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

Yin He1*, Dong Han2*, Maojing Shi3, Zhifu Sun4, Jiayi Xie2, Jihong Zhu1, Yuansheng Liu1

1Department of Emergency, Peking University People’s Hospital, 11 South Xi Zhi Men Street, Xicheng District, Beijing 100044, The People’s Republic of China; 2National Center for Nanoscience and Technology, Beijing, The People’s Republic of China; 3Department of Emergency, Peking University International Hospital, Beijing 100044, The People’s Republic of China; 4Otorhinolaryngology Head and Neck Surgery, Beijing Anzhen Hospital, Capital Medical University, Beijing, The People’s Republic of China. *Equal contributors.

Received May 19, 2016; Accepted August 1, 2016; Epub November 1, 2016; Published November 15, 2016

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
High-fat diet affects cerebral microvasculature

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.
High-fat diet affects cerebral microvasculature

**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).
High-fat diet affects cerebral microvasculature

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-
High-fat diet affects cerebral microvasculature

...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 vasodilatory 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 dyslipidemia 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...
High-fat diet affects cerebral microvasculature

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.

Acknowledgements

We thank the National Natural Science Foundation of China for valuable support [NO. 11372012].

Disclosure of conflict of interest

None.

Address correspondence to: Yuansheng Liu, Department of Emergency, Peking University People's Hospital, 11 South Xi Zhi Men Street, Xicheng District, Beijing 100044, The People's Republic of China. E-mail: lyspku@126.com

References

High-fat diet affects cerebral microvasculature


