In-vivo assessment of retinal vessel diameters and observer variability in mice: A methodological approach

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Central retinal arteriolar (CRAE) and venular (CRVE) diameter equivalents are predictive for cardiovascular and all-cause mortality in humans. The aim of this study was to investigate the inter- and intraobserver variability for the assessment of CRAE and CRVE in mice using fluorescein contrast enhancement as compared to crude analysis.; Three high quality images with (F) and without fluorescein (NF) of eight mice (type C57BL) were recorded and analysed by two independent experienced investigators to investigate interobserver variability. In addition, one investigator analysed 20 F and 20 NF images twice to investigate intraobserver variability. The time course of CRAE and CRVE vessel responses after fluorescein injection were recorded in one mouse every 30 seconds for 15 minutes.; The interobserver variability was lower in F images compared to NF images for CRAE ($r = 0.99$, $p < 0.001$ vs. $r = 0.65$, $p = 0.083$) and CRVE ($r = 0.99$, $p < 0.001$ vs. $r = 0.79$, $p = 0.019$). Intraobserver variability for CRAE ($r = 0.99$, $p < 0.001$ vs. $r = 0.48$, $p = 0.032$) and CRVE ($r = 0.98$, $p < 0.001$ vs. $r = 0.86$, $p < 0.001$) were lower in F compared to NF images. Fluorescein injection induced vascular staining mimicking vessel dilation (+14%) followed by a long-lasting stable staining phase well suited for precise measurements.; Measurement variability can be optimized by use of fluorescein as contrast enhancement in mice. Standardization for time of image acquisition after fluorescein injection is advisable. Translation of static retinal vessel analysis into a rodent model has the potential to bridge the research gap between proof of concept studies in animals and clinical studies in humans.
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