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

Steve Meaney

*Technological University Dublin, [steve.meaney@tudublin.ie](mailto:steve.meaney@tudublin.ie)*

Maura Heverin

*Karolinska Institute*

Ingemar Bjorkhem,

*Karolinska Institute*

*See next page for additional authors*

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**Authors**

Steve Meaney; Maura Heverin; Ingemar Bjorkhem,; Dorotea Religa; John Wahren; and Ulf Diczfalusy



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Steve Meaney

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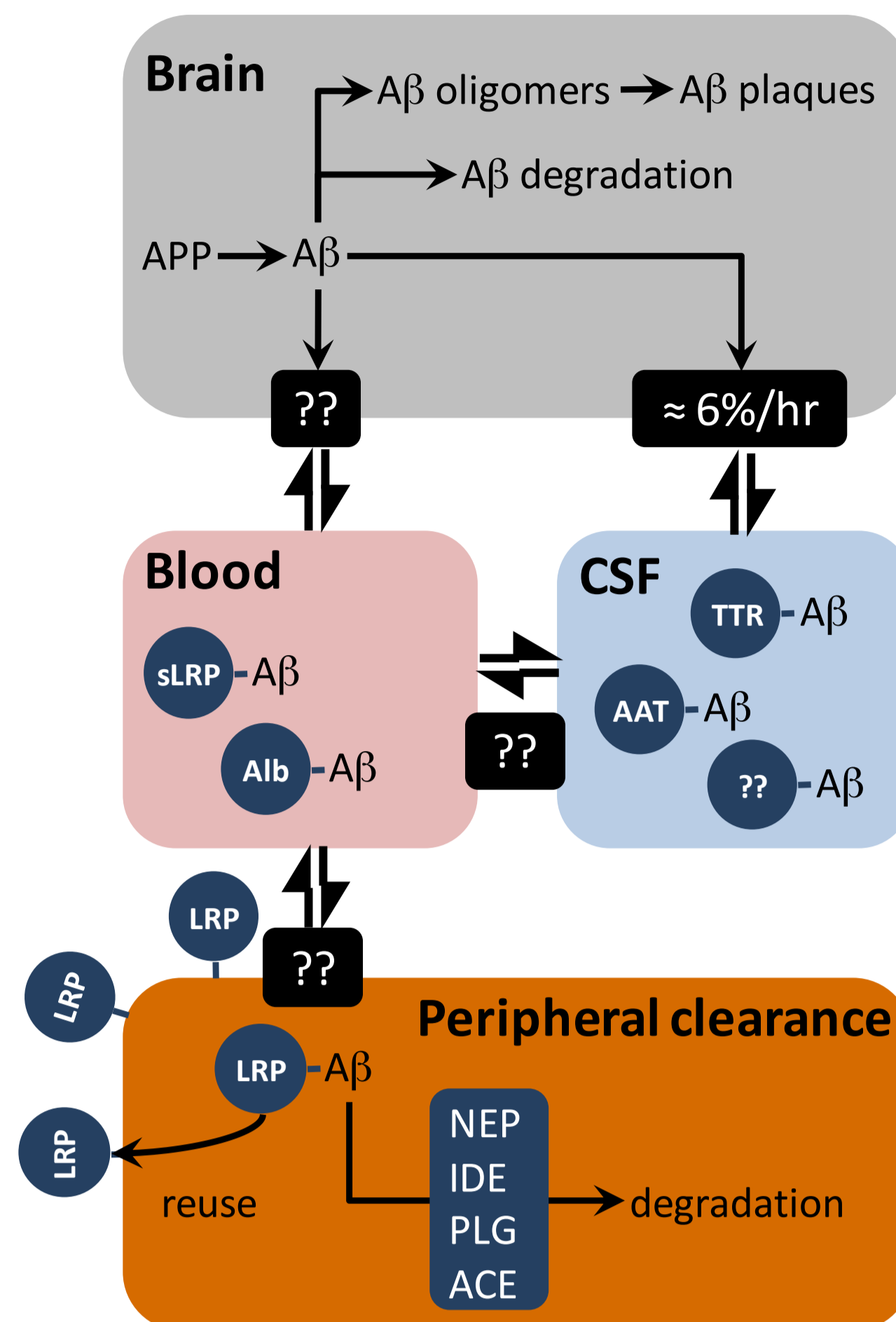
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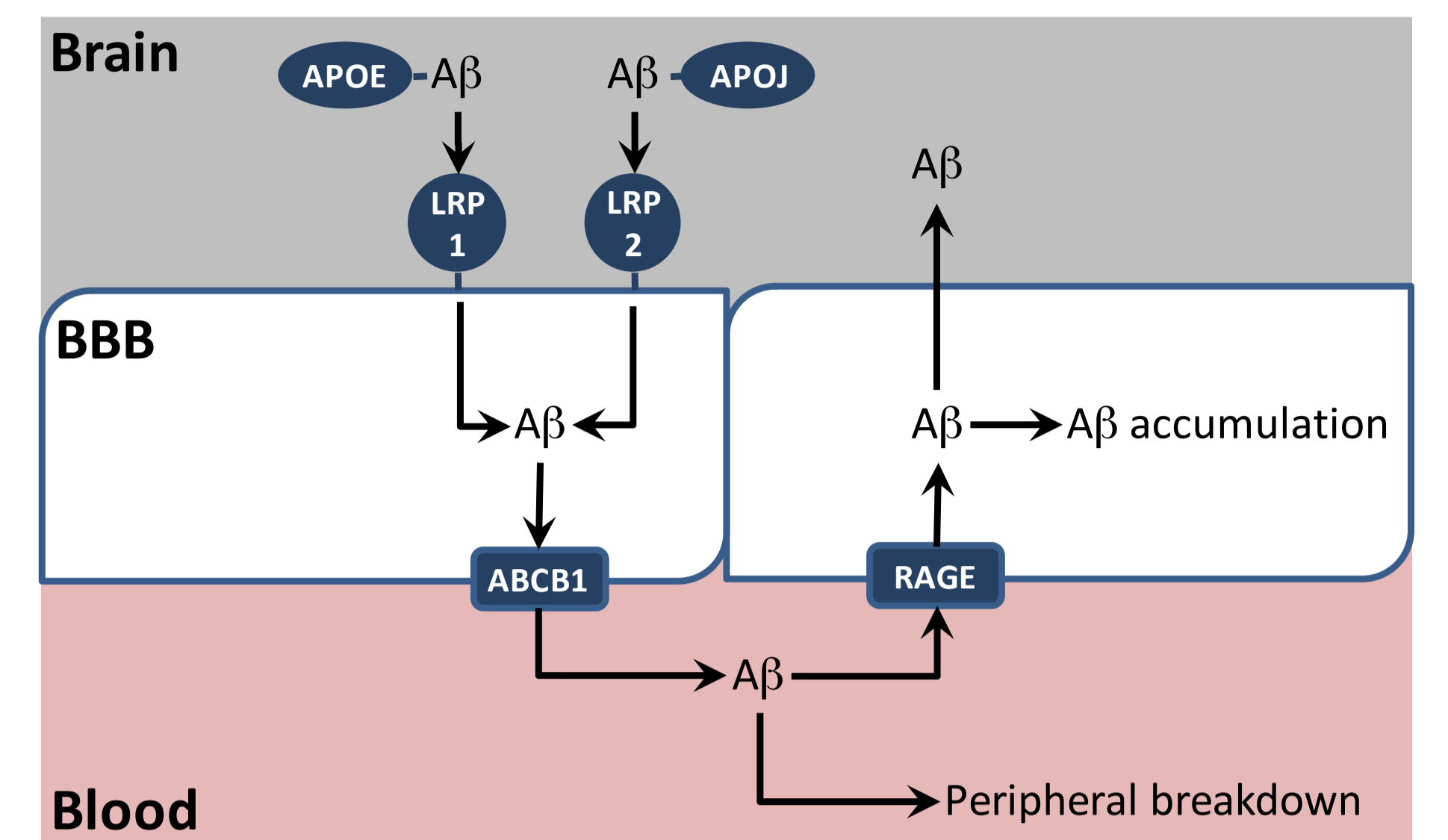


## Background to the study

- Alzheimer's disease (AD) is the most common cause of dementia and affects nearly 40,000 individuals in Ireland.
- The  $\beta$ -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 disease<sup>1</sup>.
- Flux of  $A\beta$  from the brain is believed to contribute to the overall level of  $A\beta$  within in brain<sup>2</sup> and antibody mediated brain-to-blood efflux has been observed in animal models<sup>3</sup>.
- Clearance of from the blood is believed to be mainly via the liver, kidney and spleen<sup>4</sup>.
- Data from human studies indicate that the about 6% of the  $A\beta$  pool present in the cerebrospinal fluid is cleared per hour<sup>5</sup>.
- 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.



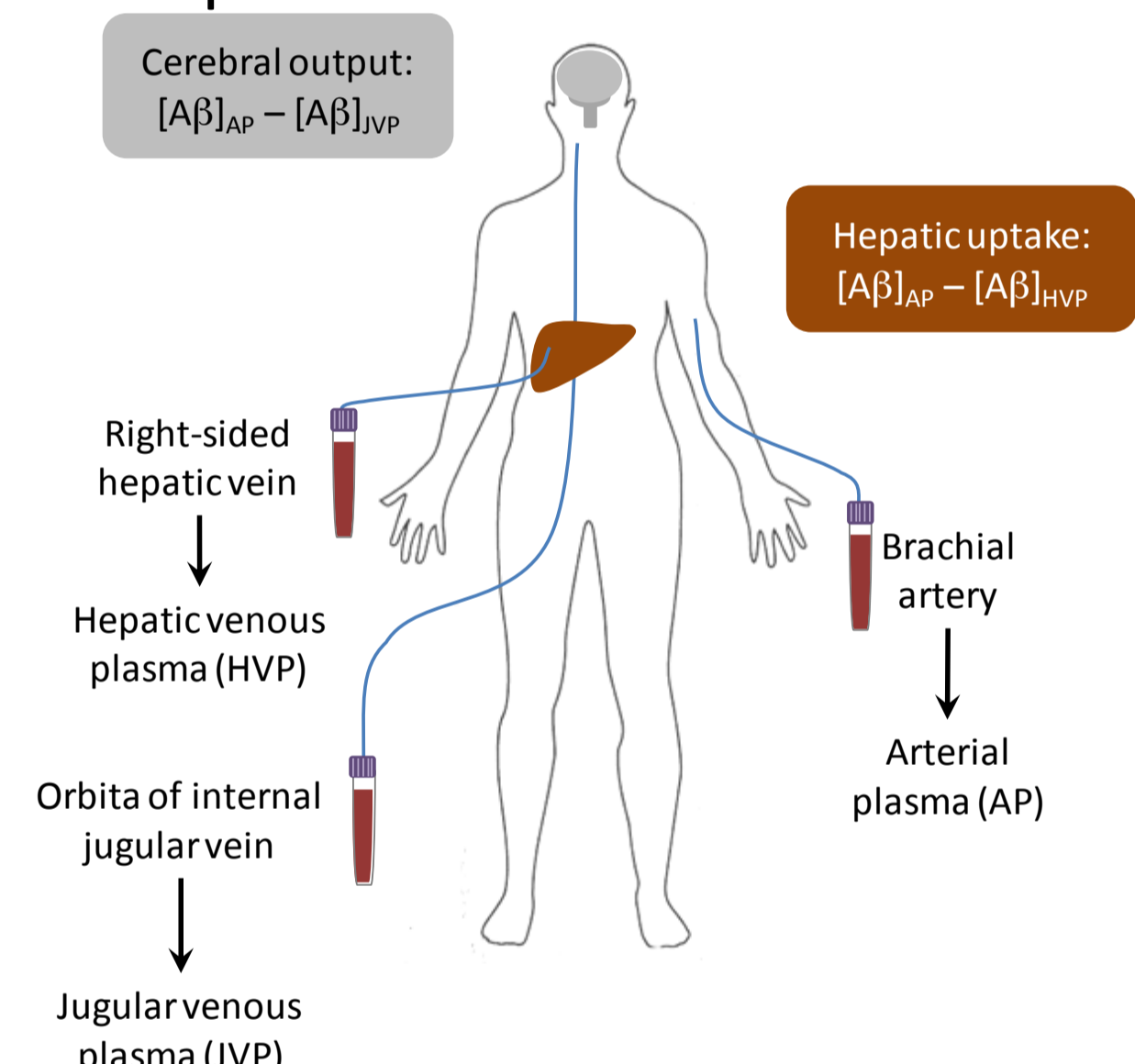
**Figure 1. Model of  $A\beta$  fluxes in man.**  $A\beta$  produced in the brain passes into the blood, either directly across the blood-brain barrier or via the cerebrospinal fluid (CSF). It is carried in a complex with numerous different proteins. The liver, kidney and spleen can take up and metabolise  $A\beta$  via various proteases. LRP1=low density lipoprotein receptor related protein 1; Alb=albumin; TTR=transthyretin; AAT= $\alpha$ 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 described<sup>6</sup>.



**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 fluxes<sup>6</sup>. 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  $-80^{\circ}\text{C}$  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:

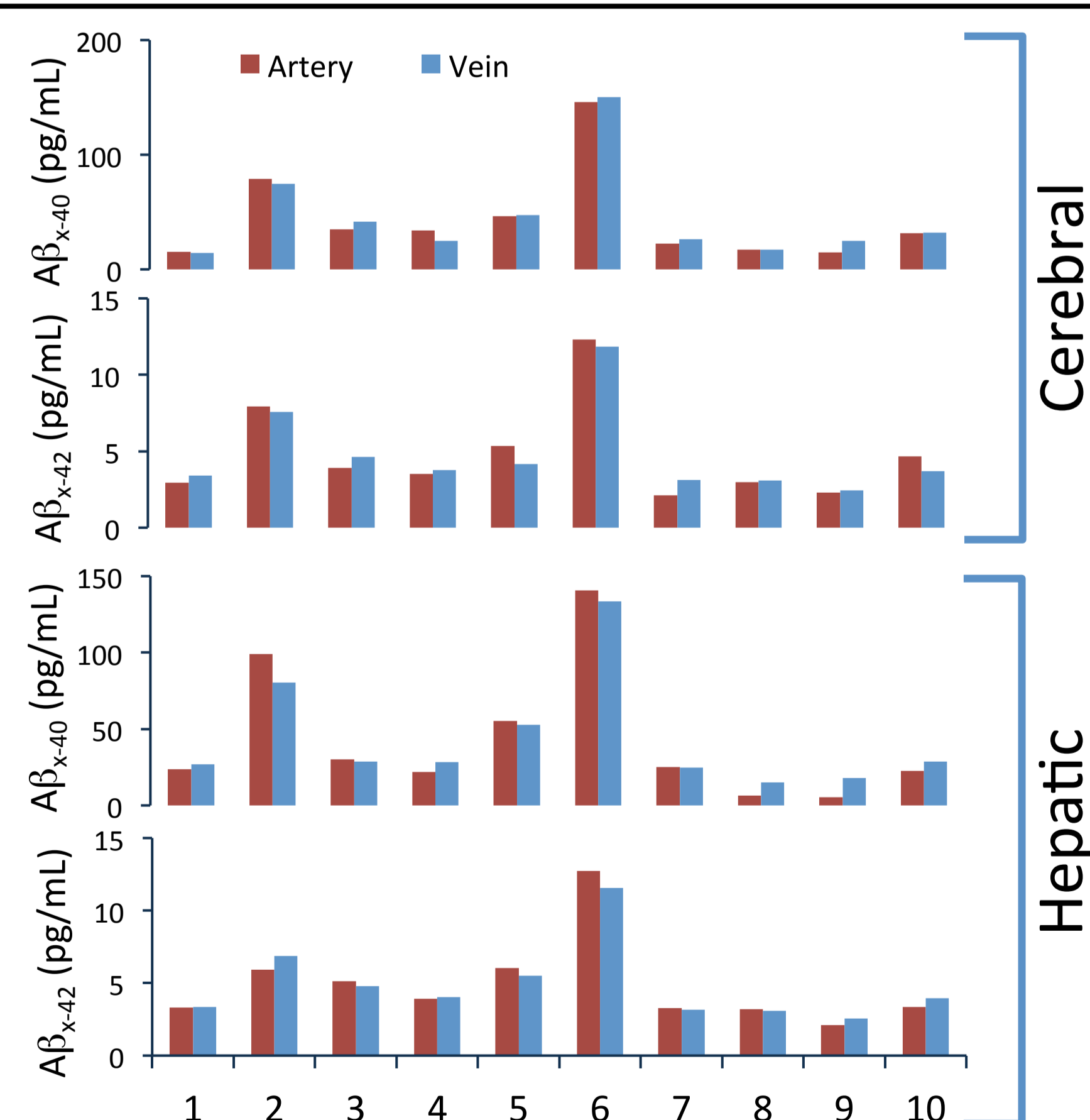
$$\text{Percent extraction} = [(C_a - C_v) / C_a] \times 100$$

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

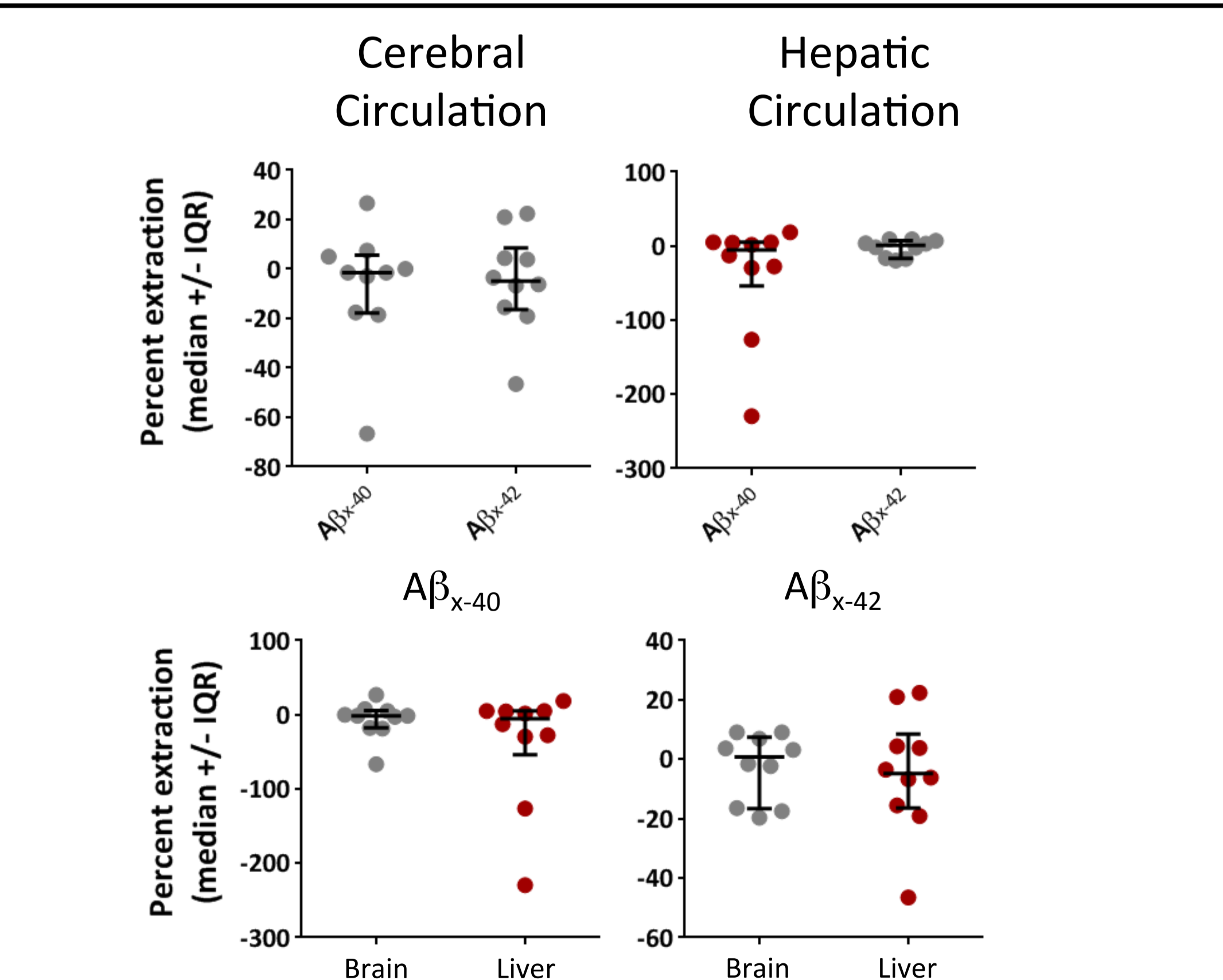
$$\text{Daily extraction} = (C_a - C_v) \times \text{organ plasma flow}$$

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:

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

ii) 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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