Published in final edited form as:

Neuroscience. 2013 December 19; 254: . doi:10.1016/j.neuroscience.2013.09.015.

Dietary Cholesterol Increases Ventricular Volume and Narrows Cerebrovascular Diameter in a Rabbit Model of Alzheimer's Disease

Bernard G. Schreurs¹, Carrie A. Smith-Bell², and Susan K. Lemieux, Ph.D.³

¹Department of Physiology and Pharmacology and the Blanchette Rockefeller Neurosciences Institute, West Virginia University, Morgantown, WV

²Department of Physiology and Pharmacology and the Blanchette Rockefeller Neurosciences Institute, West Virginia University, Morgantown, WV

³Social, Life, and Engineering Sciences Imaging Center, The Pennsylvania State University, University Park, PA

Abstract

Using structural magnetic resonance imaging in a clinical scanner at 3.0 Tesla, we describe results showing that following twelve weeks on a diet of 2% cholesterol, rabbits experience a significant increase in the volume of the third ventricle compared to rabbits on a diet of 0% cholesterol. Using time-of-flight magnetic resonance angiography, we find cholesterol-fed rabbits also experience a decrease in the diameter of a number of cerebral blood vessels including the basilar, posterior communicating, and internal carotid arteries. Taken together, these data confirm that, despite the inability of dietary cholesterol to cross the blood brain barrier, it does significantly enlarge ventricular volume and decrease cerebrovascular diameter in the rabbit – effects that are also seen in patients with Alzheimer's Disease.

Keywords

Alzheimer's Disease; animal model; ventricle; MRI; cerebrovasculature; copper

1.0 Introduction

Alzheimer's disease (AD) has significant effects on the brain including changes in volume, structure, vasculature, extracellular content, intracellular composition and alters functional properties including synaptic plasticity and cognition (de la Torre, 2012; Leung et al., 2013; Lim et al., 2012; Nestor et al., 2008; Ondrejcek et al., 2010; Roher et al., 2012; Tampellini and Gouras, 2010; Yarchoan et al., 2013). Animals including transgenic and cholesterol-fed mice, cholesterol-fed rats, transgenic and cholesterol-fed rabbits, aged dogs and primates have all been proposed as models of AD that recapitulate one or more aspects of this disease (Gotz and Ittner, 2008; Philipson et al., 2010; Woodruff-Pak, 2008).

© 2013 IBRO. Published by Elsevier Ltd. All rights reserved.

Corresponding author. Bernard G. Schreurs, Ph.D.: Tel: 304-293-0497. Fax: 304-293-7536. bschreurs@hsc.wvu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Among the most consistent and conspicuous anatomical changes found in AD is an increase in ventricular volume (Leung et al., 2013; Nestor et al., 2008). Although a hallmark in humans, surprisingly little has been reported on changes in ventricular volume in animal models of AD. In one imaging study, Andjus and colleagues found that a trimethyltin-treated rat model of AD appeared to have enlarged ventricles compared to sham-treated controls when imaged by magnetic resonance imaging (MRI) at 1.5 Tesla (Andjus et al., 2009). In another study, Dror and colleagues observed ventricle enlargement in a thiamine-deficient rat model of neurodegeneration using a 7.0 Tesla magnet (Dror et al., 2010). Xie and co-workers found that a transgenic mouse model of AD exhibited ventricular dilation compared to wild-type mice examined at 4.7 Tesla (Xie et al., 2010). Interestingly, Chen and co-workers noted ventricular enlargement in normal mice as a function of age using a 7.0 Tesla MRI scanner (Chen et al., 2010) suggesting enlargement may simply be a function of age in this model (Fjell et al., 2009). A study by Ramesh and colleagues showed that intracisternal injection of beta amyloid into the brains of aged rabbits reduced cortical thickness and increased the thickness of the lateral ventricles when measured 45 days after treatment (Ramesh et al., 2011).

Another consistent change found in AD is an alteration of the cerebrovasculature including the narrowing of vessels and the deposition of beta amyloid known as cerebral amyloid angiopathy (Bell, 2012; Roher et al., 2012; Sagare et al., 2012). In fact, there is considerable evidence suggesting that neurovascular damage may antedate other signs of pathology including beta amyloid accumulation in the brain (de la Torre, 2012), and may even be the cause of poor clearance of beta amyloid from the brain (Deane et al., 2009). One important index of cerebrovascular change that is thought to be a major contributor to Alzheimer's Disease pathology is the narrowing of vessel diameter that can lead to reductions in cerebral blood flow and hypoperfusion (de la Torre, 2012; Roher et al., 2012).

Cholesterol is important for cell structure, repair and signaling, hormone production and bile acid synthesis but is also a well-known risk factor for cardiovascular disease (Reis et al., 2013; Sprecher et al., 2007) and has been associated with AD and cognitive impairment (Purnell et al., 2009; Tolppanen et al., 2012; Whitmer et al., 2005). We and others have shown significant effects of cholesterol on learning and memory (Darwish et al., 2010; Dufour et al., 2006; Sparks and Schreurs, 2003; Ya et al., 2012) and cholesterol has been correlated with cognitive impairment (Reis et al., 2013; Solomon et al., 2007; Yaffe et al., 2002). A large body of data now confirms a strong link between cardiovascular disease and cognitive impairment (Grodstein, 2007; Nash and Fillit, 2006; Whitmer et al., 2005).

In the present study, we used both semi-automated measurements of the lateral and third ventricles and manual measurements of the diameter of individual blood vessels visualized using time-of-flight magnetic resonance angiography in the same rabbits to determine the effects of cholesterol on the brain. We found a significant change in third ventricle volume as a result of the high cholesterol diet and report that several cerebral arteries including the basilar, the posterior communicating, and the internal carotid arteries were narrowed as a function of the cholesterol diet. The size of these effects are comparable to those seen in patients with Alzheimer's Disease (Nestor et al., 2008; Roher et al., 2012).

2.0. Experimental Procedures

2.1. Animals

A total of 54 male New Zealand White rabbits (*Oryctolagus cuniculus*) 3-4 months of age and weighing approximately 2 kg upon arrival were housed individually, with access to one cup (128 grams) of Purina rabbit chow per day and *ad libitum* water, maintained on a 12-h light/12-h dark cycle and treated following United States National Institutes of Health

guidelines in experiments approved by the West Virginia University Animal Care and Use Committee.

The rabbits received one of four possible treatment conditions in a 2x2 factorial design in which food (cholesterol vs. normal chow) and water (copper vs. distilled water) were manipulated. Rabbits received either high-fiber Purina 5326 chow (containing 21% fiber and 0% cholesterol) and distilled water (n=15), Purina 5326 chow and copper in distilled water (n=13), Purina 5326 chow plus 2% cholesterol (Test Diet, Richmond, IN) and distilled water (n=9), or Purina 5326 chow plus 2% cholesterol and copper in distilled water (n=17). Rabbits given copper received copper sulfate in their distilled drinking water with a final copper concentration of 0.12 ppm (0.12 mg/liter). Rabbits were kept on their respective diets for 12 weeks. The high fiber diet was used to decrease the rate of cholesterol absorption and the consequent hepatotoxicity that develops with cholesterol feeding in rabbits (Kainuma et al., 2006; Song et al., 2000; Sparks et al., 2007).

2.3. MRI Methods

Animals were anesthetized using 27.7 mg/kg ketamine and 5.7 mg/kg of xylazine injected subcutaneously 15 minutes before imaging. A Verio 3.0 Tesla clinical scanner (Siemens Medical Solutions USA, Inc., Malvern PA) was used with the body coil used for radio-frequency transmission and the Siemens 8 channel foot and ankle coil used for signal reception. The rabbit's head was supported on a Plexiglas table to position each rabbit in the same prone position. The rabbit's body was extended onto foam supports with a heated Delta Phase Thermal pad (Braintree Scientific, Braintree, MA) under the torso to maintain a core temperature of 36-38°C. Each rabbit MRI image data set was collected in less than 30 minutes.

The rabbits underwent 3 MRI scans: 1) a 3-plane localizer scan to check animal positioning and prescribe the axial slices, 2) an axial T1-weighted 3D magnetization prepared reduced angle gradient echo sequence (MPRAGE) for anatomical scanning with good gray/white matter differentiation and dark cerebrospinal fluid (CSF); 3) a three-dimensional (3D) time-of-flight (TOF) magnetic resonance angiography sequence to image vessels. All axial image sets covered from below the most caudal end of the cerebellum to a slice beyond the rostral end of the olfactory bulb.

The MRI image acquisition parameters were as follows. The MPRAGE images were used for the current ventricle volume analysis and were 1.2 mm in slice thickness. The MPRAGE images had an in-plane resolution of 0.176 mm × 0.176 mm with image acquisition parameters as follows: slices = 104; TR/TE/TI = 1570ms./4.3ms/900ms, flip angle = 25 degrees, bandwidth = 130 Hz/pixel, number of acquisitions=2. The 3D TOF images had spatial resolution of 0.3125 mm × 0.3125 mm. × 1.0 mm. The image acquisition parameters were; flow compensation in the slice direction, 3D TOF – TR/TE = 25ms/4.1ms, flip angle = 25 degrees, bandwidth = 266 Hz/pixel, 1 acquisition. Maximum intensity projection (MIP) images were created from the axial TOF images. MIP views were calculated for the axial, sagittal, and coronal planes.

2.4. Image Analysis

To make ventricular volume measurements, the following image processing steps were performed. The MRI image contrast was enhanced using imaging software (ImageJ 1.46r, NIH) to saturate the pixels and normalize all images of each rabbit in the dataset. The lateral ventricles and the third ventricle were located with reference to a rabbit brain atlas (Girgis and Shih-Chang, 1981; Shek et al., 1986). Using the editing function of 3D Slicer – an MRI three-dimensional reconstruction program (Gering et al., 2001), the ventricles were

manually selected using intensity-based threshold painting. The lateral ventricles were fully selected. The third ventricle was selected from the anterior edge of the cerebellum through the cerebral cortex. The statistics module of 3D Slicer was used for quantification of ventricular volume. The module derives the volume from the number of pixels selected with the editing module multiplied by the image spacing and volume per pixel. These volumes were measured three times and averaged by an investigator blind to the treatment condition of the rabbits.

The vessel diameters were measured on the source images following the full-width half-maximum signal method as previously described (Lemieux et al., 2010) with the following modifications. The axial TOF source images were loaded into ImageJ (NIH) imaging software. In-plane resolution was 0.32 mm; slice resolution was interpolated to 1.0 mm. The common carotids, mid-basilar, posterior cerebral communicating, and internal carotid arteries were identified as shown in Figure 4. Data from the images were extracted using a line drawing tool to obtain a profile of the signal intensity values across each vessel. The vessel diameter was measured as the full width half maximum of the TOF signal intensity using ImageJ. Care was taken to ensure that vessel diameter measurements were not affected by intensity variation in adjacent tissues. Diameter measurements were repeated three times by an investigator blind to the treatment conditions to determine the reproducibility of the measurements.

2.5. Statistical Analysis

Lateral and third ventricle volumes as well as the diameter of the basilar artery and the summed diameters of the left and right common carotid, posterior communicating, and internal carotid arteries for all rabbits were subjected to analysis of variance with factors of food (cholesterol vs. normal chow) and water (copper vs. distilled water) with the significance level set at $p < .05$ (SYSTAT, Version 8, Chicago, IL). Where appropriate, a linear trend across the four treatment conditions was calculated using an orthogonal contrast (SYSTAT, Version 8, Chicago, IL).

3.0. Results

3.1. Ventricular Volume

The panels of Figure 1 show sample sequential MRI axial scans from left to right through the brain between the superior colliculus and temporal cortex of a rabbit that received chow and distilled water (A), chow and 0.12 ppm copper in distilled water (B), chow plus 2% cholesterol and distilled water (C), or chow with 2% cholesterol and 0.12 ppm copper in distilled water (D). Compared to the controls, the MRI scans show ventricular enlargement that was produced by the cholesterol diet. Between-subject variations in the representative MRI scans are due to slight differences in the position of each rabbit in the scanner.

The four panels of Figure 2 depict a sample MRI image from a rabbit in each of the four treatment groups to illustrate the ventricular volume differences. The enlarged inset in each panel shows the area of the third ventricle in white selected using the intensity-based threshold detection method. The insets illustrate clearly the larger third ventricle volume in rabbits fed cholesterol (Panels C and D) compared to those fed normal chow (Panels A and B). The difference in volume of the lateral and third ventricles is quantified in the bar graph of Figure 3 which shows means and standard errors of the mean ventricular volumes for each treatment group. The figure shows clear differences in third ventricular volume but not in the lateral ventricle for rabbits given cholesterol in their food without there being any significant effect of adding copper to their drinking water. Analysis of variance of the 2×2 factorial design with the factors of cholesterol and copper confirmed a significant main

effect of cholesterol for the third ventricle volume ($F(1, 50) = 20.63, p < .001$) without any other significant effects (F 's < 1.1). There were no significant differences in the volumes of the lateral ventricle (F 's < 2.3).

3.2. Vessel diameter

The panels of Figure 4 show sample TOF MRI images used to measure the diameter of blood vessels in the brains of rabbits in the four treatment groups. Panel A shows the maximum intensity projection image of the blood vessels throughout the head of a rabbit. The scale bar at top left is 2 mm. The remaining panels are different axial TOF images that allow visualization of the common carotid arteries (arrows in panel B), the internal carotid arteries (arrows in panel C), the posterior communicating arteries (arrows in panel D), and basilar artery (arrow head in panel D).

The bar graph in Figure 5 shows means and standard errors of the mean for vessel diameters for the common carotid, basilar, posterior communicating and internal carotid arteries for each of the four treatment groups. The figure shows a decrease in vessel diameter as a function of each treatment with the largest vessels in control rabbits and the narrowest vessels in the rabbits fed 2% cholesterol and given copper in the drinking water. Analysis of variance of the 2×2 factorial design yielded highly significant differences between cholesterol-fed rabbits and controls for the basilar artery ($F(1, 50) = 7.81, p < .01$), posterior communicating arteries ($F(1, 50), p < .001$), and the internal carotid arteries ($F(1, 50) = 8.71, p < .01$) but not for the common carotid arteries ($F(1, 50) = 2.61, p > 0.1$). An analysis of linear trend across the four treatment groups yielded a significant negative slope in vessel diameter from control rabbits to rabbits given 2% cholesterol and copper for all four sets of arteries including the common carotid arteries (F 's $(1, 50) > 4.57, .05 < p$'s $< .001$).

4.0. Discussion

The principal findings of our research are that rabbits fed a cholesterol diet show significant increases in ventricular volume and significant decreases in the diameter of the cerebrovasculature that are consistent with effect sizes seen in patients with Alzheimer's Disease. Specifically, rabbits fed a high-fiber, 2% cholesterol for twelve weeks showed an increase in the volume of the third ventricle and a decrease in the diameter of the basilar, posterior communicating and internal carotid arteries. Taken together, these findings extend our initial observations (Deci et al., 2012; Lemieux et al., 2010), recapitulate changes seen in Alzheimer's Disease, and add to the other indices of Alzheimer's Disease that we and others have found in the cholesterol-fed rabbit (Jiang et al., 2012; Lemieux et al., 2010; Prasanthi et al., 2010; Prasanthi et al., 2008; Woodruff-Pak, 2008; Zheng et al., 2009). As a result, the cholesterol-fed rabbit is becoming an important model for assessing mechanisms of and treatments for Alzheimer's Disease (Chen et al., 2008; Coico and Woodruff-Pak, 2008; Jiang et al., 2012; Prasad et al., 2012; Prasanthi et al., 2010; Rojas et al., 2010; Woodruff-Pak, 2008). Elevated cholesterol causes a number of changes in brain including ventricular enlargement, blood vessel narrowing, and the deposition of beta amyloid. The time course of CNS changes in the cholesterol-fed rabbit is quite rapid and can take place in a matter of weeks. First, there is an increase in the density of cortical cholesterol transporter apolipoprotein E (apoE) that is detectable in the frontal cortex four weeks after the start of a cholesterol diet (Sparks et al., 1995). At six to eight weeks, apoE staining continues to increase in the frontal cortex and is also detectable in the hippocampus (Sparks et al., 1995). Second, the density of beta-amyloid immunoreactivity becomes significantly higher than controls in the frontal cortex six weeks into the cholesterol diet and reaches a maximum in the hippocampus and frontal cortex at eight weeks (Sparks et al., 1994). Consequently, beta-amyloid accumulation appears to lag apoE density increases by about 2 weeks (Sparks et al., 1995). In contrast, increases in ventricular volume and cerebrovascular narrowing do not to

change significantly until rabbits are on a 2% cholesterol diet for at least ten weeks (Deci et al., 2012; Lemieux et al., 2010). The present findings show that these effects are more pronounced if rabbits are on a cholesterol diet for twelve weeks.

It is at present unclear how increases in dietary cholesterol and the subsequent increase in plasma lipid level are signaled to the CNS to bring about cholesterol-mediated changes in the brain (Dietschy and Turley, 2001). One possibility is that there may be different roles played by cholesterol and cholesterol esters and the enzymes that control the dynamic equilibrium between them (Puglielli et al., 2001). Another possibility is that there may be small, as yet undetectable, amounts of cholesterol that are taken up by the CNS from plasma enabling crosstalk between the periphery and CNS across the blood brain barrier (Dietschy and Turley, 2001; Refolo et al., 2001). For example, Ghribi and colleagues reported that a 7-month, 1% cholesterol diet produced a significant elevation in the cholesterol content of rabbit hippocampal neurons (Ghribi et al., 2006). There is some evidence that high cholesterol can disrupt the blood brain barrier (Chen et al., 2008; Jiang et al., 2012; Sparks et al., 2000) and that caffeine can reverse these disruptive effects (Chen et al., 2008).

The decreases in vessel diameter induced by a 2% cholesterol diet extend our initial report and are consistent with two other studies in the literature (Deci et al., 2012; Kanamaru et al., 1989; Koruzumi et al., 1978). Although it is possible that narrowing was due to cholesterol-induced amyloid deposits or fatty streaks, it is more likely that it was the result of a loss of vascular relaxation (Stokes, 2006; Sun et al., 2000; Verbeuren et al., 1986; Yang et al., 2003). In fact, there is considerable evidence for endothelial dysfunction that results in a loss of potassium channel-mediated vascular relaxation in the carotid arteries of cholesterol-fed rabbits (Moroe and Honda, 2006; Verbeuren et al., 1986).

The effect sizes we see in the current experiment are remarkably similar to those seen in humans. In terms of vessel diameter, a 10 percent decrease in diameter leads to a 20% decrease in blood flow (for the same given velocity) suggesting the diameter does not have to change much to show a significant effect on blood flow. Roher et al. (2012) found a 20% decrease in mean total regional blood flow in patients with AD compared to normal controls when measuring basilar, internal carotid, and middle cerebral arteries using TOF MRI techniques. This suggests a reduction in arterial diameter of about 10%. In the present experiment, we found a 10% decrease in three different rabbit vessel diameters suggesting an effect size very similar to that found in AD patients by Roher et al. (2012). Importantly, some of the same vessels were measured in both experiments. In terms of ventricular volume, Nestor et al. (2008) found an increase of 30% in mean ventricular volume of Alzheimer's Disease patients compared to normal age-matched controls. Our data show a 35% increase in ventricular volume suggesting a similar effect size in cholesterol-fed rabbits.

Finally, the larger volume of the third ventricle compared to the lateral ventricle in our MRI studies are different from anatomical atlases (Girgis and Shih-Chang, 1981; Shek et al., 1986) and casting methods (Levinger, 1971) which show the lateral ventricle to be considerably larger than the third ventricle. This issue turns upon how little of the lateral ventricle is visible in the MRI scans (Deci et al., 2012). For example, although Levinger (1971) documents the rabbit lateral ventricle to be between 20 and 25 mm in length when measured with a polyester resin cast, in a rabbit MRI study, Fellows-Mayle et al. (2005) were only able to see the lateral ventricle in one or two axial MRI scans that were spaced a millimeter apart. In our studies we were able to see parts of the lateral ventricle in up to three axial scans spaced 1.2 millimeters apart. In contrast, the third ventricle was completely and clearly visible in at least four to five axial scans. The second aspect of this issue concerns the thickness of the ventricle that can be resolved in the MRI image. In contrast to

the third ventricle which is relatively wide with clearly defined edges, the lateral ventricles in our MRI images are thinner with ill-defined edges. Thus, each individual volume element (voxel) in the MRI image may contain white matter, grey matter or ventricular cerebrospinal fluid in varying proportions depending on the angle of the slice, the lateral ventricle width and its curvature.

Elevated cholesterol, in addition to hypertension, diabetes, obesity and smoking, has been linked strongly to cardiovascular disease which, in turn, is a risk factor for cognitive impairment and dementia. Cholesterol has been implicated in neurodegenerative diseases more generally – not just in Alzheimer's Disease but in vascular dementia, and possibly Huntington's and Parkinson's Disease. Given the critical role that cholesterol plays in normal CNS function particularly membrane structure, cell communication and synaptic plasticity, it may not be surprising that alterations in cholesterol metabolism could contribute to different forms of degeneration and malfunction in the CNS.

The peripheral effects of feeding cholesterol to rabbits are atherosclerosis, inflammation, and liver toxicity. As a result of elevated cholesterol, the liver produces increased lipoproteins rich in cholesterol esters that stay in the bloodstream and lead to atherosclerotic lesions. High levels of LDL trigger the endothelial cell expression of adherence molecules that mediate attachment of monocytes and lymphocytes to the rabbit artery wall that then migrate into the wall and result in fatty streaks (Jessup et al., 2004; Rader and Daugherty, 2008). Oxidized LDL in the artery wall accumulates in macrophages that have differentiated from monocytes and develop into foam cells. Other macrophages are activated by pro-inflammatory cytokines in the artery wall and release more pro-inflammatory mediators including reactive oxygen and nitrogen species, interleukin-1 and tumor necrosis factor alpha (Hansson et al., 2008). Cholesterol in the rabbit induces hepatotoxicity by activating hepatic stellate cells, producing fibrosis, fat deposition and ballooning degeneration leading to focal necrosis, inflammatory reactions and lipogranuloma (Kainuma et al., 2006).

The rabbit is proving to be an important non-transgenic model of AD because it recapitulates a large number of the features of the disease but it does have limitations. The diet causes hepatotoxicity that can become lethal unless the cholesterol is removed from the diet. This requires careful titration of the diet and monitoring of the animals with the need for veterinary support. This practical issue has been addressed in a number of ways including the use of lower concentrations of cholesterol (e.g., 1.0, 0.5, or 0.25%) fed for a longer period of time in some cases as long as six months or more (Bocan et al., 1993). We have reported elsewhere on the effects of cholesterol concentration and duration on memory (Schreurs et al., 2012). Manipulation of the fiber content of the chow is another approach which tends to reduce the rate of cholesterol absorption and hence the rate of hepatotoxicity and was used here to extend the duration of diet compared to an initial report (Deci et al., 2012).

5.0. Conclusions

Rabbits fed cholesterol show consistent increases in ventricular volume and decreases in cerebrovasculature diameter. These findings replicate and extend initial observations and add to the other indices of Alzheimer's Disease that have been reported in the cholesterol-fed rabbit. Although a promising model of Alzheimer's Disease, systemic effects of cholesterol present limitations that must be managed.

Acknowledgments

Preparation of the manuscript and the described experiments was supported by National Institutes of Health/ National Institute on Aging grant AG023211. The contents of this manuscript are solely the responsibility of the

authors and do not necessarily represent the official views of the National Institute on Aging. The National Institute on Aging had no input in the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Reference List

- Andjus PR, Bataveljic D, Vanhoutte G, Mitrecic D, Pizzolante F, Djogo N, Nicaise C, Gankam KF, Gangitano C, Michetti F, van der LA, Pochet R, Bacic G. In vivo morphological changes in animal models of amyotrophic lateral sclerosis and Alzheimer's-like disease: MRI approach. *Anat Rec*. 2009; 292:1882–1889.
- Bell RD. The imbalance of vascular molecules in Alzheimer's disease. *Journal of Alzheimer's Disease*. 2012; 32:699–709.
- Bocan TMA, Mueller SB, Mazur MJ, Uhlendorf PD, Brown EQ, Kieft KA. The relationship between the degree of dietary-induced hypercholesterolemia in the rabbit and atherosclerotic lesion formation. *Atherosclerosis*. 1993; 102:9–22. [PubMed: 8257456]
- Chen C-CV, Tung Y-Y, Chang C. A lifespan MRI evaluation of ventricular enlargement in normal aging mice. *Neurobiol Aging*. 2010; 32:2299–2307. [PubMed: 20137831]
- Chen X, Gawryluk JW, Wagener JF, Ghribi O, Geiger JD. Caffeine blocks disruption of the blood brain barrier in a rabbit model of Alzheimer's disease. *Journal of Neuroinflammation*. 2008; 5:1–14. [PubMed: 18171484]
- Coico R, Woodruff-Pak DS. Immunotherapy for Alzheimer's disease: harnessing our knowledge of T cell biology using a cholesterol-fed rabbit model. *Journal of Alzheimer's Disease*. 2008; 15:657–671.
- Darwish DS, Wang D, Konat GW, Schreurs BG. Dietary cholesterol impairs memory and memory increases brain cholesterol and sulfatide levels. *Behav Neurosci*. 2010; 124:115–123. [PubMed: 20141286]
- de la Torre JC. Cerebral hemodynamics and vascular risk factors: setting the stage for Alzheimer's Disease. *Journal of Alzheimer's Disease*. 2012; 32:553–567.
- Deane R, Bell RD, Sagare A, Zlokovic BV. Clearance of amyloid- across the blood-brain barrier: implications for therapies in Alzheimer's disease. *CNS & Neurological Disorder - Drug Targets*. 2009; 8:16–30.
- Deci S, Lemieux SK, Smith-Bell CA, Sparks DL, Schreurs BG. Cholesterol increases ventricular volume in a rabbit model of Alzheimer's Disease. *Journal of Alzheimer's Disease*. 2012; 29:283–292.
- Dietschy JM, Turley SD. Cholesterol metabolism in the brain. *Curr Opin Lipidol*. 2001; 12:105–112. [PubMed: 11264981]
- Dror V, Eliash S, Rehavi M, Assaf Y, Biton IE, Fattal-Valevski A. Neurodegeneration in thiamine deficient rats - a longitudinal MRI study. *Brain Res*. 2010; 1308:176–184. [PubMed: 19857469]
- Dufour F, Liu Q-Y, Gusev P, Alkon DL, Atzori M. Cholesterol-enriched diet affects spatial learning and synaptic function in hippocampal synapses. *Brain Res*. 2006; 1103:88–98. [PubMed: 16814755]
- Fjell AM, Walhovd KB, Fennema-Notestine C, McEvoy LK, Hagler DJ, Holland D, Brewer JB, Dale AM. One-year brain atrophy evident in healthy aging. *J Neurosci*. 2009; 29:15223–15231. [PubMed: 19955375]
- Gering DT, Nabavi A, Kikinis R, Hata N, O'Donnell LJ, Grimson WE, Jolesz FA, Black PM, Well WM III. An integrated visualization system for surgical planning and guidance using image fusion and an open MR. *J Magn Reson Imaging*. 2001; 13:967–975. [PubMed: 11382961]
- Ghribi O, Larsen B, Schrag M, Herman MM. High cholesterol content in neurons increases BACE₁, - amyloid, and phosphorylated tau levels in rabbit hippocampus. *Exp Neurol*. 2006; 200:460–467. [PubMed: 16696972]
- Girgis, M.; Shih-Chang, W. *Stereotaxic Atlas of the Rabbit Brain*. Warren H. Green, Inc; St Louis, MO: 1981.
- Gotz J, Ittner LM. Animal models of Alzheimer's disease and frontotemporal dementia. *Nature Reviews Neuroscience*. 2008; 9:532–544.

- Grodstein F. Cardiovascular risk factors and cognitive function. *Alzheimer's & Dementia*. 2007; 3:S16–S22.
- Hansson GK, Robertson A-KL, Soderberg-Naucler C. Inflammation and atherosclerosis. *Annual Review of Pathology: Mechanisms of Disease*. 2008; 1:297–329.
- Jessup W, Kritharides L, Stocker R. Lipid peroxidation in atherogenesis: an overview. *Biochem Soc Trans*. 2004; 32:134–138. [PubMed: 14748732]
- Jiang X, Guo M, Su J, Lu B, Ma D, Zhang R, Yang L, Wang Q, Ma Y, Fan Y. Simvastatin blocks blood-brain barrier disruptions induced by elevated cholesterol both in vivo and in vitro. *International Journal of Alzheimer's Disease*. 2012; 2012 dci:10.1155/2012/109324.
- Kainuma M, Fujimoto M, Sekiya N, Tsuneyama K, Cheng C, Takano Y, Tersawa K, Shimada Y. Cholesterol-fed rabbit as a unique model of nonalcoholic, nonobese, non-insulin-resistant fatty liver disease with characteristic fibrosis. *J Gastroenterol*. 2006; 41:971–980. [PubMed: 17096066]
- Kanamaru K, Waga S, Tochio H, Nagatani K. The effect of atherosclerosis on endothelium-dependent relaxation in the aorta and intracranial arteries of rabbits. *J Neurosurg*. 1989; 70:793–798. [PubMed: 2709120]
- Koruzumi T, Tanaka K, Yae Y. Hypertension-induced cerebral atherosclerosis in the cholesterol-fed rabbit. *Atherosclerosis*. 1978; 30:137–145. [PubMed: 678315]
- Lemieux SK, Smith-Bell CA, Wells JR, Ezerioha NM, Carpenter JS, Sparks DL, Schreurs BG. Neurovascular changes measured by TOF-MRA in cholesterol-fed rabbits with cortical amyloid beta-peptide accumulation. *J Magn Reson Imaging*. 2010; 32:306–314. [PubMed: 20677255]
- Leung KK, Barlett JW, Manning EN, Ourselin S, Fox NC. Cerebral atrophy in mild cognitive impairment and Alzheimer disease. *Neurology*. 2013; 80:1–7.
- Levinger IM. Special features of the rabbit cerebroventricular system, studied by the casting method. *J Anat*. 1971; 109:527–533. [PubMed: 5153805]
- Lim YY, Ellis KA, Harrington K, Pietrzak RH, Gale J, Ames D, Bush AI, Darby D, Martins RN, Masters CL, Rowe CC, Savage G, Szoek C, Villemagne VL, Maruff P. Cognitive decline in adults with amnesic mild cognitive impairment and high amyloid-beta: Prodromal Alzheimer's Disease? *Journal of Alzheimer's Disease*. 2012;xxx. in press.
- Moroe H, Honda H. Comparison of endothelial function in the carotid artery between normal and short-term hypercholesterolemic rabbits. *Comp Biochem Physiol C*. 2006; 144:197–203.
- Nash DT, Fillit H. Cardiovascular disease risk factors and cognitive impairment. *Am J Cardiol*. 2006; 97:1262–1265. [PubMed: 16616038]
- Nestor SM, Rupsingh R, Borrie M, Smith M, Accomazzi V, Wells JL, Fogarty J, Bartha R. Ventricular enlargement as a possible measure of Alzheimer's disease progression validated using the Alzheimer's disease neuroimaging initiative database. *Brain*. 2008; 131:1443–2454.
- Ondrejcek T, Klyubin I, Hu N-W, Barry AE, Cullen WK, Rowan MJ. Alzheimer's disease amyloid - protein and synaptic function. *NeuroMolecular Medicine*. 2010; 12:13–26. [PubMed: 19757208]
- Philipson O, Lord A, Gumucio A, O'Callaghan P, Lannfelt L, Nilsson LNG. Animal models of amyloid- related pathologies in Alzheimer's disease. *FEBS Journal*. 2010; 277:1389–1409. [PubMed: 20136653]
- Prasad K, McNair ED, Qureshi AM, Casper-Bell G. Vitamin E slows the progression of hypercholesterolemia-induced oxidative stress in heart, liver, and kidney. *Mol Cell Biochem*. 2012; 368:181–187. [PubMed: 22714834]
- Prasanthi JR, Dasari B, Marwarha G, Larson T, Chen X, Geiger JD, Ghribi O. Caffeine protects against oxidative stress and Alzheimer's disease-like pathology in rabbit hippocampus induced by cholesterol-enriched diet. *Free Radical Biology & Medicine*. 2010; 49:1212–1220. [PubMed: 20638472]
- Prasanthi JRP, Schommer E, Thomasson S, Thompson A, Feist G, Ghribi O. Regulation of -amyloid levels in the brain of cholesterol-fed rabbit, a model system for sporadic Alzheimer's disease. *Mech Ageing Dev*. 2008; 29:649–655.
- Puglielli L, Konopka G, Pack-Chung E, Ingano LAM, Berezovska O, Hyman BT, Chang TY, Tanzi R, Kovacs D. Acyl-coenzyme A: cholesterol acyltransferase modulates the generation of the amyloid -peptide. *Nature Cell Biology*. 2001; 3:905–912.

- Purnell C, Gao S, Callahan CM, Hendrie HC. Cardiovascular risk factors and incident Alzheimer disease. *Alzheimer's Disease and Associated Disorders*. 2009; 23:1–10.
- Rader DJ, Daugherty A. Translating molecular discoveries into new therapies for atherosclerosis. *Nature*. 2008; 451:904–913. [PubMed: 18288179]
- Ramesh BN, Raichurkar KP, Shamsaundar NM, Rao TSS, Rao KSJ. A (42) induced MRI changes in aged rabbit brain resembles AD brain. *Neurochem Int*. 2011; 59:637–642. [PubMed: 21723897]
- Refolo LM, Pappolla MA, LaFrancois J, Malester B, Schmidt SD, Thomas-Bryant T, Tint GS, Wang R, Mercken M, Petanceska SS, Duff KE. A cholesterol-lowering drug reduces β -amyloid pathology in a transgenic mouse model of Alzheimer's disease. *Neurobiol Dis*. 2001; 8:890–899. [PubMed: 11592856]
- Reis JP, Loria CM, Launer LJ, Sidney S, Liu K, Jacobs DR Jr, Zhu N, Lloyd-Jones DM, He K, Yaffe K. Cardiovascular health through young adulthood and cognitive functioning in midlife. *Ann Neurol*. 2013; 73:170–179. [PubMed: 23443990]
- Roher AE, Debbins JP, Malek-Ahmadi M, Chen K, Pipe JG, Maze S, Belden C, Maarouf CL, Thiyyagura P, Mo H, Hunter JM, Kokjohn TA, Walker DG, Kruchowsky JC, Belohlavek M, Sabbagh MN, Beach TG. Cerebral blood flow in Alzheimer's disease. *Vascular Health and Risk Management*. 2012; 8:599–611. [PubMed: 23109807]
- Rojas B, Ramirez AI, Salazar JJ, de HR, Redondo A, Raposo R, Mendez T, Tejerina T, Trivino A, Ramirez JM. Low-dosage statins reduce choroidal damage in hypercholesterolemic rabbits. *Acta Ophthalmol (Copenh)*. 2010; 89:660–669.
- Sagare AP, Bell RD, Zlokovic BV. Neurovascular dysfunction and faulty amyloid β -peptide clearance in Alzheimer disease. *Cold Spring Harbor Perspectives in Medicine*. 2012; 2:a011452. [PubMed: 23028132]
- Schreurs BG, Wang D, Smith-Bell CA, Burhans LB, Bell R, Gonzales-Joekes J. Dietary cholesterol concentration and duration degrade long-term memory of classical conditioning of the rabbit's nictitating membrane response. *International Journal of Alzheimer's Disease*. 2012; 2012:doi:10.1155/2012/732634.
- Shek, JW.; Wen, GY.; Wisniewski, HM. *Atlas of the Rabbit Brain and Spinal Chord*. Krager; Basel, Switzerland: 1986.
- Solomon A, Kareholt I, Ngandu T, Winblad B, Nissinen A, Tuomilehto J, Soininen H, Kivipelto M. Serum cholesterol changes after midlife and late-life cognition. *Neurology*. 2007; 68:751–756. [PubMed: 17339582]
- Song S-H, Min B-I, Lee J-H, Cho KS. Chronological effects of atherogenic diets on the aorta, liver and spleen of rabbits. *J Korean Med Sci*. 2000; 15:413–419. [PubMed: 10983689]
- Sparks DL, Kuo Y-M, Roher AE, Martin TA, Lukas RJ. Alterations of Alzheimer's disease in the cholesterol-fed rabbit, including vascular inflammation. Preliminary observations. *Annals New York Academy of Sciences*. 2000; 903:335–344.
- Sparks DL, Liu H, Gross DR, Scheff SW. Increased density of cortical apolipoprotein E immunoreactive neurons in rabbit brain after dietary administration of cholesterol. *Neurosci Lett*. 1995; 187:142–144. [PubMed: 7783962]
- Sparks DL, Martin T, Stankovic G, Waggoner T, Van Adel R. Influence of water quality on cholesterol induced systemic pathology. *The Journal of Nutrition, Health & Aging*. 2007; 11:189–193.
- Sparks DL, Scheff SW, Hunsaker JC III, Liu H, Landers T, Gross DR. Induction of Alzheimer-like β -amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. *Exp Neurol*. 1994; 126:88–94. [PubMed: 8157129]
- Sparks DL, Schreurs BG. Trace amounts of copper in water induce β -amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease. *Proceedings of the National Academy of Sciences, USA*. 2003; 100:11065–11069.
- Sprecher DL, Schaefer EJ, Kent KM, Gregg RE, Zech LA, Hoeg JM, McManus B, Roberts WC, Brewer HB Jr. Cardiovascular features of homozygous familial hypercholesterolemia: analysis of 16 patients. *Am J Cardiol*. 2007; 54:20–30. [PubMed: 6331147]

- Stokes KY. Microvascular responses to hypercholesterolemia: the interactions between innate and adaptive immune responses. *Antioxidants & Redox Signaling*. 2006; 8:1141–1151. [PubMed: 16910762]
- Sun Y-P, Lu NC, Parmley WW, Hollenbeck CB. Effects of cholesterol diets on vascular function and atherogenesis in rabbits. *Proc Soc Exp Biol Med*. 2000; 224:166–171. [PubMed: 10865232]
- Tampellini D, Gouras GK. Synapses, synaptic activity and intraneuronal A β in Alzheimer's disease. *Frontiers in Aging Neuroscience*. 2010;2. doi: 10.3389/fnagi.2010.00013. [PubMed: 20552042]
- Tolppanen A-M, Solomon A, Soininen H, Kivipelto M. Midlife vascular risk factors and Alzheimer's disease: evidence from epidemiological studies. *Journal of Alzheimer's Disease*. 2012 in press;doi: 10.3233/JAD-2012-120802.
- Verbeuren TJ, Jordaens FH, Zonnekeyn LL, Van Hove CE, Coene M-C, Herman AG. Effect of hypercholesterolemia in vascular reactivity in the rabbit. I. Endothelium-dependent and endothelium-independent contractions and relaxations in isolated arteries of control and hypercholesterolemic rabbits. *Circ Res*. 1986; 58:552–564. [PubMed: 3486053]
- Whitmer RA, Sidney S, Selby J, Johnston SC, Yaffe K. Midlife cardiovascular risk factors and risk of dementia in late life. *Neurology*. 2005; 64:277–281. [PubMed: 15668425]
- Woodruff-Pak DS. Animal models of Alzheimer's disease: therapeutic implications. *Journal of Alzheimer's Disease*. 2008; 17:507–521.
- Xie Z, Yang D, Stephenson D, Morton D, Hicks C, Brown T, Bocan T. Characterizing the regional structural difference of the brain between tau transgenic (rTg4510) and wild-type mice using MRI. *Medical Image Computing and Computer Assisted Intervention*. 2010; 13:308–315. [PubMed: 20879245]
- Ya BL, Liu WY, Ge F, Zhang YX, Zhu BL, Bai B. Dietary cholesterol alters memory and synaptic structural plasticity in young rat brain. *Neurological Science*. 2012 DOI 10.1007/s10072-012-1241-4.
- Yaffe K, Barret-Connor E, Lin F, Grady D. Serum lipoprotein levels, statin use, and cognitive function in older women. *Arch Neurol*. 2002; 59:378–384. [PubMed: 11890840]
- Yang A-L, Jen CJ, Chen H-I. Effects of high-cholesterol diet and parallel exercise training on the vascular function of rabbit aortas: a time course study. *J Appl Physiol*. 2003; 95:1194–1200. [PubMed: 12754174]
- Yarchoan M, Xie SX, Kling MA, Toledo JB, Wolk DA, Lee EB, Van D,V, Lee VM, Trojanowski JQ, Arnold SE. Cerebrovascular atherosclerosis correlates with Alzheimer pathology in neurodegenerative dementias. *Brain*. 2013; 135:7349–7356.
- Zheng H, Zhang C, Yang W, Wang Y, Lin Y, Yang P, Yu Q, Fan J, Liu E. Fat and cholesterol diet induced lipid metabolic disorders and insulin in rabbit. *Exp Clin Endocrinol Diabetes*. 2009; 117:400–405. [PubMed: 19301228]

Highlights

- Rabbits fed cholesterol develop increased ventricle volume and decreased vascular diameter.
- These indices of pathology are similar to those seen in Alzheimer's Disease.
- Cholesterol may induce hepatotoxicity requiring careful titration or even cessation of the diet.

Figure 1.

Sample magnetic resonance imaging scans of the brains of rabbits fed different cholesterol or control diets. Sample sequential magnetic resonance imaging axial scans from left to right through the brain from the temporal cortex back to the colliculus of a rabbit that received chow and distilled water (A), chow and 0.12 parts per million copper in distilled water (B), chow plus 2% cholesterol and distilled water (C), or chow with 2% cholesterol and 0.12 parts per million copper in distilled water (D).

Figure 2.

A cholesterol diet enlarges the third ventricle of the rabbit brain. Panels show analysis of ventricular volume in a sample MRI axial scan for a rabbit that received chow and distilled water (A), chow and 0.12 parts per million copper in distilled water (B), chow plus 2% cholesterol and distilled water (C), or chow with 2% cholesterol and 0.12 parts per million copper in distilled water (D). The inset of each panel shows the third ventricle in white selected using the intensity-based threshold painting method.

Figure 3.

Measurements of the volume of the lateral ventricle and third ventricle in rabbits fed different cholesterol or control diets. Mean ($\pm$ standard errors of the mean) volume in cubic millimeters of the lateral and third ventricle for rabbits that received chow and distilled water (Purina dH₂O, n=15), chow and 0.12 parts per million copper in distilled water (Purina Cu, n=13), chow plus 2% cholesterol and distilled water (Cholesterol dH₂O, n=9), or chow with 2% cholesterol and 0.12 parts per million copper in distilled water (Cholesterol Cu, n=17). *** $p < .001$.

Figure 4.

Blood vessels in the heads of rabbits fed different cholesterol or control diets. Sample time-of-flight magnetic resonance imaging images used to measure blood vessel diameters. Panel A shows the maximum intensity projection image of the blood vessels throughout the head of a rabbit. The scale bar is 2 mm. The remaining panels are different axial time-of-flight images showing the common carotid arteries (arrows, B), the internal carotid arteries (arrows, C), and the posterior communicating arteries (arrows, D) and basilar artery (arrow head, D).

Figure 5.

Measurements of the diameter of blood vessels in the brains of rabbits fed different cholesterol or control diets. Means and ($\pm$ standard errors of the mean) diameters for the common carotid, basilar, posterior communicating and internal carotid arteries for rabbits that received chow and distilled water (Purina dH₂O, n=15), chow and 0.12 parts per million copper in distilled water (Purina Cu, n=13), chow plus 2% cholesterol and distilled water (Cholesterol dH₂O, n=9), or chow with 2% cholesterol and 0.12 parts per million copper in distilled water (Cholesterol Cu, n=17). ** p < .01, *** p < .001.