# Assessment of cerebrovascular reactivity as a function of age in a transgenic mouse model of Alzheimer's Disease displaying significant vascular pathology using acetazolamide.

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**INTRODUCTION:** The most specific and exclusive pathological hallmark of Alzheimer's Disease (AD) is the extracellular occurrence of amyloid plaques, consisting of accumulated β-peptides, a downstream product of the amyloid precursor protein (APP). β-Amyloid deposition can occur in the brain parenchyma as well as around cerebral blood vessels (cerebral amyloid angiopathy CAA). Amyloid deposition is associated with compromised functional CNS responses to various types of stimuli in transgenic mouse models as assessed by fMRI [1,2]. To what extent this is due to impaired neural activity or a consequence of the angiopathy is currently not known. arcAβ mice, a transgenic mouse model overexpressing human APP695 containing both the Swedish and the Arctic mutation, develop a high vascular plaque load in an age dependent manner from the age of 7 month on [3]. The progressive course of CAA in this mouse model is therefore well suited to investigate its functional consequence on the fMRI response using vasodilatory stimulation.

**METHODS:** Animals: two age groups of arcAβ (tg) and age-controlled littermates (wt) were used for this study: 16 month ($N_{(tg)}$=9, $N_{(wt)}$=10) and 23 month old ($N_{(tg)}$=6, $N_{(wt)}$=8). Animals were anesthetized with isofluorane, intubated and artificially ventilated during the MR experiment. The mice were placed on a water-heated cradle and all agents were injected via cannula into the tail vein. All animal experiments were performed in strict adherence to the Swiss Law for Animal Protection. fMRI protocol: experiments were performed on a Pharmascan 47/16 (Bruker BioSpin GmbH, Karlsruhe, Germany). Scan parameters of the RARE sequence [4] were: temporal resolution: 40s, repetition time (TR): 2500ms, effective echo time ($TE_{eff}$): 80.2ms, field of view (FOV): 20x13mm$^2$, RARE factor: 32, matrix dimension: 128x128, slice thickness: 0.7mm, voxel dimension: 156x156x700µm$^3$, interslice distance: 1.2mm, number of averages: 4, number of slices: 5. The fMRI protocol comprised 3 phases: 8 baseline images ($S_{pre}$) were acquired as reference for the determination of the relative cerebral blood volume ($CBV_{rel}$) changes. Thereafter scanning was interrupted and contrast agent (Endorem® 55mg/kg) was injected as a bolus. After 15min to allow for contrast agent to reach steady state concentration, 35 postcontrast images (S(0)) were acquired. Injection of acetazolamide was followed by acquisition of 51 images (S(t)). Stimulation paradigm: acetazolamide (Diamox®), a carbon anhydrase inhibitor, is used in the clinics to determine cerebral vasoreactivity [5]. In mice administration of 30mg/kg acetazolamide has been shown to lead to an increase in CBV in cortical regions [6]. Data analysis was carried out using the Biomap software (M. Rausch. Novartis Institutes for Biomedical Research). Changes of $CBV_{rel}$ in percent of baseline values ($\Delta CBV_{\%}$) were computed on a pixel by pixel basis according to $\Delta CBV_{\%}(t)=\Delta CBV(t)/CBV(0)*100 = (\ln\{S(t)/S(0)\})/(\ln\{S(0)/S_{pre}\})*100$. Further analysis were performed on OriginPro 7.5 SR6, USA.

**RESULTS:** Injection of acetazolamide at time t=0 leads to an increase in CBV in tg and wt mice at both ages (Fig.1). The $\Delta CBV_{\%}$ response in wt animals levels off at approximately 30% in both age groups. The respective maximal $\Delta CBV_{\%}$ value was approximately 20% in 16 month old and 15% in 23 month old tg mice. For further quantitative comparison, $\Delta CBV_{\%}$ versus time curves have been integrated for two time windows: over first 4min (Fig.2a) and over last 10min (Fig.2b). Statistical comparison of integral values for tg and wt mice (independent t-test) revealed the following p-values: a) first 4min: p=0.042 for 16 month old mice ($N_{(tg)}$=9, $N_{(wt)}$=10) and p=0.099 for 23 month old mice ($N_{(tg)}$=6, $N_{(wt)}$=8); b) last 10min: the respective values were p=0.085 for 16 and p=0.137 for 23 month old animals. For the first integration period the analysis for age effects indicated a deterioration of CBV response from 16 to 23 month old tg animals at p=0.08; the respective p-value for the long integration period was p=0.34. No age related differences in the CBV response has been observed in wt animals.

**CONCLUSIONS:** The hypothesis that CAA in arcAβ mice may interfere with the vascular response to a vasodilatory stimulation has been addressed using fMRI. Two parameters of cerebrovascular reactivity can be derived from dynamic CBV profiles: the rate of vasodilation and the CBV value corresponding to maximally dilated vessels. We suggest that integration of CBV-profiles over the initial phase of the response (Fig 2a) reflects vascular reactivity, i.e. capability of the blood vessel to react to a vasodilatory challenge with rapid vasodilation. Integration over the late period (Fig.2b) provides information about the maximal vasodilation (vascular reserve capacity). The difference in the initial CBV response between arcAβ and control mice at both ages indicates impaired vascular reactivity in tg mice displaying CAA. In addition, there is a tendency that this effect deteriorates with age, i.e. with increasing severity of the angiopathy. No age-related effect was observed in wt animals, indicating a potential role of CAA in this finding. The maximal CBV values tended to be lower in 16 months arcAβ as compared to age-matched wt animals; in 23 month old animals the difference in average values was even larger, yet failed to reach statistical significance due to large variability in data. The analysis for age-related effects on maximal CBV values did not reveal statistically significant difference neither for tg nor for wt mice. In summary: arcAβ mice display a reduced CBV-increase indicative of compromised blood vessel properties potentially due to CAA. Combining fMRI readouts with tg models displaying various degrees of parenchymal versus vascular amyloid deposits and suitable stimulation paradigms allows elucidating the role of the two forms cerebral amyloid deposits in controlling the hemodynamic response. This is of interest for investigation of functional consequences of immunotherapies known to modulate plaque load [6].

**REFERENCES: [1]** Mueggler et al.,Journal of Neurosciences,2002; **[2]** Kranz et al., Proc ISMRM 2007, **[3]** Knobloch et al., Neurobiol. of Aging,2006; **[4]** Hennig et al., Magn.Reson.Med.,1986; **[5]** Grossmann et al., Cerebrovas.Dis.,2000; **[6]** Wilcock et al., J. Neuroinflammation, 2004.

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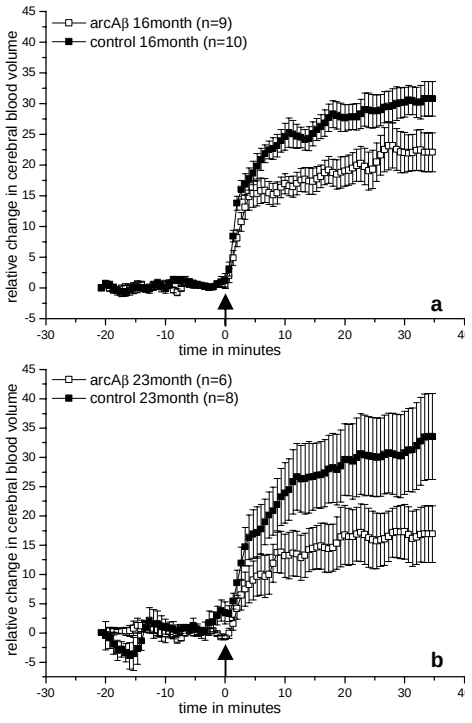


**Figure 1**: change in cerebral blood volume (CBV) in parietal cortex as a function of time after injection of acetazolamide in (**a**) 16 month and (**b**) 23 month old arcAβ and control mice (values: mean±SEM).

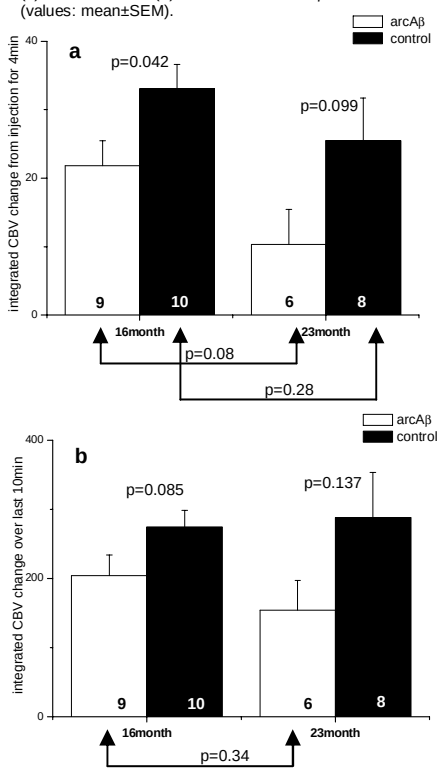


**Figure 2**: integrated $\Delta CBV\%$ response in parietal cortex. Integration after acetazolamide-injection for (**a**) first 4min and (**b**) last 10min in 16 and 23 month old arcAβ and control mice (values:mean+SEM).