Modulation of Cerebral Blood Perfusion by Cocoa Flavanols in Aged Mice

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Abstract

Cocoa-derived flavanols, and in particular (-)-epicatechin, have been extensively shown to exert beneficial effects on vascular function in humans. More recently, there has been a growing interest in understanding to what extent such beneficial vascular effects in the periphery might benefit the central nervous system and further impact cognitive function, particularly in aging. In the present study, we examined the long-term impact (6 months) of oral supplementation with either i) cocoa-flavanols or ii) pure (-)-epicatechin on cerebral blood perfusion as assessed by 99m-Tc-HMPAO Single Photon Emission Computed Tomography (SPECT), in an aged mice model (16 to 22 months old). Preliminary data suggests that long-term chronic supplementation (6 months) with cocoa is more effective at maintaining cerebral blood perfusion in aging (parallel design, n=8; age x treatment effect: p=0.019), than pure (-) -epicatechin when delivered at equivalent doses. We are currently examining the effects of the dietary interventions on specific regions across the brain to identify the areas which were significantly affected by flavanol supplementation. Furthermore, we have observed that changes in blood perfusion are unlikely to be mediated by increases in density of blood vessels, as shown by our preliminary data depicting no differences in density of blood vessels across the hippocampal formation. Collectively, these data support a positive modulation of cerebral blood perfusion by cocoa flavanols and may have the potential to counteract age-associated decreases in brain health and cognition.

Introduction

Aging-associated cognitive decline is affecting an increasing proportion of the population and can have a devastating impact on the quality of life of the aging population. It has become apparent that a substantial contribution to this cognitive impairment during aging comes from deficits in cerebral blood flow and vascularization of the Central Nervous System (CNS) (1). More alarmingly, vascular deficits in the brain during aging may lead to more severe neurological disorders, such as vascular dementia and even Alzheimer’s disease (AD). Currently, there are no treatments in place for such neurological disorders, so it is important to identify lifestyle interventions, such as physical exercise and nutrition that may have the potential to prevent or delay the onset of cognitive decline. In particular, interventions that target the vascular systems may be an effective strategy to delay the onset of age related cognitive impairments and improve the quality of life. Cocoa-derived flavanols, in particular the flavanol (-) epicatechin, have been extensively shown to exert beneficial effects on vascular function in humans (2). More recently, there has been a growing interest in understanding to what extent such beneficial effects in the peripheral vascular system might benefit the CNS and cognitive function, particularly in aging (3).

To investigate the role of cocoa flavanols on vascular function in the CNS, we supplemented 16 month-old mice with either i) cocoa flavanols, ii) pure (-)-epicatechin, or iii) a control AIN93M diet (Figure 1A).

	CONTROL	COCOA	(-)-EPICATECHIN
FLAVANOLS (ug/g food)			
(-) Epicatechin	0	148	948
(+) Catechin	0	49	0
Procyanidins	0	751	0
Total	***0***	***948***	***948***
Other ingredients (mg/g food)			
Caffeine	0.024	0.024	0.024
Theobromine	0.209	0.209	0.209
Macronutrient breakdown (% by weight)			
Protein	12.4	12.4	12.4
Carbohydrate	68.3	68.1	68.2
Fat	4.1	4.1	4.1

Figure 1: *Compositional analysis of the flavanols' chronic interventions. Both cocoa and (-)-epicatechin interventions are matched for total levels of flavanols (948 ug/g food, approx. 102 mg/kg BW), with cocoa intervention containing the naturally occurring monomers, (-)-epicatechin and (+)-catechin, and procyanidins (oligomers), whilst the (-)-epicatechin intervention is composed of matching levels of the pure monomer (-)-epicatechin. All three interventions were matched for levels of caffeine and theobromine, as well as all other macronutrients and micronutrients.*

Global and regional blood perfusion were assessed using Single-Photon Emission Computed Tomography (SPECT-CT) before (16 months) and after (22 months) the chronic dietary interventions (Figure 1B). A radioactive tracer, 99m-Tc-labelled hexamethyl-propyleneamine oxime (99m-Tc-HMPAO), was injected 20 mins prior to imaging on i) structural X-ray CT and ii) HMPAO-SPECT. Hippocampal blood-vessel density was measured in individual regions of the hippocampus (CA1, CA2, CA3, DG) by performing an immunohistochemistry for Collagen IV.

Figure 2: *SPECT-CT imaging system for small rodents.*

Results

Cocoa Flavanols Prevent Decrease in Global Cerebral Blood Perfusion in Aging

Following the 6 month dietary interventions, we found that cocoa flavanols, but not the equivalent levels of pure (-)-epicatechin, prevent a decrease in blood brain perfusion associated with aging (Figure 3). The group given the control diet showed a significant decrease in global HMPAO brain uptake from 16 months to 22 months, indicating that there is a natural decline in global cerebral blood perfusion with age. The global HMPAO brain uptake for the pure (-)-epicatechin group showed a decline with age similar to the control group, which leads us to

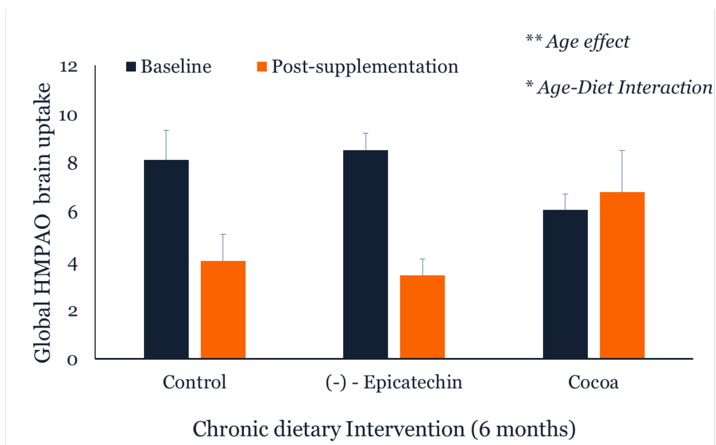

Figure 3: *Global cerebral brain perfusion measured by HMPAO brain uptake before and after dietary supplementation with either control, (-)-epicatechin and cocoa (N=7-10, parallel design). A significant effect of aging (p=0.002) and interaction age x diet (p=0.019) was detected.*

believe that pure (-)-epicatechin does not prevent the decrease in blood brain perfusion due to aging. However, the cocoa flavanol group did not show a decline in global HMPAO brain uptake due to age, which shows that it was able to prevent the age-related decline in global blood perfusion.

Pure (-)-Epicatechin and Cocoa Flavanols Do Not Affect Blood Vessel Density in the Hippocampus

In the granule layer (Figure 5A) and molecular layer (Figure 5B) of the dentate gyrus of the hippocampus, there were no significant differences in the area fraction covered by blood vessels due to dietary interventions.

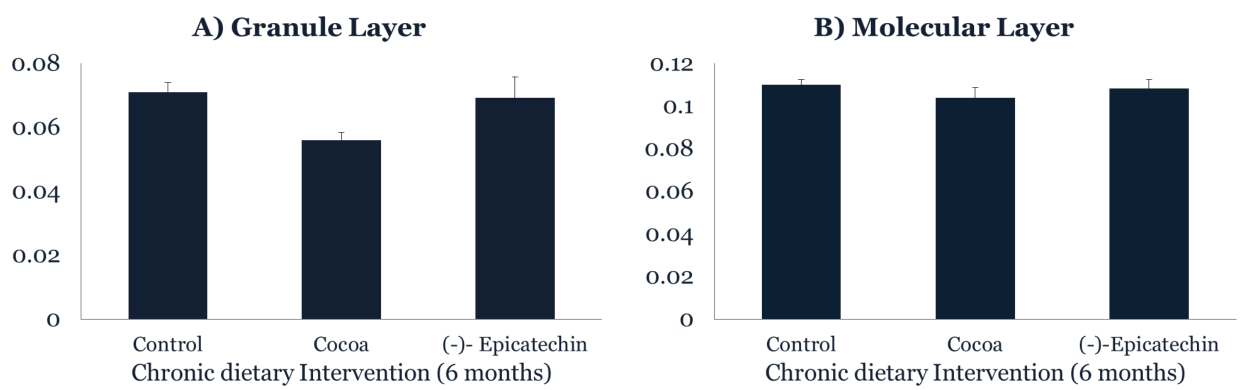

Figure 4: *Area fraction covered by blood vessels in the A) granule layer and B) molecular layer of the dentate gyrus of the hippocampus measured by collagen IV immunohistochemistry shows no significant changes following chronic supplementation with either control, cocoa flavanol or pure (-)-epicatechin diet (N=7-10).*

Regional Blood Perfusion

We are currently in the process of analyzing regional blood perfusion across the central nervous system to determine if there are any differences in blood flow in certain areas of the brain due to diet. Specifically, we are looking to see if the protective effects that we observed in animals fed cocoa flavanols is specific to certain regions of the brain, or if this effect is a global phenomenon that occurs all over the brain. To obtain this data, we are analyzing the SPECT-CT images using 3DSlicer, which is a program that allows us to measure the blood perfusion in specific areas of the brain. We have divided the brain into 15 distinct regions, and will be looking to see if the diets have any effect on blood perfusion in these areas.

Discussion

These results show that chronic intake of cocoa flavanols, but not pure (-)-epicatechin, appears to rescue from an age-related decline in cerebral blood perfusion globally. We also discovered that neither cocoa flavanols nor pure (-)-epicatechin improves the blood vessel density in regions of the hippocampus relative to the control. This leads us to believe that cocoa flavanols do not increase the number of blood vessels, but rather they maintain the function of the blood vessels in aged animals, allowing them to more efficiently pump blood throughout the brain. Once we finish collecting the data for regional blood perfusion, we will be able to determine whether the protective effect that cocoa flavanols have in preventing the decline in blood perfusion due to aging is purely a global effect, or if there are certain areas of the brain that are protected by the cocoa flavanols. Overall, the data collected from this study shows that a diet containing cocoa flavanols could possibly be a lifestyle intervention used to delay certain aspects of aging, specifically the decline in cerebral blood perfusion.

References

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