

Serum IGF-I levels are associated with improved white matter recovery after TBI

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Serum IGF-I levels are associated with improved white matter recovery after TBI

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ABSTRACT

Objective: Traumatic brain injury (TBI) is a common disabling condition with limited treatment options. Diffusion tensor imaging (DTI) measures recovery of axonal injury in white matter (WM) tracts after TBI. Growth hormone deficiency (GHD) after TBI may impair axonal and neuropsychological recovery, and serum IGF-I may mediate this effect. We conducted a longitudinal study to determine the effects of baseline serum IGF-I concentrations on WM tract and neuropsychological recovery after TBI.

Methods: Thirty-nine adults after TBI (84.6% male; age median 30.5y; 87.2% moderate-severe; time since TBI median 16.3 months, n=4 with GHD) were scanned twice, 13.3 months (12.1-14.9) apart, and 35 healthy controls scanned once. Symptom and quality of life questionnaires and cognitive assessments were completed at both visits (n=33). Our main outcome measure was fractional anisotropy (FA), a measure of WM tract integrity, in *a priori* regions of interest: splenium of corpus callosum (SPCC), and posterior limb of internal capsule (PLIC).

Results: At baseline FA was reduced in many WM tracts including SPCC and PLIC following TBI compared to controls, indicating axonal injury, with longitudinal increases indicating axonal recovery. There was a significantly greater increase in SPCC FA over time in patients with serum IGF-I above vs. below the median-for-age. Only the higher IGF-I group had significant improvements in immediate verbal memory recall over time.

Interpretation: WM recovery and memory improvements after TBI were greater in patients with higher serum IGF-I at baseline. These findings suggest that GH/IGF-I system may be a potential therapeutic target following TBI.

INTRODUCTION

Traumatic brain injury (TBI) is a common and disabling disease causing severe disability worldwide in 150-200 million people annually.¹ Many more suffer long-term cognitive and psychological problems with variable natural history.² A major pathological hallmark of TBI is diffuse axonal injury (DAI), resulting from shearing forces sustained during the injury.^{3,4} This is a key determinant of poor clinical outcome after TBI, through the disruption of large-scale brain network function.⁵ There are currently no drug treatments available to limit axonal injury or promote its recovery.⁶

Diffusion tensor imaging (DTI) provides a sensitive way of quantifying and localizing DAI damage by measuring white matter (WM) and axonal structural integrity, which is difficult using conventional structural MRI imaging.⁷ Fractional anisotropy (FA) has been widely used as a measure of WM damage and its recovery.⁸ We and others have demonstrated reductions in FA in many WM tracts after TBI that correlate with cognitive deficits including memory impairment and sustained attention.⁹⁻¹² Furthermore changes in FA within the splenium of the corpus callosum (SPCC) and posterior limb of the internal capsule (PLIC) have been shown to predict clinical outcome after TBI.⁹

It is unclear what factors contribute to the wide variation WM recovery after TBI. One potential factor is post-traumatic hypopituitarism, which can be produced by TBI and can influence brain repair.¹³⁻¹⁵ Studies using gold-standard dynamic testing have reported a prevalence of the most common endocrinopathy, growth hormone deficiency (GHD), of 2.7-11.8% in patients >1 year after TBI.^{14,16,17} The prevalence increases with TBI severity, certain TBI mechanisms and findings on neuroimaging,^{18,19} with a particularly high prevalence of pituitary dysfunction (32.0%) and GHD (15.8%) after moderate-severe blast TBI in soldiers.²⁰ The exact diagnostic criteria and use of body mass index-dependent cut-offs for endocrine tests is however important when determining risk factors especially for GHD.^{15,17,20,39}

GH has several targets within the brain.²¹⁻²³ Effects are direct or mediated through its downstream hormone insulin-like growth factor-I (IGF-I). Non-TBI related GHD is associated with impaired cognitive

function, as well as WM tract abnormalities (lower FA) in the corpus callosum and corticospinal tract in children.²⁴ GH replacement in this group increases serum IGF-I and improves attention, memory, and psychological well-being.²⁵⁻²⁷ Variations in serum IGF-I concentrations have been shown to be related to brain structure and cognitive function.²² Following stroke, higher serum IGF-I concentrations at baseline are associated with better neurological and functional outcomes.^{28,29} Mechanistically, GH/IGF-I deficiency reduces oligodendrocyte turnover in the corpus callosum,³⁰ whereas GH/IGF-I administration increases the formation of new neurons in the hippocampus.^{28,31,32}

Following TBI, GH replacement also improves cognition, psychological function and quality of life in patients with GHD.³³⁻³⁷ This could be produced through an effect on WM tract recovery and changes in the levels of IGF-I might mediate this effect. However, the effects of GHD/IGF-I status on WM recovery following TBI are unknown.

We hypothesised that IGF-I status would impact on recovery after TBI such that improvement in WM tract FA, neuropsychological and cognitive measures over time would be greater in those with a higher serum IGF-I at baseline. MRI imaging including DTI was performed twice along with neuropsychological and cognitive testing in a longitudinal study of patients after TBI, and improvements compared by IGF-I/GH status.

METHODS AND MATERIALS

Participants

Thirty-nine patients with persistent neuropsychological symptoms were recruited in the post-acute phase following a single TBI (>6 weeks post-injury). All patients had the following data recorded: age, sex, body mass index (BMI), mechanism of injury, date and severity of TBI using Mayo classification.³⁸ Research ethics committee approval (Ealing and West London REC 09/H0707/82) and informed written consent were obtained, in accordance with the Declaration of Helsinki. The following exclusion criteria were applied: (i) diabetes mellitus, (ii) pre-TBI history of psychiatric disorder or neurological disease, including learning disability or attention deficit hyperactivity disorder (ADHD), (iii) current or previous drug abuse or excess alcohol use, (iv) craniotomy following TBI (to avoid image registration difficulties in cases of severe structural changes). All participants had completed endocrine testing prior to enrolment in the study.

Participants had standard structural brain MRI, DTI, neuropsychological and cognitive assessment at baseline and at follow-up at least 6 months later. Thirty-five age and sex-matched healthy volunteers underwent an identical neuroimaging visit at a single time point as a control group. Baseline scans only from 13 of the patients with TBI had previously been presented in the BIOSAP study, that found greater cognitive dysfunction and WM tract damage in soldiers after blast TBI subjects with vs. without pituitary dysfunction at baseline.²⁰

Endocrine assessment

Patients had full endocrine testing at baseline as part of their routine clinical care, as previously described.²⁰ Measurements of anterior pituitary hormones (serum IGF-I, prolactin, TSH, free thyroxine, LH, FSH, testosterone/oestrogen, SHBG) were taken in the late morning/early afternoon in the non-fasted state. Blood sampling was on average within a week of their baseline MRI scan (median 0.8 weeks, IQR 0.3-3.1). Serum IGF-I was measured by immunoassay using the Immulite 2500 assay (Siemens, Erlangen, Germany), with reference ranges given in Supplementary Table 1. Serum IGF-I gave

stable measurements over time with good reliability on test-retest. Using this assay in $n=195$ patients after TBI, the intraclass correlation coefficient of serum IGF-I was 0.90 (time since TBI: median [IQR] 17.0 weeks [9.7, 45.1], time between IGF-I measurements: 4.0 weeks [2.4, 6.3]).

Subsequently, patients had comprehensive dynamic testing of the GH (and ACTH-cortisol) axis to look for GH (or ACTH) deficiency using endocrine stimulation tests with serial GH measurement after acute administration of glucagon, GHRH-Arginine or insulin-induced hypoglycaemia, as previously described.³⁴ Initial screening for GH (and ACTH) deficiency was performed using the glucagon stimulation test using peak GH cut-off <5 mcg/L.³⁴ The diagnosis of GHD was then confirmed using the GHRH-Arginine test, with age and BMI-adjusted cut-offs,³⁹ and/or insulin tolerance test (peak GH <3 mcg/L), in all patients. Serum GH was assayed using the Immulite 2000 assay. The protocol for diagnosis of other pituitary dysfunction e.g. ACTH or gonadotropin deficiency, was as previously described.²⁰ Although limited by the low prevalence, an exploratory sub-group analysis of FA recovery was made for those patients after TBI with ($n=4$) versus without ($n=35$) confirmed GHD. However, since only 2 subjects with GHD completed neuropsychological and cognitive testing at both time points, these endpoints were not analysed by GHD status.

Serum IGF-I status

To account for the fact that IGF-I level decline naturally with age, an age-adjusted IGF-I ratio was calculated for each patient. Absolute IGF-I was divided by the median of the age-related reference range (Supplementary Table 1). Patients were then assigned to an 'above median-for-age' or 'below median-for-age' IGF-I group if this ratio was ≥ 1 or <1 respectively, indicating patients with an absolute IGF-I in the upper half or lower half of their age-related reference range.

Structural neuroimaging

To assess macrostructural abnormalities, each patient had standard high-resolution T1 and gradient-echo T2* magnetic resonance imaging on a Philips 3T Achieva scanner (Philips Medical Systems,

Netherlands) using an 8-channel head coil. Scans were reported by a consultant neuroradiologist with extensive expertise in reporting structural abnormalities following TBI, looking specifically for the number of contusions, presence of microbleeds, diffuse axonal injury (DAI) and superficial siderosis. The 4 patients with confirmed GHD also had dedicated gadolinium-enhanced pituitary MRI scans as part of routine clinical care.

In addition, structural DTI was acquired on each participant to assess directionality of water diffusion as a measure of WM tract integrity through extraction of the primary outcome measure of interest, FA, a measure of uni-directionality (0 = isotropic, 1 = anisotropic, lower number indicates lower uni-directionality and hence greater WM tract damage). Diffusion-weighted volumes with gradients applied in 16 non-collinear directions were collected in four runs, with a total of 64 directions, using the following parameters: 73 contiguous slices, slice thickness 2mm, field of view 224mm, matrix 128×128 (voxel size $1.75 \times 1.75 \times 2 \text{mm}^3$), b -value 1000s/mm^2 , four images with no diffusion weighting ($b0$). DTI images were registered to the $b0$ image by affine transformations to minimize distortion due to motion and eddy currents and then brain-extracted using the Brain Extraction Tool⁴⁰ from the FMRIB Software Library.^{41,42} The Diffusion Toolbox was used to fit diffusion tensors to the data in order to generate FA diffusion maps.⁴³

DTI and FA analysis

FA was analysed specifically in two *a priori* WM tract ROIs that have previously been shown to be abnormal after TBI and where normalization of FA predicted clinical outcome, namely the SPCC and the PLIC.⁹ Bilateral ROI masks were defined using the John Hopkins University WM tractography atlas with a cut-off threshold $>20\%$ (Figure 1), averaging mean FA values in left and right ROIs. FA differences and changes over time were also analysed across all WM tracts in the whole brain using tract-based spatial statistics (TBSS) in the FMRIB Software Library using threshold-free cluster enhancement (TFCE) to correct for multiple comparisons at cluster-wise threshold $P < 0.05$. The TBSS steps included: (i) non-linear alignment of all subjects' FA images into common FMRIB58 FA template space; (ii) affine-

transformation of aligned images into standard MNI152 1mm space; (iii) averaging of aligned FA images to create a 4D mean FA image; (iv) thinning of the mean FA image to create a mean FA 'skeleton' representing the center of all WM tracts, to remove partial-volume confounds; and (v) thresholding of the FA skeleton at >0.2 to suppress areas of extremely low mean FA and exclude those with considerable inter-individual variability.⁴⁴ Non-parametric permutation-based statistics were employed using randomize and 5000 permutations.⁴⁵

FA comparisons by group

Analyses and design matrices were constructed to compare FA cross-sectionally: (i) between patients after TBI (baseline scan 1) versus controls: to examine effects of TBI (ROIs, whole brain); and (ii) in patients only between groups at scan 1 only: to examine the effects of GHD and IGF-I status at baseline only (whole brain). Longitudinal analyses were then performed in patients only: (iii) between follow-up scan 2 vs. scan 1: to examine overall recovery over time after TBI (ROI, whole brain); (iv) using an interaction design analysis to examine the effect of GHD and IGF-I status at baseline and FA recovery over time (ROIs); and (v) using the subtracted FA difference between scan 2 and scan 1: to examine the interaction between GHD and IGF-I status at baseline and recovery in FA over time (whole brain WM tracts).

Cognitive and neuropsychological assessment

Patients underwent a detailed cognitive testing battery to examine measures previously found to be sensitive to impairments after TBI:^{10,20,46} (i) Weschsler Memory Scale-Third edition (WMS-II) to test immediate and delayed verbal recall (Logical Memory I and II) and working memory (Digit Span); (ii) People test from Doors and People test battery to measure associative learning and recall; (iii) verbal and letter fluency and Colour-Word (Stroop) tests from Delis-Kaplan Executive Function System to assess cognitive flexibility, inhibition and set-shifting; (iv) Trail Making Test to assess executive function; (v) Wechsler Test of Adult Reading and Wechsler Abbreviated Scale of Intelligence Similarities and Matrix-Reasoning subsets to assess pre-morbid verbal and non-verbal reasoning ability at the first visit

only.

In addition, patients completed the following symptom, psychological and quality of life (QoL) questionnaires at both visits that we have used previously in TBI studies: Beck Depression Inventory II Score (BDI-II), Epworth Daytime Sleepiness Scale, and Assessment of GH Deficiency in Adults (AGHDA) and Short Form 36 Health Survey (SF-36) QoL questionnaires.²⁰

Relationship between DTI FA and cognitive findings

To assess potential causative relationships between the influence of IGF-I status on recovery of FA in WM tracts and cognitive function, significant findings were further examined cross-sectionally at a single time point in a separate cohort. This consisted of patients after moderate-severe TBI (n=82) and healthy controls of similar age and sex (n=40), who underwent similar DTI scanning and an identical neurocognitive assessment. Comparison was made between patients after TBI and controls, and correlations were made between WM FA and cognitive function, both in whole brain WM tract analysis, and in any individual ROIs which were affected by IGF-I status in the longitudinal study, correcting for age and sex.

DTI scans were performed on a Siemens Verio 3T scanner with diffusion-weighted volumes with gradients applied in 16 non-collinear directions were collected in four runs, with a total of 64 directions, using the following parameters: 64 contiguous slices, slice thickness 2mm, field of view 256mm, matrix 128 x 128 (voxel size 2x2x2mm³), TR 9500 ms, TE 103 ms, GRAPPA 2, *b*-value 1000s/mm², four images with no diffusion weighting (*b*0). The DTI processing and analysis pipeline was identical to the longitudinal study.

Statistical analyses

Analysis of demographics and ROI FA values was performed using SPSS (v22, IBM). Comparison between groups used Mann-Whitney U test, chi-squared test (with Yates' correction), 2-way unpaired or paired

Student t-test, and one-way ANCOVA or one-way repeated measures ANCOVA with post-hoc Fisher LSD test, as appropriate with statistical threshold $P < 0.05$.

Comparison of FA in ROIs between controls and TBI patients at baseline using ANCOVA included age and sex as co-variables. Comparison of FA in ROIs between groups in TBI patients at baseline using ANCOVA included age, sex, TBI severity, time since TBI as co-variables. Repeated measures ANCOVA examined improvement in FA in ROIs, neuropsychological and cognitive function over time in TBI patients, and differences between groups to establish effects of group, time and group x time interaction without and with addition of the above co-variables of the following co-variables that might influence recovery: age, sex, TBI severity, time since TBI, and time between DTI scans. Examination of relationships between variables used Pearson or Spearman correlation coefficients r_p or r_s . Time variables were $\log_{10}$ -transformed as they were not normally distributed.

RESULTS

Patient characteristics

Thirty-three of 39 patients (84.6%) were male with median age 30.5y (interquartile range (IQR) 24.5-47.0). Thirteen patients (33.3%) had sustained their TBI as a result of a blast exposure from an improvised explosive device, 10 (25.6%) road traffic accident, 7 (17.9%) assault, 6 (15.4%) fall and 3 (7.7%) through a sporting injury. Thirty-four patients (87.2%) had a moderate-severe TBI by Mayo classification.³⁸ The median time between TBI and first scan was 16.3 months (IQR 3.6-24.5).

Further demographic data and structural abnormalities in the baseline MRI are given for the whole cohort and by IGF-I group in Table 1 (and by GHD status in Supplementary Table 2). No patients had craniotomy as these subjects had been excluded from the study.

Thirty-five healthy controls were studied with the following demographics: 23 (65.7%) male ($P=0.11$ vs. TBI), and median age 30.7y (IQR 25.9-38.2, $P=0.22$ vs. TBI).

GHD and pituitary dysfunction

Four (10.2%) patients after TBI had confirmed untreated GHD. Overall, 7/39 (17.9%) had pituitary dysfunction (3 isolated GHD, 1 ACTH deficiency, 2 hyperprolactinemia, 1 combined ACTH/GH/gonadotropin deficiencies). Six were soldiers who had sustained blast TBI.²⁰ None of these patients had yet started any hormone replacement therapy at the time of their first or second scan. There were no significant differences in age, sex, TBI severity, time since injury, time between scans, prevalence of blast TBI, or prevalence of structural MRI abnormalities at baseline, between patients with and without GHD (Supplementary Table 2). Although dedicated pituitary MRI imaging was only performed in those with GHD, there was no evidence of hypothalamic-pituitary damage in any patient.

Absolute serum IGF-I and age-adjusted IGF-I ratio tended to be lower (median 19.0% lower, $P=0.11$; median 18.2% lower, $P=0.11$ respectively) in those patients with vs. without GHD (Supplementary Table

2). Seventy-five per cent of GHD subjects had a serum IGF-I below the age-related median.

IGF-I status

Seventeen (43.6%) patients were in the below median-for-age IGF-I group and 22 in the above median-for-age IGF-I group. The below and above median-for-age numbers were unequal as they were defined according to age-matched reference ranges (Supplementary Table 1). In the below median-for-age IGF-I group 1/17 (5.6%) had a serum IGF-I <5th percentile (age-adjusted normal range), and in the above median-for-age IGF-I group 4/22 (18.2%) had a serum IGF-I >95th percentile. There were no significant differences in age, sex, TBI severity, time since injury, BMI, or prevalence of blast TBI (below vs above median-for-age IGF-I group 29.4% vs. 36.3%, $P=0.90$), structural MRI abnormalities at baseline or post-menopausal females, between IGF-I groups (Table 1). There was a trend towards a greater length of time between the two scans in the below median IGF-I group compared to the above median IGF-I group (median 14.4 vs. 12.5 months, $P=0.08$).

Only 3/17 (17.6%) of patients in the below median-for-age IGF-I group had GHD confirmed on dynamic testing, compared to 1/22 (4.5%) in the above median-for-age IGF-I group ($P=0.15$). Of the 4 patients with confirmed GHD, none had serum IGF-I <5th percentile, 3 had IGF-I between 5th percentile and median, and one had IGF-I between median and 95th percentile.

Effect of TBI on WM tracts

TBI patients show evidence of axonal injury: In whole brain analysis, FA was significantly lower in many WM tracts including splenium, body and genu of corpus callosum, the PLIC, cerebellar peduncles, anterior region of the corona radiata and the internal capsule in patients following TBI at baseline scan 1 ($n=39$), compared to healthy controls ($n=35$) (Figure 2A). As expected, compared to the healthy control group, the TBI group overall had significantly lower FA in the *a priori* ROIs SPCC (effect size mean $\pm$ SEM - 0.021 ± 0.009 , 95% CI [-0.003, -0.040], $t(72)=-2.26$, $P=0.027$) and PLIC (effect size - 0.026 ± 0.006 , [-0.039, -0.013], $t(72)=-3.90$, $P<0.001$) (Figure 3).

WM tract recovery over time

Increased FA over time suggest axonal recovery following TBI: Across the whole TBI group, FA increased between scan 1 to follow-up scan 2 in a number of WM tracts including splenium, body and genu of corpus callosum, subcortical regions, right frontal region and widespread ventral regions (Figure 2B). This suggests an overall recovery of axonal injury in these tracts. This was also observed in the *a priori* ROIs SPCC (effect size 0.015 ± 0.007 , [0.001, 0.281], $t(38)=2.2$, $P=0.035$), and PLIC (effect size 0.016 ± 0.004 [0.003, 0.020], $t(38)=3.1$, $P=0.005$). There were no significant effects of age ($P=0.35-0.51$), sex ($P=0.14-0.60$), TBI severity ($P=0.11-0.23$), time since TBI ($P=0.33-0.73$), or time between scans ($P=0.58-0.90$) on the change in FA over time (co-variate x time interaction), when including each factors separately as a within subject co-variate in a repeated measures ANCOVA model.

Effect of IGF-I group on WM tract FA

No cross-sectional influence of IGF-I group on FA at baseline: Within the TBI group, there was no significant effect of IGF-I group on FA at baseline in whole brain analysis, with or without inclusion of co-variates. This lack of any effect was determined by the absence of any voxels within any WM tract reaching the cluster-wise TFCE threshold $P<0.05$.

Influence of IGF-I group on recovery in FA over time: Within the TBI group, there was a significant IGF-I group x time interaction ($F(1,37)=4.62$ $P=0.038$). This resulted from a significant increase in FA in the *a priori* SPCC ROI over time in the above median-for-age but not in the below median-for-age IGF-I group (above median-for-age IGF-I group effect size $+0.027 \pm 0.008$, 95% CI [0.009, 0.044], $F(1,37)=9.82$, $P=0.03$; below median-for-age IGF-I group effect size -0.001 ± 0.100 , [-0.021, 0.190], $F(1,37)=0.01$, $P=0.92$). Similar results were seen when including co-variates in the repeated measures ANCOVA (IGF-I group x time interaction $F(1,32)=5.66$, $P=0.023$, above median-for-age IGF-I group effect size $+0.030 \pm 0.009$, [0.011, 0.048], $F(1,32)=10.60$, $P=0.003$; below median-for-age IGF-I group effect size $-0.011 \pm$

0.010, [-0.026, 0.017], $F(1,32)=0.21$, $P=0.65$ (Figure 3B). There was no significant difference in SPCC FA between IGF-I groups at baseline. A similar interaction was seen for FA improvement in SPCC over time was also seen including blast vs. non-blast TBI in the ANOVA model (IGF-I group x time interaction ($F(1,36)=4.27$, $P=0.046$), with no significant effect of blast TBI overall ($F(1,36)=0.28$, $P=0.60$), or blast TBI x time interaction ($F(1,36)=1.94$, $P=0.17$).

A similar interaction was seen for FA improvement in SPCC over time, when excluding the 4 subjects without moderate-severe TBI (IGF-I group x time interaction ($F(1,29)=3.74$, $P=0.063$), or when excluding 2 subjects with a prolonged time since TBI >100 months (IGF-I group x time interaction ($F(1,31)=5.06$, $P=0.032$, including age, sex, severity and time between scans).

There was however no significant effect of IGF-I adjusted for age as a continuous rather than categorical variable on FA recovery in the SPCC (time x IGF-I age-adjusted ratio interaction: $F(1,37)=0.25$, $P=0.62$; Spearman's correlation coefficient for delta SPCC FA $r=+0.20$, $P=0.22$).

There was no significant effect of IGF-I group on increase in FA over time in the *a priori* PLIC ROI either without any covariates (IGF-I group x time interaction $F(1,37)=0.57$, $P=0.46$), or when including covariates (IGF-I group x time interaction $F(1,32)=1.31$, $P=0.26$) (Figure 3C).

In whole brain subtraction analysis, there was no significant effect of IGF-I group on the change in FA between scans 2 and 1, with or without inclusion of co-variates. A similar lack of effect of IGF-I group was also seen when restricting the skeleton in whole brain analysis to only those voxels showing a significant increase in FA between scans (Figure 2B).

Effect of IGF-I group on neuropsychological and cognitive variables

Within the TBI group, there was a significant IGF-I group x time interaction ($F(1,26)=4.38$, $P=0.046$). This resulted from a significant increase in the logical memory I total score (measure of immediate recall)

over time in the above median-for-age but not the below median-for-age IGF-I group (above median-for-age IGF-I group effect size $+4.19 \pm 2.01$, 95% CI [0.06, 8.31], $F(1,26)=4.34$, $P=0.047$; below median-for-age IGF-I group effect size -2.42 ± 2.22 , [-0.699, 2.15], $F(1,26)=1.19$, $P=0.29$), when including covariates (age, sex, severity, time since injury and time between scans) in the repeated measures ANCOVA, despite scores being similar between groups at baseline ($F(1,26)=0.60$, $P=0.45$) (Figure 4).

There was no significant correlation between the improvement in SPCC FA over time and the improvement in logical memory I total score over time in patients after TBI, either alone ($r_p=+0.20$, $P=0.28$), or when correcting for age, sex, severity, time since injury and time between scans (partial $r_p=+0.26$, $P=0.18$).

There was no significant IGF-I group x time interactions or effects of time for any of the other cognitive measures, or symptom (BDI-II, Epworth Daytime Sleepiness Scale) or quality of life (SF-36, AGHDA) questionnaire scores (Supplementary Table 3). There were however significant improvements over time in associative memory (People Test) ($F(1,32)=6.12$, $P=0.019$) and cognitive flexibility (Color Word Stroop) ($F(1,29)=5.67$, $P=0.024$), independent of IGF-I group.

Relationship between DTI FA and neurocognitive findings in cross-sectional cohort

Lower SPCC FA and worse memory in patients after TBI and controls in separate cohort: Both SPCC FA and logical memory I total score (measure of immediate recall) were significantly lower in a separate cross-sectional cohort of patients after moderate-severe TBI ($n=82$), than in controls of similar age and sex ($n=40$) (Table 2, Figure 5A).

Correlation between lower SPCC FA and worse memory in patients after TBI and controls: Furthermore, SPCC FA (average in whole ROI) was positively correlated with logical memory I total score in patients after TBI and controls ($r_s= +0.25$, $P=0.006$, Figure 5A), and when adjusting for age and sex ($r_s= +0.23$, $P=0.012$).

In whole brain WM tract analysis, a positive correlation between logical memory I total score in patients after TBI and FA was seen only in the SPCC (large arrow), and the left inferior longitudinal fasciculus (arrowhead), using cluster-corrected TFCE $P < 0.05$, including age and sex as co-variates (Figure 5B).

Effect of GHD on WM tract FA

Only 4/39 subjects had confirmed GHD. However, we performed exploratory investigations to determine whether there was an effect of GHD on WM recovery despite the underpowered nature of such analyses.

No cross-sectional influence of GHD on FA at baseline: Within the TBI group, there was no significant effect of GHD on FA at baseline in whole brain WM tract analysis, with or without inclusion of co-variates (age, sex, TBI severity, time since TBI). This lack of any effect was determined by the absence of any voxels within any WM tract reaching the cluster-wise TFCE threshold $P < 0.05$.

No longitudinal influence of GHD status on change in FA over time: Within the TBI group, there was no significant effect of GHD on the increase in FA over time in either the SPCC or PLIC ROIs (GHD status x time interaction $P = 0.29-0.45$). This remained non-significant when including the co-variates: age, sex, TBI severity, time since TBI, and time between DTI scans (GHD status x time interaction $P = 0.22-0.45$) (Supplementary Figure 1).

Similarly, in whole brain WM tract subtraction analysis, there was no significant effect of GHD on the change in FA between scans 2 and 1, with or without inclusion of co-variates. A similar lack of effect of GHD was also seen when restricting the skeleton in whole brain WM tract analysis to only those voxels showing a significant increase in FA between scans (Figure 2B).

DISCUSSION

Our results show that recovery of diffuse axonal injury after TBI, measured using DTI, appears influenced by serum IGF-I concentration at baseline. We focused on the corpus callosum as this region is frequently damaged by TBI and its recovery has been shown to predict clinical outcome.⁹ A greater increase in FA over time in the corpus callosum, indicative of WM recovery, was seen in patients who had serum IGF-I above the median for their age, compared to those with IGF-I below median. We also found that immediate memory recall improved to a greater extent in those with serum IGF-I above the median for their age compared to those below. The association of IGF-I with improved WM and memory recovery after TBI requires further exploration but, if a direct neuroendocrine effect on brain recovery is confirmed, this may have important therapeutic implications.

Human serum IGF-I, a 70 amino acid polypeptide, is thought to mediate many of the trophic effects of GH through both hepatic and local production. The effects of both systemic IGF-I and GH on the brain are not completely clear or disentangled, however both peptides are able to penetrate the blood brain barrier and peripheral IGF-I and GH administration can improve spatial learning and memory in animal studies, suggesting that similar pathways mediate these effects.^{22,47}

At a mechanistic level, GH receptors are ubiquitous throughout the brain and GH/IGF-I can have a direct neurogenic effect on proliferation of neurons, oligodendrocytes and new blood vessels.³¹ Deficiency of either hormone can also decrease neuronal survival time suggestive of a neuroprotective effect.⁴⁷ GH also influences the hippocampal NMDA receptor system with effects on memory acquisition. GH/IGF-I may also promote brain repair through the local induction and release of a number of neurotrophic factors such as VEGF, EGF and BDNF.⁴⁸ Other putative mechanisms may be directly through reduction of neuroinflammation or improvement in peripheral metabolic factors such as metabolic syndrome.

GHD following TBI is a well-recognised post-traumatic phenomenon but appears not be as common as initially reported. More recent larger studies have reported a prevalence of GHD of 2.7-11.8% of 112

patients in a Dutch cohort (using insulin tolerance test, or GHRH-arginine test if contra-indicated),¹⁶ and 4.5-11.8% of 439 patients in a Danish cohort (using insulin tolerance test or GHRH-arginine/GHRH-pyridostigmine test),¹⁷ both including patients >1 year after TBI. In the Imperial TBI clinic, we have found a prevalence of GHD in 7.4% of 176 patients after TBI of mixed severity (median 0.47 years since TBI), and 8.5% of 258 patients after moderate-severe TBI only, with diagnosis requiring inadequate GH response to both glucagon, and GHRH-Arginine testing or insulin-induced hypoglycaemia.²⁰ In this study we found only 4 of 39 patients (10.3%) had GHD using these criteria, a prevalence enhanced by one third of patients having blast TBI, which is associated with a particularly high risk of pituitary dysfunction.²⁰ GHD at baseline did not influence increase in FA in either ROI, although these results should be interpreted with caution since only 4 patients had GHD in this cohort, meaning that we are likely to have significant type II errors in examining the effects of WM tract recovery.

We therefore chose to primarily study serum IGF-I as a biomarker given the potential convergence of the GH/IGF-I axis on brain recovery. Indeed serum IGF-I is the biomarker used to titrate GH replacement therapy in subjects who are GHD, with a target IGF-I in the upper half of the age-related reference range being the usual target for dose adjustment.⁴⁹ We were not powered to look for effects of extremely low or high serum IGF-I concentrations since only 5 of 39 patients (12.8%) had serum IGF-I outside the 95% CI for their age (1 above, 4 below).

Another limitation of our study is that we did not measure serum IGF-binding proteins that may alter free IGF-I concentrations, though none of our patients were malnourished, and there were no significant differences in other factors that might influence IGF-I concentrations such as BMI and post-menopausal status between IGF-I groups.

Finally, we had included 4 patients with mild TBI in our cohort, and 2 with a very long time from injury (>100 months). In these patients, we may expect that the dynamic WM changes over time may be reduced and therefore could have influenced our overall result. However, in two separate analyses

excluding these patients, the effect of IGF-I on WM recovery remained similar. Furthermore, there was a high prevalence of blast TBI in our cohort. However, there was no difference in the prevalence of blast TBI between IGF-I groups, while inclusion of blast TBI category did not influence the finding of greater FA recovery in SPCC over time in the above median-for-age IGF-I group. This makes it unlikely that the results were influenced by the inclusion of many subjects with blast TBI.

We chose to focus on FA as a measure of WM tract recovery as this is a reliable measure of WM integrity.^{10,11} Given DTI measures water diffusion in WM tracts and FA is a measure of diffusion directionality, we cannot be certain that this specifically relates to underlying WM damage and repair in these patients. However, we do show a striking reduction in FA across the whole brain in patients after TBI compared to controls and that FA increases over time after TBI.

Although interpretation is limited by the relative small sample size and multiple neuropsychological and cognitive tests used, we also found that only the group with IGF-I above the median for age had a significant improvement in immediate memory recall over time. We have previously shown that after TBI, FA measurements in particular WM tracts relate to multiple neuropsychological domains.^{10,11,46} Indeed in a separate cross-sectional cohort of patients after moderate-severe TBI and controls who were scanned once, we confirmed both lower SPCC FA and immediate verbal memory recall in patients after TBI compared to controls. Furthermore, SPCC FA was positively correlated with immediate verbal memory recall.

This association may reflect a direct role of the SPCC in immediate verbal memory. The SPCC contains fibres from the ventral, retrosplenial, subsplenial and dorsoposterior cingulate cortex (Brodmann's areas (BA) 23, 29-31), in addition to sensory and visual association cortices (BA 5,7,18,19).⁵⁰ Diffusivity measures in the SPCC, indicating greater WM tract damage, have been negatively correlated with functional connectivity of the posterior cingulate cortex (PCC) after TBI.⁵¹ Infarction of the SPCC and retrosplenial region can lead to verbal memory problems.⁵²⁻⁵⁴ In patients with multiple sclerosis, verbal

learning performance was inversely associated with lesions in the SPCC, while lower FA in SPCC predicted a greater progression of disability, and lower FA in the corpus callosum predicted worse verbal memory, longitudinally over five years.⁵⁵⁻⁵⁶

Alternatively, the SPCC-memory results may be a marker of related damage to nearby WM tracts, or overall severity of TBI and associated WM damage, with the larger size and higher FA of the SPCC providing greater sensitivity to detect damage. Indeed, damage to the neighbouring cingulum bundle, the WM tract connecting the parahippocampal gyrus to the PCC, has been associated structurally and functionally with the development of acute post-traumatic amnesia after TBI, where there is pronounced inability to encode new memories.⁵⁷

However, in the longitudinal study, we could not demonstrate any correlation of the improvement in SPCC FA, with improvement in immediate verbal memory recall, nor any associated change in any other cognitive, symptom or QoL measures, which may reflect the smaller numbers. Future larger studies will need to confirm these preliminary findings, see if it is also found in other WM tracts, and address whether any greater increase in FA seen over time in those with higher IGF-I concentrations is also associated with greater improvements in other neuropsychological functions, and whether other neuroimaging markers such as brain atrophy are also influenced by IGF-I status.

Our ROI analysis was restricted to areas expected to see a change in FA based on previous studies.^{9,58} Whole brain WM tract analyses using TBSS showed a striking effect of time on FA recovery across many WM tracts. However, we did not find any significant interaction with IGF-I group. This may be due to several factors including small numbers given the need for robust statistical correction for multiple comparisons in whole brain analysis, and variability in the time since TBI and time between scans in our cohort, though these did not appear to be important factors influencing FA recovery in ROI analysis. Future studies should utilise larger cohorts with more consistent time periods. Unlike the categorical classification, we did not see any influence of serum IGF-I as a continuous variable on SPCC FA recovery.

However, this assumes that there is a straightforward relationship between IGF-I status and WM tract recovery and there may be a cut-off level on the IGF-I dose response curve. Measurement of cerebrospinal fluid IGF-I and serum IGF-I at serial time points would also be helpful in future studies.

In conclusion, we have presented preliminary work demonstrating that higher concentrations of serum IGF-I for age in adults following a TBI is associated with higher WM recovery in the SPCC, and greater improvements in immediate verbal memory over time, irrespective of the presence of GHD. Furthermore, in a larger expanded cross-sectional cohort, greater SPCC WM tract damage was correlated with worse immediate verbal memory in patients after TBI, consistent with a possible direct relationship. There are limited treatments to improve recovery from TBI and relatively little is known about what predicts the prognosis for patients. Future larger studies are needed to confirm and extend these findings. If confirmed this reveals the possibility that it may be worthwhile investigating whether GH treatment to raise serum IGF-I into the upper part of the age-related reference range might aid recovery following TBI, regardless of whether GHD is present using dynamic tests.

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AUTHOR CONTRIBUTIONS

Study concept and design (CF, DJS, MM, APG), data acquisition and analysis (CF, DJS, PJH, AEJ, JHC, GS, DB, SJ, ER, TEH, POJ, LML, NG, APG), drafting the manuscript and figures (CF, DJS, APG), review and editing of manuscript (all authors).

CONFLICTS OF INTEREST

There are no conflicts of interest to declare

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TABLES

Table 1. Patient characteristics by IGF-I group

	All TBI Group	IGF-I Above Age-Related Median	IGF-I Below Age-Related Median	P Above vs. Below ^a
n	39	22	17	
Age at first scan (yrs)	30.5 [24.5-47.0]	30.2 [24.3-47.6]	34.5 [25.2-48.8]	0.43
Range	19.6-66.9	19.6-62.2	21.3-66.9	
Male n (%)	33 (84.6%)	17 (77.3%)	16 (94.1%)	0.32
Post-menopausal women n (%)	3 (7.7%)	2 (9.1%)	1 (5.9%)	0.82
BMI (kg/m ²)	25.3 [23.1-29.8]	23.6 [19.6-29.0]	25.3 [24.4-30.2]	1.00
Range	17.0-35.0	17.0-32.3	21.3-35.0	
Moderate-severe TBI n (%)	34 (87.2%)	20 (90.6%)	14 (82.4%)	0.75
Blast TBI n (%)	13 (33.3%)	8 (36.3%)	5 (29.4%)	0.90
Time since TBI (months)	16.3 [3.6-24.5]	12.5 [4.0-25.9]	17.2 [3.1-33.5]	0.89
Range	1.47-571.0	1.5-571.0	1.5-102.9	
Time between scans (months)	13.3 [12.1-14.9]	12.4 [10.8-13.4]	14.4 [13.1-15.1]	0.08
Range	6.3-24.6	6.3-24.6	8.9-17.8	
Absolute IGF-I nmol/L ^b	23.8 [19.2-29.5]	28.2 [23.3-35.1]	19.9 [16.8-23.7]	0.02
Range	12.8-74.5	19.2-74.5	12.8-29.0	
Age-adjusted IGF-I ratio	1.1 [0.9-1.2]	1.2 [1.1-1.5]	0.8 [0.8-0.9]	<0.001
Range	0.6-3.0	1.0-2.9	0.6-1.0	
GHD n (%)	4 (10.2%)	1 (4.5%)	3 (17.6%)	0.42
Any pituitary deficiency n (%)	7 (17.9%)	3 (13.6%)	4 (23.5%)	0.71
Any structural brain abnormality n (%)	30 (76.9%)	19 (86.4%)	11 (64.7%)	0.23
≥ 1 contusion	20 (51.3%)	11 (28.2%)	9 (52.9%)	0.89
No. of contusions ^c	1 [0-2]	1 [0-1]	1 [0-2]	0.40
Range	0-5	0-2	0-5	
≥ 1 microbleed n (%)	10 (25.6%)	8 (36.4%)	2 (11.8%)	0.17
Superficial siderosis n (%)	13 (33.3%)	6 (27.3%)	7 (41.2%)	0.57
Diffuse axonal injury (DAI) n (%)	5 (12.8%)	3 (13.6%)	2 (11.8%)	0.76

Data given as n (%) or median [IQR] and range (minimum-maximum). IGF-I groups refer to patients with an absolute IGF-I concentration above the median-for-age or below the median-for-age.

^a P value for comparison serum IGF-I group using chi-squared test with Yates' correction for categorical variables and Mann-Whitney U test for continuous variables.

^b To convert nmol/L to ng/mL divide by 0.131.

^c including those without any contusions

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Table 2. Comparison between splenium fractional anisotropy and immediate memory recall in expanded cross-sectional cohort of controls and patients after TBI.

	Controls	TBI	Statistic	P
n	40	82		
Age (years)	42.1 ± 12.6 (21-72)	42.4 ± 12.0 (20-72)	t -0.14	0.89
Male n (%) ^a	30 (75.0%)	69 (84.1%)	χ ² 0.93	0.33
Time since TBI (months)	n/a	30.5 [12.5, 153.8]	n/a	n/a
SPCC FA ^b	0.8037 [0.7760, 0.8203] (0.7603-0.8408)	0.76671 [0.6989, 0.7926] (0.4072-0.8221)	Z -5.13	<0.001
Logical Memory I total	50.00 ± 8.39 (33-68)	39.24 ± 12.15 (13-66)	t 5.70	<0.001

Comparison of demographics, average fractional anisotropy (FA) in splenium of corpus callosum (SPCC) and Logical Memory I total score (immediate recall) in expanded cross-sectional cohort of healthy controls and patients after moderate-severe traumatic brain injury. Data given as mean ± SD (range) except for ^a n (%) and ^b median [interquartile range] (range). Comparison between groups by 2-tailed unpaired Student’s t-test, except for ^a chi-squared test, and ^b Mann-Whitney test as data not normally distributed.

FIGURE LEGENDS

Figure 1. Posterior limb of internal capsule and splenium of corpus callosum white matter tract regions of interest.

Group average FA skeleton (green, threshold >0.2) from all patients after TBI ($n=39$) and healthy controls ($n=35$), overlaid onto standard MNI152 1mm T1 MRI brain scan. White matter (WM) tract defined using the JHU white matter tractography atlas with a cut-off threshold of 20% indicated in red. Mean fractional anisotropy (FA) values in posterior limb of internal capsule and splenium of corpus callosum WM tract regions of interest were calculated from the average of the voxels indicated by the overlap of the green skeleton and red mask. Co-ordinates given in Montreal Neurological Institute (MNI) standard space. R indicates right.

Figure 2. White matter tract fractional anisotropy in patients after TBI.

A) Lower white matter tract fractional anisotropy in patients after TBI. Group average statistical maps showing voxels displaying lower fractional anisotropy (FA) in patients with TBI ($n=39$) compared to healthy controls ($n=35$) displayed in red (TFCE cluster-wise threshold $P<0.05$ and correcting for age and sex), overlaid on combined whole group FA skeleton (green), displayed on standard MNI125 1mm T1 MRI brain scan. Co-ordinates given in Montreal Neurological Institute (MNI) standard space. R indicates right.

B) White matter tract FA improvement between scans. Group average statistical maps showing voxels displaying higher fractional anisotropy (FA) in TBI patients at follow-up scan (in red) compared to baseline scan overlaid on combined whole group FA skeleton (green) using TFCE cluster-wise threshold $P<0.05$ and correcting for age, time since injury, sex, TBI severity and time between scans. Results displayed on standard MNI125 1mm T1 MRI brain scan. Co-ordinates given in Montreal Neurological Institute (MNI) standard space. R indicates right.

Figure 3. White matter tract FA in regions of interest in patients after TBI at baseline and recovery over time by IGF-I group.

(A) Lower FA in splenium of corpus callosum (SPCC) and posterior limb of internal capsule (PLIC) ROIs in patients after TBI (black bar) compared to controls (white bar) at baseline scan, adjusting for age and sex (* $P < 0.05$, ANCOVA with post-hoc Fisher LSD test).

(B) Greater improvement in FA in SPCC, but (C) no difference in improvement in FA in PLIC, between scan 1 (dotted bar) and scan 2 (striped bar) in above median-for-age IGF-I group compared to below median-for-age IGF-I group, adjusting for age, sex, TBI severity, time since TBI and time between scans (* $P < 0.05$, repeated measures ANCOVA with post-hoc Fisher LSD test). Data given as mean $\pm$ SEM. Statistics for repeated measures ANCOVA: in (B) effect of Time: $F(1,32)=0.15$, $P=0.70$; Group: $F(1,32)=0.12$, $P=0.74$; Time $\times$ Group interaction: $F(1,32)=5.72$, $P=0.023$; and (C) effect of Time: $F(1,32)=0.34$, $P=0.56$; Group: $F(1,32)=1.01$, $P=0.32$; Time $\times$ Group interaction: $F(1,32)=1.31$, $P=0.26$.

Figure 4. Recall memory in patients after TBI at baseline and recovery over time by IGF-I group.

Greater improvement in Logical Memory I (LMI) total score (immediate recall) in above median-for-age IGF-I group compared to below median-for-age IGF-I group, adjusting for age, sex, TBI severity, time since TBI and time between scans (* $P < 0.05$, repeated measures ANCOVA with post-hoc Fisher LSD test). Significant increase in LM1 score between scan 1 (dotted bar) and scan 2 (striped bar) for IGF-I group above but not below median at baseline, and no significant difference between IGF-I groups at baseline. Data given as mean $\pm$ SEM. Statistics for repeated measures ANCOVA: effect of: Time: $F(1,26)=1.33$, $P=0.26$; Group: $F(1,26)=2.56$, $P=0.12$; Time $\times$ Group interaction: $F(1,26)=4.38$, $P=0.046$.

Figure 5. Relationship between recall memory and splenium fractional anisotropy in patients after TBI and controls

(A) Relationship between mean splenium of corpus callosum (SPCC) fractional anisotropy (FA) as independent variable and Logical Memory I (LMI) total score (immediate recall) as dependent variable, in patients after moderate-severe TBI ($n=82$, red circles) and controls of similar age and sex ($n=40$, green circles), without adjustment for any co-variates. r_s represents Spearman correlation coefficient for combined groups. This represents a separate cross-sectional cohort of subjects to the longitudinal

cohort.

(B) Group average statistical maps showing voxels (in red) in SPCC (long arrow), and inferior longitudinal fasciculus (arrowhead), white matter (WM) tracts whose fractional anisotropy (FA) is positive correlated with Logical Memory I (LMI) total score (immediate recall), in TBI patients (n=82). Results overlaid on combined group FA skeleton (green, threshold >0.2), using TFCE cluster-wise threshold $P < 0.05$ correcting for age and sex. Results displayed on standard MNI125 1mm T1 MRI brain scan. SPCC WM tract defined using the JHU WM tractography atlas with a cut-off threshold of 20% is indicated in yellow. Mean FA values in the SPCC WM tract region of interest in Figure 5A were calculated from the average of the voxels indicated by the overlap of the green skeleton and yellow mask. Co-ordinates given in Montreal Neurological Institute (MNI) standard space. R indicates right.

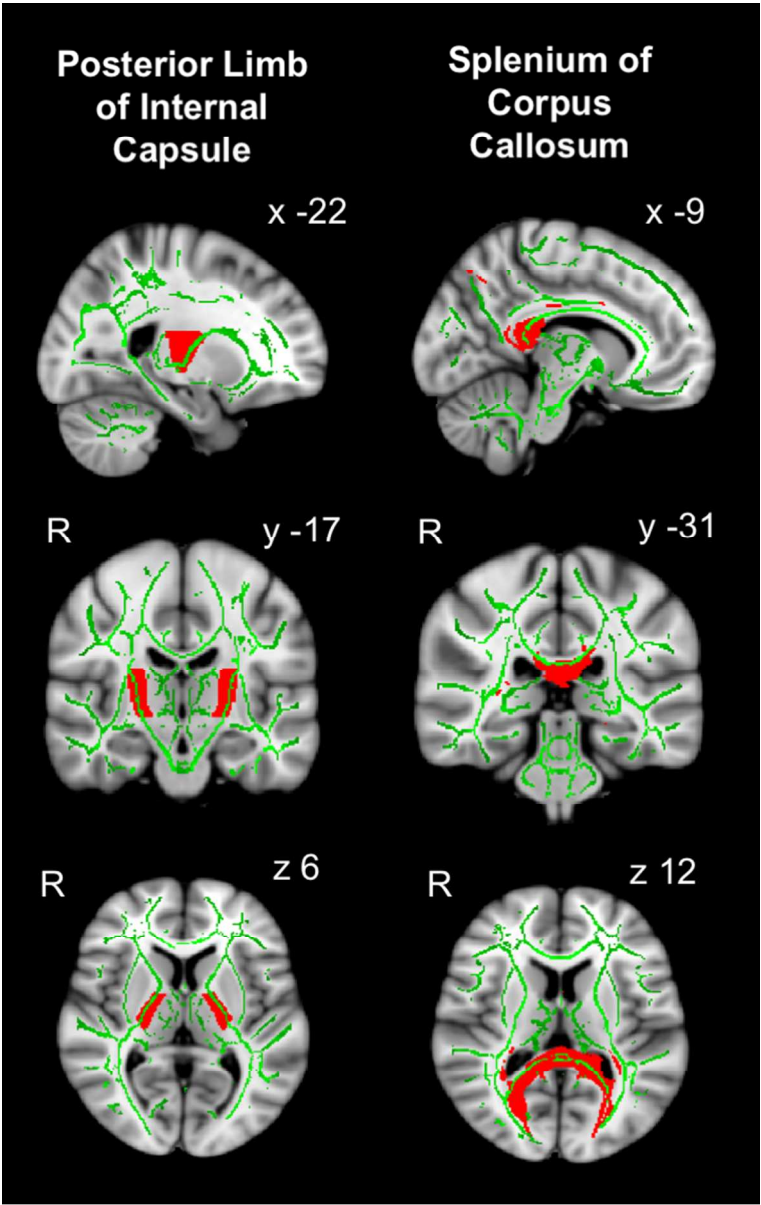


Figure 1. Posterior limb of internal capsule and splenium of corpus callosum white matter tract regions of interest.

Group average FA skeleton (green, threshold >0.2) from all patients after TBI (n=39) and healthy controls (n=35), overlaid onto standard MNI152 1mm T1 MRI brain scan. White matter (WM) tract defined using the JHU white matter tractography atlas with a cut-off threshold of 20% indicated in red. Mean fractional anisotropy (FA) values in posterior limb of internal capsule and splenium of corpus callosum WM tract regions of interest were calculated from the average of the voxels indicated by the overlap of the green skeleton and red mask. Co-ordinates given in Montreal Neurological Institute (MNI) standard space. R indicates right.

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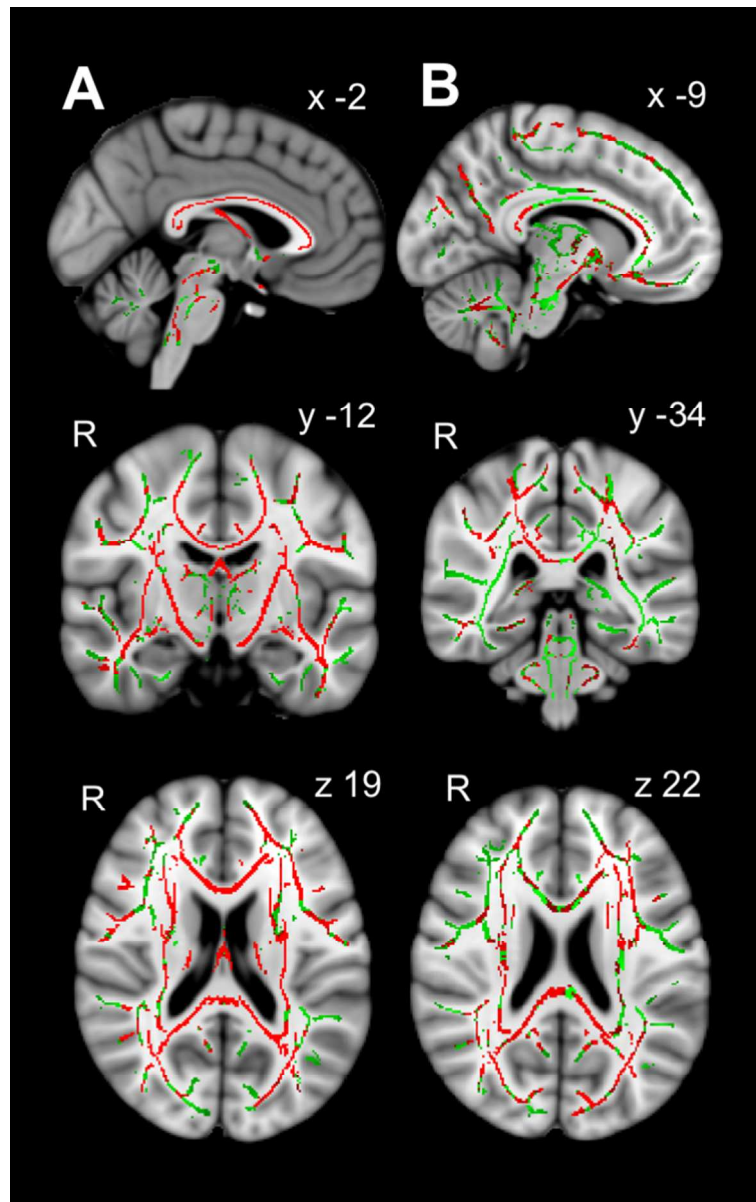


Figure 2. White matter tract fractional anisotropy in patients after TBI.

A) Lower white matter tract fractional anisotropy in patients after TBI. Group average statistical maps showing voxels displaying lower fractional anisotropy (FA) in patients with TBI ($n=39$) compared to healthy controls ($n=35$) displayed in red (TFCE cluster-wise threshold $P<0.05$ and correcting for age and sex), overlaid on combined whole group FA skeleton (green), displayed on standard MNI125 1mm T1 MRI brain scan. Co-ordinates given in Montreal Neurological Institute (MNI) standard space. R indicates right.

B) White matter tract FA improvement between scans. Group average statistical maps showing voxels displaying higher fractional anisotropy (FA) in TBI patients at follow-up scan (in red) compared to baseline scan overlaid on combined whole group FA skeleton (green) using TFCE cluster-wise threshold $P<0.05$ and correcting for age, time since injury, sex, TBI severity and time between scans. Results displayed on standard MNI125 1mm T1 MRI brain scan. Co-ordinates given in Montreal Neurological Institute (MNI) standard space. R indicates right.

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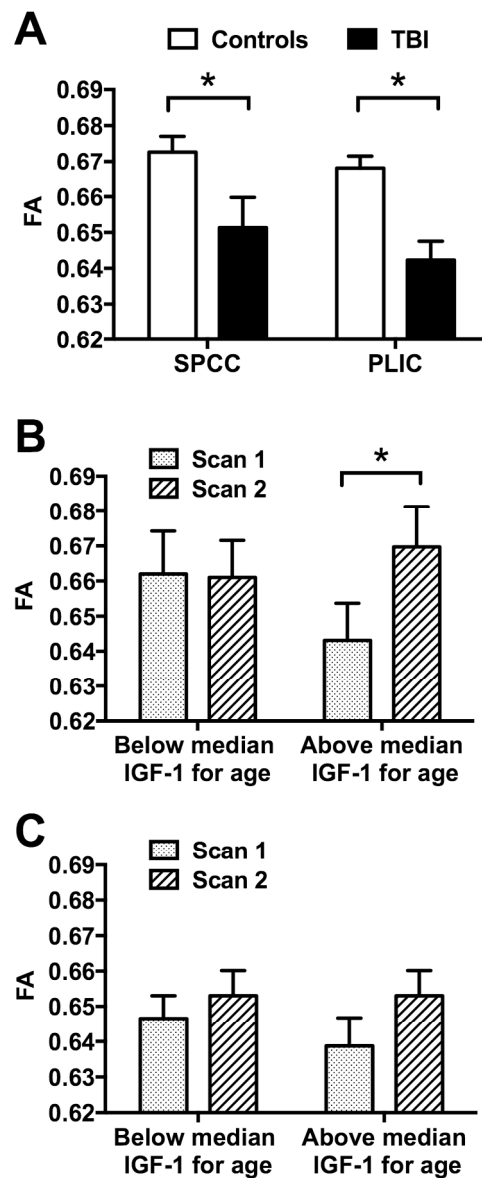


Figure 3. White matter tract FA in regions of interest in patients after TBI at baseline and recovery over time by IGF-I group.

(A) Lower FA in splenium of corpus callosum (SPCC) and posterior limb of internal capsule (PLIC) ROIs in patients after TBI (black bar) compared to controls (white bar) at baseline scan, adjusting for age and sex (* $P < 0.05$, ANCOVA with post-hoc Fisher LSD test).

(B) Greater improvement in FA in SPCC, but (C) no difference in improvement in FA in PLIC, between scan 1 (dotted bar) and scan 2 (striped bar) in above median-for-age IGF-I group compared to below median-for-age IGF-I group, adjusting for age, sex, TBI severity, time since TBI and time between scans (* $P < 0.05$, repeated measures ANCOVA with post-hoc Fisher LSD test). Data given as mean $\pm$ SEM. Statistics for repeated measures ANCOVA: in (B) effect of Time: $F(1,32)=0.15$, $P=0.70$; Group: $F(1,32)=0.12$, $P=0.74$; Time $\times$ Group interaction: $F(1,32)=5.72$, $P=0.023$; and (C) effect of Time: $F(1,32)=0.34$, $P=0.56$; Group: $F(1,32)=1.01$, $P=0.32$; Time $\times$ Group interaction: $F(1,32)=1.31$, $P=0.26$.

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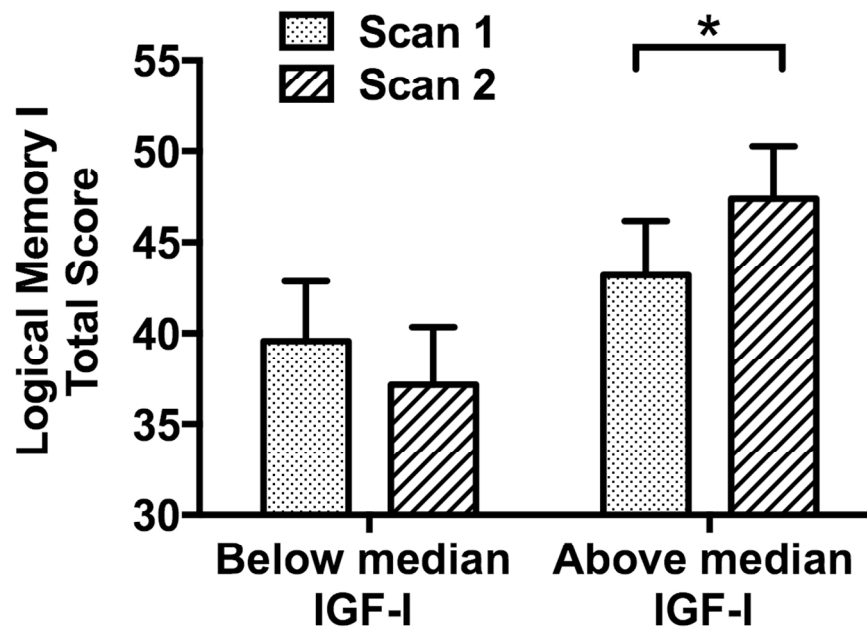


Figure 4. Recall memory in patients after TBI at baseline and recovery over time by IGF-I group. Greater improvement in Logical Memory I (LMI) total score (immediate recall) in above median-for-age IGF-I group compared to below median-for-age IGF-I group, adjusting for age, sex, TBI severity, time since TBI and time between scans (* $P < 0.05$, repeated measures ANCOVA with post-hoc Fisher LSD test). Significant increase in LM1 score between scan 1 (dotted bar) and scan 2 (striped bar) for IGF-I group above but not below median at baseline, and no significant difference between IGF-I groups at baseline. Data given as mean $\pm$ SEM. Statistics for repeated measures ANCOVA: effect of: Time: $F(1,26)=1.33$, $P=0.26$; Group: $F(1,26)=2.56$, $P=0.12$; Time $\times$ Group interaction: $F(1,26)=4.38$, $P=0.046$.

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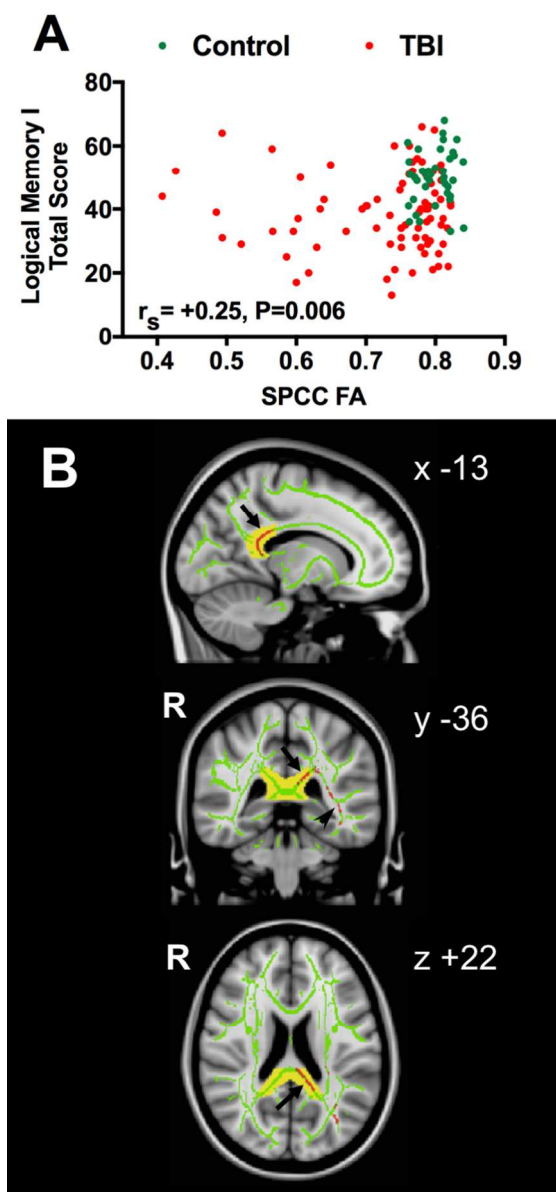


Figure 5. Relationship between recall memory and splenium fractional anisotropy in patients after TBI and controls

(A) Relationship between mean splenium of corpus callosum (SPCC) fractional anisotropy (FA) as independent variable and Logical Memory I (LMI) total score (immediate recall) as dependent variable, in patients after moderate-severe TBI ($n=82$, red circles) and controls of similar age and sex ($n=40$, green circles), without adjustment for any co-variables. r_s represents Spearman correlation coefficient for combined groups. This represents a separate cross-sectional cohort of subjects to the longitudinal cohort.

(B) Group average statistical maps showing voxels (in red) in SPCC (long arrow), and inferior longitudinal fasciculus (arrowhead), white matter (WM) tracts whose fractional anisotropy (FA) is positive correlated with Logical Memory I (LMI) total score (immediate recall), in TBI patients ($n=82$). Results overlaid on combined group FA skeleton (green, threshold >0.2), using TFCE cluster-wise threshold $P<0.05$ correcting for age and sex. Results displayed on standard MNI125 1mm T1 MRI brain scan. SPCC WM tract defined using the JHU WM tractography atlas with a cut-off threshold of 20% is indicated in yellow. Mean FA values in the SPCC

WM tract region of interest in Figure 5A were calculated from the average of the voxels indicated by the overlap of the green skeleton and yellow mask. Co-ordinates given in Montreal Neurological Institute (MNI) standard space. R indicates right.

254x543mm (72 x 72 DPI)

For Peer Review

SUPPLEMENTARY INFORMATION**Serum IGF-I levels are associated with improved white matter recovery after TBI**

Feeney C, Sharp DJ, Hellyer PJ, Jolly AE, Cole JH, Scott G, Baxter D, Jilka S, Ross E, Ham TE, Jenkins PO, Li LM, Gorgoraptis N, Midwinter M, Goldstone AP

SUPPLEMENTARY TABLES

Supplementary Table 1. Age-related reference ranges for serum IGF-I assay

Supplementary Table 2. Patient characteristics by GH deficiency status

Supplementary Table 3. Neuropsychological outcome measures in patients after TBI at baseline and recovery over time by IGF-I status.

SUPPLEMENTARY FIGURES

Supplementary Figure 1. White matter tract FA in regions of interest in patients after TBI at baseline and recovery over time by GHD status

Supplementary Table 1. Age-related reference ranges for serum IGF-I assay

Age yr	IGF-I median nmol/L	IGF-I lower nmol/L	IGF-I upper nmol/L
18	40.0	21.2	75.9
19	33.9	18.3	62.8
20	30.2	16.5	55.1
21-25	26.4	15.1	46.5
26-30	25.5	15.2	42.8
31-35	24.4	15.0	39.9
36-40	22.9	14.2	36.9
41-45	21.3	13.1	34.7
46-50	20.0	12.2	32.8
51-55	18.7	11.3	30.9
56-60	17.6	10.5	29.3
61-65	16.4	9.8	27.6
66-70	15.3	9.0	26.0
71-75	14.3	8.3	24.4
76-80	13.3	7.7	23.0
81-90	12.4	7.2	21.6

Median, lower (<5% CI) and upper (>95% CI) values normal range for serum IGF-I assay (Immulin 2500) from Dept. of Biochemistry, University College London Hospital, London, UK. To convert nmol/L to ng/mL divide by 0.131.

Supplementary Table 2. Patient characteristics by GH deficiency status

	All	No GHD	With GHD	P with vs. without GHD ^a
n	39	35	4	
Age at first scan (yrs)	30.5 [24.5, 47.0]	30.4 [24.1, 49.2]	39.4 [29.7, 44.4]	0.50
Range	19.6-66.9	19.6-66.9	27.8-44.7	
Male n (%)	33 (84.6%)	29 (82.9%)	4 (100%)	0.87
Post-menopausal women n (%)	3 (7.7%)	3 (8.6%)	0 (0.0%)	0.70
BMI (kg/m ²)	25.3 [23.1-29.8]	25.0 [21.7-28.6]	29.3 [21.7-28.2]	0.10
Range	17.0-35.0	17.0-25.0	25.3-33.7	
Moderate-severe TBI	34 (87.2%)	30 (85.7%)	4 (100%)	1.00
Blast TBI n (%)	13 (33.3%)	10 (28.6%)	3 (75.0%)	0.19
Time since TBI (months)	16.3 [3.6, 24.5]	11.4 [3.2, 25.7]	26.8 [7.7-34.3]	0.38
Range	1.5-571.0	1.5-571.0	3.6-34.5	
Time between scans (months)	13.3 [12.1, 14.9]	13.1 [12.1, 14.5]	14.6 [10.3-15.1]	0.58
Range	6.3-24.6	6.3-24.6	9.1-15.1	
Absolute IGF-1 nmol/L ^b	23.8 [19.2-29.5]	24.2 [19.5, 29.5]	19.6 [16.7, 23.2]	0.11
Range	12.8-74.5	12.8-74.5	16.5-23.7	
Age-adjusted IGF-1 ratio	1.1 [0.9, 1.2]	1.1 [0.9, 1.3]	0.9 [0.8-1.0]	0.11
Range	0.6-3.0	0.6-2.9	0.8-1.0	
Any structural brain abnormality n (%)	30 (76.9%)	28 (80.0%)	2 (50.0%)	0.47
≥ 1 contusion	20 (51.3%)	19 (54.3%)	1 (25.0%)	0.56
No. of contusions ^c	1 [0-2]	1 [0-2]	0 [0-0.8]	0.53
Range	0-5	0-5	0-1	
≥ 1 microbleed n (%)	10 (25.6%)	10 (28.6%)	0 (0.0%)	0.53
Superficial siderosis n (%)	13 (33.3%)	11 (31.4%)	2 (50.0%)	0.85
Diffuse axonal injury (DAI) n (%)	5 (12.8%)	4 (11.4%)	1 (25.0%)	1.00

Data given as n (%) or median [IQR] and range (minimum-maximum).

^a P value for comparison of groups with and without GHD using chi-squared test with Yates' correction for categorical variables and Mann-Whitney U test for continuous variables.

^b To convert nmol/L to ng/mL divide by 0.131.

^c including those without any contusions

Supplementary Table 3. Neuropsychological outcome measures in patients after TBI at baseline and recovery over time by IGF-I status.

Symptom domain		Max score	n	First Visit	n	Second visit	Effect of Time ^a	Effect of IGF-I Group ^a	Time*Group Interaction ^a
Assessment of GH Deficiency in Adults Quality of Life (AGHDA-QoL)		25	25	9 [2.5-16.5] 0-23	25	7.0 [1-12.5] 0-22	0.99	0.71	0.89
Beck Depression Inventory II Score (BDI-II)		63	25	13.0 [4.0-23.0] 0-27	25	11.0 [2.5-18.5] 0-32	0.46	0.75	0.63
Epworth Daytime Sleepiness Scale		24	22	3.0 [0.9-5] 0-19	15	4.0 [1.0-10.0] 0-14	0.69	0.45	0.53
SF-36 QoL	Physical functioning	100	16	70.0 [22.5-98.8] 0-100	24	87.5 [62.3-95.0] 0-100	0.32	0.82	0.53
	Role limitations due to physical health	100	17	75.0 [0-100] 0-100	24	50.0 [6.25-100] 0-100	0.58	0.37	0.58
	Role limitations due to emotional problems	100	16	100 [66.7-100] 0-100	24	100 [41.7-100] 0-100	0.88	0.59	0.77
	Energy/Fatigue	100	16	45.0 [45.0-70.0] 15-85	24	67.5 [47.5-83.8] 5-100	0.41	0.41	0.12
	Emotional well being	100	16	70.0 [54-83.0] 28.0-92.0	24	80.0 [61.0-92.0] 24.0-100	0.52	0.75	0.96
	Social functioning	100	17	62.5 [50.0-75.0] 12.5-100.0	24	81.3 [50.0-100] 25-100	0.22	0.77	0.87
	Pain	100	17	45.0 [39.0-83.8] 22.5-100	24	77.5 [55.6-100] 42.5-100	0.74	0.82	0.93
	General health	100	17	60.0 [42.5-80.0] 20-100	24	65.0 [45.0-91.3] 15-100	0.27	0.59	0.18
Cognitive domain	Cognitive variable								
Pre-morbid intelligence: reading ability	WTAR raw score		32	41.0 [35.3-45.0] 0-50	32	42.0 [31.8-46.8] 9-50	0.88	0.83	0.60
Intellectual ability	WASI similarities (verbal)		33	36.0 [32.5-38.0] 21-46	33	39.0 [35.5-41.0] 23-46	0.61	0.96	0.42
	WASI matrix reasoning (non-verbal)		32	26.5 [20.5-30.0] 9-34	32	28.5 [25.3-30.8] 12-35	0.08	1.00	0.20
Memory: associative memory	People test (total score)		33	22.0 [14.5-29.0] 4-38	33	27.0 [18.0-31.0] 6-38	0.010	0.19	0.78
Memory: immediate recall	Logical memory I Total		33	28.0 [20.5-32.0] 10-40	33	43.0 [33.0-51.5] 19-62	0.26	0.12	0.046
	Logical memory II Total		33	26.0 [18.5-32.5] 0-42	33	27.0 [19.5-33.0] 10-43	0.63	0.20	0.57
Memory: delayed recall	Logical memory retention		32	86.7 [78.9-94.3] 33.3-107.7	33	90.3 [82.9-98.0] 45-190	0.41	0.34	0.93
Processing speed: visual search/complex	Trail Making Test Trail A (s)		32	26.0 [21.0-32.0] 14-67	33	22.0 [16.5-28.5] 13-87	0.82	0.60	0.65
	Trail Making Test Trail B (s)		32	52.0 [43.3-68.5] 22-192	33	47.0 [30.5-71.0] 25-166	0.62	0.32	0.81
Processing speed: naming/reading	Stroop Colour Naming (s)		31	32.0 [28.0-41.0] 20-101	33	32.0 [30.0-35.0] 19-51	0.56	0.90	0.96
	Stroop Word Reading (s)		31	23.0 [19.0-32.0] 15-62	32	23.0 [20.0-25.8] 14-43	0.53	0.25	0.34
Executive function: alternating-switch cost	Trail Making Test Trail B minus A (s)		32	22.5 [13.0-38.8] 4-125	33	19.0 [15.0-42.5] 7-102	0.57	0.24	0.62
Cognitive flexibility	Color Word Stroop Inhibition/switching (s)		32	66.0 [55.5-76.5] 38-128	33	57.0 [51.5-66.5] 37-119	0.003	0.33	0.50
Word generation fluency	DKEFS Letter Fluency F+A+S total		33	36.0 [27.0-45.0] 11-65	33	41.0 [35.5-48.5] 15-60	0.15	0.57	0.59

^a corrected for all covariates (time since injury, time between scans, gender, age at first scan, TBI severity)

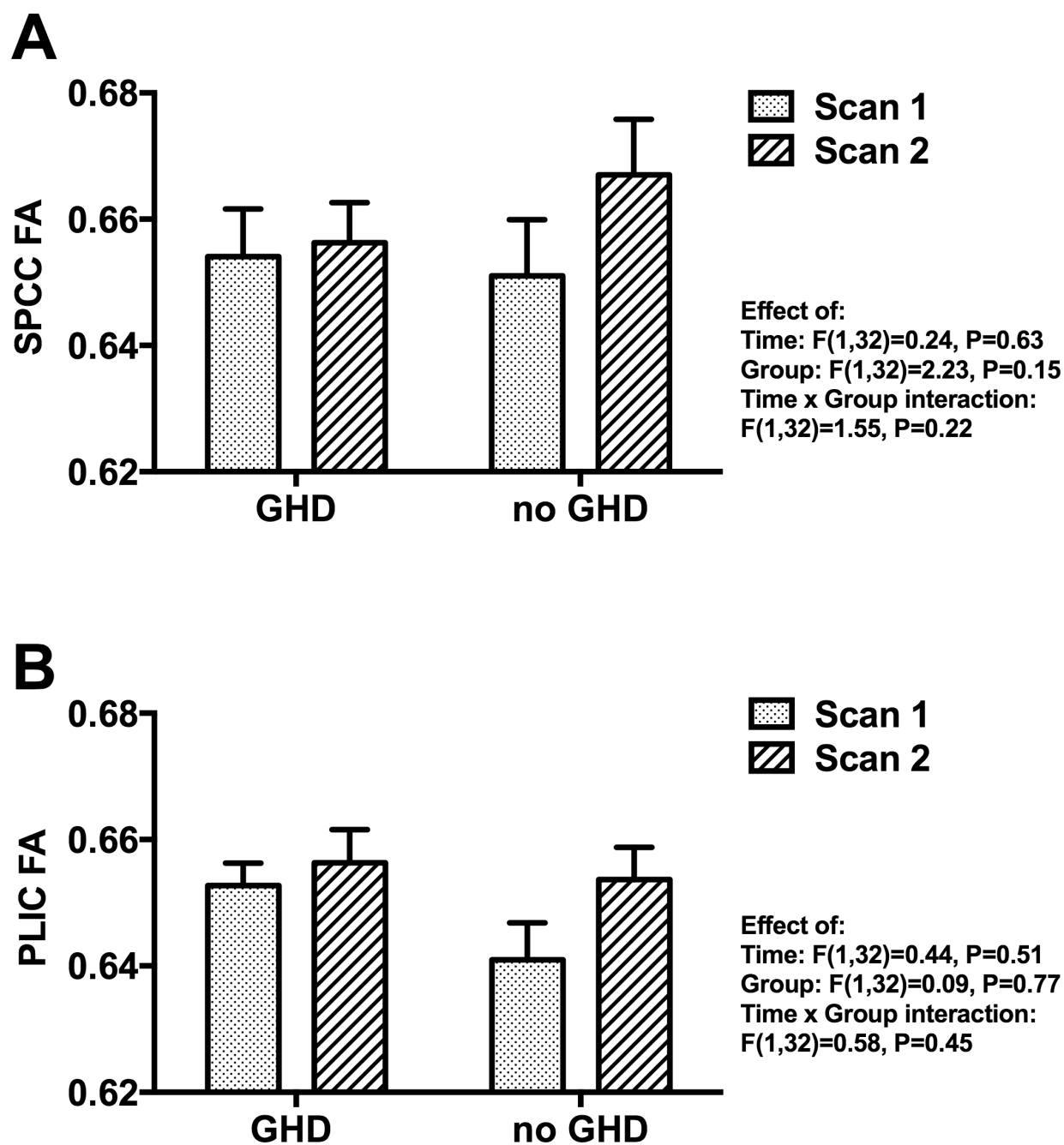
Data displayed as median, [IQR], range.

Note: AGHDA, BDI-II, Epworth: higher score equals worse symptoms; SF-36: lower score equals worse quality of life; WTAR, Similarities, Matrix reasoning, People test, Logical memory: higher score equals better performance; Trail-making, Stroop, letter fluency: lower score equals better cognition.

Abbreviations: DKEFS, Delis-Kaplan executive function system; (s), seconds; SF-36, Short Form 36 Health Survey; WASI, Wechsler Adult Intelligence Scale; WTAR, Wechsler Test of Adult Reading.

SUPPLEMENTARY FIGURES

Supplementary Figure 1. White matter tract FA in regions of interest in patients after TBI at baseline and recovery over time by GHD status.



No significant difference in improvement in FA in either (A) splenium of corpus callosum (SPCC) or (B) posterior limb of internal capsule (PLIC) between scan 1 (dotted bar) and scan 2 (striped bar) in patients after TBI between those without ($n=35$) and with ($n=4$) GH deficiency (GHD), adjusting for age, gender, TBI severity, time since injury and time between scans.