



BRAIN MICROVASCULATURE OF CAFETERIA DIET-FED RATS

Emily Schaefer¹, Mariana Gomez-Smith^{2,3}, Adrienne Dorr⁴, Bojana Stefanovic^{3,4}, Dale Corbett^{2,3}

1: University of Ottawa, 2: Cellular and Molecular Medicine, University of Ottawa, 3: Canadian Partnership for Stroke Recovery, 4: Sunnybrook Research Institute, 5: Medical Biophysics, University of Toronto



uOttawa

Introduction

The prevalence of metabolic syndrome (MetS) in Canada has rapidly increased from 15% in 2009 [1] to 20% in 2013 [2]. This surge in rate of MetS needs to be taken seriously as it is one of the strongest risk factors for cardiovascular disease, itself a leading cause of death in Canada [3]. MetS is caused by poor lifestyle, with insufficient amounts of exercise and intake of a diet rich in fat, sugar and salt. According to the NCEP ATP III definition, a patient is considered to have MetS when at least three of the following five criteria are present: increased waist circumference and triglyceride levels, reduced HDL cholesterol, elevated blood pressure and raised fasting blood glucose [4]. Although many studies have detailed the peripheral symptoms of MetS, few have examined its effects on the brain, particularly on the small blood vessels, or the microvasculature. Aberrant changes in blood vessel structure are linked to impaired blood flow and increased resistance which is detrimental for vascular health [5]. In our study, a Cafeteria (CAF) diet was used to mimic the effects of the human "Western diet". The CAF diet consisted of high fat, sugar and salt content human grocery store-purchased "junk" foods. Rats fed the CAF diet were compared to rats fed a standard diet (SD) and their brain vasculature was studied. Analysis included the quantification of penetrating vessel tortuosity, red blood cell stases, string vessels and enlarged cortical vessels (diameter $\geq 30\mu\text{m}$). Blood vessel density was also measured to examine angiogenesis. Gaining a better understanding of structural changes occurring in the microvasculature consequent to an unhealthy diet is the first step in better understanding the mechanisms of disease progression.

Methods

Rats were fed a SD and a CAF diet (Figure 1) for three months after which craniotomies were done to implant a cranial window in the skull of each rat (Figure 2). Following this surgery, *in vivo* two-photon fluorescent microscopy was performed to image the surface cerebral microvasculature. 3D images of the vasculature were analyzed using Imaris software to manually quantify vessel tortuosity, number of branch points, and vessel diameter. In a separate experiment, stroke was induced in CAF and SD diet animals using the ET-1 (endothelin-1) stroke model, following 3 months on the diet. Ten days later, animals were perfused with 4% PFA. Brains were removed, cryoprotected and cryosectioned to perform immunohistological examination of the vasculature using an antibody against RECA-1, an endothelial cell marker. Analysis of vascular structure included the quantification of red blood cell stases, string vessels and enlarged cortical vessels using stereology, as well as measuring blood vessel density using ImageJ.



Figure 1: Exposure of rats to standard (SD) and Cafeteria (CAF) diets. SD rats are given standard chow (Harlan 1818) while CAF diet rats are given the option of the standard chow and three types of human "junk" food items daily, as well as two bottles containing water or 12% sucrose (used to mimic the sugar content in soda).



Figure 2: Craniotomy (left) and two-photon microscope apparatus (right). Anaesthetized rats underwent craniotomy surgery in which a 5 mm cranial window was placed above the sensory-motor cortex to visualize cortical microvasculature. Afterwards, rats were secured in a stereotaxic frame and placed underneath the objective of a two-photon microscope.

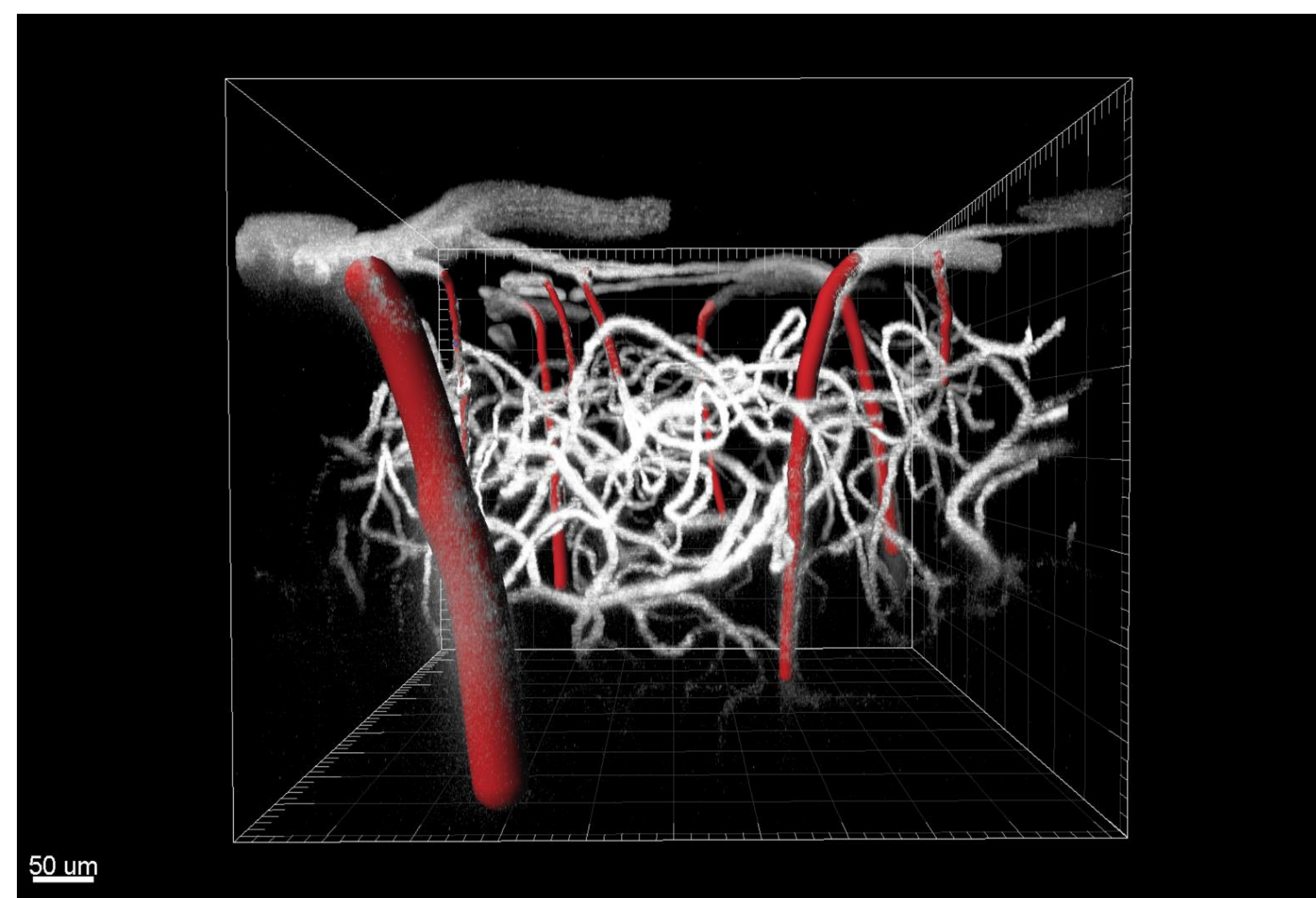


Figure 3: 3D vascular image taken using a two-photon microscope. Vessel diameter, number of branch points and tortuosity of penetrating vessels (red vessels) were manually quantified using Imaris software. Penetrating vessels were imaged up to 500 μm in depth from the surface of the brain.

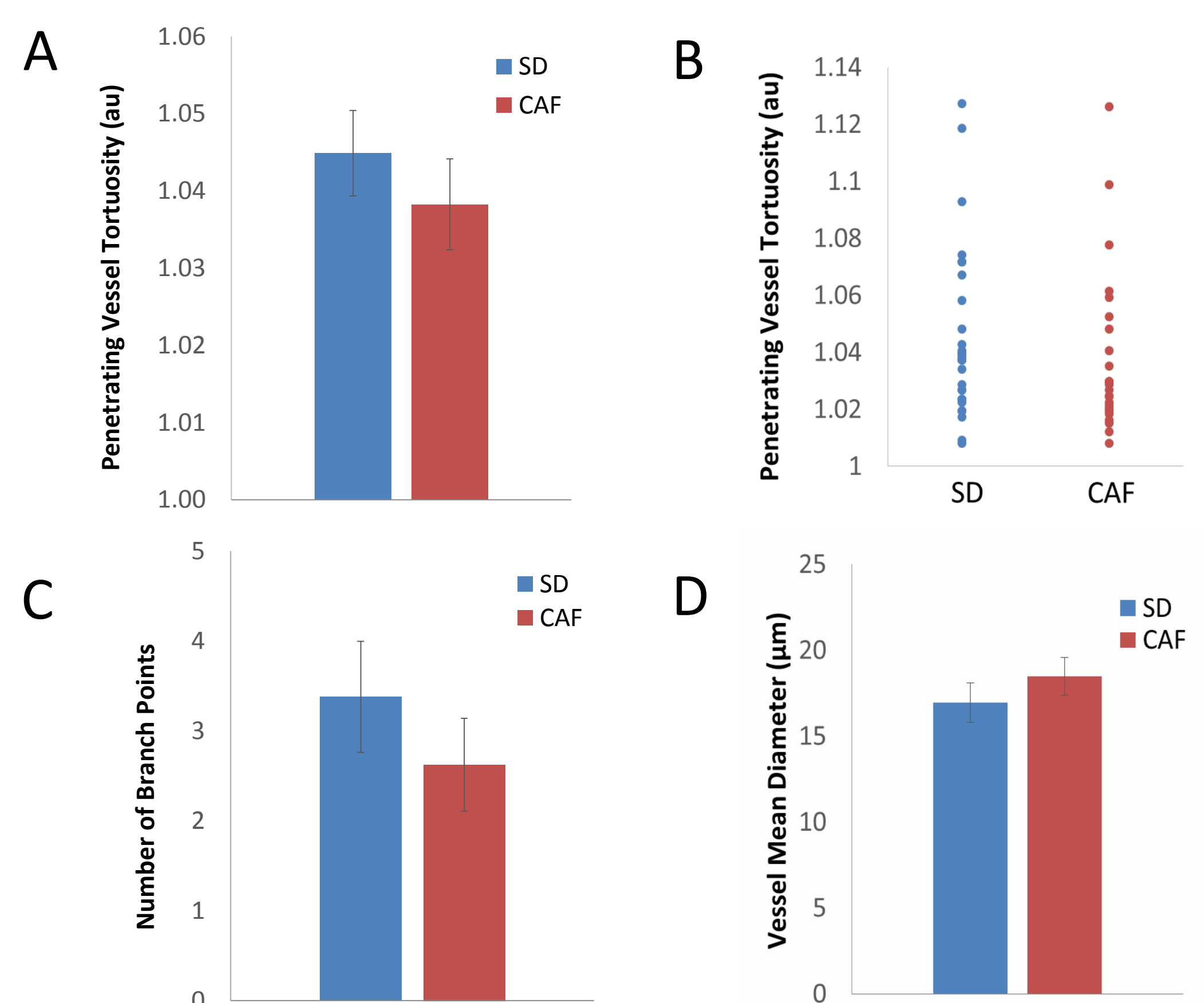


Figure 4: Quantitative analysis of vessel tortuosity, number of branch points and mean diameter of penetrating vessels in SD ($n = 4$) versus CAF ($n = 4$) rats. A + B: Penetrating vessel tortuosity measured in arbitrary units represented as a bar graph (A) and as a scatterplot (B) ($p = 0.417$). C: Number of vessels branching from each penetrating vessel ($p = 0.366$). D: Penetrating vessel mean diameter measured in μm ($p = 0.146$). Tortuosity was calculated using the following formula: vessel length/(vessel length x vessel straightness).

Results

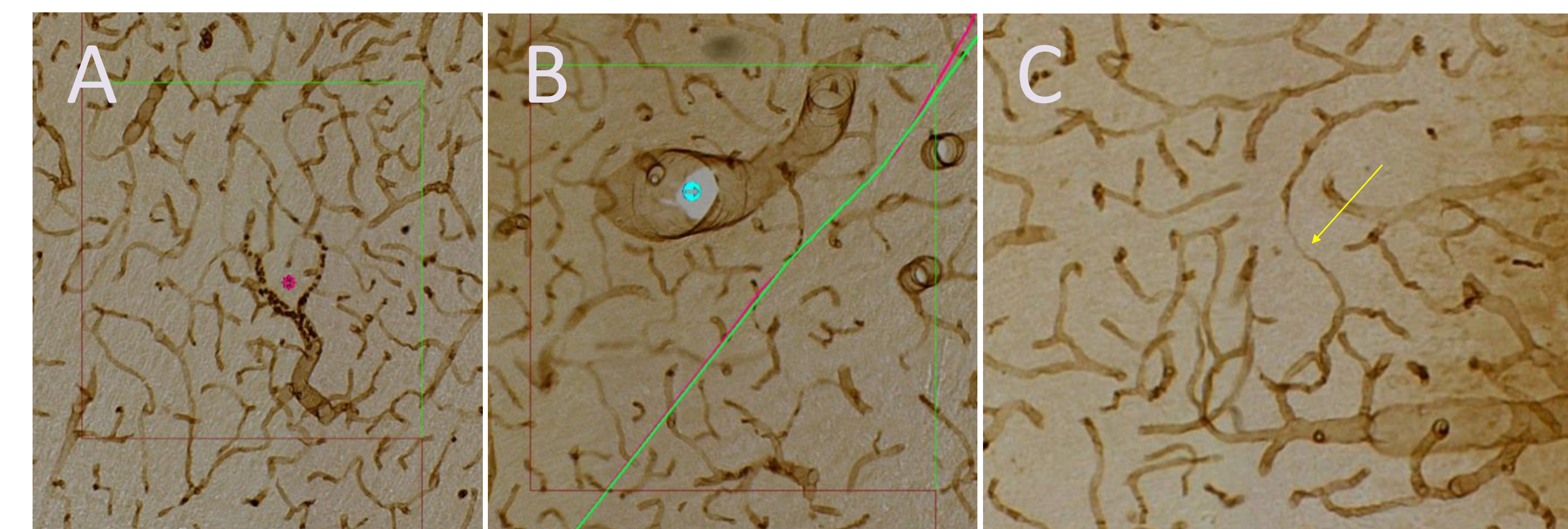


Figure 5: Representative structural pathologies in RECA-1 stained brain tissue. A: Red blood cell stasis, pink star. B: Enlarged cortical vessel, blue arrow. C: String vessel, yellow arrow. Structures were manually quantified using StereoInvestigator software from 50 μm free-floating brain slices stained with anti-RECA-1 antibody. Vessels with a diameter greater than 30 μm were considered enlarged cortical vessels. Clusters of two or more red blood cells were considered stases. Two connected vessels separated by a segment of notably reduced diameter were considered string vessels.

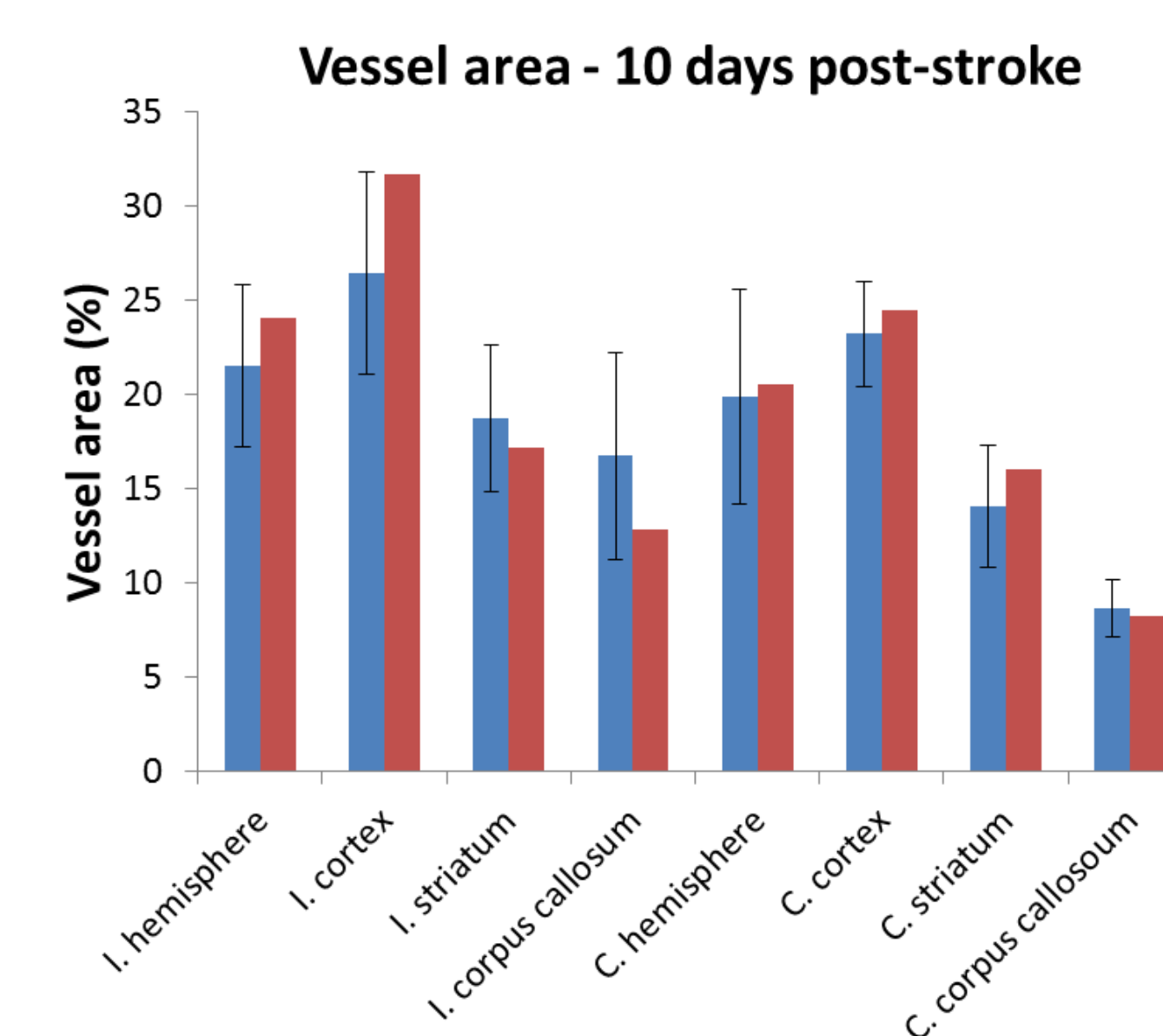
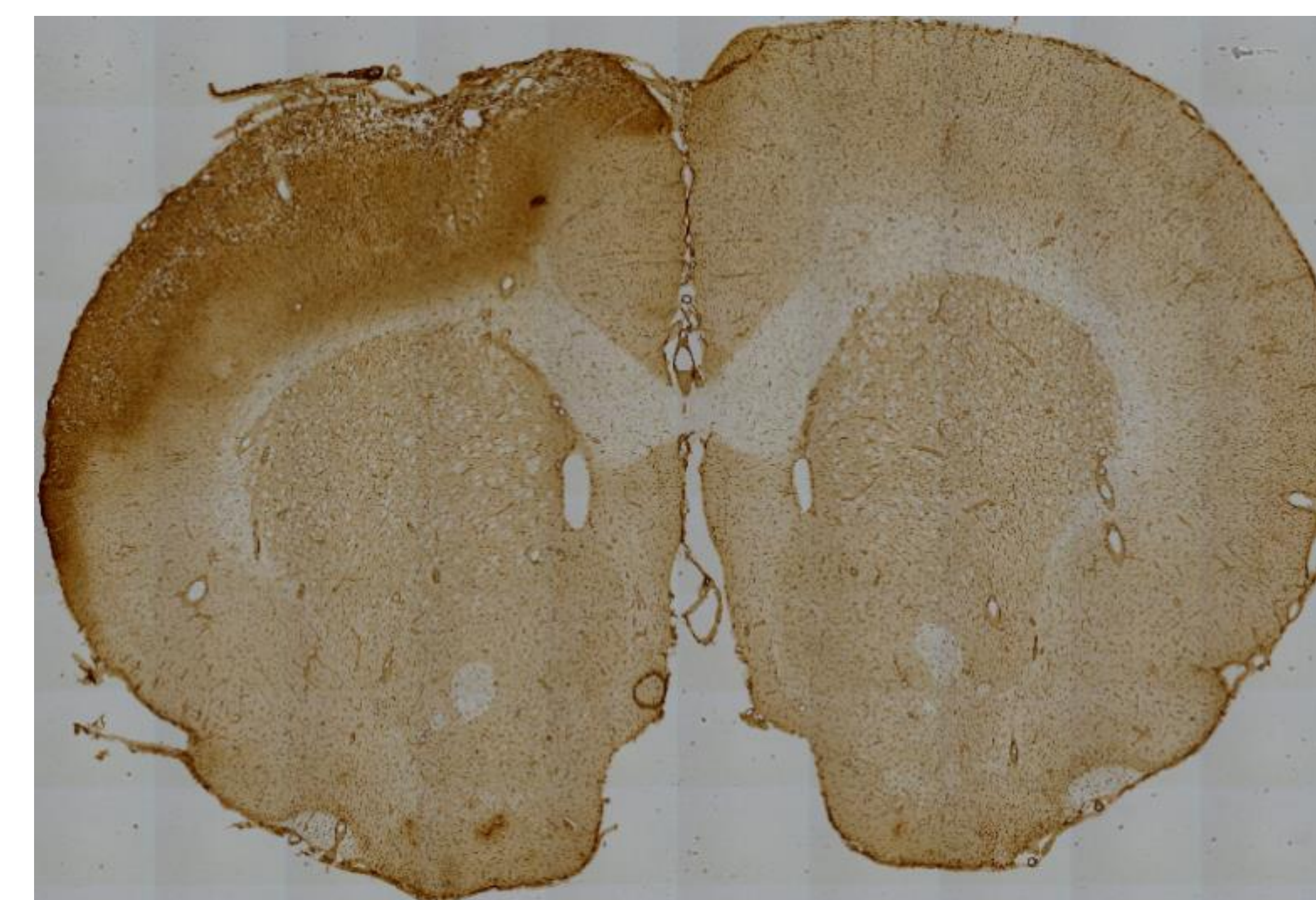


Figure 6: Blood vessel density in the brains of SD and CAF animals following ET-1-induced stroke. Image of coronal sections stained with RECA-1 were binarized from their original RGB colour format (above) and different brain regions were manually traced. Vessel density was quantified using pixel density within the traced areas (left).

Conclusions

- This project aimed to study changes in microvascular structure due to consumption of an unhealthy diet high in fat, sugar and salt. Better understanding the effects of "junk" food on the brain may help to elucidate the mechanisms of disease progression.
- Assessed with two-photon fluorescent microscopy, penetrating vessel tortuosity, mean vessel diameter and number of branch points were not significantly different between SD and CAF rats.
- Further work needs to be done to complete the analysis of red blood cell stases, enlarged cortical vessels, string vessels as well as vessel density using traditional histology. A larger sample size is needed to accurately describe these outcome measures.
- Future directions could include analysis of blood flow in live animals using bolus tracking and MRI to determine whether any structural changes are reflected at the functional level.

References

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