dark cycle room, at 22°C and with free access to water and food. The control low-fat (LF) cholesterol-free group of mice was fed a semi-purified diet (AIN-93M; Glen Forrest Stockfeeds, Perth, Western Australia) containing 4 % (w/w) as total fat. Cholesterol was incorporated at 2 %

(w/w) into the chow pellets in the sterol-supplemented group (SF06-056; Glen Forrest Stockfeeds). Mice treated with ezetimibe (Ezetrol; Schering-Plough Pty Limited, Baulkham Hill, NSW, Australia) also had the drug incorporated into chow at 12 mg/kg food and atorvastatin (Lipitor; Pfizer, West Ryde, NSW, Australia) was included at a dose of 20 mg/kg at the time of feed manufacture.

Tissue collection and sample preparation

The mice were fed with their respective diets for a period of 4 weeks and were weighed weekly. At the end of the intervention period, mice were anaesthetised with phenobarbitone (45 mg/kg intraperitoneally) and exsanguinated by cardiac puncture. Blood was collected into EDTA tubes and stored in ice. Plasma was separated by short speed centrifugation at 4°C and stored at − 80°C.

The small intestine was isolated and flushed with chilled PBS (pH 7·4). A 2 cm segment of the small intestine distal to the duodenum was fixed in 10 % buffered formal saline for a minimum of 24 h, processed and longitudinal segments embedded in paraffin wax. Serial sections of 5 μm thick were cut on a microtome and mounted on silanised slides for histology and immunohistochemistry.

Immunohistochemistry

Intestinal tissue sections (5 μm) were deparaffinised, rehydrated and immunohistochemistry analysis was done as previously described[14]. Briefly, the sections were exposed to 3 % H_2O_2 in methanol for 30 min to quench endogenous peroxidase activity, washed and incubated in blocking serum (20 % goat serum) before overnight incubation at 4°C with polyclonal rabbit anti-human Aβ$_{1−40/42}$ antiserum (AB5076; Chemicon, Temecula, CA, USA), diluted to 1:1000 with 10 % goat serum. We previously established specificity by replacing the primary antibody with an irrelevant serum or with PBS and by competition immunohistochemistry analysis. For the latter, the primary antisera were pre-mixed with solubilised Aβ. Cerebral tissues from transgenic mice (Tg2576sw) expressing familial human APP$_{695}$ with established plaques were used as positive controls. Slides were washed in PBS and incubated with biotinylated goat anti-rabbit secondary antibody (1:1000 dilution) (E 0432; Dako, Carpinteria, CA, USA), followed by avidin–biotin–peroxidase complex (ABC/HRP) (K 0377; Dako) for 45 min at room temperature. Positive immunostaining was established with liquid diaminobenzidine plus (DAB +) substrate chromogen kit (K 3467; Dako). Sections were then counterstained with Harris's haematoxylin.

Quantitative immunohistochemical analysis

The intensity of immunostaining for Aβ was quantified as previously described[14,34]. Stained sections were observed with an AxioVert 200M microscope (Zeiss, Jena, Germany). Six mice per group were investigated with duplicate tissue blocks prepared for each group. The absorptive epithelial cells of the small intestine were assessed by a blinded investigator from twenty randomly selected villi per intestine, and at least 100 cells in each villus were counted. The intensity of Aβ immunostaining was graded as negative (0), mild

(+1), moderate (+2) and intense (+3) at ×200 magnification. The number of cells with different staining intensity was counted for each villus.

Imaging

Digital images for photomicroscopy were acquired by an AxioCam HRc camera (Zeiss, Jena, Germany). Images were captured under identical settings utilising AxioVision software (version 4.5).

Cholesterol and triacylglycerol analysis

Plasma cholesterol and TAG were determined in duplicate by enzymic assays (Randox Laboratories Ltd, Crumlin, Co. Antrim, UK) and according to the manufacturer's instructions.

Statistical analysis

Statistical analysis of correlation between the intensity of Aβ staining and the feeding groups was determined by the χ^2 test. Plasma lipid data were analysed by ANOVA to assess the main effects of dietary cholesterol, cholesterol absorption inhibition (by ezetimibe) and cholesterol biosynthesis inhibition (by atorvastatin) and their two-way interactions. *Post hoc* comparison of means was done if the associated main effect or interaction was statistically significant within the ANOVA procedure. *P* values <0·05 were considered to be statistically significant.

Results

Body weight and plasma cholesterol and triacylglycerol levels

The body weights post-dietary and drug intervention and plasma lipids are given in Table 1. The diet and drug interventions were well tolerated. Weight gain was similar for all groups of mice; however, the final body weight of LF mice given atorvastatin was modestly less than the control LF group (*P*=0·026). Furthermore, LF mice given atorvastatin and ezetimibe also had lower final body weights when compared with the cholesterol-supplemented group (high-cholesterol; HC). Plasma lipids were not increased in response to dietary cholesterol supplementation, nor significantly influenced by either ezetimibe or atorvastatin. However, the TAG concentration for the HC + atorvastatin group was slightly lower than the control LF group (*P*=0·01).

The pattern of amyloid-β distribution in absorptive epithelial cells of the small intestine of mice

Staining for Aβ in the small intestine is shown in Fig. 1. Positive Aβ staining was observed in absorptive epithelial cells for all groups of mice. Aβ immunostaining was found throughout the villi, increasing with proximity to the intestinal lumen. The Aβ was enriched within the perinuclear region of the enterocytes consistent with the sites of the Golgi apparatus and the rough endoplasmic reticulum, and the overall pattern of Aβ distribution between treatments was similar. A decreasing gradient of Aβ staining was evident from the perinuclear region through the cytoplasm and lacteals.

Table 1. Effect of various feeding regimens on average weight, and serum cholesterol and TAG levels in wild-type mice (C57BL/6J)*

(Mean values with their standard errors for six mice per group)

Feeding regimen	Weight (g)		Total serum cholesterol (mM)		Serum TAG (mM)	
	Mean	SEM	Mean	SEM	Mean	SEM
LF (df 5)	19·20	0·60	2·50	0·16	0·87	0·12
HC (df 5)	20·53	0·67	2·43	0·12	0·53	0·04
LF + atorvastatin (df 5)	18·91†‡	0·20	2·25	0·05	0·55	0·02
LF + ezetimibe (df 5)	19·10‡	0·24	1·94	0·29	0·61	0·13
HC + atorvastatin (df 5)	19·98	0·34	2·70	0·26	0·50†	0·07
HC + ezetimibe (df 5)	20·27	0·57	1·95	0·21	0·72	0·07

LF, low-fat; HC, high-cholesterol.
* Wild-type mice (C57BL/6J) were randomised to the six different feeding regimens and were fed their respective diets for 4 weeks. Weights and total serum cholesterol and TAG levels at the end of the experiment were compared between the groups with *post hoc* comparison of means within the ANOVA procedure. The *F* statistic for weight between all groups is 10·044; for total serum cholesterol, *F* 2·458; for serum TAG, *F* 2·843.
† Mean value was significantly lower than that of the LF group (*P*<0·05).
‡ Mean value was significantly lower than that of the HC group (*P*<0·05).

The effect of dietary cholesterol and the cholesterol absorption inhibitor ezetimibe on amyloid-β abundance in small-intestinal enterocytes

Enterocytic Aβ determined in mice given a sterol-free diet (LF) or a diet containing 2 % cholesterol is shown as Figs. 1 (A) and (B), respectively, and quantitative analysis for the intensity of Aβ immunostaining is indicated in Fig. 2. Cholesterol-supplemented mice showed a significant reduction in Aβ staining in the perinuclear regions of the absorptive enterocytes in comparison with the LF group (*P*<0·0001; Pearson's χ^2 85·206; df 3) and indeed a larger percentage of absorptive epithelial cells had no discernible staining (Fig. 2). The control group had a greater proportion of cells with mild and moderate staining (Fig. 2). In contrast, the majority of absorptive epithelial cells in the cholesterol-fed group showed no Aβ staining.

The inhibition of enterocytic Aβ staining as a consequence of dietary cholesterol supplementation could be reversed by co-treatment with ezetimibe (Fig. 1 (C)). The intensity of perinuclear Aβ expression was enhanced in comparison with the HC group (*P*<0·0001; Pearson's χ^2 80·215; df 3) but was not significantly different from the control LF group. Essentially all cells showed mild–moderate staining intensity and the pattern of distribution was unchanged (Fig. 2). To exclude pleiotropic effects independent of cholesterol absorption inhibition, mice were given ezetimibe in the absence of dietary cholesterol supplementation. No significant difference in intensity or distribution of staining was seen when compared with the LF control group (Fig. 1 (F)).

The effect of cholesterol biosynthesis inhibition by atorvastatin and cholesterol supplementation on amyloid-β abundance in small-intestinal enterocytes

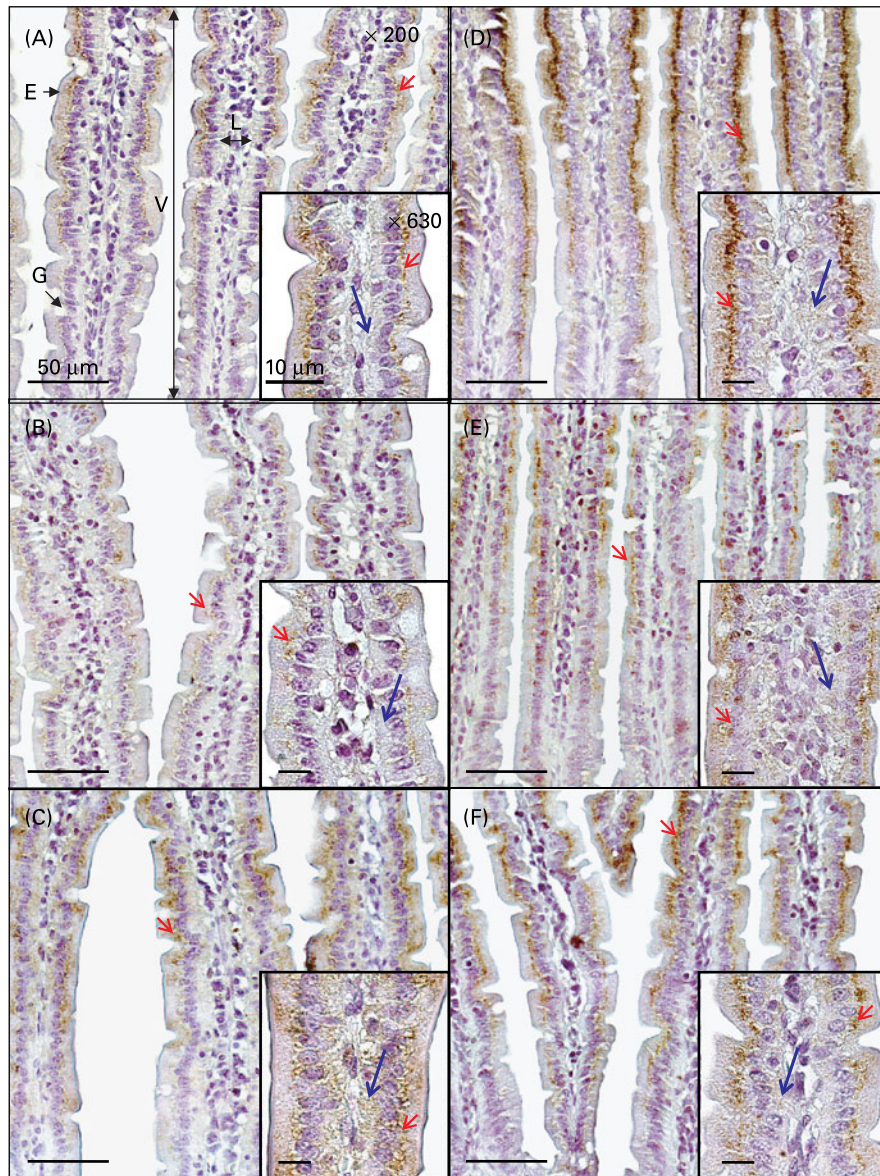To explore whether endogenous cholesterol biosynthesis regulates Aβ abundance in enterocytes, control LF-fed mice were

Fig. 1. Amyloid-β (Aβ) expression in enterocytes of wild-type mice fed low-fat (LF) (A), high-cholesterol (2 %; HC) (B), HC + ezetimibe (C), LF + atorvastatin (D), HC + atorvastatin (E) and LF + ezetimibe (F) dietary regimens for 4 weeks. Intestinal sections showing villi at increasing magnification show the pattern of Aβ distribution in the absorptive epithelial cells of the small intestine. A significant amount of Aβ (red arrow) was found concentrated within the perinuclear region of the absorptive enterocytes. Aβ staining was also visible in the lacteals (blue arrow). Aβ concentration was attenuated in HC-fed mice when compared with the LF mouse group, free of drug treatment (see also Fig. 2). The intensity of Aβ staining was enhanced in the HC + ezetimibe group compared with the HC group. Furthermore, atorvastatin increased Aβ immunostaining in the LF group. Large frames: magnification × 200; scale bar 50 μm. Inset frames: magnification × 630; scale bar 10 μm. E, enterocyte; G, goblet cell; L, lacteal; V, villus.

treated with atorvastatin (Fig. 1 (D)). We found a pronounced increase in staining intensity as a consequence of atorvastatin treatment, with approximately 20 % of enterocytes having intense colouration and the remainder with moderate to mild staining ($P = 0.016$; Pearson's χ^2 7·409; df 3) (Fig. 2). To indirectly explore if the atorvastatin-induced effect in Aβ staining was as a consequence of decreased cellular cholesterol abundance, another group of mice was given atorvastatin concomitant with dietary cholesterol. Exogenous cholesterol was found to significantly attenuate but not completely normalise the atorvastatin-mediated effect (Fig. 1 (E)). A large proportion of cells showed no Aβ staining (60 %), with essentially the remainder being classified as mild intensity (Fig. 2).

The effect of saturated fat feeding on amyloid-β abundance in small-intestinal enterocytes

To explore whether it was the SFA component of the diet which induced enterocytic Aβ accumulation previously reported[14], another group of mice were fed sterol-free chow supplemented with 20 % saturated fats. Figure 3 shows substantially exaggerated Aβ in the enterocytes of the mice given saturates.

Discussion

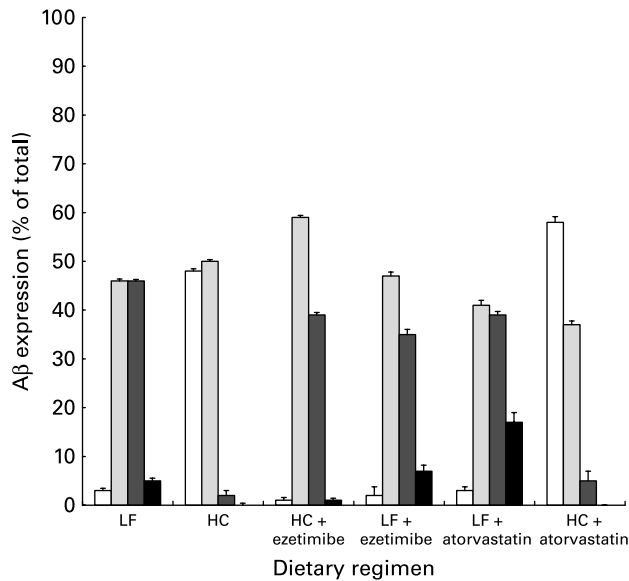We reported that wild-type mice given a diet enriched in saturated fat and cholesterol had substantially greater

Fig. 2. Semi-quantitative analysis of enterocytic amyloid-β (Aβ) expression in six mouse groups fed individual dietary regimens. The histogram shows the number of enterocytes (y axis), as a percentage of the total cell count, and the intensity of Aβ signal (x axis) in mice fed low-fat (LF), high-cholesterol (HC), HC + ezetimibe, LF + ezetimibe, LF + atorvastatin and HC + atorvastatin dietary regimens. Values are means, with their standard errors represented by vertical bars. *P* value was determined with the χ^2 test and $P<0.05$ considered significant. Staining intensity was considered negative when there was no immunostaining (0; ☐) and positive when mild (1+; ☐), moderate (2+; ▥) and intense (3+; ■) Aβ staining was generated. Mice fed cholesterol had attenuated Aβ expression when compared with the LF group ($P<0.0001$; Pearson's χ^2 85·206; df 3). Aβ expression was restored in cholesterol-supplemented mice given ezetimibe (HC + ezetimibe *v.* HC). Moreover, the atorvastatin-mediated increase in Aβ immunostaining in LF mice, compared with the LF diet group, was abolished by exogenous cholesterol feeding.

enterocytic Aβ[14]. To explore if this observation was specifically in response to dietary cholesterol, in the present study we determined enterocytic Aβ expression in 6-week-old female wild-type mice given a LF diet free of saturated fat but supplemented with cholesterol. The effect of cholesterol on enterocytic Aβ homeostasis was also investigated by pharmacologically blocking dietary cholesterol absorption and endogenous cholesterol biosynthesis.

For all groups of mice, the majority of Aβ immunostaining was found concentrated within the perinuclear region of the enterocytes as previously reported[14]. The distribution was reminiscent of cell-culture studies, which showed substantial Aβ within the endoplasmic reticulum and the Golgi apparatus[7–9]. The enterocytic perinuclear Aβ distribution is
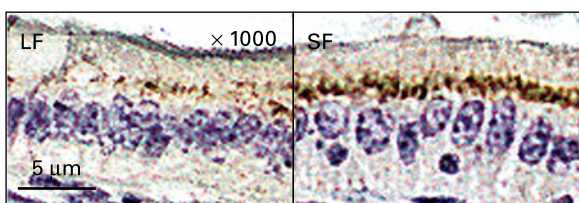

Fig. 3. Amyloid-β (Aβ) expression in enterocytes of wild-type mice fed low-fat (LF) and 20 % saturated fat (SF) diets. The enterocytes of mice given a LF diet had modest Aβ abundance within the perinuclear region of the cell compared with mice given a SF-enriched diet. Staining for Aβ is shown at high magnification (× 1000; scale bar 5 μm) in brown and the cell nuclei in blue.

consistent with the sites of chylomicron production and we previously hypothesised that high-fat feeding stimulates Aβ secretion in association with nascent lymph chylomicrons[14].

In contrast to our hypothesis, in the present study we demonstrate that enterocytic Aβ concentration was attenuated in response to cholesterol feeding. Our findings are consistent with that of Howland *et al.* [35], who established that exposure to increased dietary cholesterol resulted in a significant reduction in the brain level of $A\beta_{1-40/42}$ in AβPP gene-targeted mice. In the present study, reduced enterocytic Aβ abundance as a consequence of cholesterol feeding may be a reflection of attenuated Aβ biosynthesis and/or enhanced secretion, probably associated with intestinally derived lipoproteins[30]. Consistent with the latter, exogenous cholesterol has been found to stimulate chylomicron biogenesis and secretion[28]. Our previous studies showed increased cellular Aβ[14] in response to high saturated fat and cholesterol feeding. In the present study, we confirm that saturated fat feeding induces enterocytic Aβ accumulation.

The attenuation of enterocytic Aβ accumulation in cholesterol-supplemented mice could be abolished by the administration of ezetimibe, which effectively suppresses cholesterol absorption[36]. Changes in plasma cholesterol concentration were not observed with cholesterol feeding and ezetimibe treatment; however, enterocytic abundance of cholesterol may nonetheless have occurred. There was no difference in enterocytic Aβ staining intensity in mice given cholesterol-free chow plus ezetimibe, suggesting that no pleiotropic effects occurred with this agent.

The suppressive effect of dietary cholesterol on enterocytic Aβ abundance occurred in the absence of significant changes of plasma cholesterol, indicating that the dose of sterol provided was within physiologically tolerable limits. This contrasts with our previous studies with mice given saturated fats plus cholesterol[14] and studies by others[15,35] where higher doses of sterol supplementation significantly increased plasma cholesterol concentration. The absence of significant changes in plasma cholesterol homeostasis with an attenuation of enterocytic Aβ is consistent with the concept of reduced Aβ production, rather than enhanced secretion associated with chylomicrons. Collectively, these data support the notion that exogenous cholesterol plays an important role in Aβ homeostasis in the absorptive epithelial cells of the small intestine.

To explore whether endogenous cholesterol biosynthesis regulates enterocytic Aβ homeostasis, mice were given atorvastatin, a potent cholesterol biosynthesis inhibitor. Consistent with the findings of exogenous cholesterol attenuating Aβ concentration in enterocytes, we found that inhibiting cholesterol synthesis with atorvastatin significantly enhanced Aβ. Our findings are an extension on the findings by Park *et al.* [37] who demonstrated cholesterol biosynthesis inhibition by lovastatin increased Aβ generation in the brain tissues of female transgenic mice with familial Alzheimer's disease.

In the present study, we cannot ascertain the mechanism for the atorvastatin-induced effect. Plasma cholesterol, whilst not changed in LF mice given atorvastatin, is a poor surrogate marker of epithelial cell cholesterol homeostasis, because plasma cholesterol is mainly of hepatic origin. However, clues as to whether the atorvastatin effect was pleiotropic are indicated when the drug was co-administered with

exogenous cholesterol (HC + atorvastatin). We observed that dietary cholesterol abolished the atorvastatin effect on enterocytic Aβ homeostasis, consistent with this agent regulating enterocytic Aβ concentration *via* modulation of enterocytic pools of cholesterol.

Mechanisms by which dietary cholesterol inhibits enterocytic Aβ concentration are not readily explained. Frears *et al.* [38] observed that in the presence of cholesterol, human AβPP-transfected human embryonic kidney (HEK) cells secrete greater quantities of Aβ. In contrast, Abad-Rodriguez *et al.* [39] showed that upon lowering cholesterol, Aβ generation was increased in primary cell cultures of rat embryo hippocampal neurones and also identified that a moderate reduction in membrane cholesterol resulted in increased β-secretase. When cholesterol was added back to the cell-culture medium, β-secretase level returned to a similar level as the control. In animal model studies, enhanced intracellular Aβ accumulation was evident in brain tissues of cholesterol-fed rabbits [18], but this probably reflected deposition rather than intracellular abundance. Increased cerebral Aβ deposition was also reported in TgAPPsw mice with dietary induced hypercholesterolaemia [17], but intestinal Aβ expression in this animal model system has not been reported. Our findings suggest that reduced cholesterol availability enhances enterocytic Aβ abundance by enhancing its production.

Increased cellular cholesterol could act to increase membrane rigidity of intracellular compartments and thereby block accessibility of secretases to AβPP [39]. Furthermore, intracellular distribution between non-esterified cholesterol in the membrane and cholesteryl esters in the cytoplasm may be important in modulating Aβ homeostasis by alternative mechanisms. Inhibition of cholesterol trafficking in neuronal cells decreased β-secretase but enhanced γ-secretase processing of AβPP [40]. The substantial increase in γ-secretase resulted in an increased concentration of intracellular Aβ [40]. It is possible that an alteration in subcellular cholesterol distribution might induce changes in the cell membranes of intracellular compartments, endoplasmic reticulum and Golgi apparatus, and re-localise the enzymes responsible for Aβ production. Cholesteryl esters also appear pivotal to chylomicron biogenesis [24,25] and may influence secretion of Aβ by modulating release of the native particles. Moreover, there is some evidence of reciprocal modulation of acyl-coenzyme A:cholesterol acyltransferase and Aβ biosynthesis in cultured cell models [41].

The findings of the present study could suggest that cholesterol absorption inhibitors and 3-hydroxy-3-methylglutaryl CoA reductase inhibitors would lead to higher intestinal Aβ concentrations and therefore to a higher risk of Alzheimer's disease. However, the present study is not designed to consider the potential benefits or risks associated with the use of cholesterol-modulating agents on Alzheimer's disease risk. Inhibitors of 3-hydroxy-3-methylglutaryl CoA reductase reduce CVD and exhibit pleiotropic effects independent of lipid modification and, similarly, some lipid-modulating agents may improve outcome or ameliorate symptoms of neurological disorders [23,42]. Nonetheless, the appropriateness of initiating statin therapy is not presently established [43]. Elevated total cholesterol, LDL and TAG with normal HDL and total-cholesterol:HDL ratio characterise the lipid profile in Alzheimer's disease. However, scores on the mini mental-state examination do not correlate with lipid parameters, suggesting no interaction between cholesterol and cognition in Alzheimer's disease [44]. On the other hand, population studies support a role of dietary fats in Alzheimer's disease. Laitinen *et al.* [45] reported that intake of unsaturated fats is protective, whereas intake of saturates increases the risk of Alzheimer's disease. In the Framingham study, the top quartile of plasma DHA was associated with a 47 % reduction in risk of all-cause dementia [46]. Furthermore, evidence continues to come from animal studies including that of Oksman *et al.* [47], which confirmed that saturates increased, while DHA decreased, Aβ levels compared with a soya oil diet. Also, in cell-culture studies, fatty acids increased presenilin 1, γ-secretase and Aβ independent of any increase in cellular cholesterol [48]. Investigating the putative effects of dietary fatty acids on intestinal Aβ homeostasis may be worthwhile exploring.

Collectively, the findings of the present study indicate that enterocytic Aβ concentration is differentially regulated by dietary cholesterol and saturated fats. Dietary induced changes in production and secretion of Aβ may influence the net circulating pool of Aβ and, possibly, bi-directional kinetics of Aβ across the blood–brain barrier.

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