

1 ORIGINAL RESEARCH

2 Short running header: Retinal biomarkers of head trauma in Olympic boxers using OCT

3 Childs *et al*

4 Investigating possible retinal biomarkers of head trauma in
5 Olympic boxers using optical coherence tomography (OCT)

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27

28 **Abstract:**

29 **Purpose:** Changes to retina have been reported after a number of neurodegenerative conditions. The
30 purpose of this study was to investigate retinal structures in Olympic boxers exposed to frequent head
31 blows.

32 **Methods:** Retinal imaging offers potential as a non-invasive biomarkers of neuropathology. Macula and
33 retinal nerve fibre layer (RNFL) thickness was measured using optical coherence tomography (OCT) in
34 UK Olympic boxers attending two mandatory eye screening programs, 18 months apart. Data from the
35 two eye-screenings provide longitudinal data of retinal change over time. Sedentary healthy subjects
36 (controls) without past or present history of concussion were also screened at the time of the second
37 boxer screening to provide comparison cross-sectional data.

38 **Results:** Sixteen Olympic boxers aged 20-33 years and 20 sedentary healthy controls, aged 24-45 years
39 were recruited. Significant macula thickening was observed over time (18 months) in 75% of right and
40 50% of left eye sectors. For RNFL, left eye quadrants thickened. For right eye RNFL quadrants,
41 thickening *and* thinning of this layer was observed. Cross-sectional results showed thinner macula
42 sectors *and* RNFL quadrants in Olympic boxers compared to controls.

43 **Conclusion:** Significant change to macula and RNFL densities, occurring over an 18 month interval is
44 an unexpected finding in otherwise healthy elite sportsmen. In addition, macula and RNFL were thinner
45 than healthy sedentary controls. OCT may prove clinically useful as a candidate retinal biomarker of
46 neuropathological change after mild traumatic brain injury and/or repeat head blows.

47

48 **Keywords:** [optical coherence tomography \(OCT\)](#), mild traumatic brain injury, macula, retinal nerve fibre
49 layer, imaging, concussion, eye

50 **Introduction**

51 Retinal imaging is a well-established, non-invasive, technique for capturing eye and systemic disease.
52 The eye and neurosensory retina, as an extension of brain tissue during embryonic development is a part
53 of the central nervous system and can be visualized relatively easily. Pathological change to eye
54 structures and retinal blood vessels may arguably serve as a *proxy* of brain change where other imaging
55 techniques (e.g. computed tomography, magnetic resonance imaging) are not so readily accessible.

56 The retinal nerve fibre layer (RNFL) constitutes axonal fibres of retinal ganglion cells (RGC) forming the
57 optic nerve. RNFL conveys visual information to the brain, and appears sensitive to pathological brain
58 change. Studies have shown thinning of the RNFL in multiple sclerosis (MS), a degenerative brain
59 condition causing white matter atrophy¹⁻³. Similarly, RNFL *and* total macular volumes are thinner in
60 Alzheimer disease and in Parkinson's disease when comparisons are made with normal ranges, the
61 changes correlating with disease severity⁴⁻⁶.

62 Alterations to the retinal vasculature have also been reported after mild traumatic brain injury (mTBI). Our
63 *first in man* study showed significantly greater arteriolar and venular tortuosity in patients presenting with
64 two or more post-concussive symptoms after mild-moderate TBI compared to age-, gender-, race-
65 matched-controls⁷. Beyond these preliminary findings it is presently unclear whether mTBI in humans
66 affects retinal tissue with any correspondence to that which occurs in demyelinating and degenerative
67 conditions. However, this is a plausible consideration given the dual impact of coup, contra-coup
68 acceleration/deceleration forces to the brain together. This, together with the secondary damage due to a
69 wave of excitotoxic, metabolic and inflammatory cascades which follow impact individually contribute to
70 worsening outcome⁸⁻⁹. This, combined with disruption to retinal blood supply might deleteriously affect
71 the retina, the most metabolically tissue of the body¹⁰.

72 The clinical consequences of mild to moderate TBI, concussion and repeated head blows is recognition
73 of a change in cognitive function¹¹. Often lingering for weeks and months but without a definite diagnosis,
74 subtle histological, biochemical and neuronal structural changes evade capture with conventional brain
75 imaging techniques, making it impossible to monitor progressive pathological brain change. In contact
76 sports, repeated head impact occurs frequently, yet little is known as to whether early-stage head impact
77 or mild brain trauma is the start of progressive pathological disease processes that could be detected or
78 even halted with screening and surveillance *in vivo*. For amateur (Olympic) boxers, professional boxers
79 and for those playing football, rugby or mixed martial arts, the cumulative effects of brain injury are largely
80 uncharted because quantifying lifetime incidence of brain damage is a major challenge¹². Post-mortem
81 evaluation is currently the only conclusive method to evaluate cumulative concussive damage to brain
82 and now more usually termed Chronic Traumatic Encephalopathy (CTE) of the brain.

83 CTE was first described in the 1970's based on clinical and neuropsychological features of 15 retired
84 boxers¹³ and more recently has been reported in sportsmen and women exposed to head injuries *and* in
85 blast-exposed military veterans¹⁴⁻¹⁵, CTE may also be influenced by genetic and behavioral factors¹⁴.
86 However, common to all known cases of CTE is a history of repeated mild brain trauma with CTE stage
87 correlating with symptom progression¹⁴.

88 As initial brain changes in CTE are unremarkable¹⁴ they are likely to be overlooked or go undetected with
89 current imaging methods so there is a pressing need for new *in vivo* technology to allow monitoring of
90 brain health over lifetime exposure to head trauma (especially so for children and youths as they begin to
91 encounter exposure to TBI risk when taking up contact sports). Being able to forewarn of neurocognitive
92 problems and neurodegeneration could have life-long health benefits. Retinal imaging offers one
93 possible solution but data are now needed to show the performance of the technique of OCT for this new
94 application. If changes to retinal parameters could, in the future, fruitfully aid prognosis, management and
95 treatment when overt brain changes are undetectable by current methods, this technology would reap
96 significant benefits for maintaining brain health in those engaged in contact sports.

97 This paper represents the first report of OCT in elite athletes (Olympic boxers) where risk of head blows
98 and mTBI would be expected to be greater than in a sedentary group of similarly aged, healthy men and
99 women.

100 As a start point, the objective of the study was to determine retinal tissue density of presumptive
101 candidate biomarkers, macula and RNFL, in Olympic boxers compared with healthy controls and to
102 examine whether retinal measurements remain stable or change over time.

103 **Material and methods**

104 Institutional ethics approval from Sheffield Hallam University Ethics committee was obtained to access
105 secondary anonymized data of Olympic boxers from who underwent regular eye screening [and vision](#)
106 [testing](#). In the present study data are presented for two time-points, separated by an interval of 18 months
107 (longitudinal data). As data for Olympic boxers was obtained from a secondary, anonymized source, [all](#)
108 [boxers who attended the screening sessions were included. For the second screening, only those boxers](#)
109 [attending first and second screenings were included to enable 'matched' pairs for repeated measures](#)
110 [longitudinal analyses](#). Figures for the number of head blows sustained were not available [in this](#)

111 [anonymized sample](#) but an estimation was calculated from number of bouts for the 18-months interval
112 between first and second eye-screenings, Time 1 and Time 2; T1 and T2 respectively. With a median of
113 22 bouts during this period, a typical Olympic Boxer receives approximately eight head blows during a
114 spar session with 35% of boxers receiving 10 or more head blows. As a conservative estimate, sustained
115 head blows would equate to approximately 500 over a five-year period based on a presumption of five
116 head blows per bout and 10 bouts per year over 5 years. Over a period of 18 months to 2 years, this
117 would equate to an estimated 200 head blows.¹⁶

118 We recruited sedentary healthy controls not engaged in contact sports to compare to Olympic boxers.
119 Controls responded to an advertisement for the study and were recruited at the time of the second eye
120 screening of Olympic boxers. Participants were only included if there was no known history of concussion
121 or mTBI, systemic illness or eye problems. Participants were excluded if there was known history of
122 diabetes or high myopia. These data of healthy participants provide retinal values for cross-sectional
123 analyses.

124 ***Retinal Parameters***

125 [Boxing participants: Retinal imaging of right and left eyes were performed using a Topcon™, non-](#)
126 [invasive, non-contact, optical coherence tomography \(OCT\) system \(3D OCT 2000, Serial number](#)
127 [673053, Newbury, Berkshire UK\). The system is a non-mydratic trans-pupillary imaging technique](#)
128 [producing high-resolution images of the structure of the retina from the anterior segment to the posterior](#)
129 [pole. Images were acquired for mapping of retinal layers; from deep layers \(RPE; retinal pigment](#)
130 [epithelium\) to retinal nerve fibre layer \(RNFL\). Data were extracted from scan reports; macula centred](#)
131 [report and optic disc centred report, both with 3D image from 6mm x 6mm field.](#)

132

133 [Non-contact sport controls: Retinal parameters were acquired for a single retinal scan session using a](#)
134 [Topcon™ Triton, swept source \(SS\) OCT, deep range imaging \(DRI\) system \(Newbury, Berkshire\) \(Fig](#)
135 [1\). From the optic disc report \(3D 6mm x 6mm; 512x256 pixel resolution\) and the macula report \(7mm x](#)
136 [7mm, 512x256 pixel resolution\) descriptive data was extracted for retinal parameters, averaged and used](#)
137 [as a comparator for the boxer group. To observe for effects of raised intraocular pressure \(IOP\) in](#)

138 controls, measurements were obtained from each eye, at each testing session, using an ocular pressure
139 tonometer (Model TA011, Vantaa, Finland).
140 Information from Triton manufacturers' FDA report indicates that measurements taken from both Triton
141 instruments are comparable (https://www.accessdata.fda.gov/cdrh_docs/pdf17/K173119.pdf).
142 Results are reported for measurements of four outer and inner subfields of the nine subfields on the
143 optical coherence tomography (OCT) thickness map defined by the Early Treatment Diabetic Retinopathy
144 Study (EDTRS)¹⁷. Retinal imaging of right and left eyes were performed using a Topcon™ (Newbury,
145 Berkshire), non-invasive, non-contact, optical coherence tomography (OCT) system. Images were
146 acquired for mapping of retinal layers; from deep layers (retinal pigment epithelium) to retinal nerve fibre
147 layer (RNFL). Data obtained from Olympic boxers during the eye-screening (performed by AG) was
148 transferred *via* an encrypted hard disk in the form of an Excel (Microsoft™) spreadsheet and without any
149 personal identifiers. OCT values were obtained for macula thickness (microns, μm) in eight sectors of
150 each eye *and* for RNFL thickness of optic disc-centred RNFL in four quadrants (superior, inferior, nasal
151 and temporal). Data were analysed with SPSS Statistical Package for the Social Sciences (SPSS ver 24,
152 IBM) software¹⁸.

153 **Results**

154 ***Participants***

155 Secondary data from 16 Olympic boxers (12 male) mean age 24.5 (*SD* 3.5, range 20-33) years attending
156 for two screening sessions T1 and T2 and 20 healthy, sedentary, adults (8 male), mean age 30.1 (*SD*
157 6.2, range 24-45) years were recruited. *Although* the control group were older than Olympic boxers, $F(1,$
158 $34) = 7.20, p < 0.05$ (because recruitment was from a demographically random selection of interested
159 healthy participants *via* the university recruitment website), only two participants were outside of Olympic
160 boxers age range (i.e. *were* aged 43 and 45 years). The remainder fell within the 24 to 33-year age range
161 of boxers. In the control group, more females were recruited than in the Olympic boxer group (12 female
162 controls compared to four females in the Olympic boxer group). *We found no significant correlations for*
163 *age and retinal variables using Pearson's bivariate co-efficient*. We therefore conducted MANOVAs to
164 compare male with female control data for right and left eye macula sectors and right RNFL and left
165 RNFL quadrants. Using Pillai's trace, no significant effect was observed for gender on left eye macula

166 sectors, $V = 0.59$, $F(10, 9) = 1.30$, $p > 0.1$. For right eye macula sectors, Pillai's trace $V = 0.49$, $F(10, 9)$
167 $= 0.88$, $p > 0.1$. For left and right RNFL sectors, Pillai's trace $V = 0.34$, $F(10, 9) = 0.47$, $p > 0.1$. Null
168 findings indicated that our control group constituted an appropriate comparator for the Olympic boxer
169 data.

170

171 ***Longitudinal comparison of macula sectors and RNFL quadrants in Olympic boxers at T1 and T2***

172 Repeated measures statistics were conducted for right and left eye macula sectors (Table 1-2) and for
173 right and left eye RNFL quadrants (Table 3-4) to investigate any potential longitudinal change to retinal
174 layers. Tables 1 and Table 2 show the mean values and standard errors (*SE*'s), inferential statistics,
175 significance and effect size for each eye sector and quadrant. No prediction was made about potential
176 direction of findings so conservative two-tailed significance is reported throughout.

177 [Insert Tables 1 and 2 here]

178 Six of eight macula sectors of the right eye (Table 1) were consistently (shown by the direction of t-value)
179 and significantly thicker at the second T2 screening (18 months from T1) of the Olympic boxers. Large
180 effect sizes (above 0.5) indicate that the results are unlikely due to chance. For the right eye, inner
181 inferior and outer temporal sectors, and for the left eye, outer temporal and total volume did not differ
182 significantly over time.

183 For the left eye (Table 2), results show that seven of eight macula sectors of the left eye were (shown by
184 the direction of t-value) significantly thicker at second screening and with moderate effect sizes. This
185 compares with the large effect sizes observed for the right eye. It is worth noting here that it is difficult to
186 interpret this pattern of findings in this, a first study, but results may reflect the pattern and target of the
187 blows to the head (i.e. that one side of the head received more blows and this in turn affects the integrity
188 of retinal structures of that eye more than the other eye).

189 ***RNFL***

190 Results of analyses for right eye RNFL quadrants showed that superior and inferior quadrants were
191 significantly thicker at second screening, whilst the nasal quadrant was significantly thinner (Table 3).
192 The large effect size for the nasal quadrant analysis indicates this finding is robust and unlikely to be
193 attributable to error. There were no changes to temporal quadrant and total average thickness. Left eye

194 RNFL descriptive data and analyses are shown in Table 4.

195 [Insert tables 3 and 4 about here]

196 Results of analyses for left RNFL sectors showed that the inferior quadrant was significantly thicker and
197 temporal quadrant was significantly thinner at second screening. (Inferior quadrant was also significantly
198 thicker in right eye RNFL data). Again, significant findings had moderate to large effect sizes. The pattern
199 of both thinning and thickening seems specific to RNFL whereas results for macula showed only
200 thickening over time.

201 ***Cross-sectional analyses: Boxers and Controls at T2***

202 Comparisons were made for macula and RNFL thickness between Olympic boxers and controls. Controls
203 were tested during the months that the boxers' second eye screen was undertaken.

204 **Macula**

205 Independent MANOVA with group (Olympic boxer and control) as between-subjects factor, and right eye
206 macular variables as dependent variables found (Pillai's trace) a significant effect of group on right eye
207 macula variables for the boxers and controls, $V = 0.99$, $F(11,24) = 13.23$, $p < .05$. Separate univariate
208 ANOVA on outcome variables revealed significant as well as non-significant differences between groups
209 for right eye macula variables. The conventional effect size calculations for ANOVA (η^2 eta squared)
210 were computed for all outcome analyses (Table 5).

211 [insert table 5 about here]

212 Control right eye macula thickness measurements were significantly greater than Olympic boxer
213 measurements for most sectors (Table 5). Inner superior, inner nasal, outer inferior and outer nasal
214 values were higher for controls compared to the boxers but not significantly so. All other sectors were
215 significantly different with small to medium effect size (Table 5). Overall, for controls, macula density was
216 thicker than Olympic boxers for right eye, whether the difference was significant or not.

217 Left eye macula data were similarly analyzed with group as between-subjects factor and left eye macula
218 variables as dependent variables. There was a significant effect of group on left eye macula variables,
219 Pillai's trace $V = 0.85$, $F(11,24) = 12.43$, $p < 0.05$. Separate univariate ANOVA on outcome variables
220 revealed significant and non-significant differences between groups for left eye macula (Table 6), as
221 found for right eye sectors.

222 [insert table 6 about here]

223 Once again, control left eye macula measurements were consistently higher than Olympic boxer
224 measurements but not significant for inner nasal, outer inferior, outer nasal, outer temporal and average
225 thickness. All other sectors were significantly different with small to medium effect size (Table 6). Thus
226 the same pattern of thicker macula sectors for controls compared to the boxers was found.

227 Removal of one outlier (\pm a measurement of 529.6 for average thickness in the control group, skewed the
228 data to generate a large standard error of 12.3, Table 6). On removal of this outlier, total average
229 thickness was significantly different for Olympic boxers and controls $F(1, 33) = 3.93, p < 0.05$.

230 Overall, findings showed that the control group macula measurements were greater than for Olympic
231 boxer measurements for both left and right eyes.

232

233 **RNFL**

234 There was a significant effect of group on RNFL left/right eye measurements for Olympic boxers and
235 controls, Pillai's trace $V = 0.76, F(11,24) = 7.04, p < 0.05$. Post-hoc ANOVA showed significant and non-
236 significant differences between the boxers and the controls.

237 For right eye, ANOVA revealed significant differences in two RNFL quadrants (Olympic boxers vs
238 controls): inferior, mean = 127.3 (SE 3.5) vs 139.2 (SE 3.1), $F(1,34) = 6.31, p < 0.05, \eta^2 = 0.16$; nasal,
239 mean = 79.0 (SE 3.2) vs 89.0 (SE 2.8), $F(1,34) = 5.52, p < 0.05, \eta^2 = 0.14$; and for total right eye average
240 thickness mean = 100.6 (SE 1.8) vs 106.4 (SE 1.6) $F(1,34) = 5.65, p < 0.05, \eta^2 = 0.14$. Control RNFL
241 quadrant measurements were significantly greater than for the boxer measurements indicating a thicker
242 RNFL layer.

243 For left eye, once again, ANOVA revealed significant differences in one RNFL quadrant (Olympic boxers
244 vs controls): superior, mean = 120.3 (SE 2.7) vs 123.1 (SE 2.4), $F(1,34) = 8.44, p < 0.05, \eta^2 = 0.11$, total
245 volume mean = 7.8 (SE 0.09) vs 8.0 (SE 0.07), $F(1,34) = 4.22, p < 0.05, \eta^2 = 0.11$, and for total average
246 thickness mean = 100.6 (SE 1.8) vs 106.4 (SE 1.6) $F(1,34) = 5.45, p < 0.05, \eta^2 = 0.14$ with higher values
247 for controls.

248

249

250 **Summary**

251 The results of these data analyses showed that Olympic boxers undergoing eye screening at two time
252 points, 18 months apart, had thicker macula density by sector for both eyes and thicker (as well as
253 thinner) RNFL quadrants. Comparison of cross-sectional data measurements at the T2 time-point
254 showed that the Olympic boxers' macula and RNFL measurements compared to controls were lower,
255 despite the significant changes to retinal measurements shown in the Olympic boxers over the 18-month
256 period.

257 **Discussion**

258 This is a novel study exploring the possibility that OCT may provide a candidate method to capture retinal
259 biomarkers of brain injury. In this study, macula thickness and RNFL thickness values were investigated
260 in a young, fit and healthy population of Olympic boxers, and healthy non-contact controls. As a cohort,
261 this group of elite sportsmen and women are exposed to risk of TBI, as evidenced by the number of bouts
262 (median 22) over the 18 months of this study. However, at this stage of our investigations, our
263 interpretation of results are limited by lack of exact data on frequency of head blows or sub-concussive
264 events, during competition and during training (sparring), where it is expected that there would be an
265 additional exposure risk.

266 Six *right* eye and seven *left* eye macula sectors out of a total of eight on the EDTRS map were
267 significantly thicker in young, healthy, Olympic boxers over an 18-month period with moderate to large
268 effect sizes for all analyses. Effect sizes are a standardized and objective measure of effect magnitude
269 and indicate that statistically significant findings are robust. An increase in macula measurements
270 (thickening) in young healthy Olympic boxers on repeat testing over an 18-month period using the same
271 retinal imaging equipment and administered by the same examiner, has several possible interpretations.
272 The first is that there is a possibility of diurnal variation in overall retinal thickness of between 5-10% as
273 suggested by clinical opinion. Secondly, in our cohort the complex picture of change in measurement
274 values is of interest, potentially reflecting pathological influences but not consistently so. For example,
275 changes were observed in some sectors but not others, there were *different* sector and quadrant
276 thickness changes to each eye, as well as thinning *and* thickening of RNFL fibers from screening at T1
277 and T2. This casts doubt on a diurnal variation explanation as the pattern is inconsistent. [Indeed,](#)

278 Sharifipour *et al* (2016) have shown no diurnal variation in a number of measured retinal parameters¹⁹. In
279 addition, it is now known that choroid and retinal pigment epithelium (RPE) volumes are the retinal layers
280 most sensitive to diurnal fluctuations²⁰ but as these layers were not included in the study measurements,
281 the changes we have observed are unlikely to be explained by diurnal variation in macula and RNFL
282 thickness. Another possibility is that the presence of microcystic macula oedema (MME), which is not
283 condition-specific, but may nevertheless be an early sign of optic neuropathy, might contribute to eventual
284 thinning of RNFL²¹ as reported by Abegg *et al.*, (2014), but MME was not detected in this series. It might
285 be plausible that increased retinal vessel permeability or impaired lymphatic drainage might influence the
286 retinal measurements of the boxers and, since we have previously shown an effect of TBI on retinal
287 vessel parameters (tortuosity) this would be an area of future investigation⁷.

288 At the present time the mechanisms of retinal change after human mTBI remain poorly understood so few
289 solid conclusions can be drawn on potential pathological causes beyond the effects commensurate with
290 repeated head trauma. Furthermore, retinal changes in the Olympic boxers from T1 to T2 were opposite
291 to what might be expected from the literature of neurodegenerative conditions¹⁻⁶; rather than a thinning of
292 macula and/or RNFL, we report thickening over time.

293 With respect to RNFL, right eye superior and right eye inferior plus left eye inferior quadrants were
294 significantly thicker at second screening but right eye nasal quadrant and left eye temporal quadrant were
295 significantly thinner at second screening. Nasal and temporal quadrant are typically the thinnest retinal
296 nerve fibre layers in health when measured using Triton instruments (SS or SD systems
297 https://www.accessdata.fda.gov/cdrh_docs/pdf17/K173119.pdf) and we posit, the most likely affected by
298 head trauma/head blows as we report here. Again as for macula changes, this pattern of findings is not
299 easily explained by diurnal variation. The specificity of thinning to right eye nasal and left eye temporal
300 quadrants seem to align with a biomechanical account of purported change, particularly since side-to-side
301 head jarring is common in boxers during head blows. There is an extensive literature on RNFL thinning in
302 other degenerative and demyelinating conditions¹⁻⁶ and thinning also occurs naturally with age²¹. By
303 contrast, far less is known about how mTBI might impact upon retinal thickness in man, although there is
304 a growing body of evidence investigating traumatic optic neuropathy (TON) after brain injury²³⁻²⁷. Here,
305 *Indirect* TON (ITON) is specifically associated with concussion but is difficult to detect through normal eye

306 examination particularly in the early stages²³⁻²⁷. There is also evidence that RNFL and retinal ganglion
307 cell (RGC) layers thin around two weeks after mTBI in those with indirect ToN and that this continues up
308 to 20-weeks post-trauma. Visual defects however are not always present²⁸.

309 In murine models of brain injury there is evidence of selective pathological effects of mTBI on optic tract
310 that is not easily detected on histological examination. Evenson et al.,²⁹ found myelin injury, gliosis and
311 axonal degeneration to optic tract. The authors concluded that axons from retinal ganglion cells (RNFL),
312 but not retinal ganglion cell bodies, were directly injured by mTBI causing thinning. Özevren and Deveci³⁰
313 showed that experimentally induced blast injury maintained over three successive days produced a
314 selective inflammatory response to retina. Other studies in neuro-ophthalmology shows inflammation,
315 glial responses and neuronal loss to retina at 30-days post-trauma, with no indication of cognitive
316 dysfunction on tests or visible pathology to frontal brain regions³¹. Animal models therefore provide
317 insights into sequelae of blast-induced head trauma and initiation of retinal pathology but at the present
318 time there is insufficient human data to determine which mechanism is responsible for the changes we
319 have observed in Olympic boxers study over an interval of 18 months.

320 The only explanation that we are aware of for RNFL *thickening* is edema possibly associated with anterior
321 optic neuritis³². Additionally, it is unclear from the longitudinal data alone whether macula and RNFL
322 thickness levels differ significantly from expected densities. It is for this reason that we undertook to
323 compare Olympic boxer retinal measurements with healthy controls. Although the controls were slightly
324 older, sedentary ('non-sporty') individuals with no past or current history of being engaged in contact
325 sports and neither a history of TBI nor concussion, we found that overall, macula sectors and RNFL
326 quadrants were thinner in the boxers, even where those differences were not significant. OCT values for
327 healthy eyes, without glaucoma, lose approximately 0.017% density per year; an overall loss of 10–20 µm
328 (micron) over a 60-year period³³. A significant loss of thickness, especially of macula would not be
329 expected in otherwise fit and healthy young adults. If our data in Olympic boxers represents a *true* loss of
330 retinal tissue thickness, over and above *expected* loss for this age group it is entirely plausible that these
331 data reflect a pathological process not previously reported. Nevertheless, this work should stimulate
332 further International enquiry as to the lasting effects of mTBI for those engaged in all levels of contact
333 sports; a concern for brain health currently attracting intense media interest.

334 We acknowledge a number of limitations with the study; the first being the retrospective nature of the
335 boxer data. This meant that due to anonymized data, it was not possible to investigate precise
336 contributory factors that might offer further explanation for the changes reported here (i.e. history of
337 concussion and head blows). Additionally, with the variety of OCT systems available on the market, it is
338 appreciated within the industry that variations in values for thickness of retinal layers, as well as
339 reproducibility of measurements, will have some impact on results³⁴. For example, with each new
340 development in OCT technology, even for the same manufacturer, segmentation algorithms, software
341 and the operator will have an impact on measurement. For the OCT systems used in this study, this may
342 well have a bearing on our findings. The differences reported in RNFL thickness for example, between
343 Olympic boxers at the two screenings, using the same OCT system and performed by the same operator
344 are significantly different at three quadrants (right eye) and two quadrants (left eye) yet the values are
345 different by up to 6µm only. That these differences are due to variability in measurement cannot be ruled
346 out although moderate to large effect sizes reported here would not be expected in findings caused by
347 measurement error.

348 Of note is a recent publication³⁵ reporting significant differences of 4-6 µm in RNFL thickness between
349 collision sports athletes and controls using spectral domain OCT. In the current study of Olympic boxers,
350 the retinal variable values at the second screening when compared with sedentary controls, showed
351 greater differences for macula (both eyes) but also differences of up to 12 µm were observed for right eye
352 RNFL. Leong's (2018) study found a similar order of thinning in a small cohort of boxers with an average
353 of 10.8 µm of thinning compared to controls accompanied by vision-based changes.³⁵ It would seem that
354 whilst difference in the OCT systems (SD vs SS) in the current study could have had a bearing on results
355 due to segmentation effects, it is of interest that patterns of RNFL 'thinning' are consistent across the
356 studies. Combined together these data might catalyze explorations of retinal structures in contact sports
357 using OCT to identify biomarkers of repeated mild brain trauma in the same way that retinal biomarkers
358 currently signify neurodegenerative conditions.

359 **Conclusion**

360 In this study data are presented for a first-step investigation of possible macula and RNFL biomarkers of

361 mTBI in Olympic boxers exposed to repeated head trauma. Limitations include use of secondary data but
362 a deliberate step to protect the anonymity of the boxers. It is recognized also that there is a lack of
363 precise visual, behavioral and concussive indices. Such limitations will be addressed in our ongoing
364 studies. Our findings and the data of others when considered together, suggest that OCT of the retina
365 opens up a novel pathway to the effects of mild TBI on brain via detailed imaging of the neurosensory
366 tissue of the eye.

367 **Acknowledgments**

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369 Stockton for assistance with preparation of the Boxer database and all the participants in the study.

370 **Author contributions**

371 The authors have contributed equally to the design, acquisition of data, analysis and interpretation to
372 provide the results of this study. CC and LB drafted the manuscript with contributions from all authors to
373 meet the requirements for publication.

374 **Disclosure**

375 The authors report no conflicts of interest in this work.

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475 **Table 1** Boxers ($N = 16$) right eye macula data for T1-T2

Retinal sector	Time ¹ Mean (SE)	Time ² Mean (SE)	T-test statistic, two-tailed significance and effect size (r)
Inner Superior	305.0 (3.2)	310.5 (3.5)	$t(1,15) = -4.85, p < .05, r = .61^*$
Inner Inferior	301.1 (3.3)	303.5 (3.6)	$t(1,15) = -1.0, p > .05, r = .01$
Inner Nasal	304.0 (4.4)	311.3 (3.9)	$t(1,15) = -2.80, p < .05, r = .34$
Inner Temporal	288.3 (3.3)	295.7 (3.4)	$t(1,15) = -3.91, p < .05, r = .51^*$
Outer Superior	264.6 (3.7)	269.9 (3.7)	$t(1,15) = -3.21, p < .05, r = .41$
Outer Inferior	256.5 (3.1)	262.6 (4.0)	$t(1,15) = -4.21, p < .05, r = .54^*$
Outer Nasal	280.6 (3.3)	286.8 (3.8)	$t(1,15) = -5.76, p < .05, r = .69^*$
Outer Temporal	250.9 (3.5)	254.0 (2.9)	$t(1,15) = -1.94, p > .05, r = .02$
Average thickness	270.5 (2.9)	275.6 (3.0)	$t(1,15) = -6.84, p < .05, r = .76^*$
Total Volume	7.65 (0.8)	7.79 (0.8)	$t(1,15) = -6.68, p < .05, r = .75^*$

476 * r effects: small $\geq .10$, medium $\geq .30$, large $\geq .50$ (Cohen³⁶)

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492 **Table 2** Boxers (*N* = 16) left eye macula data for T1-T2

Retinal sector	Time ¹ Mean (<i>SE</i>)	Time ² Mean (<i>SE</i>)	<i>T</i> -test statistic, two-tailed significance and effect size (<i>r</i>)
Inner Superior	301.8 (4.3)	310.6 (3.6)	<i>t</i> (1,15) = -2.71, <i>p</i> < .05, <i>r</i> = .33*
Inner Inferior	298.5 (3.4)	304.4 (2.9)	<i>t</i> (1,15) = -3.37, <i>p</i> < .05, <i>r</i> = .43*
Inner Nasal	306.2 (3.4)	311.7 (4.1)	<i>t</i> (1,15) = -3.78, <i>p</i> < .05, <i>r</i> = .49*
Inner Temporal	289.2 (4.1)	294.5 (3.1)	<i>t</i> (1,15) = -2.90, <i>p</i> < .05, <i>r</i> = .36*
Outer Superior	264.3 (3.9)	270.3 (3.8)	<i>t</i> (1,15) = -2.70, <i>p</i> < .05, <i>r</i> = .33*
Outer Inferior	257.5 (3.2)	262.3 (4.1)	<i>t</i> (1,15) = -2.57, <i>p</i> < .05, <i>r</i> = .31*
Outer Nasal	278.3 (3.4)	285.9 (4.0)	<i>t</i> (1,15) = -4.14, <i>p</i> < .05, <i>r</i> = .53*
Outer Temporal	253.3 (3.9)	256.0 (3.2)	<i>t</i> (1,15) = -.956, <i>p</i> > .05, <i>r</i> = .20
Average thickness	268.3 (3.1)	275.8 (3.2)	<i>t</i> (1,15) = -3.70, <i>p</i> < .05, <i>r</i> = .47*
TOTAL VOLUME	7.68 (0.1)	7.77 (0.1)	<i>t</i> (1,15) = -1.46, <i>p</i> > .05, <i>r</i> = .12

493 **r* effects: small ≥ .10, medium ≥ .30, large ≥ .50 (Cohen³²)

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505 **Table 3** Boxers ($N = 16$) Right eye RNFL data for T1-T2

Retinal nerve fibre Layer quadrants	Time ¹ Mean (SE)	Time ² Mean (SE)	T-test statistic, two-tailed significance and effect size (r)
Superior	114.5 (2.7)	120.3 (2.7)	$t(1,15) = -3.42, p < .05, r = .44^*$
Inferior	124.3 (2.7)	127.3 (3.0)	$t(1,15) = -3.24, p < .05, r = .41^*$
Nasal	88.5 (2.1)	79.0 (2.6)	$t(1,15) = 7.50, p < .05, r = .79^*$
Temporal	75.8 (3.4)	76.0 (2.7)	$t(1,15) = -.07, p > .05, r = .004$
Total average thickness	101.0 (1.6)	100.6 (1.6)	$t(1,15) = .38, p > .05, r = .02$

506 * r effects: small $\geq .10$, medium $\geq .30$, large $\geq .50$ (Cohen³²)

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526 **Table 4** Boxers (*N* = 16) Left eye RNFL data for T1-T2

Retinal nerve fibre layer quadrants	Time ¹ Mean (<i>SE</i>)	Time ² Mean (<i>SE</i>)	<i>T</i> -test statistic, two-tailed significance and effect size (<i>r</i>)
Superior	118.5 (2.1)	119.5 (1.8)	<i>t</i> (1,15) = -.45, <i>p</i> > .05, <i>r</i> = 01
Inferior	127.1 (3.5)	131.5 (3.4)	<i>t</i> (1,15) = -2.89, <i>p</i> < .05, <i>r</i> = .35*
Nasal (<i>N</i> = 15) [†]	82.1 (2.3)	83.8 (2.0)	<i>t</i> (1,14) = -1.83, <i>p</i> > .05, <i>r</i> = 19
Temporal (<i>N</i> = 15)	70.4 (2.2)	67.0 (2.1)	<i>t</i> (1,14) = 3.98, <i>p</i> < .05, <i>r</i> = .52*
Total Average Thickness (<i>N</i> = 15)	99.8 (1.4)	100.2 (1.4)	<i>t</i> (1,14) = -.57, <i>p</i> > .05, <i>r</i> = .02

527 **r* effects: small ≥ .10, medium ≥ .30, large ≥ .50 (Cohen³²)

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547 **Table 5** Right eye macula descriptive data, ANOVA post hoc results and η^2 effect size for boxers and
548 controls

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Retinal sector	Boxers Time 2 Mean (SE) n=16	Control Time 2 Mean (SE) n=20	F-test statistic, two-tailed significance and eta squared effect size (η^2)
Inner Superior	310.5 (3.5)	318.8 (3.2)	F (1, 34) = 3.00, p > .05, η^2 = 0.08
Inner Inferior	303.5 (3.5)	316.2 (3.4)	F (1, 34) = 6.57, p < .05, η^2 = 0.16*
Inner Nasal	311.4 (3.5)	319.9 (3.8)	F (1, 34) = 2.59, p >.05, η^2 = 0.07
Inner Temporal	295.7 (3.4)	306.3 (3.4)	F (1, 34) = 4.61, p < .05, η^2 = 0.12*
Outer Superior	269.9 (3.7)	279.0 (2.3)	F (1, 34) = 4.62, p < .05, η^2 = 0.12*
Outer Inferior	262.6 (4.0)	268.3 (2.6)	F (1, 34) = 1.48, p >.05, η^2 = 0.04
Outer Nasal	286.8 (3.8)	296.1 (3.0)	F (1, 34) = 3.75, p > .05, η^2 = 0.09
Outer Temporal	254.0 (2.9)	263.0 (2.4)	F (1, 34) = 5.75, p < .05, η^2 = 0.14*
Average thickness	275.6 (3.0)	284.2 (2.4)	F (1, 34) = 4.60, p < .05, η^2 = 0.11*
Total volume	7.79 (0.8)	8.03 (0.7)	F (1, 34) = 4.60, p < .05, η^2 = 0.11*

550 * small to medium effect size

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Table 6 Left eye macula descriptive data, ANOVA post hoc results and η^2 effect size for boxers and controls

Retinal sector	Boxers Time 2 Mean (SE) n=16	Control Time 2 Mean (SE) n=20	F-test statistic, two-tailed significance and eta squared effect size (η^2)
Inner Superior	310.7 (3.6)	320.7 (2.9)	$F(1, 34) = 4.65, p < .05, \eta^2 = .12^*$
Inner Inferior	304.4 (2.9)	316.2 (3.2)	$F(1, 34) = 6.96, p < .05, \eta^2 = .17^*$
Inner Nasal	311.7 (4.2)	320.6 (3.6)	$F(1, 34) = 2.61, p > .05, \eta^2 = .07$
Inner Temporal	294.5 (3.1)	306.4 (3.3)	$F(1, 34) = 6.39, p < .05, \eta^2 = .15^*$
Outer Superior	270.3 (3.8)	279.8 (2.1)	$F(1, 34) = 5.30, p < .05, \eta^2 = .13^*$
Outer Inferior	262.3 (4.1)	267.3 (2.7)	$F(1, 34) = 1.08, p > .05, \eta^2 = .03$
Outer Nasal	285.9 (4.0)	291.3 (5.1)	$F(1, 34) = 0.62, p > .05, \eta^2 = .01$
Outer Temporal	256.0 (3.2)	262.5 (2.6)	$F(1, 34) = 2.48, p > .05, \eta^2 = .06$
Average thickness	275.8 (3.2)	297.8 (12.3) [‡]	$F(1, 34) = 2.40, p > .05, \eta^2 = .06$
Total volume	7.77 (0.1)	8.0 (0.1)	$F(1, 34) = 4.42, p < .05, \eta^2 = .11^*$

* small to medium effect size

Figure Legend

Fig1: Disc Report for healthy male subject showing good image quality for right (68%) and left eye (72%) at baseline and follow up (70% right, 71% left) eye with an interim of 5 months. Report shows minimal changes for RNFL total thickness, superior and inferior quadrants over time

3D Disc Report OU w/ Topography

Triton plus (Ver.10.07)

Print Date : .



ID : 107

Ethnicity :
Gender :
DOB :

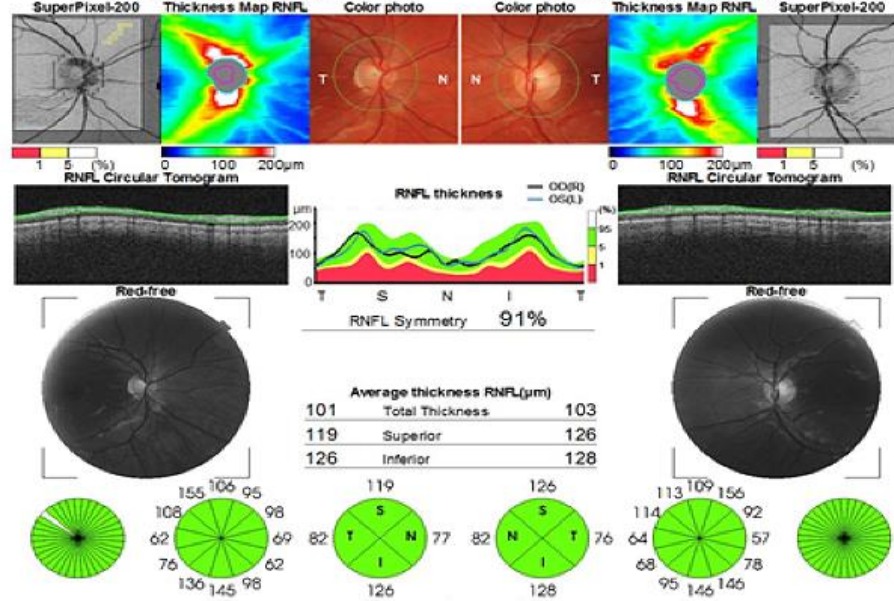
Technician :
Fixation : OD(R) Disc / OS(L) Disc
Scan : 3D(6.0 x 6.0mm - 512 x 256)

Name : RO15

OD(R)

TopQ Image Quality: 68 mode: Fine(2.0.7)
Capture Date: 24/02/2016 O.E.M 1.000x

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3D Disc Report OU w/ Topography

Triton plus (Ver.10.07)

Print Date : 15/06/2016



ID : 107A

Ethnicity :
Gender :
DOB :

Technician :
Fixation : OD(R) Disc / OS(L) Disc
Scan : 3D(6.0 x 6.0mm - 512 x 256)

Name : RO15 or RP no 135

OD(R)

TopQ Image Quality: 70 mode: Fine(2.0.7)
Capture Date: 16/05/2016 O.E.M 1.000x EVV:

mode: Fine(2.0.7) TopQ Image Quality: 71 OS(L)
Capture Date: 16/05/2016 O.E.M 1.000x EVV:

