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Original Article

Effects of high-fat diet on the morphological characteristics of cerebral microvasculature without hyperlipidemia in Wistar rat

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Abstract: A high-fat diet (HFD) is associated with cerebrovascular disorders, however; its effect on the microvasculature in the brain is unknown. The aim of this study was to investigate the cerebral microvessel pathology by scanning electron microscope. Ten male Wistar rats were randomly divided into two groups: standard diet (n=5) and HFD (n=5). All of the rats were fed either standard rodent chow diet or HFD for 10 weeks and the brains were examined for microvascular pathology at the end of experiment. Coronal sections of a region of the cortex were observed by employing scanning electron microscope. Cerebral microvessels in the standard diet group exhibited the luminal endothelial surface with microvilli, indentations, and occasional vacuoles. The microvessels in the HFD group showed varying degrees of endothelial cell pathology including altered nuclear swelling as well as degenerative changes in the luminal wall. There was also thickening of the vascular basal laminae and expanded perivascular spaces often filled with amorphous debris. On a HFD, the luminal endothelial surface had more microvilli, indentations, and vacuoles. A HFD can affect the morphology of cerebral microvasculature and arteries in the Wistar rat in the absence of hyperlipidemia and lead to a series of pathological changes and microvascular remodeling, which can increase the likelihood of blood flow retardation and platelet aggregation, and ultimately thrombosis. The effects of HFD on microvasculature is more serious than that on large and medium-sized artery. Scanning electron microscope is a tool for studying microvasculature and artery pathology.

Keywords: High-fat diet, microvasculature, cerebrovascular disorder, hyperlipidemia, scanning electron microscope

Introduction

Hyperlipidemia, an established risk factor for cardiovascular diseases, is also implicated in cerebrovascular disorders [1]. It is well established that hyperlipidemia can cause atherosclerosis in medium to large sized arteries; however, its role on cerebral microvasculature is less studied [2]. There is a growing body of evidence that hypercholesterolemia also leads to microvascular dysfunction long before the appearance of atherosclerotic lesions in large vessels and that the altered microvascular function during hypercholesterolemia produces exaggerated tissue injury responses to ischemia and reperfusion [3]. RL Reddick et al demonstrated that apolipoprotein E (apoE)-deficient mice had spontaneous elevations of total plasma cholesterol and triglycerides and reduced high-density lipoprotein. Atherosclerosis was centered in the aortic sinus in young mice and the lesions were widely distributed throughout the arterial tree at 8 to 9 months of age [4]. Scanning electron microscope (SEM) provided a three-dimensional view of the vascular bed along the entire thickness of the cerebral cortex. The SEM analysis in the different cortical areas showed an angioarchitectonic distribution of vessels [5]. SEM can be applied to observe vascular intima, including the integrity of the endothelial cell layer, presence of lateral thrombus, collagen fiber, debris, lipid droplets,

and vacuoles, and so on. Through bisecting the blood vessels, the vascular muscle layer can be observed to determine whether there is vascular muscle layer thickening, muscular fibrosis with or without muscle cell hypertrophy, atheromatous plaque, or calcification plaque.

The microvasculature, a network of blood vessels less than 100 μm in diameter, is a part of circulation and its main function is to transport oxygen and nutrients, hormones, and metabolic products through the exchange of circulating blood and parenchyma cells [6]. According to anatomical features and the direction of the blood flow, the microvasculature is subdivided into arterioles, capillaries, and venules. Adequate blood flow in capillaries can ensure the normal perfusion and organ function. Capillaries consist of a single layer of epithelium and a basement membrane, which allow the exchange of molecules between blood and tissues. Endothelial dysfunction (ED) is a disorder, which is defined as reduction of bioavailability of vasodilators, mainly nitrous oxide, and is characterized by a state of endothelial activation where a pro-inflammatory, proliferative and pro-coagulant milieu is predominant [7]. It is an early stage of the occurrence and development of atherosclerosis [8].

However, at present there are few reports on the effects of hyperlipidemia for cerebral microvasculature, and even fewer studies on the effect of a high-fat diet on the cerebral microvasculature. Based on the previous studies, in our research, the hypothesis is that without hyperlipidemia, high-fat diet can damage the cerebral microcirculation. The aim of this study was to investigate the effects of high-fat diet on morphological characteristics of the cerebral microvasculature and vasculature in the Wistar rat in the absence of hyperlipidemia through scanning electron microscopy.

Materials and methods

Dietary and environmental manipulations

Male Wistar rats were purchased from Vital River Laboratories. All procedures were in accordance with the Guide for Care and Use of Animal Experimentation of the Peking University People's Hospital. All rats were randomly assigned to one of two groups: standard diet (n=5) or high-fat diet (n=5). The standard diet rats were fed a chow (Research Diets, D12451) that contained 11.2% fat, 55.5% carbohydrate, and 33.3% protein (kcal); whereas the high-fat diet rats received a chow which contained 45.2% fat, 28.6% carbohydrate, and 26.2% protein (kcal). The high-fat diet was high calorie food (high-fat diet =4.5 kcal/g *versus* standard diet =3.3 kcal/g). All rats were housed in individual cages in a climate-controlled room with purified air (23 ± 3°C; 60 ± 5% relative humidity) with a 12 h light/dark cycle (lights on at 6:00 am). The rats in each group were fed the indicated diet with sufficient water and food *ad libitum* for 10 consecutive weeks. Food consumption was calculated daily; while water consumption was evaluated once a week.

Measurement the levels of serum index analysis

After the experimental period of 10 weeks, the rats were sacrificed and blood samples were collected and centrifuged at 3,000 rpm at 4°C for 15 min and the supernatants were stored at -70°C for biochemical analysis. The levels of triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) in the serum were measured by the enzymatic colorimetric method using commercial reagents assay kits (Beijing Biosino Bio-technology & Science Inc, China) according to the manufacturer's instructions.

Scanning electron microscope

Five Wistar rats on a standard rodent chow diet or high fat diet were anaesthetized and a cannula was inserted in the ascending aorta. The brain was perfused with 1 ml of heparinized saline solution to prevent blood clotting, following by an injection of 500 ml of saline to complete the wash out of the remaining blood from the vascular bed. The vascular bed was fixed by a slow injection of 100 ml of combined stock solution (0.25% glutaraldehyde and 0.25% paraformaldehyde in 0.1 M buffer at pH=7.35) to prevent the leakage of the resin and reduce the modifications occurring to the endothelial cells during the injection of the medium. The cannula was then removed from ascending aorta. Brains were then removed and the specimens were prepared for scanning electron microscope observations by dehydration in graded alcohol. 5 μm-thick coronal sections

were cut using a region of the cortex. The specimens were then observed through scanning electron microscope Philips XL-30 FEG at 10 kV.

Statistical analysis

The serum triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) in the standard diet groups and the HFD group were collected, and the levels (means ± SD for three independent experiments) were analyzed. Statistical significance of observed differences among these groups was calculated using a two-tailed unpaired Student's *t* test. A *p* value of less than 0.05 was considered to be statistically significant. The statistical calculations were performed with SPSS 13.0 for Windows (SPSS, Inc, Chicago, IL, USA).

Results

The levels of serum index

We analyzed the values of triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) in the serum and observed no statistically significant differences between standard and high-fat diet (HFD) chows ($P>0.05$) (data not shown).

HFD changes cerebral microvessel morphological characteristics

Microvessels of a defined lumen diameter were included in the analysis (rodent: d<7 μm, human d<12 μm). In the HFD group, we observed the reduction of functional capillary density and microvascular rarefaction (**Figure 1** a *versus* a1) with a significant increase in macromolecular permeability and cerebral microvessels exhibiting finger-like endothelial processes protruding the vessel lumen (microvilli). Furthermore, the luminal endothelial surface appeared to have more microvilli, indentations, and vacuoles compared with the standard diet group (**Figure 1** b *versus* b1, d *versus* d1). Additional changes observed in the HFD group include microthrombus caused by red blood cell and platelet aggregation formed in the microvasculature, thinner vascular walls, more pronounced gaps among vascular endothelial cells, and visible binding of collagen fibers in the capillary lumen that results in luminal stenosis (**Figure 1C**).

At the ultrastructural level, microvessels in the HFD group showed varying degrees of endothelial cell pathology including altered nuclear swelling as well as degenerative changes in the luminal wall. We also observed increased thickness of the vascular basal laminae and expanded perivascular spaces often filled with amorphous debris. The luminal endothelial surface appeared to contain more microvilli, indentations, and vacuoles in the HFD group (**Figure 2**).

Cerebral vascular

In contrast with the standard diet, a high fat diet can lead to cerebral vascular pathology, including arterial wall thickening, stiffness, and loss of elasticity. Furthermore, HFD leads to stenosis, a rougher cerebral vascular endothelial surface, which would result in hemodynamic change and increase the risk of microcirculation blood clots, with more microvilli, indentations, occasional vacuoles, and more frequent and wider gaps between endothelial cells. In addition, more folds were present on chamber surface with the HFD than the standard diet. At the ultrastructural level, the HFD cerebral vasculature had more apparent nuclear swelling and a rougher, disordered surface as well as increased collagen fibers, scattered lipid drops and thrombocytes; however, the cerebral vasculature of the standard diet group appeared smoother and neater (**Figure 2**).

Discussion

A high-fat diet can change the morphology of cerebral microvasculature and arteries in the Wistar rat in the absence of hyperlipidemia and lead to a series of pathological changes. It causes microvascular remodeling, especially compromising the integrity of endothelium and increasing the incidence of related cerebrovascular disease. Our results indicate that a high-fat diet is involved in cerebral microvasculature and artery pathology, with more serious pathological changes occurring in microvasculature than in large- and medium-sized arteries. In the HFD group, the luminal endothelial surface of cerebral microvessels had a higher presence of microvilli, indentations, and vacuoles in addition to evidence of endothelial cell pathology

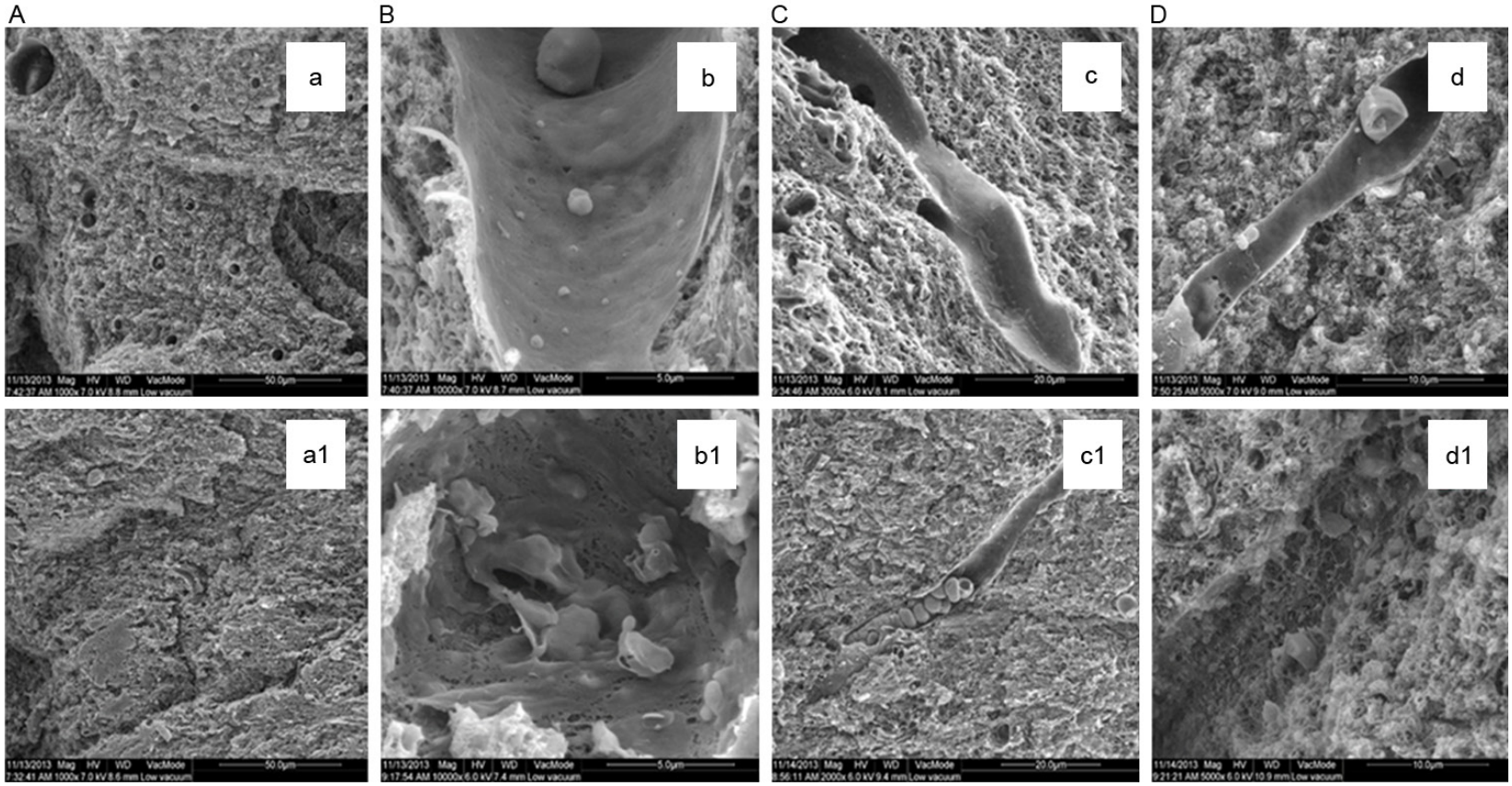

Figure 1. Changes in cerebral microvessels in the standard diet group (a-d) *versus* the HFD group (a1-d1). A. Functional capillary density and microvascular rarefaction was reduced in the HFD group (a1) compared to standard chow (a). B, D. The luminal endothelial surface of microvessels in the HFD group have more microvilli, indentations, vacuoles, and wider gaps among vascular endothelial cell compared to standard chow (b1, d1 *versus* b, d). C. Luminal stenosis resulting from collagen fibers binding together in the capillary lumen (c1 *versus* c).

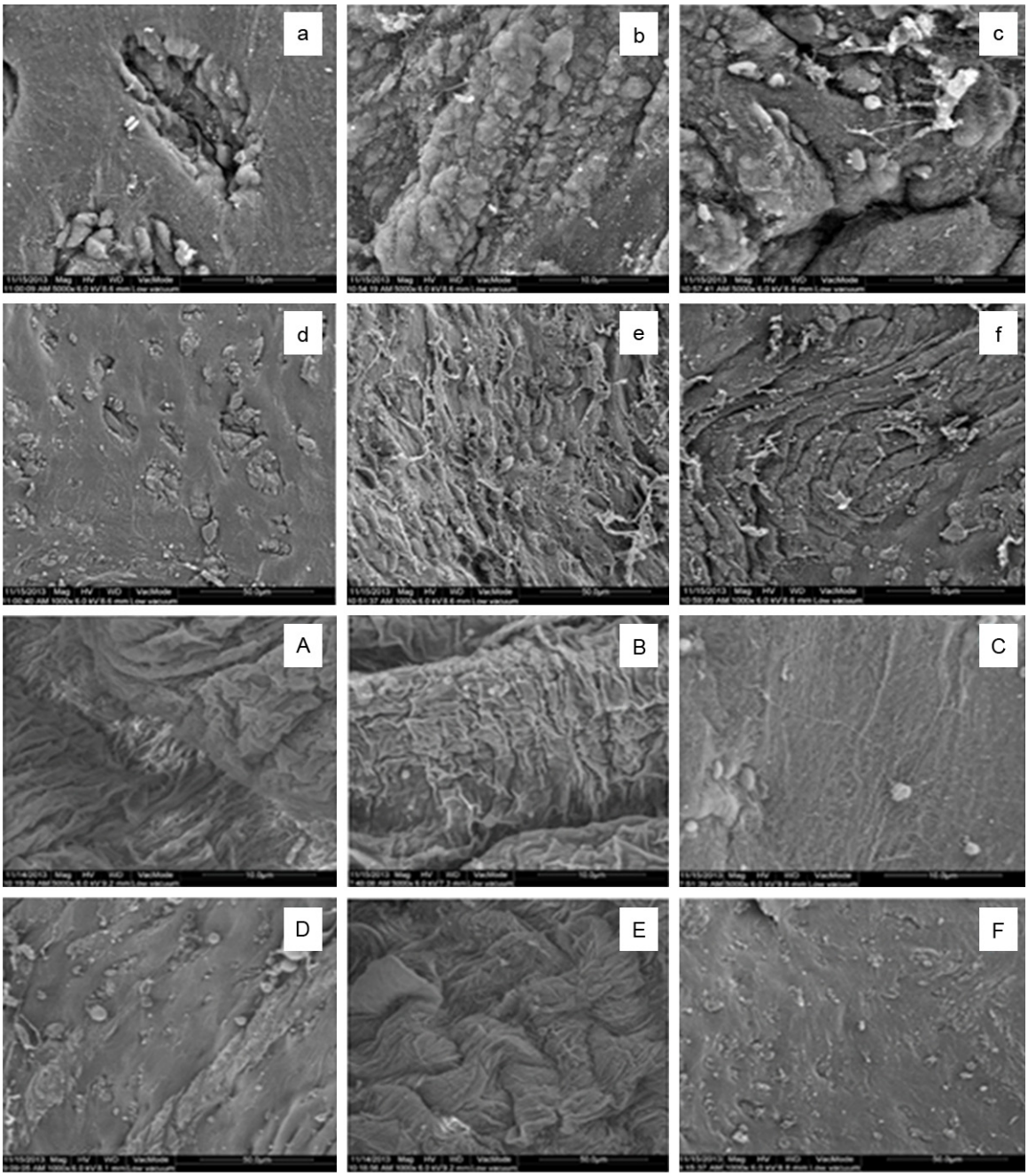

Figure 2. The changes to the cerebral vascular surface on different rat chows. The HFD group (a-f) *versus* the standard diet (A-F) under different magnification (×800: a-c, A-C; ×500: d-f, D-F). With the HFD, the surface of the cerebral vascular endothelial became rougher and had more microvilli, indentations, and vacuoles, and wider gaps between endothelial cells compared with the standard diet (b, e *versus* B, E). In addition, the chamber surface was more folded with HFD (c, f versus C, F). The surface became more disorderly and rougher and the appearance of collagen fibers increased (b, e *versus* B, E), in addition to more scattered lipid drops (a, d *versus* A, D) and thrombocytes (a, d *versus* A, D). On the HFD, the vascular endothelium is damaged and compromised, resulting lipid droplets, and cell infiltration (these can be observed at a-f *versus* A-F). Compared with the HFD group, the vasculature of the standard diet group appeared smoother and neater. On the standard chow, the vascular endothelium is intact without lipid droplets and cell infiltration. No microvilli, indentations, vacuoles, and fewer collagen fibers were present on the endothelial surface (A-F).

including the thickening of the vascular basal laminae and expanded perivascular spaces filled with amorphous debris. The intima not only plays key roles in the selective permeabili-

ty between blood and vessel wall, secretion of effector molecules, as well as regulation of blood vessel tone and blood flow, but also participates in the processes of inflammation, immunity, thrombogenesis, and angiogenesis [9-12]. All endothelial pathology can increase the likelihood of blood flow retardation and platelet aggregation, and ultimately thrombosis. Furthermore, the HFD group has reduced functional capillary density and microvascular rarefaction compared with the standard diet group. Before hyperlipidemia, microvascular remodeling and rarefaction occurs in brain tissue (**Figure 2**). Oxidative stress, endothelial dysfunction, and apoptosis are possible mechanisms implicated on microvascular rarefaction [13].

Previous research also showed that a high fat diet can lead to microcirculation dysfunction, including endothelial dysfunction characterized by blunted vasodilatatory responses and increased shear stress by raising blood flow, reduction of functional capillary density and microvascular rarefaction, a significant augmentation of macromolecular permeability as well as prostanoid-mediated vasoconstriction in conductance vessels of skeletal muscles [14]. Increased cholesterol levels induced by a high-fat diet activate a number of cerebral vascular events, including oxidative stress, endothelial dysfunction, blood-brain barrier disturbances and vascular inflammation, which as a result worsens stroke mortality [15]. Actually, an obvious induction of CYP2E1 was caused by hyperglycemia in the HFD rats combined with cerebral ischemia-reperfusion injury [16]. Consequently, the induction of CYP2E1 oxidizes mitochondrial DNA, proteins, and lipids and triggers pro-inflammatory cytokines, such as TNF-α, IL-1, IL-6, TGF-β, which further increases mitochondrial ROS (reactive oxygen species) [16, 17]. On the other hand, oxidative low-density lipoprotein (ox-LDL) could enhance the expression of multiple adhesive factors and cytokines in cells through activating the vascular endothelial cells. Furthermore, ox-LDL could significantly elevate the level of TNF-α, IL-6, and sICAM-1 in the supernatant after the intervention with ox-LDL [18]. Which will lead to endothelial injury and increase the risk of atherosclerosis. Besides, oxidative stress can lead to severe and immediate damage to cellular proteins, DNA, and lipids by causing overproduction of free radicals that overwhelms the detoxification capacity of cellular antioxidant enzymes [19]. Other research showed that hyperlipidemia could exacerbate cerebral ischemia-reperfusion (I/R)-induced injury by the synergistic effect on CYP2E1 induction, as a result, reactive oxygen species formation, oxidative stress, inflammation, and neuronal apoptosis by coexistence of hyperlipidemia and cerebral I/R have been induced [20]. At present, there has not been related research on effects of a high-fat diet on morphological characteristics of cerebral microvasculature in the absence of hyperlipidemia. The purpose of this experiment was to approach the subject. A HFD increases O-GlcNAc-modified proteins which contribute to intensifying contractile responses and cerebrovascular dysfunction [21]. Hyperlipidemia is best known for its role in atherosclerotic vascular disease, a process that is the most apparent in larger arteries such as the aorta or the coronary arteries [4]. However, the processes involved in atherosclerosis (arteriosclerosis affecting arteries) may be somewhat different to those in arteriolosclerosis (arteriosclerosis affecting smaller arteriolar branches) and the relationship between dyslipidaemia and arteriolosclerosis is less clear. There is some evidence that hypercholesterolemia may also cause both functional and structural changes in the peripheral microvasculature [22]. Cholesterol is generally not thought to cause pathology in the microvasculature. In particular, how a high-cholesterol diet induces microvascular pathology in brain is not known since arterial lesions develop in apoE-deficient mice fed regular mouse chow.

Atherosclerotic vascular disease of larger arteries has been demonstrated. There are four major features on the lumenal surface of the aorta: 1) blood cells including leukocytes adhering to the endothelial surface; 2) abundant cholesterol-ester crystals in extracellular spaces; 3) cave-like structures possibly suggesting new capillarization in the thrombotic atherosclerotic plaques; and 4) a de-endothelialized surface showing both elastogenesis and elastolysis [23]. In our experiment, we found that the high fat diet without hyperlipidemia rat the microcirculation has similar change in endothelial surface. Vessels with a diameter of 7 μm or less form the cortical capillary network, which is arranged parallel to the apical surface [24]. As a general rule, capillary density in the gray matter of the brain was found about three times as

much as that of the white matter. The endothelial cells are surrounded by a 30- to 40-nm-thick basement membrane, which is often a target of investigation due to frequently observed malformations under pathophysiological conditions [25]. While we observed increased microvessel diameter, microvascular density and length were not consistently affected. However, degenerative changes and thickened vascular basement membranes were present ultrastructurally [4]. In the cerebral cortex of wild-type mice, the vascular walls of microvessels are composed of flat endothelium and smooth muscle cells rich in fine myofilaments [26]. Oh Young Bang et al showed that cerebral micro- and macroangiopathy often coexist and share common risk factors, including atherosclerosis [27]. Obesity and high fat intake could cause functional and structural changes in the vasculature [28-32]. High intake of fat in the presence or absence of obesity can also lead to vascular dysfunction [33, 34].

Taken together, a high-fat diet can change the morphology of cerebral microvasculature and arteries in Wistar rat in the absence of hyperlipidemia. Additionally, it can lead to a series of pathological changes and microvascular remodeling which can increase the likelihood of blood flow retardation and platelet aggregation, and ultimately thrombosis. This causes increased incidence of related cerebrovascular disease. Furthermore, the effects of a high-fat diet are more serious on the microvasculature than that on large and medium-sized artery.

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Disclosure of conflict of interest

None.

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References

[1] Constantinescu E, Safciuc F and Sima AV. A hyperlipidemic diet induces structural changes in cerebral blood vessels. Curr Neurovasc Res 2011; 8: 131-144.

[2] Franciosi S, Gama Sosa MA, English DF, Oler E, Oung T, Janssen WG, De Gasperi R, Schmeidler J, Dickstein DL, Schmitz C, Gandy S, Hof PR, Buxbaum JD and Elder GA. Novel cerebrovascular pathology in mice fed a high cholesterol diet. Mol Neurodegener 2009; 4: 42.

[3] Ishikawa M, Stokes KY, Zhang JH, Nanda A and Granger DN. Cerebral microvascular responses to hypercholesterolemia: roles of NADPH oxidase and P-selectin. Circ Res 2004; 94: 239-244.

[4] Reddick RL, Zhang SH and Maeda N. Atherosclerosis in mice lacking apo E. Evaluation of lesional development and progression. Arterioscler Thromb 1994; 14: 141-147.

[5] Rodriguez-Baeza A, Reina-de la Torre F, Poca A, Marti M and Garnacho A. Morphological features in human cortical brain microvessels after head injury: a three-dimensional and immunocytochemical study. Anat Rec A Discov Mol Cell Evol Biol 2003; 273: 583-593.

[6] Lymphatic system and microcirculation. Proceedings of the Third International Symposium on Daflon 500 held during the First Mediterranean Congress of Angiology. Corfu, Greece, May 31, 1988. Int Angiol 1989; 8: 1-75.

[7] Anderson TJ. Assessment and treatment of endothelial dysfunction in humans. J Am Coll Cardiol 1999; 34: 631-638.

[8] Hirase T and Node K. Endothelial dysfunction as a cellular mechanism for vascular failure. Am J Physiol Heart Circ Physiol 2012; 302: H499-505.

[9] Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med 1999; 340: 115-126.

[10] Lusis AJ. Atherosclerosis. Nature 2000; 407: 233-241.

[11] Falk E. Morphologic features of unstable atherothrombotic plaques underlying acute coronary syndromes. Am J Cardiol 1989; 63: 114E-120E.

[12] Sumpio BE, Riley JT and Dardik A. Cells in focus: endothelial cell. Int J Biochem Cell Biol 2002; 34: 1508-1512.

[13] DeLano FA, Parks DA, Ruedi JM, Babior BM and Schmid-Schonbein GW. Microvascular display of xanthine oxidase and NADPH oxidase in the spontaneously hypertensive rat. Microcirculation 2006; 13: 551-566.

[14] Costa RR, Villela NR, Souza M, Boa BC, Cyrino FZ, Silva SV, Lisboa PC, Moura EG, Barja-Fidalgo TC and Bouskela E. High fat diet induces central obesity, insulin resistance and microvascular dysfunction in hamsters. Microvasc Res 2011; 82: 416-422.

[15] Ansell BJ. Cholesterol, stroke risk, and stroke prevention. Curr Atheroscler Rep 2000; 2: 92-96.

[16] Tiku ML, Liesch JB and Robertson FM. Production of hydrogen peroxide by rabbit articular chondrocytes. Enhancement by cytokines. J Immunol 1990; 145: 690-696.
[17] Becker KJ. Targeting the central nervous system inflammatory response in ischemic stroke. Curr Opin Neurol 2001; 14: 349-353.
[18] Jiang YR, Miao Y, Yang L, Xue M, Guo CY, Ma XJ, Yin HJ, Shi DZ and Chen KJ. Effect of chinese herbal drug-containing serum for activating-blood and dispelling-toxin on ox-LDL-induced inflammatory factors' expression in endothelial cells. Chin J Integr Med 2012; 18: 30-33.
[19] Chan PH. Reactive oxygen radicals in signaling and damage in the ischemic brain. J Cereb Blood Flow Metab 2001; 21: 2-14.
[20] Cao XL, Du J, Zhang Y, Yan JT and Hu XM. Hyperlipidemia exacerbates cerebral injury through oxidative stress, inflammation and neuronal apoptosis in MCAO/reperfusion rats. Exp Brain Res 2015; 233: 2753-2765.
[21] Lima VV, Giachini FR, Matsumoto T, Li W, Bressan AF, Chawla D, Webb RC, Ergul A and Tostes RC. High-fat diet increases O-GlcNAc levels in cerebral arteries: a link to vascular dysfunction associated with hyperlipidaemia/obesity? Clin Sci (Lond) 2016; 130: 871-880.
[22] Leung H, Wang JJ, Rochtchina E, Wong TY, Klein R and Mitchell P. Dyslipidaemia and microvascular disease in the retina. Eye (Lond) 2005; 19: 861-868.
[23] Song SH and Roach MR. Surface features of human aortic atherosclerosis as seen with scanning electron microscopy. Yonsei Med J 1998; 39: 430-438.
[24] Reina-De La Torre F, Rodriguez-Baeza A and Sahuquillo-Barris J. Morphological characteristics and distribution pattern of the arterial vessels in human cerebral cortex: a scanning electron microscope study. Anat Rec 1998; 251: 87-96.
[25] Farkas E and Luiten PG. Cerebral microvascular pathology in aging and Alzheimer's disease. Prog Neurobiol 2001; 64: 575-611.
[26] Mato M, Ookawara S, Mashiko T, Sakamoto A, Mato TK, Maeda N and Kodama T. Regional difference of lipid distribution in brain of apolipoprotein E deficient mice. Anat Rec 1999; 256: 165-176.
[27] Bang OY, Chung JW, Ryoo S, Moon GJ, Kim GM, Chung CS and Lee KH. Brain microangiopathy and macroangiopathy share common risk factors and biomarkers. Atherosclerosis 2016; 246: 71-77.
[28] Wilde DW, Massey KD, Walker GK, Vollmer A and Grekin RJ. High-fat diet elevates blood pressure and cerebrovascular muscle Ca(2+) current. Hypertension 2000; 35: 832-837.
[29] Li W, Prakash R, Chawla D, Du W, Didion SP, Filosa JA, Zhang Q, Brann DW, Lima VV, Tostes RC and Ergul A. Early effects of high-fat diet on neurovascular function and focal ischemic brain injury. Am J Physiol Regul Integr Comp Physiol 2013; 304: R1001-1008.
[30] d'Uscio LV, Smith LA and Katusic ZS. Hypercholesterolemia impairs endothelium-dependent relaxations in common carotid arteries of apolipoprotein e-deficient mice. Stroke 2001; 32: 2658-2664.
[31] Osmond JM, Mintz JD, Dalton B and Stepp DW. Obesity increases blood pressure, cerebral vascular remodeling, and severity of stroke in the Zucker rat. Hypertension 2009; 53: 381-386.
[32] Kitayama J, Faraci FM, Lentz SR and Heistad DD. Cerebral vascular dysfunction during hypercholesterolemia. Stroke 2007; 38: 2136-2141.
[33] Naderali EK, Brown MJ, Pickavance LC, Wilding JP, Doyle PJ and Williams G. Dietary obesity in the rat induces endothelial dysfunction without causing insulin resistance: a possible role for triacylglycerols. Clin Sci (Lond) 2001; 101: 499-506.
[34] Naderali EK and Williams G. Effects of short-term feeding of a highly palatable diet on vascular reactivity in rats. Eur J Clin Invest 2001; 31: 1024-1028.