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Pilot Data on Brain-to-Blood Efflux of B-Amyloid Peptides in Man

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Background to the study

- Alzheimer's disease (AD) is the most common cause of dementia and affects nearly 40,000 individuals in Ireland.
- The β -amyloid peptide ($A\beta$) plays a key role in the pathogenesis of the AD and the presence of $A\beta$ plaques in the brain is diagnostic.
- The hypothesis posits that $A\beta$ deposition is a critical factor in the disease process and that production and clearance of $A\beta$ are key drivers of the disease¹.
- Flux of $A\beta$ from the brain is believed to contribute to the overall level of $A\beta$ within in brain² and antibody mediated brain-to-blood efflux has been observed in animal models³.
- Clearance of from the blood is believed to be mainly via the liver, kidney and spleen⁴.
- Data from human studies indicate that the about 6% of the $A\beta$ pool present in the cerebrospinal fluid is cleared per hour⁵.
- There are no data available on the magnitude of the cerebral output of $A\beta$ peptides in man or the hepatic uptake.
- The aim of this work was to investigate if the concentration $A\beta$ peptides is different in jugular venous plasma and arterial plasma and so estimate direct values for both brain-to-blood $A\beta$ efflux and hepatic clearance in man.

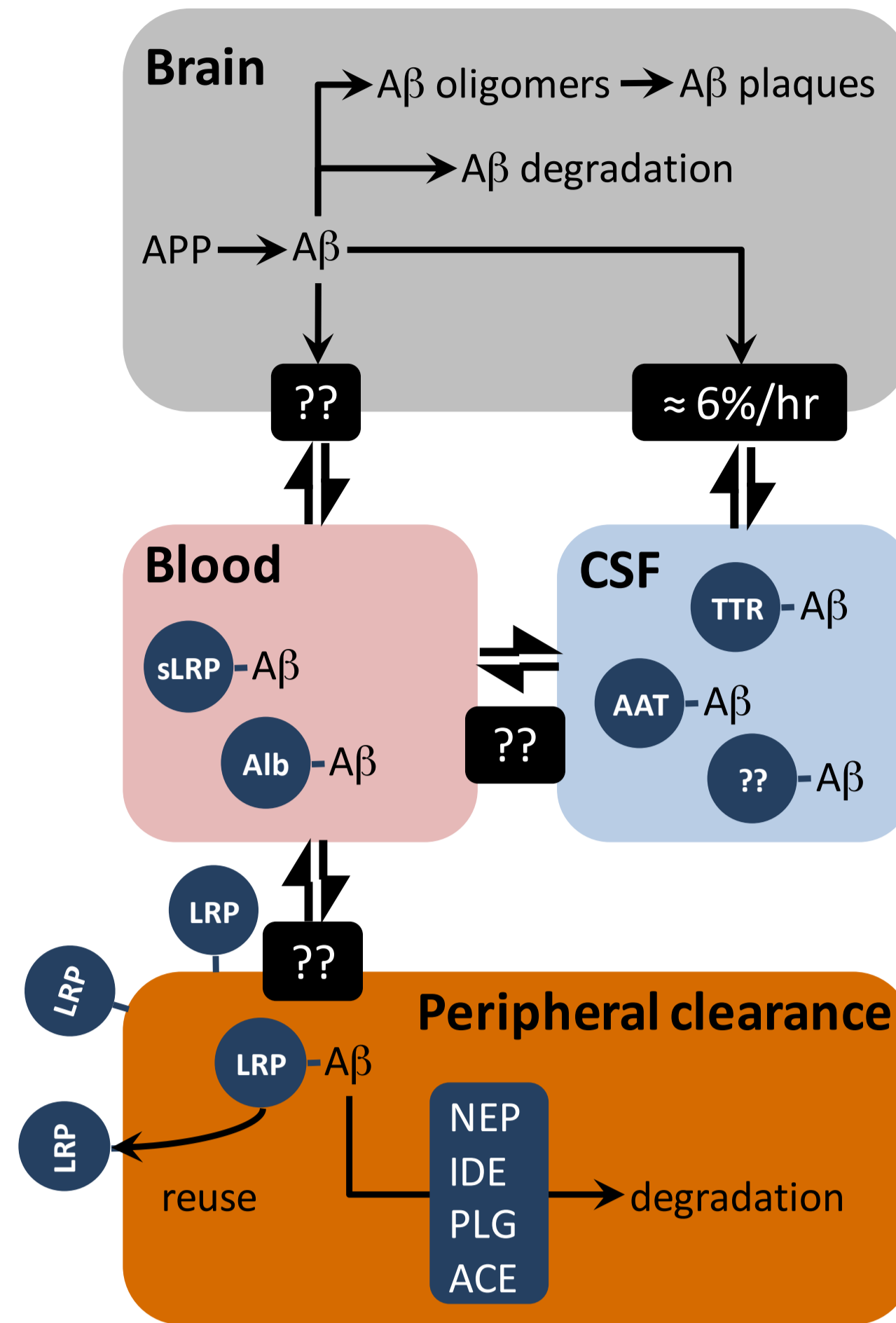


Figure 1. Model of $A\beta$ fluxes in man. $A\beta$ produced in the brain passes into the blood, either directly across the blood-brain barrier or via the cerebrospinal fluid (CSF). It is carried in a complex with numerous different proteins. The liver, kidney and spleen can take up and metabolise $A\beta$ via various proteases. LRP1=low density lipoprotein receptor related protein 1; Alb=albumin; TTR=transthyretin; AAT= α 1-antiprotease; NEP= nephylilisin; IDE=insulin degrading enzyme; PLG=plasmin; ACE=angiotensin converting enzyme

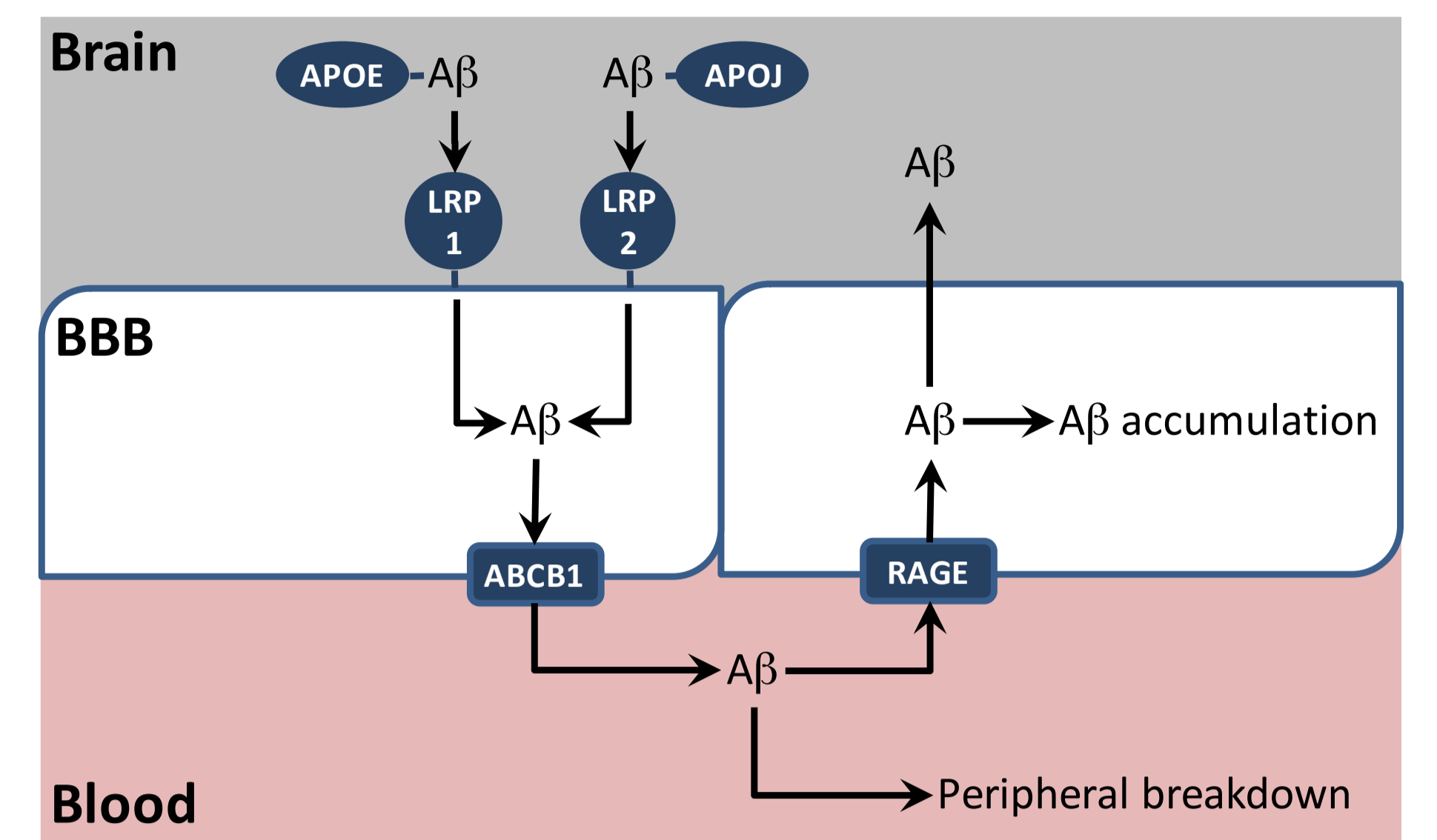


Figure 2. Mechanism of transport of $A\beta$ across the blood-brain barrier. $A\beta$ produced in the brain becomes bound to apolipoproteins within the brain interstitial fluid. Brain microvascular endothelial cells can take up APOE- $A\beta$ and APOJ- $A\beta$ complexes via mechanisms dependent on LRP1 or LRP2, respectively. Intracellular $A\beta$ may then be effluxed into the circulation via a process involving ABCB1 where the $A\beta$ may either be degraded peripherally or become subjected to RAGE dependent uptake. This latter process leads to the re-entry of $A\beta$ into the brain and is effectively a futile cycle. APOE=apolipoprotein E; APOJ=apolipoprotein J; LRP1=low density lipoprotein receptor related protein 1; LRP2=low density lipoprotein receptor related protein 2; ABCB1=ATP binding cassette transporter B1; RAGE=receptor for advanced glycation endproducts.

Experimental Methods

- Blood samples were obtained as described⁶.

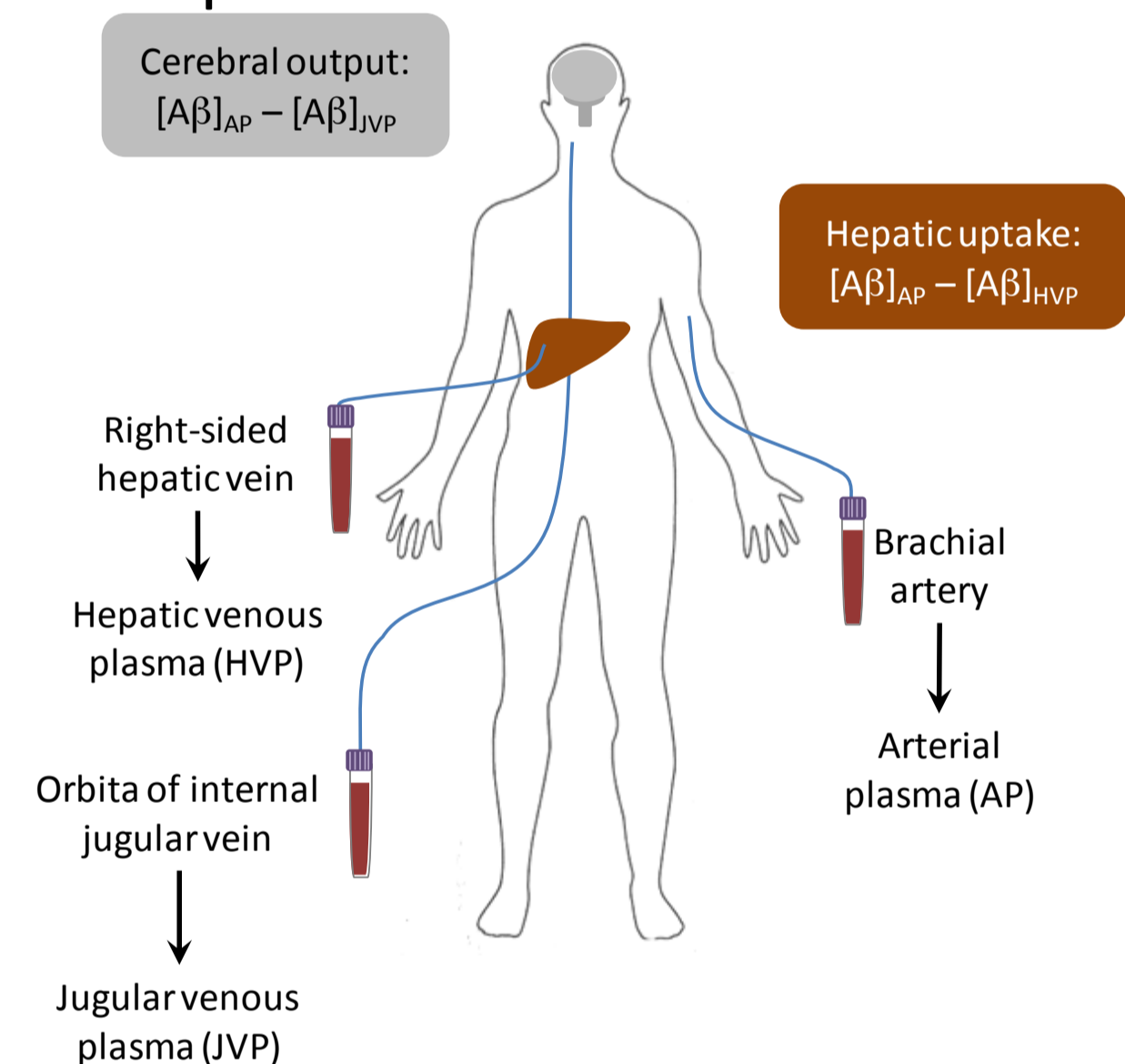


Figure 3. Sampling strategy for organ specific arterial and venous plasma. Blood samples were taken simultaneously from catheters inserted percutaneously and positioned as above.

- Plasma samples:** These were available in connection with a previous study on brain sterol fluxes⁶. Briefly, ten healthy males, mean age 29 years (range, 21–38 years) were recruited for this study and blood samples were taken after an overnight fast. Plasma was frozen at -80°C until required for analysis.

- Ethics:** All experiments involving human volunteers were reviewed and approved by the ethics committees at the Huddinge Hospital and the Karolinska Hospital. Participants gave informed consent to participate in the study

- ELISA for $A\beta$:** Specific antibodies against $A\beta_{x-40}$ and $A\beta_{x-42}$ were used as primary antibodies. The reporter antibody was horseradish-peroxidase-linked anti-rabbit IgG and colour was developed with o-phenylenediamine. The detection limit for synthetic $A\beta_{x-40}$ and $A\beta_{x-42}$ was 1 pM. All samples were analyzed in the linear range of the ELISA.

- Data Analysis:** The concentration of $A\beta_{x-40}$ and $A\beta_{x-42}$ in the arterial and venous plasma was compared using a non-parametric approach. The significance level was set at 0.05. The molar concentrations of $A\beta_{x-40}$ and $A\beta_{x-42}$ were calculated. The percent extraction of individual $A\beta$ species was calculated according to:

$$\text{Percent extraction} = [(C_a - C_v) / C_a] \times 100$$

Negative values were considered to represent a net output. Daily fluxes were estimated according to:

$$\text{Daily extraction} = (C_a - C_v) \times \text{organ plasma flow}$$

For the purposes of this calculation the cerebral and hepatic plasma flow were set at $650\text{L}\cdot\text{d}^{-1}$ and $1000\text{L}\cdot\text{d}^{-1}$ respectively.

Results

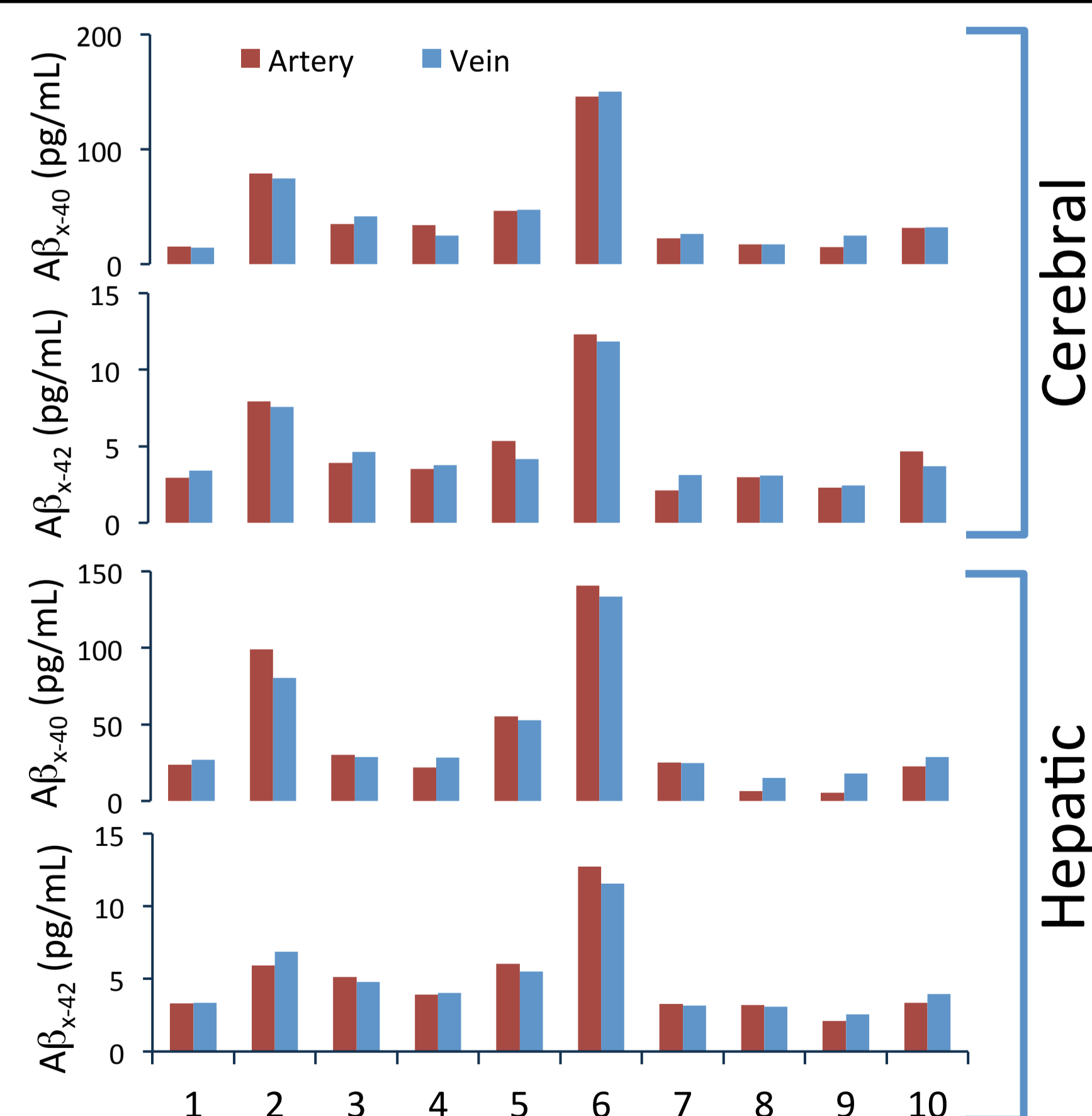


Figure 4. Paired absolute values of $A\beta_{x-40/42}$ in cerebral and hepatic plasma. In the cerebral circulation, greater concentration in the vein is consistent with output while the opposite applies in the hepatic circulation. Each number corresponds to an individual participant.

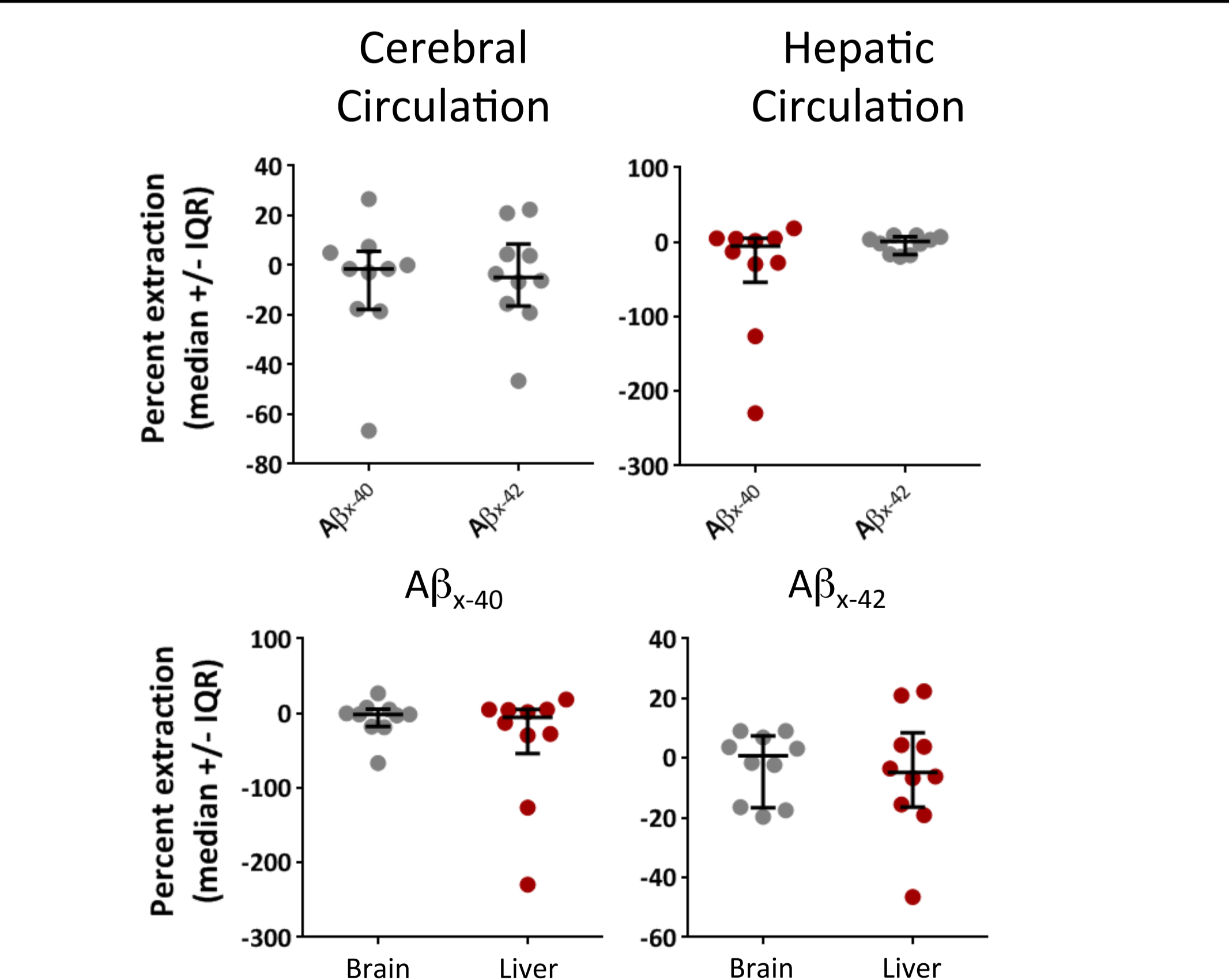


Figure 5. Absolute values of $A\beta_{x-40/42}$ in cerebral and hepatic plasma and inter organ fluxes. In the cerebral circulation a negative percent extraction is equivalent to an output while in the hepatic circulation the opposite applies. No statistically significant difference were found using the Wilcoxon matched-pairs signed rank test.

Discussion

- This is the first attempt to directly quantify the brain-to-blood passage of $A\beta$ in man. The daily cerebral output of $A\beta_{x-40}$ was estimated to be $\approx 1\text{ng}\cdot\text{d}^{-1}$ and that of $A\beta_{x-41}$ was estimated to be $\approx 3\text{ng}\cdot\text{d}^{-1}$.

- Although the data was not statistically significant the values are in reasonable agreement with data from a transgenic rat model of $\approx 1.6\text{ng}\cdot\text{d}^{-1}$ for $A\beta_{x-40}$.
- There are two main limitations to this work:

- The main limitation in the current study is the small number of samples available which has affected the power of the study.

- A further limitation is that the material analysed was collected in connection with a previous study on brain sterol homeostasis.

- Given the paucity of the data available we considered it prudent to commence these investigations on a pilot basis and use the data to design larger studies.

- Based on the data available in connection with this study we estimate that a sample size of 40 would be required to have an 80% power to detect a difference in percent extraction of 13.5%.

- While this is an ambitious number of participants for a relatively invasive procedure we believe that the data generated would be very valuable for the field

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