

ASTAXANTHIN ENHANCES LRP-1 MODULATED INSULIN SENSITIVITY AND AMYLOID-BETA CLEARANCE IN AN IN VITRO BLOOD-BRAIN BARRIER MODEL

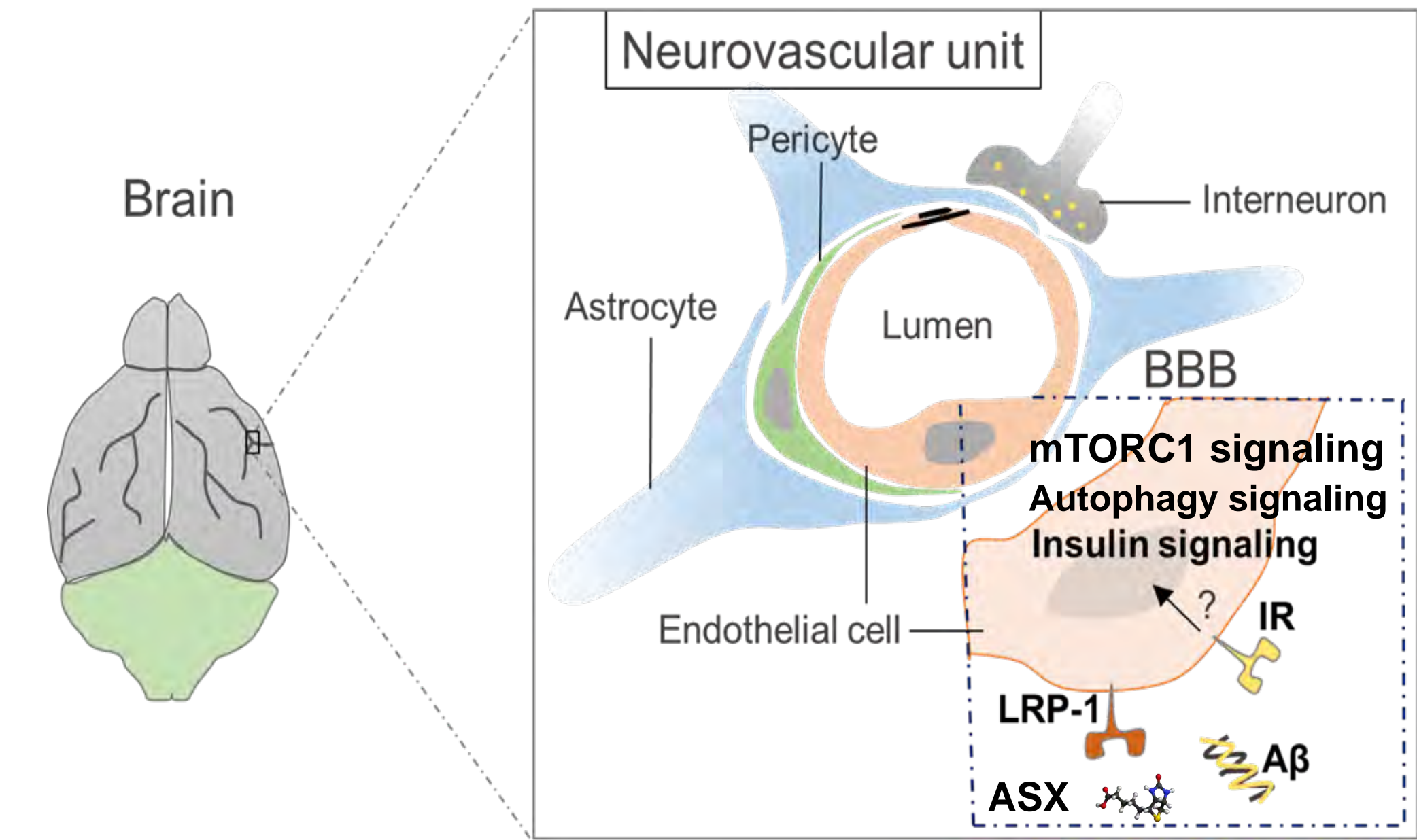
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BACKGROUND

Amyloid- β ($A\beta$) burden in Alzheimer's Disease (AD) leads to impairment in cerebrovascular insulin signaling by disrupting two potentially linked key receptors Low density lipoprotein receptor related protein-1 (LRP-1) and Insulin Receptor-beta ($IR-\beta$), involved in $A\beta$ homeostasis and insulin signaling. Dysfunctional insulin signaling results in tau hyper-phosphorylation, defective autophagic and mTORC1 signaling thereby impairing $A\beta$ degradation and clearance. This study aim to investigate if modulating LRP-1 activity via Astaxanthin (ASX), a lipid-soluble xanthophyll beta-carotenoid may be a therapeutic candidate for improved $A\beta$ clearance and insulin mediated signaling in AD and other related dementia.



MATERIALS and METHODS

By using the established *in vitro* porcine brain capillary endothelial cell (pBCEC) model of the Blood-Brain Barrier (BBB), we analyzed the effects of astaxanthin on LRP-1 expression, $A\beta$ clearance and tau hyper-phosphorylation associated with AD at the protein and mRNA level. We also examined the pBCEC ultra-structures by electron microscopy.

RESULTS

pBCECs showed enhanced expression of LRP-1 when treated with astaxanthin. Increased expression of LRP1, autophagy and reduced expression level of mTOR signalling markers were observed when pBCECs pre-incubated with astaxanthin were further treated with amyloid beta peptides. Preliminary micrographs demonstrated that there are autolysosomes and autophagosomes visible in the pBCECs.

RESULTS

Astaxanthin increases LRP-1 expression in pBCECs

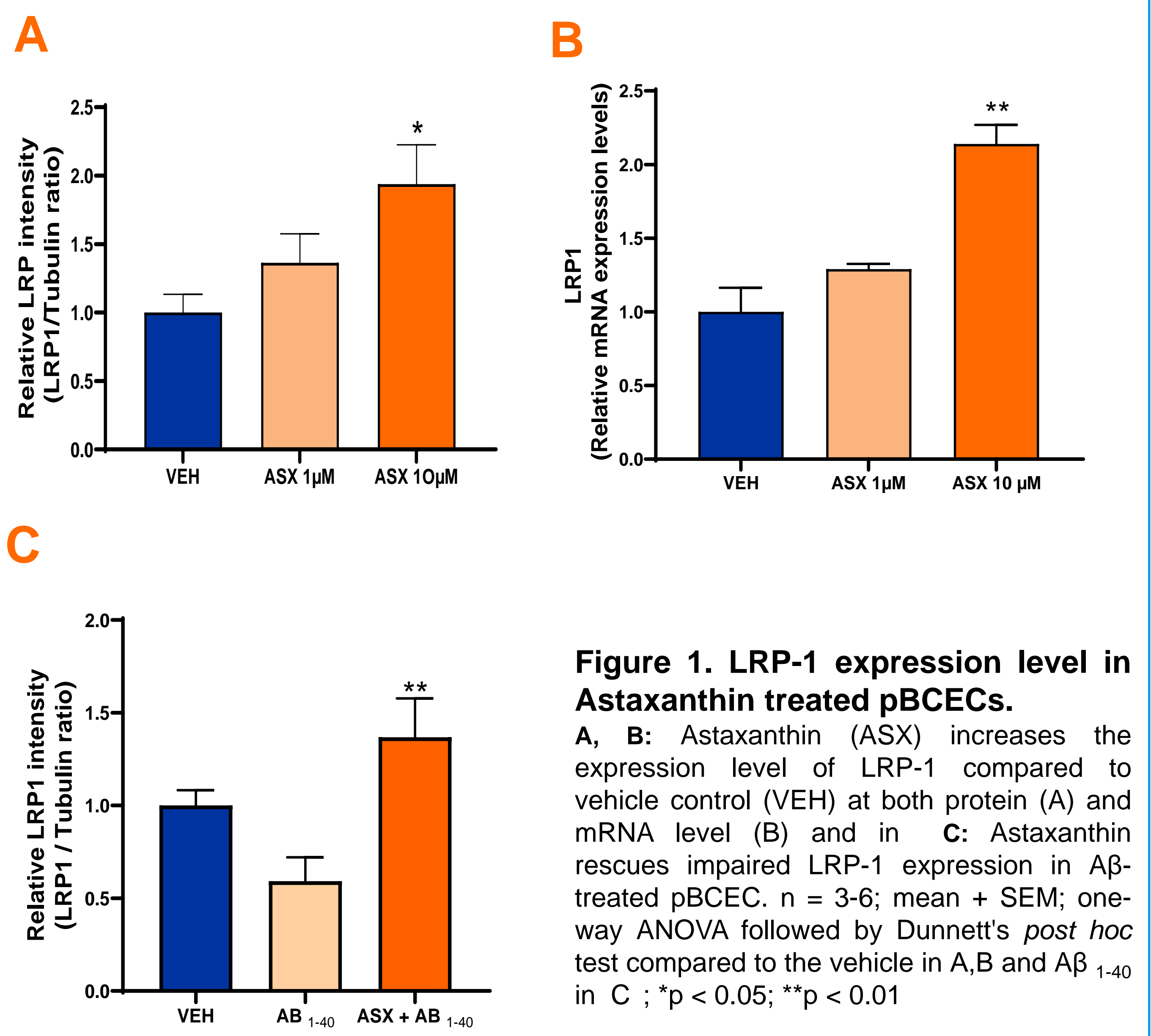


Figure 1. LRP-1 expression level in Astaxanthin treated pBCECs.
A, B: Astaxanthin (ASX) increases the expression level of LRP-1 compared to vehicle control (VEH) at both protein (A) and mRNA level (B) and in C: Astaxanthin rescues impaired LRP-1 expression in $A\beta$ -treated pBCEC. n = 3-6; mean + SEM; one-way ANOVA followed by Dunnett's *post hoc* test compared to the vehicle in A,B and $A\beta_{1-40}$ in C ; *p < 0.05; **p < 0.01

RESULTS

Astaxanthin induces autophagy and inhibits mTORC1 pathway signaling

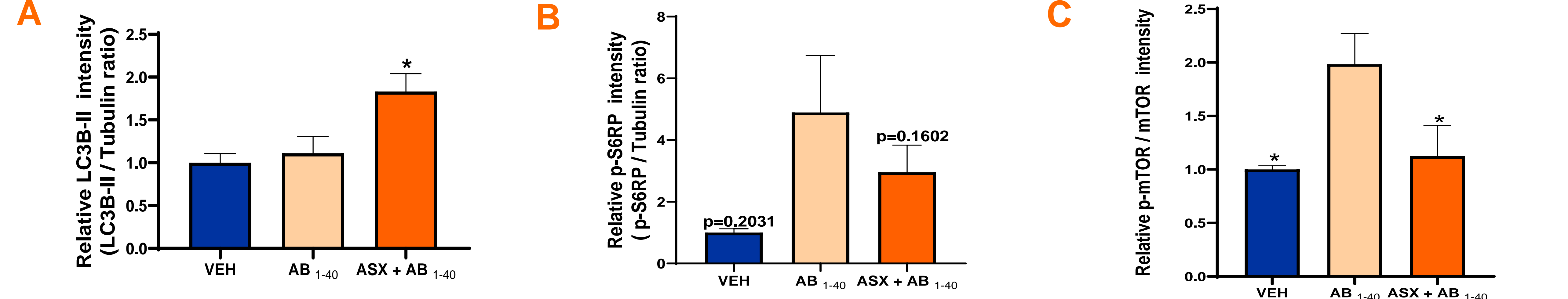


Figure 2. Autophagy and mTORC1 Signaling in $A\beta$ -treated pBCEC.
Densitometric evaluation of LC3B-II (A), p-S6RP (B) and p-mTOR/mTOR (C) in $A\beta$ -treated pBCEC, n=3-6; mean + SEM; one-way ANOVA followed by Dunnett's *post hoc* test compared to $A\beta_{1-40}$; *p < 0.05.

Autophagic structures in Astaxanthin treated pBCECs

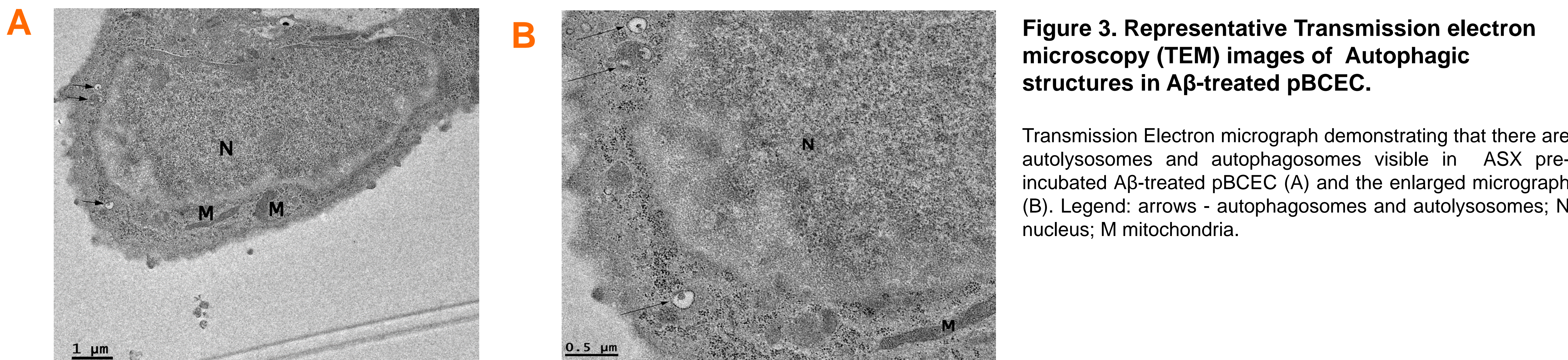


Figure 3. Representative Transmission electron microscopy (TEM) images of Autophagic structures in $A\beta$ -treated pBCEC.
Transmission Electron micrograph demonstrating that there are autolysosomes and autophagosomes visible in ASX pre-incubated $A\beta$ -treated pBCEC (A) and the enlarged micrograph (B). Legend: arrows - autophagosomes and autolysosomes; N nucleus; M mitochondria.

Astaxanthin enhances the phosphorylation of GSK3 α/β

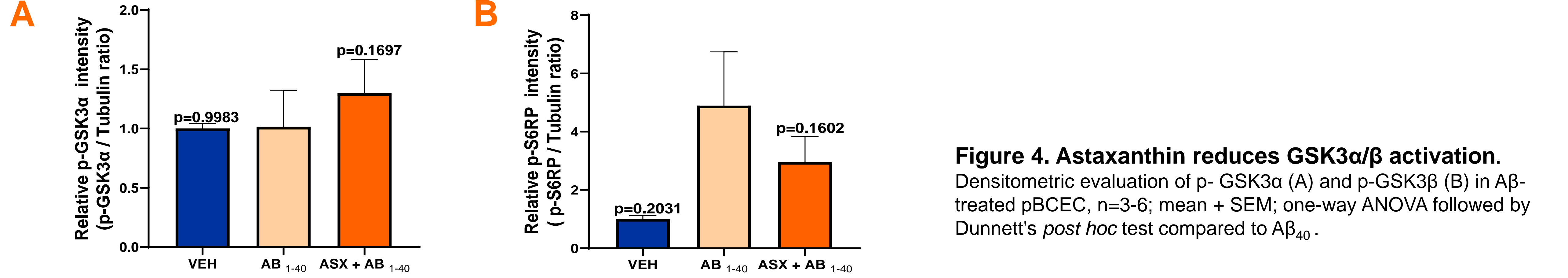


Figure 4. Astaxanthin reduces GSK3 α/β activation.
Densitometric evaluation of p- GSK3 α (A) and p-GSK3 β (B) in $A\beta$ -treated pBCEC, n=3-6; mean + SEM; one-way ANOVA followed by Dunnett's *post hoc* test compared to $A\beta_{40}$.

CONCLUSION

Our results suggest that increased LRP1 expression by Astaxanthin enhances insulin sensitivity, autophagy induction and improves $A\beta$ clearance. Astaxanthin could thus be a promising therapeutic candidate for Alzheimer's disease.