Investigating the neuroprotective effect of exercise; the role of redox homeostasis

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Abstract

The balance between oxidant production and the antioxidant defence system is essential to brain health. First-episode psychosis (FEP) and schizophrenia (SZ) are psychiatric disorders that affect the patients' contact with reality, with unique presentation of symptoms and experiences. Both FEP and SZ can be characterised by perturbed redox status, notably via antioxidant depletion. Previous research, undertaken in healthy populations, has highlighted the ability of exercise training to regulate redox balance.

One particular biomarker of redox status is the antioxidant glutathione (GSH), which has a principal role of removing toxic substances from the cell, especially in the brain.

This thesis presents novel data investigating the drawbacks of exercise study design in youth mental health, the efficacy of a training intervention in 'normalising' measures of antioxidant capacity, oxidative stress, inflammation and functionality in FEP. Studies presented go on to interrogate some of the redox-related mechanisms of the neurodevelopmental aspect of psychosis pathophysiology. Assessment of GSH in the brain, blood and in patient-derived cell models offers significant gateways into elucidating the pathological mechanisms of mental ill-health.

In order to address some of the principle weaknesses in previous study design which have aimed to provide exercise to a FEP cohort, chapter 2 of this thesis used personalised heart rate zones (50-70% VO_2max) to standardise bouts of activity across a range of exercise modalities, in a healthy but sedentary group of young people. The study was successful in provoking a redox response

during each acute bout, which typically initiates the stimulation of adaptive processes. This study achieved 100% retention, which also demonstrated that engagement in an exercise intervention is possible in a sedentary group, when choice of activity and variety of exercise mode are offered to the participant. Chapters 3 and 4 highlight the therapeutic benefits of regular exercise training (12 weeks, 3-4 times/week, 40-60 minutes) for FEP patients in a randomised trial. The study highlighted the positive impact of increased physical activity on peripheral and brain indices of GSH, markers of oxidative stress, and patient-centred measures of psychotic symptomology and daily function. In a more in-depth assessment of the GSH cycle and indices of cell damage, chapter 5 describes the characterisation and generation of mature astrocytes and neurons from SZ patient-derived induced pluripotent stem cells (iPSCs), as a model of the neurodevelopmental aspect of disease pathogenesis. It was found that the de-toxifying ability of GSH is impaired in SZ cerebral cells, offering a cogent target for further investigation in this novel approach to assessing the mechanisms of mental ill-health, in the brain.

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MRS 'internship' at Orygen Centre of Excellence for Youth Mental Health, Melbourne

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Designing a feasible exercise intervention in first-episode psychosis: exercise quality, engagement and effect (2020). (Fisher, E., Wood, S.J., Upthegrove, R., Aldred, S.) *Psychiatry Research* 248

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Exercise as a protective mechanism against the negative effects of oxidative stress in first-episode psychosis: a biomarker-led study (2020). (Fisher, E., Wood, S. J., Elsworthy, R.J., Upthegrove, R., Aldred, S.) *Translational Psychiatry* 273

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Abbreviations

1RM	1 repetition maximum
AAPH	Dihydrochloride
ACSM	American College of Sports Medicine
AE	Aerobic exercise
AIM	Astrocyte induction media
AMM	Astrocyte maturation media
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BCA	Bicinochonic acid
BD	Bipolar disorder
BDNF	Brain derived neurotrophic factor
bFGF	Fibroblast growth factor-basic
BHT	Buytlated hydroxytoluene
BMI	Body mass index
BP	Blood pressure
BSA	Bovine serum albumin
BSO	Buthionine sulphoxamine
CAT	Catalase
CDNB	1-chloro-2,4-dinotrobenzene
CGIS	Clinical global impression scale
CRP	C-reactive protein
CSF	Cerebrospinal fluid
Cu^{+}	Copper ion

Cys	Cysteine
DAPI	4’.6-diamidino-2-phenylindole
ddH_2O	double distilled water
df	Degrees of freedom
DMEM	Dulbecco’s modified Eagle’s medium
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DTNB	5.50-dithio-bis-(2-nitorbenzioc acid), Ellman’s reagent
DTT	Dithiothreitol
DNPH	Dinitrophenylhydrazine
EB	Embryoid body
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbent assay
EtOH	Ethanol
FBS	Fetal bovine serum
Fe^{2+}	Ferrous ion
$FeCl_3$	Ferric chloride
FEP	First-episode psychosis
FGF	Fibroblast growth factor
FID	Free induction decay
FRAP	Ferric Reducing Ability of Plasma
g	Centrifugal force
GABA	Gamma aminobutyric acid
GCL	Glutamate-cysteine ligase

GCLC	Glutamate-cysteine ligase catalytic subunit
GCLM	Glutamate-cysteine ligase modifier subunit
GFAP	Glial fibrillary acidic protein
GHQ	General health questionnaire
Gln	Glutamine
Glu	Glutamate
Gly	Glycine
GSH	Glutathione
GSSG	Glutathione disulphide
GST	Glutathione-S-transferase
GPx	Glutathione peroxidase
GR	Glutathione reductase
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
HC	Healthy control
HCl	Hydrochloric acid
HDRS	Hamilton depression rating scale
HIIT	High intensity interval training
HR	Heart rate
HRmax	Maximal heart rate
HRP	Horseradish peroxidase
ICD-10	International Classification of Diseases (Version 10)
IgE	Immunoglobulin E
IGF-1	Insulin-like growth factor
IgG	Immunoglobulin G

IL	Interleukin
In Min	Intensity minute
IPAQ	International physical activity questionnaire
iPSC	Induced pluripotent stem cell
Kg	Kilogram
Kg/m^2	Kilogram per metre squared
Klf4	Kruppel-like factor 4
Km	Kilometre
KO	Knock-out
KOSR	Knock-out serum replacement
LDN	LDN193189
LMA	Low melting point agarose
LOOH	Lipid hydroperoxide
M	Molar
Map2	Microtubule-associated protein 2
MEF	Mouse embryonic fibroblast
MEGAPRESS	Mescher-Garwood point resolved specroscopy
min	minute
MDA	Malonyldialdehyde
MDD	Major depressive disorder
mg	milli gram
MHC	Major histocompatibility complex
MHI	Mental health inventory
mL	millilitre
mmol	millimole

MPA	Metaphosphoric acid
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
n	Number of participants
NAA	N-acetyl aspartate
NAA:Cr	N-acetyl aspartate: creatine ratio
Na_2HPO_4	Disodium phosphate
NaCl	Sodium chloride
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
NaOH	Sodium hydroxide
Nestin	Neuroectodermal stem cell marker
NGF	Nerve growth factor
NIHR	National Institute for Health and Research
NFκB	Nuclear factor kappa beta
NMDA	N-methyl-D-aspartate
nmol	Nanomole
NMM	Neural maintenance media
NO	Nitric oxide
NPC	Neural progenitor cell
O_2^-	Superoxide radical
Oct4	Octamer-binding transcription factor 4
OH^-	Hydroxyl radical
PA	Physical activity
PACES	Physical activity enjoyment scale

PANSS	Positive and negative symptom scale
PAT	Patient group
PATC	Patient control group
Pax6	Paired box protein 6
PBS	Phospho-buffered saline
PDGF	Platelet-derived growth factor
PET	Positron emission tomography
PFA	Paraformaldahyde
pg	Picogram
PUFA	Poly-unsaturated fatty acid
ppm	Parts per million
PRS	Polygenic risk score
QoL	Quality of life
RATS	Robust Alignment to a target Spectrum
RBC	Red blood cell
REC	Research ethics committee
REDOX	Reduction/ oxidation
RHR	Resting heart rate
RF	Radiofrequency
ROCKi	Rho kinase inhibitor
RONS	Reactive oxygen and nitrogen species
ROS	Reactive oxygen species
ROUT	Robust regression and outlier removal
RPM	Revolutions per minute
RT	Room temperature

S100- β	S100 calcium binding protein β
SB	SB431542
SD	Spontaneous differentiation
SD	Standard deviation
SE	Standard error
Sig	Significant
SMD	Standardised mean difference
SOD	Superoxide dismutase
Sox1	SRY box transcription factor 1
Sox2	Sex-determining region Y
SPANT	Spectroscopy ANanlysis Tools package
SSEA-4	Surface-antigen expression of stem cell markers
SZ	Schizophrenia
TAC	Total antioxidant capacity
TARQUIN	Totally automatic robust quantitation in nuclear MR
TBARS	Thiobarbituric acid reactive substances
TBS	Tris-buffered saline
TCA	Tricarboxylic acid
TGF-β	Transforming growth factor beta
TPTZ	2, 4, 6-Tris (2-pyridyl)-S-triazine
TNF- α	Tumour necrosis factor alpha
TNR	Trinucleotide repeat
Tris	Trisaminomethane
Tuj1	Class III β tubulin
VO_2max	Maximal oxygen consumption

VO_2peak	Peak oxygen consumption
VOI	Volume of interest
WHO	World Health Organisation
WHODAS	World Health Organisation disability assessment scale
WHOQOL	World Health Organisation quality of life scale
XAV	XAV939
µg	Microgram
µL	Microlitre
µmol	Micromole

CHAPTER 1: BACKGROUND AND LITERATURE REVIEW

1.1 Pathophysiology of first-episode psychosis

First-episode psychosis (FEP) is a psychiatric condition that usually manifests during early adulthood, and is characterised by positive, negative and cognitive symptoms. The American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (13) broadly defines FEP as "*gross impairment in reality testing*", and "*loss of ego boundaries*" that interferes with the capacity to meet the ordinary demands of life. However, the clinical manifestations of psychosis vary enormously between cases. FEP is typically preceded by a retrospectively identified 'prodromal' period, during which, people display some of the hallmarks of the FEP state, such as neuroses, disturbances in attention and perception, altered motor function, depressed mood and anxiety (14). Archetypal symptoms and phenomenological experiences observed during a first-psychotic episode are summarised in figure 1.1.

Typically, patients presenting with symptoms of psychosis are prescribed neuroleptic treatment (7, 15). To further promote functional recovery, many care-teams offer cognitive and behavioural psychotherapy (12, 16), family education and support (17-19) and educational and vocational rehabilitation (20, 21) alongside pharmacological intervention. FEP and schizophrenia (SZ) pare not mutually exclusive; with a 60% transition rate (22).

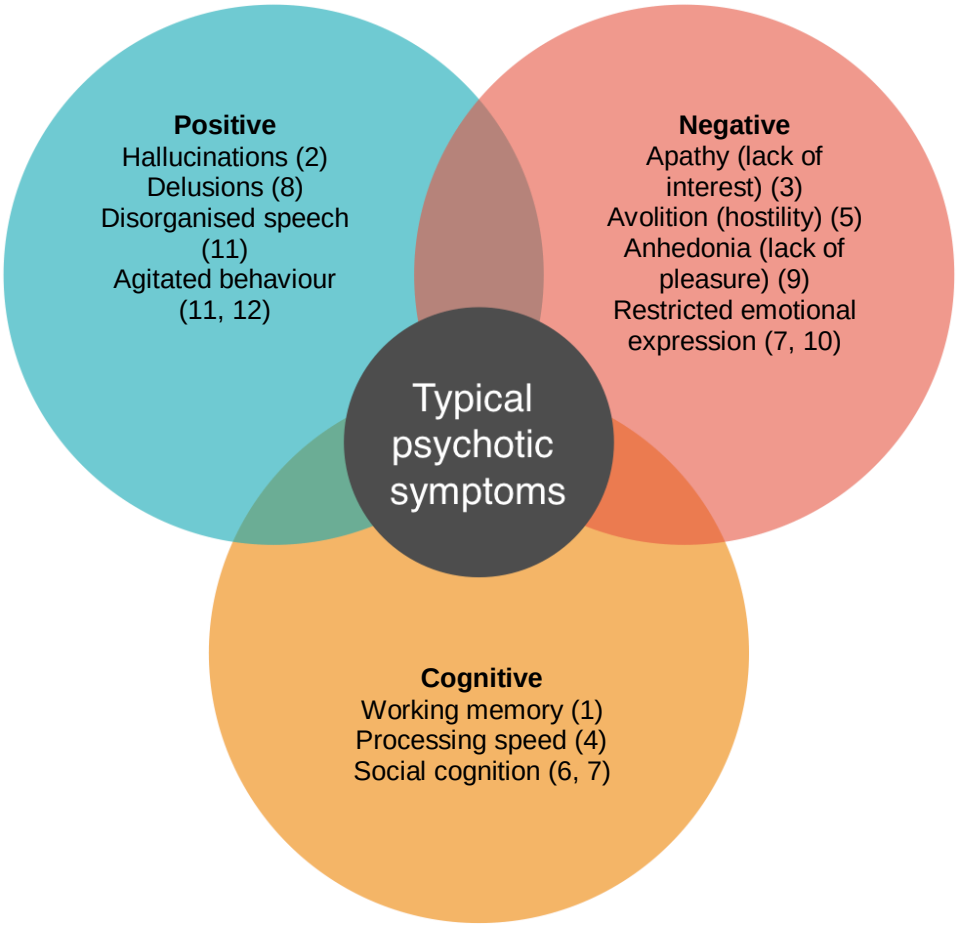


Figure 1.1 Typical symptoms that are characteristic of psychosis. The symptom profile consists of three 'sub-groups': positive, negative and cognitive.

However, psychosis can be described as a continuum of psychopathology, with many shared symptomatic and mechanistic profiles. Complete clinical and functional remission is rare, with one study reporting sustained evidence of recovery, over a 10-year period, of 12.5% (23).

The global burden of mental ill-health is a strong argument for provision of early treatment in FEP as it raises the possibility of future prevention, or reduction of the chronicity of mental illness, and associated morbidity. It is proposed that mental illness accounts for 7.7% of disability-adjusted life years and is 5th on

the list of disease types that comprise the global burden of disease (24). Co-morbid conditions are commonly found in a clinically heterogeneous FEP population. In a review by Buckley et al (25), the most prevalent co-diagnoses with SZ were identified as: substance abuse (47%), anxiety, panic disorder (15%) post-traumatic stress disorder (29%) and depression (50%). Alongside these psychological illnesses, a diagnosis of FEP is often associated with and increased obesity (26), poor diet (27), sedentariness (28), smoking (29) and increased rates of suicide (30) when compared to the general population. All of these listed characteristics of FEP and SZ contribute to the early mortality rate reported in chronic mental illness, with the life-expectancy of a person with SZ 20 years less than that of the average population (31).

Antipsychotic medication is closely associated with significant weight gain, particularly in the first few months after initial prescription. This immediate change is associated with increased fat deposition in visceral adipose tissue (32), which is an important risk factor for dyslipidaemia, hyperglycemia, development of type 2 diabetes mellitus, hypertension and cardiovascular disease. One longitudinal study assessed weight gain over an average treatment duration of 7.6 years (33). The average gain of body weight was 24.6kg, at a rate of 5.81kg per year. Hyperglycemia is well-reported in FEP following medication initiation, however, studies have shown that glucose disturbances are reversible when antipsychotics are discontinued (34). Altered lipid metabolism is also highly associated with treatment status. Hypertriglyceridemia is elevated as a result of second generation antipsychotic medication (35), which leads to excess release of free-fatty acids, and can ultimately cause insulin resistance. Additionally, the psychosocial

consequences of weight gain often result in medication discontinuation (36). It should be noted however, that impaired glucose and lipid metabolism may not solely be a consequence of medication status. Studies assessing drug-naïve FEP patients have reported insulin resistance (37, 38) and impaired glucose tolerance (39). Dysregulation of glucose may be an intrinsic impairment in the pathogenesis of FEP, and may pre-dispose the risk of a psychotic episode. Metabolic dysfunction is well-documented as a consequence of antipsychotic medication and sedentariness associated with illness, but may also represent a key aspect of disease pathophysiology.

It is estimated that the global prevalence of FEP is 86 per 100,000 members of the population for ages 15-29, and 46 per 100,000 for ages 30-59 (40). For SZ, the incidence is reduced, since not all cases of FEP transition to a SZ diagnosis (41). This emphasizes the opportunity for early intervention in FEP, with the potential to reduce the incidence of disease progression and future chronicity. There are a number of risk factors that increase the probability of FEP becoming a diagnosis of SZ. Longer first admission in hospital, younger age and being male have all been associated with increased risk of transition (42). Further investigation of the transition from FEP to SZ observed that there was a 12.6% transition after 1.7 years of FEP diagnosis, 80% after 2.8 years, and 90% after 4.6 years. These statistics highlight the importance of early intervention for improved diagnostic outcome.

1.2 Nature or nurture?

1.2.1 Genetic contribution

FEP and SZ are highly polygenic in genetic contribution, which means that the predictive power of any one genetic variant is extremely small. FEP and SZ risk are multifactorial conditions, rather than a single-gene conferring disease causality. Familial inheritance describes an estimated 60-80% genetic contribution (43). There are no known familial subtypes of psychosis and SZ, and offspring of affected parents do not demonstrate a completely penetrant genetic phenotype (44). The polygenic risk score (PRS) is based on variation in multiple genetic loci, and is commonly calculated for neurodegenerative diseases. In FEP, the PRS was found by one study to have good discriminative ability to predict the development of SZ from FEP (40). From 710 individuals, and 290,871 genotyped markers, studies estimated that the heritability of SZ was 65-85% (45, 46), and a genome-wide association study associated 108 genetic loci, at independent regions, that conferred elevated SZ risk (47). There are several recent studies that have identified specific rare gene variants that may be linked with psychosis and SZ propagation. The Psychiatric Genomics Consortium validated six genomic deletions and two duplications that conferred a 60-fold increased risk of SZ (48). Exome and whole-genome studies have identified some very rare single-gene mutations that were associated with increased risk of psychosis (49, 50). Some variants have been linked to

treatment response and side-effects; for example alleles in the dopamine D2 receptor are thought to determine antipsychotic response (51), and a number of variants have also been linked to pre-disposed antipsychotic related weight-gain (52). The heterogeneous nature of psychosis and SZ genetic contribution is difficult to characterise, but, when combined with assessment of environmental contribution, a more coherent picture of disease pathology can be drawn.

1.2.2 Environmental risk factors

There is no doubt that the role of environmental factors can contribute towards precipitation of a psychotic episode, either in conjunction with, or exclusive of, genetic predisposition. Propagation of a psychotic episode and potential future chronic mental ill health is hypothesized to be neurodevelopmental (53, 54), with obstetric complications posing important contributory pathway for the pathogenesis of FEP. A meta-analysis of prospective population-based studies revealed that three groups of complications had significant association with each other; complications of pregnancy (bleeding, diabetes, rhesus incompatibility, pre-eclampsia), abnormal fetal growth and development (low birth weight, congenital malformations, reduced head circumference) and complications with delivery (uterine atony, asphyxia, emergency cesarean section) (55).

Fearon et al (56) reviewed studies that investigated populations with a higher incidence rate of psychosis. Migrant groups from developing countries (57), and countries where the majority population is black (African-Caribbean and

black Africans) (58) have a substantially greater incidence of SZ. A study by Harrison et al (59) in Nottingham (UK) found the rate of SZ was raised more than 12-fold in the African-Caribbean community, compared with the general population. This association is likely due to factors such as social isolation and social disadvantage; unemployment, low education status, being single, lower income and being childless, as all of these factors have been associated with greater risk of psychosis (11). Krabbendam (60) proposed that genetic predisposition, alongside the impact of living in an urban environment, may have an additive effect on the risk of psychosis. It is increasingly likely that any genetic contribution to the incidence of psychosis is conditional on environmental encounters (61), and the observed increase in global psychotic episode incidence may be partially accounted for by the increase in urbanicity (62).

It has been reported that drug abuse prior to first hospital admission for psychosis is 27.5%, which is twice that of the general population and indicates a strong association between FEP and drug abuse (63). A study by Giordano et al (64) examined the effect of cannabis abuse prior to an initial diagnosis of SZ. It was found that cannabis abuse was strongly associated with a subsequent diagnosis (odds ratio 10.44), however population based estimates of cannabis-schizophrenia comorbidity substantially overestimate their casual association. As described in detail in section 3, dopamine dysregulation is a hallmark of FEP (65). Intermittent stimulation of dopamine receptors leads to sensitization (66), and the propagation of the positive symptoms of psychosis (67). Drugs of abuse, such as cocaine, function as agonists of dopamine receptors (68), and may be key precipitators of FEP.

1.3 Mechanistic theories of psychosis

FEP and SZ are multifactorial and heterogeneous in aetiology, and although some of the factors that cause these conditions are unique, they also share a number of common factors. These commonalities seem to propagate during neurodevelopment and may be responsible for the psychotic symptoms which are seen in both FEP and SZ (69). There are several evidence-based hypotheses relating factors that contribute to the pathology of psychosis, including neuro-inflammation, neurotransmitter perturbation and redox-imbalance, which are further described below.

1.3.1 Inflammatory hypothesis of psychosis

Perturbed inflammatory cytokine expression is a hallmark of FEP. Increases in peripheral markers of inflammation have been described, and are linked to the severity of psychosis-related cognitive impairment and psychopathology (70, 71). PET imaging of the mitochondrial 18kDa translocator protein (TPSO), which measures microglial activation, is the most valid approach for studying neuro-inflammation *in vivo* (72). Using this method, upregulation of inflammatory mechanisms has been observed in the brain (73-76), via tagged-ligand binding of activated microglia. Several genome-wide association studies have assessed genes associated with the immune system in relation to FEP and SZ to ascertain if there is a genetic basis for disease. The most commonly implicated region is the major histocompatibility complex (MHC) island on

chromosome 6. Many of the gene products associated with the MHC are linked to components of the immune response, and have a strong allelic association with SZ (77, 78).

In a recent meta-analysis of studies investigating neuro-inflammation in FEP (79), it was shown that the inflammatory markers homocysteine, IL-6 and TNF-α were elevated in blood samples from patient groups compared with healthy controls. Some research groups have suggested that treatment with neuroleptic medication may, in part, elicit its effect via an anti-inflammatory mechanism which involves the modification of cytokine levels (80, 81). TNF-α may have an antecedent role in psychosis, since it is consistently elevated in FEP; even after acute exacerbations of symptoms are controlled. IL-6 and TNF-α cytokine levels are elevated in general and antipsychotic naïve FEP groups, which implies that increases in pro-inflammatory markers are present before the onset of symptoms; a potential biomarker for clinical high-risk groups (79). One study even noted that several inflammatory cytokines (CRP, gliadin and pentraxin-3) were predictive of conversion to FEP from a 'clinical high risk' state, before the onset of any diagnosable illness (82). In a review paper by Upthegrove et al (83), low-grade inflammation observed in many FEP cases has been associated with poor response to antipsychotic medication, alongside the theory that chronic upregulation of the immune response is a causal feature of FEP, rather than a consequence of illness. Despite the heterogeneity of findings relating to the immune infrastructure in psychosis, it is well understood that non-specific alteration of the immunological response is a hallmark of disease pathology. Activation of cytokines related to both innate and adaptive

immunity suggests that reciprocal influences of neuro-inflammation are present during the ‘prodromal’ period of time before symptoms emerge.

1.3.2 Neurotransmitter hypotheses of psychosis

One of the principle hypotheses in FEP aetiology is that both dopamine and glutamate neurotransmitter systems are dysfunctional (84, 85). The following summary presents an up-to-date stance on the role of dopamine and glutamate systems in psychosis and SZ.

Dopamine hypothesis

The dopamine hypothesis of psychosis is a model that attributes positive symptoms to dysfunctional and hyperactive dopaminergic signal transduction (86). The original dopamine hypothesis became popular after observations that administration of drugs like amphetamines, that increase dopamine neurotransmission, can induce psychotic symptoms (87). Other early studies observed increased striatal dopamine levels, and increased density of dopamine D2 receptors in SZ patients (88). Indeed, the efficacy of antipsychotic medications is directly related to their affinity of the medication for dopamine receptors (89), since all antipsychotics act by blockade of the dopamine D2 receptors. More recent studies have assessed the function of other dopamine receptors to be able to further understand the role of this pathway in psychotic disorders. For example, one study observed a 6-fold increase in the D4 receptor density in a SZ group (90). Additional evidence that

the dopamine pathway is instrumental in SZ disease pathology is provided by the observation that further upstream, there is an increased production of tyrosine hydroxylase in the substantia nigra of SZ patients. This enzyme catalyses a rate-limiting step in the production of dopamine (91). It has also been noted that dopamine D2 auto-receptor density at the pre-synaptic cleft is greater in SZ compared with healthy controls (92). One of the etiological characteristics of psychosis and SZ is an increased capacity for dopamine production, as well as increased pre-synaptic dopamine availability and release. A further link for dopamine to the pathology of psychotic disease is provided by the metabolism of dopamine. This process is a potent source of reactive oxygen species (ROS) and given that metabolism and auto-oxidation of neurotransmitters in the brain, e.g. dopamine, increases H_2O_2 (hydrogen peroxide) production (93) this will likely contribute to the altered redox status seen in psychosis. As a product of the disease process, there is increased dopamine content in the brain, which subsequently leads to increased ROS, and can further result in enhanced striatal glutamatergic neurotransmission (94). Altered dopamine neurotransmission represents one of the most well-characterised facets of the pathology of psychosis, describing a propagation of positive symptoms.

Glutamate hypothesis

Excitatory neurotransmission in the brain is primarily glutamatergic; and accounts for 60-80% of the brain's metabolic activity (95). The glutamate hypothesis of psychosis and SZ was proposed by Coyle et al (96), and

implicates dysregulation of the NMDA (N-methyl-D-aspartate) receptor (NMDAR) in particular (97). NMDAR hypofunction limits glutamate excitatory neurotransmission, and neuronal activation, and has downstream negative perturbations in synaptic plasticity and long-term potentiation (98). Antagonists of NMDARs, like ketamine, are known to induce positive and negative psychotic-like symptoms (84), and acute administration of the drug may be used as a model of early psychosis or FEP. Long-term administration of NMDAR antagonists reflects symptomology observed in chronic SZ, such as deficits in set-shifting ability (99) and functional connectivity (100). Similarly, long-term administration of ketamine to rats induces cognitive deficits that are characteristic of the SZ phenotype (101). Mechanistically, is has been proposed that antagonism of the NMDA receptor decreases GABAergic interneuron function (102) by increasing pyramidal cell firing due to disinhibition (103). The glutamate hypothesis can be closely linked to the generation and negative effects of ROS accumulation. Inhibition of ROS formation blocks the behavioural effect of NMDA receptor antagonists (104), and reduced superoxide dismutase (antioxidant enzyme) levels can prevent ketamine-induced changes in interneuron activity (105).

1.3.3 Free radical-mediated damage and oxidative stress in FEP

Oxidative stress is defined as "*a disturbance in the balance between the production of reactive oxygen species (free-radicals), and antioxidant defenses*" (106). It is this state of oxidative stress that is a hallmark of FEP pathology.

Reactive oxygen species (ROS) and reactive nitrogen species (RONs) are ubiquitous since they are a product of aerobic metabolism. Other potential sources of radical species are environmental (e.g. x-rays, cigarette smoke, air pollutants or industrial chemicals), or pathological states (107). A free-radical is any molecular species with an unpaired electron. Radical species can be de-toxified by an electron donor, or antioxidant. Radicals are highly reactive, and if they are not quenched by an antioxidant they may interact to oxidise macromolecules including lipids, proteins and DNA (108). Some of the most common ROS are superoxide (O_2^-) the hydroxyl radical (OH^-) and H_2O_2. A balance between free-radicals and antioxidants is necessary for reduction-oxidation (redox) homeostasis throughout the body.

Antioxidants can be endogenous (synthesized in the body) or exogenous (taken in through the diet). Endogenous antioxidants are most commonly enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) (109, 110). SOD is the first line of defense against oxidants and free-radicals, and catalyses the dismutation of the O_2^- anion radical into H_2O_2 by reduction. H_2O_2 is then transformed to H_2O and O_2 by either CAT or GPx. The main function of GPx is to remove H_2O_2, and it does this by oxidizing the antioxidant glutathione (GSH) to glutathione disulphide (GSSG) (111). GPx also reduces lipid and non-lipid hydroperoxides during the oxidation of GSH. GR, a flavoprotein enzyme, regenerates GSH from GSSG, with NADPH as a reducing co-factor (112). Antioxidants must be constantly restored and replenished, since they become

oxidized in the detoxification of free-radicals and oxidant molecules. When the production of ROS overwhelms antioxidant capacity then ‘damage’ to cell components results via the formation of adducts. This is termed oxidative stress. GSH is the principle non-enzymatic endogenous antioxidant in all cells, and the most abundant free-radical scavenger throughout the body, and will be described further in section 1.4.

Products of ROS-induced damage

When the antioxidant defense system is compromised, or overwhelmed, adducts form on macromolecules. Such ‘oxidative damage’ may be irreversible and can form macromolecules that have compromised function and even result in cellular apoptosis. Figure 1.2a and 1.2b describe the process of redox balance, and the state of oxidative stress.

There are three forms of oxidative protein modification (figure 1.2a): a specific amino acid may be oxidatively modified, free-radical mediated peptide cleavage can take place or protein cross-links may form as a result of a reaction with lipid peroxidation products (107). Oxidative modification of some proteins has the potential to increase the susceptibility of the cell to enzymatic proteolysis, which may ultimately affect the enzyme activity, receptor action, protein-mediated membrane transport and signal transduction. Proteins that contain cysteine or methionine amino acids as constituents are much more susceptible to oxidation because of the electron rich sulphur group within the primary amino acid structure (113).

Polyunsaturated fatty acids (PUFAs), which make up the majority of the cell membrane, are commonly oxidized to form lipid peroxides following interaction with ROS. This often results in formation of the hydroxyl radical, which can then lead to a propagation reaction where a lipid radical is converted to a diene conjugate. In the presence of oxygen, this radical interacts with other lipids to form a lipid hydroperoxide (LOOH) and a new lipid radical. This chain reaction of lipid peroxidation is propagated by the addition of a secondary free-radical (114). A number of different compounds are formed by this process; including alkanes, malonyldialdehyde (MDA) and 8-isoprostanes, which are useful biomarkers of free-radical mediated lipid damage, and can be detected in blood or urine samples.

DNA is also commonly oxidized by ROS to cause lesions to DNA, including abasic sites, base modifications, base or sugar alterations, sugar/ base cyclization, DNA-protein cross-linking and inter/intra-strand cross link formation, translocations, deletions and degradation of bases. All of these events contribute to DNA strand breaks (115, 116). The superoxide radical and hydrogen peroxide do not react with DNA under normal conditions, but they can react with each other to form hydroxyl radicals, which readily react with DNA (117). Microsatellite regions (118) and aberrant CpG island methylation (an epigenetic modification) are both targets for oxidative damage in the DNA, affecting DNA repair and cell survival. It has been suggested that DNA repair in cells has a dependence on GSH bioavailability, and that GSH, through a process called glutathionylation, may act as a modulator of DNA-repair activity (119). Aside from the principle role of GSH as an antioxidant via direct

interaction with reactive oxygen species, it also has a detoxification role alongside enzymes like GSH peroxidases and glutathione-S-transferases (120). The formation of mixed disulphides, known as S-glutathionylation, could be an essential modulating factor in response to oxidative stress in the cell, via a formation of mixed disulphides between oxidised GSH and cysteinyl residues of proteins (121). It was shown that DNA repair in the cell has a partial dependence on GSH; Rojas (122) showed that mice who had a defect in GSH metabolism demonstrated an accumulation of DNA damage as a result of low GSH levels.

Redox homeostasis is altered in many disease conditions, such as cardiovascular disease (123), autoimmune disorders (124), ageing (125), rheumatoid arthritis (126), cancer (127) and many psychological disorders (128). In particular, there is a growing evidence base to implicate altered ROS and antioxidant defences in the pathogenesis of FEP. The 'oxidative stress' model of psychosis (figure 1.2b) describes the pro-oxidative state which is often reported in this clinical population (129). In a recent meta-analysis (79), a comparison of 3002 FEP patients with 2806 healthy controls showed that in FEP, total antioxidant levels were reduced, in both general and antipsychotic naive sub-groups. Other studies assessing redox status in early psychosis have found increased plasma concentrations of MDA (lipid peroxidation

marker) (130-134) and SOD (representing an upregulation of the first line of antioxidant defence)(133, 135). There are also reports of altered CAT activity; decreased expression (136, 137), and upregulation (138). In the limited number

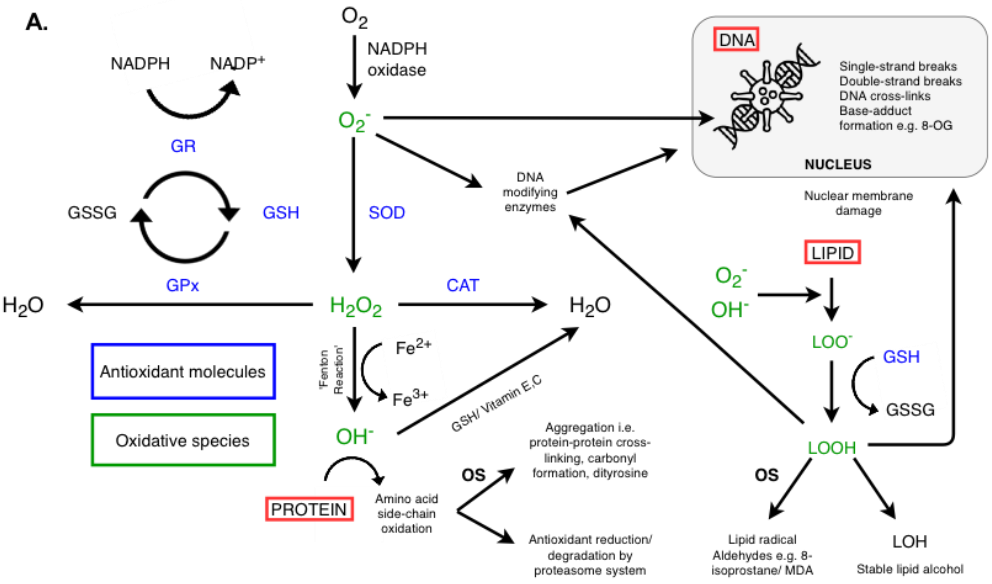


Figure 1.2A. The mechanistic processes of oxidation that form protein, DNA and lipid adducts. The action of antioxidant molecules is pictured, alongside the potential damaging effects of oxidative radicals.

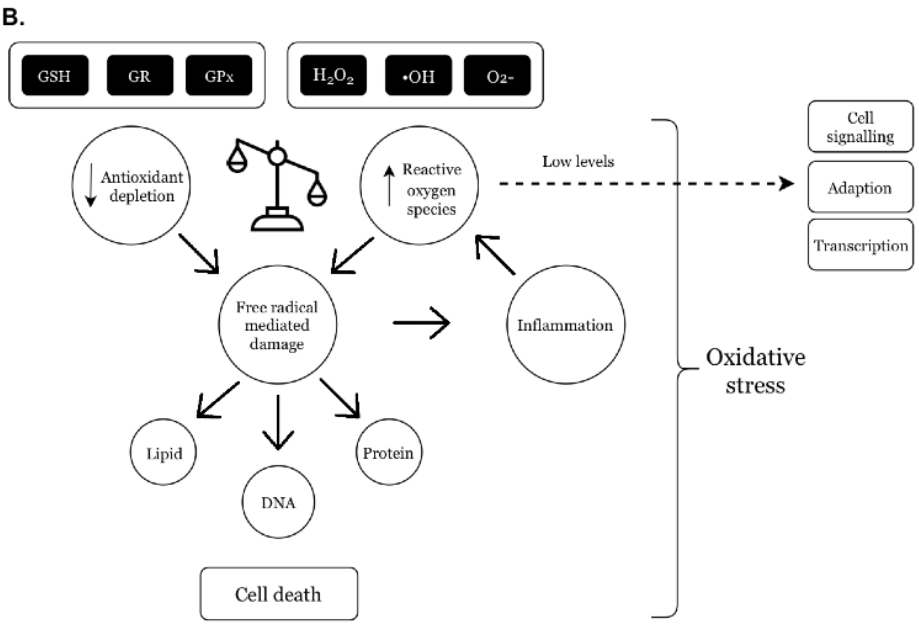


Figure 1.2B. Schematic describing the process of oxidative stress, characterised in FEP and SZ disease pathology. 8-OG: 8-oxo-guanine, Fe: iron, GPx: glutathione peroxidase, GR: glutathione reductase, GSH: glutathione, GSSG: glutathione disulphide, H_2O_2: hydrogen peroxide, LOH: stable lipid alcohol, LOO-: lipid peroxyradical, LOOH: lipid hydroperoxide, MDA: malonyldialdehyde, O_2^{-}: superoxide, OH^{-}: hydroxyl radical, OS: oxidative stress.

of studies that have assessed DNA damage in FEP, results are heterogeneous (139, 140). Oxidative stress is elevated in FEP, but DNA damage markers appear to be present only in later stages of illness chronicity, such as SZ (141). Similarly, protein carbonyl assessment in FEP is rarely described. Pedrini et al (142) observed elevated carbonyl concentration in patients within 10 years of a first psychotic episode, and Noto et al (143) observed no difference in advanced oxidized protein products between drug naive FEP patients and healthy controls. These data may suggest that damage to protein components of the cell may occur later in disease pathogenesis, rather than as a precursor to emergence of symptoms.

1.4 The neuroprotective role of glutathione

Glutathione is the most abundant antioxidant in the human body. Biosynthesis of GSH occurs in the cell cytosol, and is composed of cysteine, glutamate and glycine (figure 1.3). It is a non-protein thiol, that functions in a cycle (figure 1.4) to eliminate ROS. The sulphydryl group (-SH) on cysteine is involved in

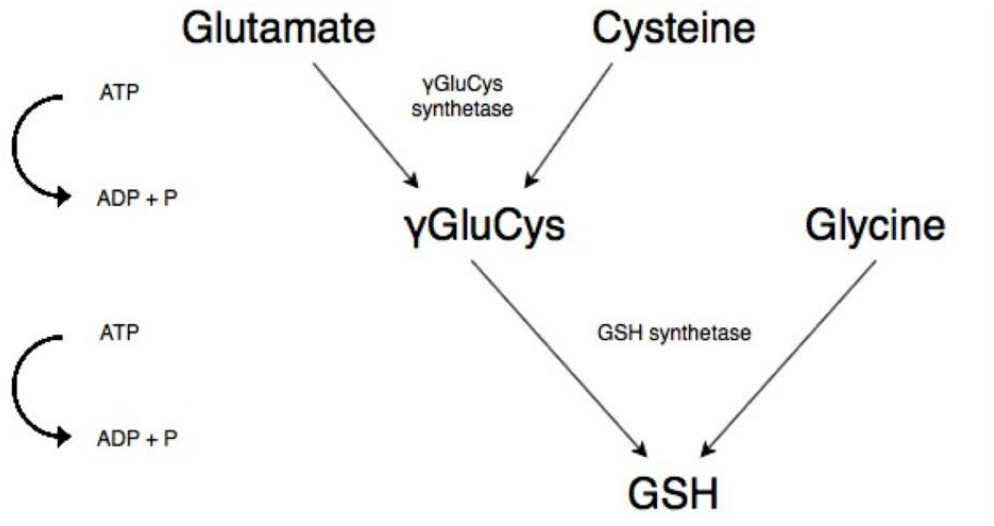


Figure 1.3 Glutathione production from three amino acid precursors; glutamate, cysteine and glycine

reduction and conjugation reactions, rendering it the most important component of the molecule.

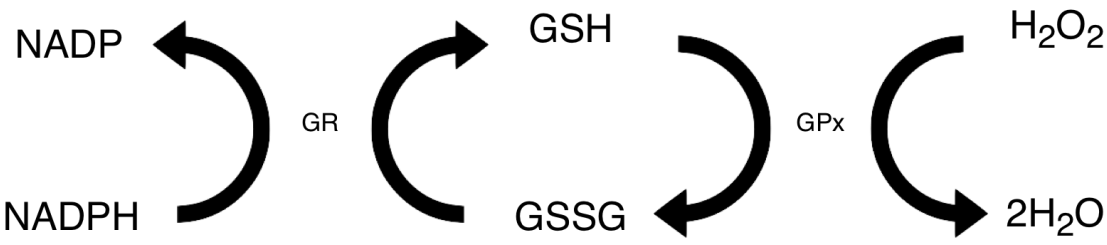


Figure 1.4 The glutathione cycle. GSH: glutathione (reduced), GSSG: glutathione disulphide (oxidised), GPx: glutathione peroxidase, GR: glutathione reductase, H_2O_2: hydrogen peroxide.

GSH production is reliant on two factors; availability of cysteine, and activity of the rate-limiting enzyme, gamma-glutamyl cysteine synthetase (γGluCys sythetase) which is composed of two subunits: GCLC (a catalytic form) and GCLM (a modifier) (144). In the GSH molecule, glutamate and cysteine are bound unusually via the γ-carboxyl group of glutamate. This bond can only be broken by specific binding to a protein outside of the cell (γ-glutamyltranspeptidase), which makes GSH resistant to intracellular degradation inside the cell. Released extracellular GSH can be then broken down into constituent amino acids, to be re-incorporated in new GSH (145). Other essential roles of GSH in the cell include detoxification of xenobiotics, regulation of cell cycle progression and apoptosis, storage of cysteine, maintenance of redox potential, modulation of immune function and fibrogenesis (146-149). In its role as an antioxidant, GSH shares the reduction of H_2O_2 with CAT. CAT is only found in the peroxisome, meaning that GSH is salient in the detoxification of reactive species, especially in the mitochondria (144).

In quantifying the amount of GSH directly in the brain, it is possible to gain a more accurate measure of antioxidant status, and therefore brain health, in a population of patients with psychosis. There are very few studies that have assessed brain GSH in FEP, and results are very mixed. A recent study employed 3T MRS (magnetic resonance spectroscopy) (method described in greater detail in section 1.6) to assess GSH in the brain, within an FEP population (150). The study saw a significant reduction in striatal GSH content in the patient group compared with healthy age-matched controls. There was also a positive correlation between GSH concentration and positive symptoms of psychosis. Other studies have reported depleted GSH concentration in the brain (79, 151), no difference compared with healthy controls (152), and elevated GSH in FEP (153). These observations may be a consequence of varied methodology (e.g. voxel of interest, size of voxel, magnetic field strength of the scanner). In SZ research, Fraguas et al (154) assessed the relationship between grey matter volume and GSH in the brains of patients. The study highlighted a progressive decline in brain GSH over the two-year study period, as well as a direct correlation between brain and declining GSH levels in the blood. This longitudinal assessment of GSH in the brain offered insight into the potential relationship between GSH and disease progression. It also highlights the potential for GSH to be studied *in vitro* as a biomarker of disease progression. Further research in SZ has reported significant decreases (52% and 50% respectively) in brain GSH compared with controls (155, 156). It appears that brain GSH may be related to duration of illness as studies have shown that it is perturbed in early psychosis, and further depleted with longer duration of psychotic illness.

Mice with a chronic GSH deficit that has been induced by knocking out GCLM have impaired parvalbumin interneuron function (157) and elevations of anterior cingulate cortical glutamate and glutamine levels (158). Both of these functional deficits are significant in the context of psychosis. Proper interneuron function is crucial for normal cognitive processes (159), and GSH deficit, which has been observed in FEP patients, may be partially responsible for the cognitive aspect of symptomology. Additionally, GSH may have a buffering role in glutamate concentration in the synaptic cleft, preventing excitotoxicity and neuronal death. Depleted GSH in the brain as a result of disease pathology elevates the toxic potential of glutamate.

1.4.1 Genetics of GSH in psychosis

The interaction between environmental and genetic factors may explain the multifactorial etiology for psychosis, particularly in the context of GSH perturbation. Chromosome 6p has been the main area of interest in several genetic studies that have associated the SZ phenotype with the region between 6p23 and 6p21 (160). Tosic et al (161) described reduced gene expression of the GCLC and GCLM subunits of the glutathione cysteine ligase enzymes. These mutations were found more commonly in SZ patients than control cases, and the resultant phenotype was a reduction in GSH. The GCLC subunit of the GCL gene sits on 6p, and a trinucleotide repeat (TNR) polymorphism of GAG nucleotides has been linked to SZ in this chromosomal region (155). Decreased expression of this enzyme leads to a downstream effect of decreased GSH, as GCL is a precursor to synthesis of GSH. Similarly, Gysin (162) described a TNR sequence that conferred incidence of SZ. This

sequence was three times more common in patients with SZ and suggests that GSH concentration is related to GCLM protein expression. Despite progress having been made in identifying SZ genetic markers, especially related to GSH metabolism and expression, the phenotypic and genetic heterogeneity across the patient population make classification of the condition very difficult (163). The pattern of inheritance does not follow a dominant or recessive pattern, since SZ represents a polygenic condition, whose effect is not completely penetrant from parent to offspring (44).

1.5 Studies assessing GSH in FEP

There are few studies that have evaluated the GSH system in FEP (table 1.1), and comparison is difficult due to a number of factors. In particular, many studies use the diagnostic labels ‘first-episode psychosis’ and ‘schizophrenia’ interchangeably, which makes it difficult to draw conclusions about the role of GSH in disease progression or pathology. Where psychosis is a symptom of SZ, it is important to distinguish the different stages of illness in order to track time-course changes that may be a result of neurodevelopment, the prodromal period, early psychosis or later illness progression. There are also far fewer studies that assess the antioxidant system in FEP, whereas this is often measured in SZ, and since FEP represents early disease pathology, the results are less consistent than findings in chronic populations. This highlights FEP as an interesting point for early detection of some of the perturbed markers in chronically ill patients. Study design is often heterogeneous, with the use of many different mediums for GSH quantification, and the umbrella term of FEP

may include psychotic illnesses that will not all be ultimately identified as SZ. Despite this, many of the underlying mechanisms that have been identified in FEP are shared by SZ. The studies in table 1.1 all reported at least one marker of perturbed redox status that was significantly altered compared with healthy controls. However, not all research studies report altered redox status. Reddy et al (164) showed no impairment of antioxidant defenses in comparison with a healthy control group, and hypothesised that disease stage is related to accumulation of oxidative stress markers. Overall, the evidence presented indicates that disruption of the entire GSH system is likely in FEP, however each study reports a different selection of outcome measures, at variable disease stages.

Duration of illness in FEP is a heterogeneous measure. This is usually based on the time since first clinical intervention, although it is estimated that the average duration of untreated psychosis is between 1 and 3 years (48, 165). Therefore, the variance in results assessing GSH and markers of oxidative stress in FEP may be partially explained by this discrepancy. As is expected, the concentration of GSH in various tissues is different. Despite this, whole blood and brain GSH regulation is related; a single infusion of N-acetyl cysteine (provides cysteine precursor for GSH synthesis) in healthy controls resulted in a concordant elevation in GSH in both tissues (166). This doesn't guarantee that GSH concentration will be altered in the same way as a result of an intervention e.g. medication. Plasma measures of GSH, however, are unreliable, since the plasma has a high concentration of –SH groups present as cysteine, the target of most GSH assays (167).

Redox status appears to be more consistently reported in SZ. Review papers (93, 135, 168-170) overviewing markers of oxidative stress in SZ report reduced GSH, GPx, total antioxidant capacity (TAC) and CAT. Lipid peroxidation is consistently increased whereas SOD data is mixed. The fact that studies in SZ present a uniform picture with regard to antioxidant status versus a much more varied situation in FEP speaks to the causal versus consequential roles of redox balance and antioxidant systems in the pathogenesis of psychosis.

Table 1.1. Comparison of human FEP study outcomes relating to the GSH cycle.

Reference	Marker	*n* (FEP, HC)	Tissue	Outcome	Duration of illness
Baumann et al (171)	GSH	42, 42	RBC	No change	21 months
Fraguas et al (154)	GSH	48, 56	Brain	Depleted	<6 months
Lesh et al (152)	GSH	36, 40	Brain	No change	*NA*
Martinez-Cengotitabengoa et al (137)	GSH	28, 28	Plasma	No change	<6 months
Martinez-Cengotitabengoa el al (71)	GSH	105, 97	Plasma	Depleted	*NA*
Mico et al (134)	GSH	102, 98	RBC	Depleted	<6 months
Parelleda et al (172)	GSH	34, 34	Plasma	No change	*NA*
Raffa et al (136)	GSH	23, 40	RBC	Depleted	<6 months
Reyes-Madrigal et al (150)	GSH	10, 9	Brain	Depleted	<5 years
Ruiz-Litago et al (173)	GSH	49, 0	Plasma	Depleted	<6 months
Wang et al (151)	GSH	81, 91	Brain	Depleted	<2 years
Wood et al (153)	GSH	30, 18	Brain	Increased	5 weeks
Raffa et al (136)	GSSG	23, 40	RBC	Increased	<6 months
Baumann et al (171)	GPx	42, 42	RBC	No change	21 months
Evans et al (138)	GPx	16, 25	RBC	No change	<1 month

Martinez-Cengotitabengoa et al (137)	GPx	28, 28	Plasma	Depleted	<6 months
Mukerjee et al (174)	GPx	14, 10	RBC	No change	4.5 days
Parelleda et al (172)	GPx	34, 34	RBC	No change	*NA*
Sarandol et al (133)	GPx	29, 25	RBC	Depleted	<6 months
Simsek et al (140)	GPx	20, 20	Plasma	No change	*NA*
Ruiz-Litago et al (173)	GPx	49 FEP, follow-up study	Plasma	Depleted	<6 months
Raffa et al (136)	GPx	30, 18	RBC	Increased	<6 months
Langbein et al (175)	GR	27 (DN), 31	Plasma	Depleted	NA

DN: drug naiive, HC: healthy control, FEP: first-episode psychosis, GPx: glutathione peroxidase, GSH: glutathione, GSSG: glutathione disulphide, GR: glutathione reductase, RBC: red blood cell

1.6 Magnetic resonance spectroscopy and its role in studying disease

The following sections of text (up to 6.4) (*Strength of a brain measure over periphery, MRS determination of metabolite concentration* and *GSH measurement in vivo)* have been taken verbatim from the following publication in which I am principle author (176).

The biological application of nuclear magnetic resonance techniques allows imaging of tissue, via magnetic resonance imaging (MRI), or quantification of key metabolites via magnetic resonance spectroscopy (MRS). The process is summarised in figure 1.5. MRS is a particularly useful method of direct, non-invasive quantification of a metabolite *in vivo*, eliminating the uncertainty of

peripheral measures. We are capable of quantifying the concentration of GSH in the brain, which represents a salient measure of GSH where it is most functional, particularly in the context of FEP.

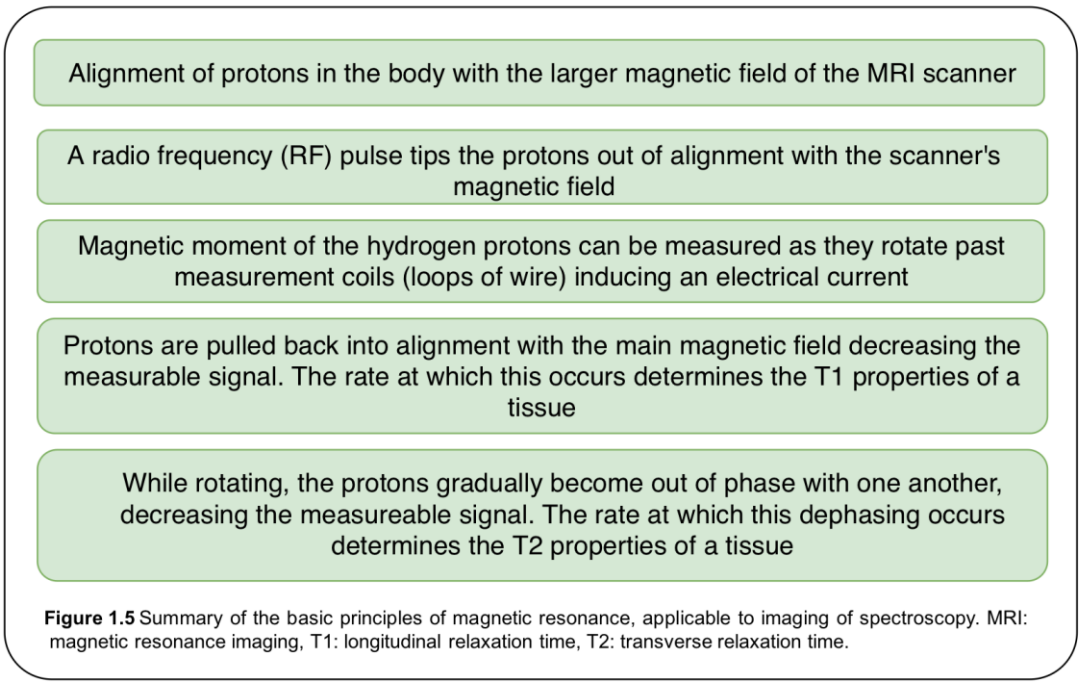


Figure 1.5 Summary of the basic principles of magnetic resonance, applicable to imaging of spectroscopy. MRI: magnetic resonance imaging, T1: longitudinal relaxation time, T2: transverse relaxation time.

1.6.1 Strength of a brain measure of GSH over periphery

Since GSH is the major free-radical scavenger in the brain, and it is known that free-radical generation is elevated in FEP, direct measurement of the thiol *in vivo* is a valuable indication of disease-related GSH perturbation. GSH is most important in the brain, since it is particularly vulnerable to oxidative insult, for a variety of reasons. Firstly, there is high oxygen use in the brain (177), around 20% of total consumption (178). Neuronal membranes are rich in poly-unsaturated fatty-acids (PUFAs), which are primary targets for oxidative damage (179), and the ratio of membrane surface area to cytoplasmic volume is high (180). Autoxidation of neurotransmitters can generate radicals (181),

alongside a modest antioxidant concentration in the brain compared with other regions of the body (182). The neurotransmitter glutamate is a major effector in causing cytotoxicity and free radical generation (183), as well as the free-radical generating capacity of activated microglia and other immune cells (184). Neuronal cells are non-replicating, and are thus sensitive to ROS (185). GSH has a primary role in quenching the brain of potentially damaging radicals, otherwise oxidative stress will prevail; a state described in many diseases, but especially important in conditions related to the brain, like FEP.

A number of studies have tried to draw comparisons between GSH in the brain and peripheral measures such as blood components or cerebrospinal fluid (CSF). Due to the variable nature of study design in the tissue assessed and the brain region of interest, the concentration of GSH across differing compartments of the human body is not well characterized (186-188). Many studies, when investigating GSH, do not differentiate between reduced, oxidized and total GSH, rendering the measure comparable only within its own intervention, and difficult to use in comparison to other studies. This makes the use of MRS as a tool for GSH quantification useful, since the measure is direct, and informative of brain GSH status. Blood measures of GSH are the most commonly reported methods, since assays are cheaper and more accessible than MRS. Plasma measures of GSH are commonly reported, but report a wide range of concentrations of GSH (0.5-759 μM) (189, 190). Plasma acts as a medium for metabolic waste products in the body, where cells excrete partial protein and lipid components for clearance. In addition, the role of GSH is to provide redox balance within a cellular system. Therefore, it is unlikely that

plasma GSH offers a reliable and accurate representation of peripheral GSH, and even more unlikely that it would be a representation of brain GSH.

1.6.2 MRS determination of metabolite concentration

MRS provides a means to explore novel chemical environments and analyse known molecular systems. Through the analysis of the free induction decay (FID) spectra of a target nucleus, the local chemical environment may be inferred. While exotic nuclei, such as ^{31}P and ^{13}C, are possible for targeting in clinical environments, ^{1}H is the most widely available (191). However, accurate quantification of some metabolites is hampered by both the low spatial resolution, due to the low chemical concentrations and the decreased spectral resolution of clinical scanners with lower field strengths. Generally, *in vivo* MRS is conducted over a single voxel placed within a target brain region (identified using a preliminary scout scan); while imaging protocols that perform spectroscopy over an array of large voxels are possible they are less common.

1.6.3 GSH measurement *in vivo*

In vivo quantification of GSH in humans is particularly challenging due to the low concentration (1.5-3 mmol/L), and the fact that all resonances overlap with stronger signals from alternate metabolites, such as water, glutamate and NAA (N-acetylaspartate) (192). However, it is possible to quantitatively isolate the spectral contribution from GSH through both refinement and calibration of standard approaches to MRS measurement as well as the development of novel sequences. Additionally, in order to accurately quantify the contribution of GSH to the measured spectra, a range of post-processing techniques, from

spectral decomposition to partial volume correction, are required. While similarities and trends do exist, studies conducted within the past 10 years vary in voxel placement, measurement sequence, acceptance bounds on signal quality and post-processing methodologies.

1.6.4 MEGAPRESS technique and glutathione quantification

Localised spectroscopy enables the observation of spectral information from a specific voxel, and one basic pulse sequence is the MEGAPRESS technique (Mescher-Garwood point resolved spectroscopy) (Figure 1.6, from one of the MRS experiments described in chapter 4 of this thesis). This method had been adapted for detection of the cysteinyl resonance of glutathione. GSH resonates at 2.95 parts per million (ppm), as a result of the strongly coupled cysteine β-CH2 at 2.95ppm, J-coupled to α-CH at 4.56ppm (193). When a selective inversion pulse is applied to the H1 frequency of the GSH cysteinyl α-CH, the GSH cysteinyl β-CH2 shows a single peak at 2.95ppm because of abolishment of the J-coupling with the cysteinyl α-CH. MEGAPRESS editing allows the 2.95ppm signal from cysteinyl β-CH2 protons to stand out over other peaks (194). It is also possible to quantify GSSG using MEGAPRESS editing; there is a distinct shift and a division of peaks due to the disulphide bond. GSH and GSSG peaks only interfere when in a ratio of 1:1- but the standard physiological range is between 100:1 and 10:1. In a study by Matsuzawa et al (155) used MEGAPRESS MRS to assess GSH in the brains of SZ patients. They observed that negative symptoms were related to reduced glutathione

levels in this group, and that a stronger relationship may have been observed if a smaller voxel had been used, but it is harder to reach sufficient signal.

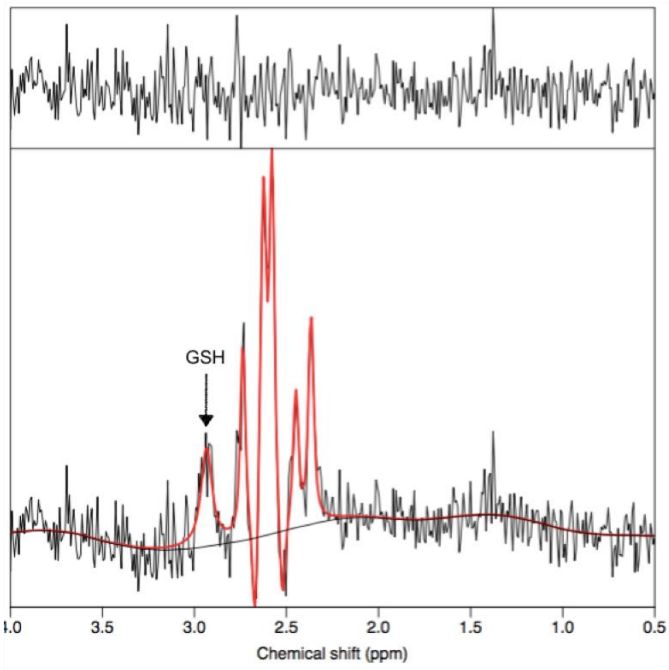


Figure 1.6 Example anterior cingulate cortex ^{1}H-MRS MEGA-PRESS chemical shift *in vivo*, after suppression of masking water signal. The y-axis represents the detected concentration of GSH, x-axis the frequency chemical shift in parts per million (ppm).

1.7 Mechanisms of exercise in the GSH system and disease

Low levels of ROS can be beneficial to adaptation and cell signalling. The theory of hormesis (195) describes the beneficial effects of a potentially toxic compound, in a dose-response manner, up to a threshold point (figure 1.7). In this case, continuous exposure to low-to-moderate concentrations of ROS can induce antioxidant enzyme expression through signalling by free radicals (196). This theory accounts for the beneficial effects of long-term exercise training; an acute bout of exercise stimulates and increases in ROS production, which

favours subsequent adaptation through upregulation of antioxidant mechanisms. Regular exercise increases activity of the proteasome complex, which is believed to be responsible for degradation of oxidised proteins (197). The damaging effects of ROS that are characteristic of many diseases are represented by the second half of the hormesis bell curve. Maintenance of adaptation in response to exercise is dependent on a balanced concentration of the xenobiotics that are attributable to disease cause. This relationship has the potential to be used as a therapy in clinical populations, since exercise training may interact with mechanisms of redox-related perturbations. Importantly, one of the most notable changes in response to exercise is upregulated antioxidant expression.

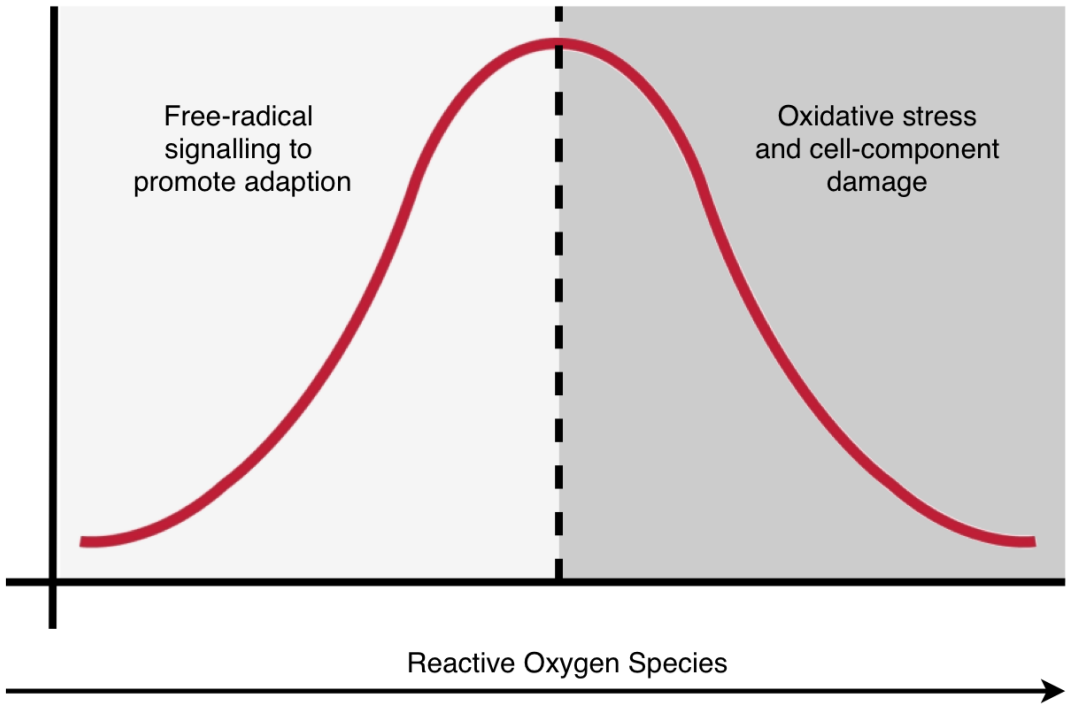


Figure 1.7 Hormesis dose-response curve demonstrating the effects of increasing ROS levels, characterised by low doses that promote signalling and adaptation, followed by the toxic effect of excessive ROS production. Exercise training modulates ROS and prevents the downward tipping of the curve.

The beneficial effects of exercise training on the antioxidant response to oxidative stress are clear, and are the subject of a growing pool of literature. The huge gap in this knowledge is the effect exercise can have in a mental health population. Exercise in healthy people and disease models has mapped the baseline from which to build new research into the potential treatment of FEP patients with exercise.

In the context of the GSH cycle, Gohil et al (198) showed that with a prolonged session of submaximal exercise (90min at 65% VO_2 peak) GSH levels were reduced by 60% immediately post exercise, and GSSG was increased 100%. This effect is temporary, and an adaptive process takes place through training. Elokda (199) demonstrated an adaptation to oxidative stress through exercise training. Using healthy sedentary volunteers, interventions of either aerobic training, or combined aerobic and circuit weight training (standardised workload) were implemented to observe the change in GSH and GSH: GSSH ratio- the criterion markers of oxidative stress. Both training programmes increased GSH, and reduced GSSG, but the combined programme had a greater effect- 45% versus 35% improvement. The subjects in this study adapted their ability to cope with greater acute stress, strengthening their response to oxidative stress. Ten-weeks of an aerobic exercise programme, with exhaustive acute bouts pre-and post-intervention resulted in an increased GPx response to exercise, and reduced GR, and GSSG (200). These results highlight the improved reducing power of GSH; with greater free-radical removing capacity. Increased GPx demonstrates the adaption to chronic exercise stress.

Exercise training also has the capacity to elicit an adaptive response to damage-marker production. One study assessed the differences in resting redox biomarkers between trained athletes and sedentary young men (201). Trained men had significantly reduced MDA (lipid peroxidation marker) and protein carbonyl concentration compared with the sedentary counterparts. Different exercise modalities appear to have different effects on redox outcomes. Bogdanis et al (202) prescribed 3-weeks of high intensity interval training, and showed that after an acute bout (pre-intervention), protein carbonyls, TBARS (thiobarbituric acid reactive substances), GPx activity, total antioxidant capacity and CAT were all elevated. After the training period, total antioxidant capacity was improved, alongside an attenuated response to TBARS production after exercise. In a comparison of three different exercise modalities, Azizbeigi et al (203) designed a study to include three groups; endurance training, resistance training and a combination of the two. All groups showed an increase in resting SOD, and reduced MDA after the intervention. For the endurance and combination groups, GPx and total antioxidant capacity were increased. Resistance and anaerobic high intensity bouts of exercise training have less requirement for oxygen, but free-radical production is elicited via a number of alternative mechanisms, including the xanthine oxidase pathway, catcholamine oxidation, local muscle ischemia, and conversion of superoxide anion to hydroxyl radical by lactate (204, 205). The decrease in lipid peroxidation products across all training types in this study may be due to an increased resistance of cell-membrane rich poly-unsaturated fatty acids to peroxidation (203).

The inflammatory pathway represents another target of exercise in FEP, and observational studies have reported lower inflammatory biomarker concentrations in those who are more habitually active (206, 207). Interventional studies have observed reductions in CRP (C reactive protein), stimulated by TNF-alpha (tumor necrosis factor alpha) and IL-6 (interleukin 6), after periods of training (208-210). Thompson et al (211) recruited sedentary middle-aged men to a 24-week exercise intervention, with a 2-week de-training assessment post-exercise. They observed that IL-6 was decreased at 24-weeks, but within the two weeks de-training period the cytokine returned to baseline level. IL-6 increased after each acute bout of exercise (212), indicating the power of acute bouts to elicit change, and the adaptive potential of exercise training. Aerobic endurance training results in increased basal IL-6 receptor expression in skeletal muscle (213), which in time results in reduced upregulation of IL-6 in response to an acute bout of exercise.
Nikseresht et al (214) assessed the effects of exercise training followed by 4-weeks detraining on inflammatory markers in inactive males. They found that after 4-weeks detraining, IL-6 was significantly increased. There was no change in TNF-α or CRP as a result of the exercise training. Another group found that long-term moderate intensity exercise training had no effect on adipokine gene expression (e.g. TNF-α)(215).

There are several mechanisms of adaptation to exercise training in reducing the raised inflammatory profile. IL-6 is principally defined as a pro-inflammatory cytokine, but also exhibits autocrine, paracrine and endocrine effects when

released by myocytes during muscular contraction (216). Many cytokines, including IL-6, and other small proteins can be described as myokines, which act as regulators of exercise-induced metabolic changes. Contrary to the belief that increases in IL-6 during exercise represent an acute-phase response initiated by local damage in the muscle (217), IL-6 release is elevated in the circulation during dynamic exercise, without muscle damage (218). This effect means that IL-6 is involved with adaption to exercise, as a signalling molecule, alongside it's pro-inflammatory role. It is also known that exercise training results in shifts in monocyte phenotype, specifically reductions on immune cell production of inflammatory mediators (205, 219). Since chronic low-grade inflammation is a potent source of ROS, the exercise-induced reduction of inflammatory cytokine reduction also reduces the concentration of ROS produced and the consequential damage to cellular components (220).

No study has assessed the effects of exercise training on redox homeostasis in a FEP or SZ group. The perturbations of redox homeostasis and the antioxidant system have been well-described in the context of FEP; highlighting the need for therapeutic treatments that have the potential to alleviate some of the products of oxidative stress. The potential for exercise as an adjunctive therapy to target the redox system is profound, alongside the other, more well-known metabolic benefits of exercise training. Improvements in BMI (221), lipid profile (222), cardiovascular fitness (223) and a reduced risk of type 2 diabetes (224) are some of the salient benefits especially since they address some of the more common negative side-effects of antipsychotic medication.

1.8 Exercise as a therapy in psychosis; potential benefits and difficulties in this clinical population

Alongside the principle aim of generating a physiological response to exercise, it is also important, in the context of patient populations, to record the response needed to elicit change in psychological factors. Depression and anxiety are two of the most common co-morbidities associated with any psychotic illness (225-227). Regular exercise has the potential to alleviate some of the symptoms associated with these diagnoses. Dunn et al (228) treated a group of patients diagnosed with depression with a 12-week programme of exercise, and the average score on the Hamilton rating scale for depression was reduced by 47%. A review in patients with depression and severe anxiety showed that physical inactivity was a risk factor for depressive symptoms, and that regular physical activity was associated with lower levels of depression, anxiety and neuroticism (229). Perceived levels of mental health and well-being, as well as reduced feelings of anxiety are heightened with regular exercise (230), and a study in European older adults correlated quality of life with increasing habitual activity (231). Acil et al (232) used a 10-week intervention of aerobic exercise as an adjunctive therapy in a SZ patient group. Exercise conferred positive and negative symptom reduction (assessed by the Positive and Negative Symptom Scale (PANSS)), as well as a significant improvement in quality of life (assessed by the World Health Organisation Quality of Life questionnaire). Beebe et al (233) employed a 16-week walking programme in a group of SZ patients, observing an average 22% reduction in

PANSS total score- clinically significant despite the relatively low intensity of training.

A meta-analysis by McAuley et al (234) compared psychological outcomes related to exercise. In a comparison of the reported studies (n=32), 6 showed no response to any of the psychological measures reported. Of these studies, either the training intensity or intervention duration was very low- providing a potential explanation for lack of improvement in measures such as mood, well-being, depression or life satisfaction. The exercise interventions for these studies comprised walking/ jogging (235-239), or in the case of higher intensity activity (70-80% HRmax (age-related maximum heart rate)) the intervention duration was only 4 weeks (240). All reported studies that provided positive results on psychological factors used exercise interventions with greater intensity, with a minimum of 8-weeks duration. With these extended parameters, improvements in anxiety, depression, well-being index, mood, quality of life, stress, life satisfaction, tension, happiness and emotional stability were reported.

Study design is the most important factor in generating the most reliable data from an exercise intervention, particularly in a clinical population. Table 1.2 describes all of the exercise trials in FEP and SZ groups, highlighting various aspects of intervention design and key outcomes. It is important to standardise bouts of exercise across an intervention, in order to observe a meaningful effect of exercise. Supervision and frequency of training have an enormous impact on study compliance and drop-out; from the studies described, supervised studies demonstrate 75% attendance and 21.3% attrition, whereas

unsupervised studies are unable to report attendance, but show 51% dropout, on average.

Table 1.2. Summary of exercise interventions in FEP and SZ

Reference	Sample size (n) PAT, PATC, HC	Exercise intervention	Frequency	Duration (weeks)	Drop-out	Attendance	Supervised?	Clinical group	Notable results
Abdel-Baki et al (241)	25,0,0	Aerobic running intervals	2 x 30 mins	14	36%	48%	Y	SZ	Decreased waist circumference and increased VO_2max
Acil et al (232)	15,15,0	Home-based AE	3 x 40 mins	10	0%	NA	N	SZ	Sig. decreased positive and negative symptoms, improved WHOQOL score
Alvarez-Jimenez et al (242)	35, 27, 0	Behavioural intervention, including AE	Flexible schedule	12	0%	NA	Y	FEP	Sig. less weight gain than controls
Archie et al (243)	10,10,0	Gym access and recommendation	Determined by patient	24	90%	30%	N	SZ	Increase in body weight in both groups
Ball et al (244)	11,11,0	Walking	3 x 5-20 mins	10	54%	NA	Y	SZ	No sig. change in body weight or symptoms
Battaglia et al (245)	18, 0, 0	Group football	2 x 100-120 mins	12	22%	80%	Y	SZ	Decreased body weight and increased mental component
Beebe et al (233)	4,6,0	Walking programme	3 x 30 mins	16	NA	67%	Y	SZ	Sig reduced body fat, and improved fitness and BMI

Behere et al (246)	Yoga 27, 0, 0 Jogging 17, 0, 0	Jogging/ yoga	Recommend ation	16	45%	NA	N	SZ	Sig. improvement in positive and negative symptoms, and socio-occupational functioning for yoga group only
Bhatia et al (247)	Yoga 104, 0, 0 Jogging 90, 0, 0	Yoga or physical exercise training	7 x 60 mins	3	17%	NA	Y	SZ	Both exercise modalities improved attention and additional cognitive domains
Bredin et al (248)	13,0, 0	AE or resistance exercise training	3 x 30 mins	12	31%	81%	Y	SZ	Non-sig. improvements in PA, aerobic fitness and BP
Curtis et al (249)	16, 12, 0	Gym access, health coaching and dietetic support	Aim to increase participation	12	62%	NA	Y	FEP	Sig. reduced weight gain in intervention group
Dodd et al (250)	8, 0, 0	Group aerobic exercise	2 x 30 mins	24	0%	73%	Y	SZ	Sig. improvements in weight, no change in symptom score
Duraiswamy et al (251)	Yoga 13, 0, 0 Jogging 30, 0, 0	Jogging/ yoga	5 x 60 mins	16	26%	NA	N	SZ	Yoga group had sig. less paychopathology than joggers
Firth et al (252)	31, 0 0	Aerobic and resistance training	2 x 45-60 mins	10	19%	50%>90min 79%>60min	Y	FEP	Sig. reduced PANSS score, psychosocial functioning and verbal short-term memory

Fogharty et al (253)	6,0,0	Individual, incremental exercise programme	NA	12	NA	NA	Y	SZ	Future participation in regular exercise was encouraged
Gholipour et al (254)	15, 15, 0	Therapeutic exercise (exercise and token behaviour therapy)	3 x 120 mins	16	%	NA	NA	SZ	Token reinforcement more effective than exercise for reducing psychotic symptoms
Heggelund et al (255)	12, 7, 0	Hight intensity interval training	3 x (4 x 4 mins)	8	25%	85%	Y	SZ	Improved VO_2 peak, no change in symptom scores
Heggelund et al (256)	6, 7, 0	Maximal strength training	3 x (4 x 4 mins)	8	17%	85%	Y	SZ	Improved 1RM, no change in symptom scores
Ho et al (257)	15, 15, 0	Thai Chi	1 x 150 mins	6	23%	NA	Y	SZ	No change in negative symptoms, thai chi reduced deterioration in movement coordination
Ho et al (258)	Thai Chi 51, 51, 0 Exercise 51, 51, 0	Thai Chi, or moderate intensity aerobic exercise	3 x 50 mins	12	5%	80%	Y	SZ	Thai Chi showed sig. decreases in motor deficits and increased cortisol. AE showed decreased motor deficits, negative symptoms, depression and increased cortisol
Ikai et al (259)	25, 24, 0	Yoga therapy	1 x 60 mins	8	10%	87%	Y	SZ spectrum	Sig. improvements in postural stability
Ikai et al (260)	25, 25, 0	Hatha yoga	1 x 60 mins	8	28%	NA	Y	SZ	No change BDNF or PANSS

Ikai et al (261)	28, 28, 0	Chair yoga therapy	2 x 20 mins	12	13%	83%	Y	SZ spectrum	Sig improvements flexibility, hand-grip strength and lower limb muscle endurance
Jayaram et al (262)	15, 28, 0	Yoga therapy	NA	4	33%	NA	Y	SZ	Improvements in socio-occupational functioning, and plasma oxytocin
Kaltsatou et al (263)	16, 15, 0	Greek traditional dancing programme	3 x 50 mins	8 months	0%	87%	Y	SZ	Improved QoL score, Global Assessment of Functioning, balance and 6-minute walk test
Kang et al (264)	118, 126, 0	Thai Chi plus social skills	2 x 120 mins per month	12 months	0%	NA	Y	SZ	Reduced PANSS negative score, improved adherence to medication
Kimhy et al (265)	16, 17, 0	AE programme with Xbox Kinect	3 x 60 mins	12	21%	79%	Y	SZ	Improved aerobic fitness and neurocognition
Lambden et al (266)	92, 0, 0	Structured group exercise; circuit training, resistance exercises, AE and stretching	5 x 60 mins	12	51%	48%	Y	FEP	Association between exercise and autonomy, representative of recovery process
Larsen et al (267)	13, 12, 0	Hight intensity training inspired by Crossfit	3 x 60 mins	8	16%	NA	Y	FEP	Participants given the chance to take part in Copenhagen Warrior obstacle race post-trial.

Lin et al (268)	Yoga 45, 39, 0 AE 40, 39, 0	Yoga or AE programme	3 x 60 mins	12	23%	Yoga 47% AE 58%	Y	FEP	Both interventions sig. improvement in working memory, and reduced depressive symptoms. Sig. Increase hippocampal volume AE group only
Loh et al (269)	52, 52, 0	Structured walking intervention	3 x 30 mins	12	8%	NA	Y	SZ	Sig. reduction PANSS domains
Manjunath et al (270)	Yoga 44, 0, 0 Jogging 44, 0, 0	Jogging/ yoga	5 x 60 mins	6	43%	NA	N	SZ	Yoga more effective for improvements in PANSS score, CGIS and HDRS
Marzolini et al (271)	7, 6, 0	Group aerobics and weights	2 x 90 mins	12	0%	72%	Y	SZ	Improved strength and MHI score
Methapatara et al (272)	32,32,0	Pedometre walking	Recommend ation	12	0%	NA	N	SZ	Decreased body weight
Nuechterlein et al (273)	7, 9, 0	AE and cognitive training	4 x 30 mins	10	NA	93.50%	50% supervised	SZ	Combined cognitive training and AE increased cognition
Oertel-Knochel et al (274)	MDD 8, 8, 0 SZ 8, 10, 0	Aerobic exercise and cognitive training	3 x 45 mins	4	32%	NA	Y	SZ & MDD	MDD sig. reduction depressive symptoms and state anxiety. SZ reduced negative symptoms
Paikkatt et al (275)	15, 15, 0	Yoga therapy	7 x 90 mins	4	7%	NA	Y	SZ	Improved PANSS rating

Pajonk et al (276)	8, 8, 8	Group aerobic exercise	3 x 30 mins	12	38%	85%	Y	SZ	Sig. increase hippocampal volume, correlating with VO_2max, NAA:Cr ratio and short-term memory
Poulin et al (277)	59,51,0	Education, diet and AE programme	2 x 60 mins	18 months	15%	85%	Y	SZ + BD	Sig. decreased BMI and cholesterol
Romain et al (278)	38, 28, 0	High intensity interval training	2 x 30 mins	6 months	50%	64%	Y	Psychotic disorder*	Compliant participants reduced waist circumference, negative symptoms, social and global functioning
Scheewe et al (279)	Exercise 31, 0, 0 OT 32, 0, 0	Aerobic and resistance training	2 x 60 mins	24	42%	79%	Y	SZ	Exercise reduced symptoms, depression, need of care and cardiovascular fitness
Silva et al (280)	Concurrent 9, 13, 0 Resistance 12, 13, 0	Resistance exercise or concurrent exercise	2 x 60 mins	20	27.65%	75%%	Y	SZ	Improved positive and negative symptoms, no change in BDNF or anthropometric measures
Strassnig et al (281)	6,0,0	Lab based treadmill running	3 x 30 mins	6	NA	60-100%	Y	SZ	Increased VO_2max
Su et al (282)	30, 27, 0	Treadmill running	3 x 40 mins	12	22.60%	77%	Y	SZ	Improved processing speed and attention. No change in cognition or fitness

Svatkova et al (283)	33, 0, 48	AE on a stationary bicycle	2 x 60 mins	6 months	NA	>50%	Y	SZ	No difference between groups, irrespective of diagnosis regular PA of an overlearned skill increases motor functioning
Takahashi et al (284)	13,10,0	AE, basketball and stretching	2 x 30-60 mins	12	0%	NA	Y	SZ	Decreased BMI and PANSS score
Varambally et al (285)	Yoga 47, 36, 0 Jogging 37, 36, 0	Jogging/ yoga	5 x 60 mins	16	41%	NA	Y/N	SZ	Yoga more effective for improved PANSS score than jogging
Visceglia et al (286)	10,9,0	Yoga	2 x 45 mins	8	NA	NA	Y	SZ	Decreased positive and negative PANSS scores
Warren et al (287)	17,0,0	Jogging, increasing throughout and educational programme	3 x 20 mins	10	11%	65%	Y	SZ	82% participation in a 5km event
Wu et al (288)	59, 0, 0	Treadmill jogging	7 x 30 mins	12	0%	72%	Y	FEP	Decreased BMI, insulin resistance, and waist circumference
Wu et al (289)	20, 0, 0	HIIT	3 x 25 mins	8	10%	NA	Y	SZ	Sig. reduced weight, BMI, BP and PANSS measures

AE: aerobic exercise, BDNF: brain derived neurotrophic factor, BMI: body mass index, BP: blood pressure, CGIS: clinical global impression scale, FEP: first-episode psychosis, HC: healthy control group, HDRS: Hamilton depression rating scale, MDD: major depressive disorder, MHI: mental health inventory, PA: physical activity, PANSS: positive and negative symptom scale, PAT: patient group, PATC: patient control group, SZ: schizophrenia, QoL: quality of life, 1RM: 1 repetition maximum

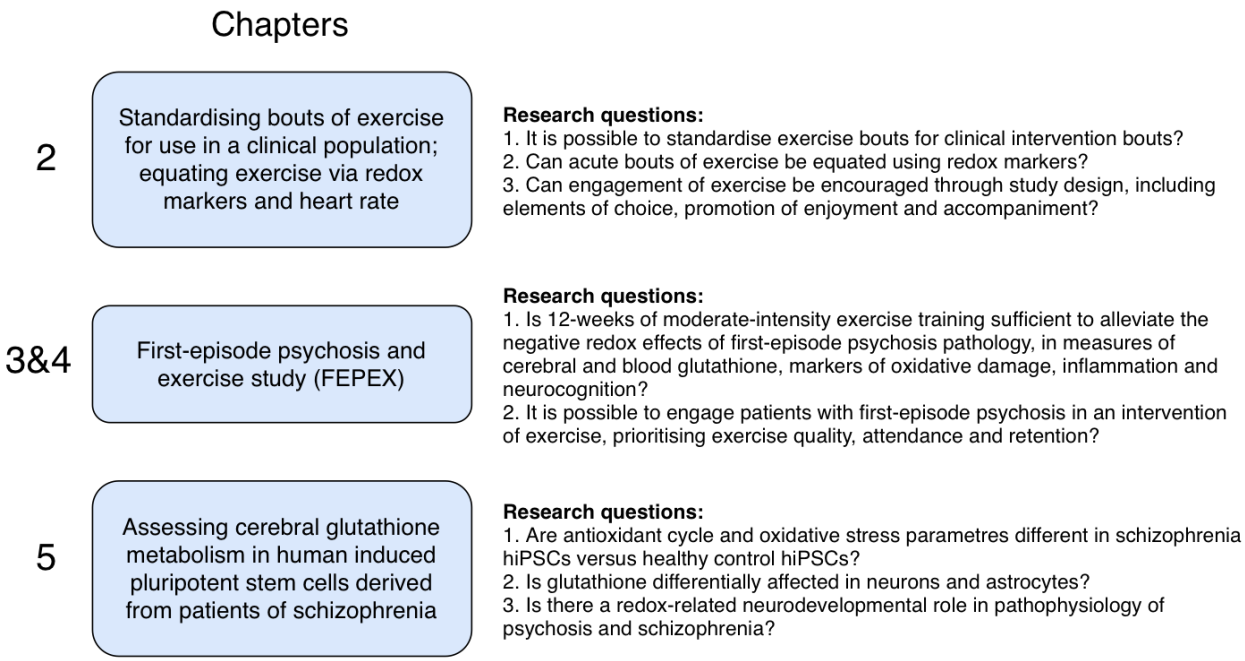
1.9 Modelling psychosis in human cerebral cells

Studying the mechanistic pathways that contribute to psychosis is very difficult in humans, and animal models have often been employed instead. There are a number of limitations of using *in vivo* models of mental illness. As described earlier, psychosis and SZ are so genetically heterogeneous that producing an animal with a genotype and metabolic regulation that represents disease is almost impossible. Since diagnoses are made based on subjective phenomenology (290), rather that physiological processes, it is reasonable to conclude that animals are unlikely to mirror the disease state well (291). Induced pluripotent stem cells (iPSCs) present an interesting model for the study of brain health, since it is possible to neurally induce these pluripotent stem cells from a human donor with a specific disease phenotype.

Since pathogenesis of FEP is hypothesised to be neurodevelopmental (53, 54), it represents a good candidate for an iPSC neural cell model. These cells reflect embryonic development, and are unique in the sense that other cell models of disease represent ‘adult’ cells in culture. iPSC-derived neurons and astrocytes are immature in comparison to the human brain (292, 293), which means that iPSC lines derived from patients represent the perfect model for the neurodevelopmental aspects of the disease phenotype. Markers of antioxidant capacity have never been assessed in SZ iPSC lines, especially in different cerebral cell-types. Additionally, redox biology is an area of iPSC research in SZ that is in its infancy. Brennand et al (294) observed increased oxidative

indices (DNA damage and protein oxidation) in iPSC neural progenitor cells, precursors to cerebral cell development, in a patient-derived SZ model.

Schematic outline of thesis



CHAPTER 2: STANDARDISING BOUTS OF EXERCISE FOR USE IN A CLINICAL POPULATION; EQUATING EXERCISE VIA REDOX MARKERS AND HEART RATE

Abstract

Regular exercise may have a therapeutic role in youth mental ill-health, but the majority of exercise studies undertaken in this population have observed poor compliance to intervention and high drop-out rates. In the context of first-episode psychosis (FEP), the antioxidant system and oxidative adducts represent cogent markers for monitoring exercise training-induced improvements, as oxidative stress and depleted antioxidant defense are a key component of disease pathology. The principle aim of this study was to characterise a variety of exercise types that could be used in an exercise intervention to ensure the quality of the exercise bout whilst promoting enjoyment for the participant. Blood markers of antioxidant capacity and oxidative stress were assessed, to determine the optimal intensity and activity type to elicit adaptation.

Ten healthy sedentary participants (aged 23.50 ± 3.70 years) were invited to complete 7 acute exercise bouts, across a range of exercise modes. A single laboratory exercise bout on a cycle ergometer was undertaken to act as a control, and following this, target heart rates were set for exercise at 50-70% VO_2max. Heart rate was continuously monitored through the session, as well

as blood glutathione (GSH) and 8-isoprostane measurements immediately prior to and post individual sessions.

The study observed an 90% completion rate of activities, and 52% of exercise sessions were within the individual target heart rate range. GSH concentration was elevated by 12.09% (± 27.51) between pre-and post-bout measurements, and the high-intensity interval running session caused a 71.40% GSH increase (*p=0.007*). There was no change in 8-isoprostane concentration as a result of any of the acute exercise bouts.

This study demonstrated that it is possible to engage and retain sedentary participants in exercise training, using a variety of different exercise modalities. The redox system was challenged, as indicated by altered GSH concentrations after exercise, which suggests these bouts of exercise are capable of stimulating an adaptive response to exercise. Exercise bouts did not elicit oxidative stress however, as no increase in 8-isoprostane concentration was detected. The findings of this pilot study inform the design of an exercise intervention as an adjunctive treatment in FEP, including bout monitoring, exercise intensity and the provision of a variety of activity types to facilitate enjoyment and study-compliance.

Key words

Heart rate, oxidative stress, glutathione, 8-isoprostane, acute exercise

2.1 Introduction

2.1.1 The sedentary lifestyle

Physical inactivity is a hallmark of youth mental illness, particularly psychosis and schizophrenia (SZ): these populations report barriers to regular exercise, which have been described by Firth et al (295) and Vancampfort et al (296); as low mood, stress and lack of support. These barriers, along with depression and anxiety, are key features of first-episode psychosis (FEP) and SZ that emphasize the incipient need for a different kind of exercise provision alongside treatment, to better aid people with mental ill health in working past these barriers and to use exercise to maximise improvements in both physiological and psychological health. Regular exercise has the potential to diminish many of the barriers presented by this population.

Severe mental illness (e.g. SZ, bipolar disorder or major depression) confers a much greater risk of chronic co-morbid-related death, up to 20 years prematurely, compared with the general population (296). This reduced life expectancy can be attributable to metabolic disorders (297) that are two-fold in origin; association with sedentariness and lack of physical activity (298), as well as impaired glucose metabolism that is linked to underlying disease pathology (299). As sedentary behaviour is an independent risk factor for a number of metabolic conditions, including cardiovascular disease, type 2 diabetes and all-cause mortality (300), it is even more important to engage young people with mental ill-health in regular physical activity.

Exercise training has the potential to elicit benefits both psychologically and physiologically, but not all exercise is equal. Exercise of different intensity, frequency and duration can equate to different physiological and psychological stimulus and there is a need to standardise exercise intervention design in youth mental health. By targeting biomarkers of illness, via more detailed prescription of exercise, the benefits of an intervention have the potential to become much more specific. Clinical recovery from FEP is often incomplete, therefore adjunctive strategies are needed alongside neuroleptic medication prescription to increase the chances of success, and reduce the number of people that progress to SZ.

2.1.2 Enjoyment and engagement

One of the principle drawbacks in exercise intervention studies in these clinical populations is the high rate of attrition and poor compliance to a training programme (301). Larsen et al (267) found that by providing a choice of exercise sessions to a cohort, adherence was improved. In another study of healthy young participants, exercise session design prioritised enjoyment, and physical activity achieved strong correlation with overall enjoyment (302). It has also been observed that enjoyment of exercise is predictive of participation in moderate to vigorous physical activity (303, 304). Therefore, it is necessary to optimise exercise interventions to prioritize session quality, engagement and enjoyment in order to achieve attendance in prospective exercise interventions.

2.1.3 Exercise and the redox response

The pro-oxidative state, and chronic antioxidant depletion in psychosis, as well as the beneficial effects of exercise training on the redox system have been well-described in chapter 1 of this thesis (pages 37-43). Briefly, the potential of exercise to reduce disease-related markers of oxidative damage, as well as upregulate the antioxidant system is profound. Exercise has an important role in reducing the damaging effects of reactive oxygen species (ROS), and exercise intensity and duration is a determinant of the magnitude of ROS production (305). An acute bout of exercise produces ROS (306), but ROS are an integral part of the process of exercise adaptation. Unaccustomed or exhaustive exercise bouts may generate excessive ROS (307), which shifts redox balance, depleting or overwhelming antioxidants to cause 'damage' to cellular components. However, in almost all cases, biomolecules carrying 'damage' as adducts will be repaired or will act as a stimulus for repair (308).

2.1.4 Using redox markers to assess the physiological stress associated with acute bouts of exercise

Redox status can be assessed by measurement of biomarkers of oxidative damage or antioxidant concentration. These markers give an indication of the physiological stress or load that an exercise bout may have placed on the system. It is salient to note that markers of oxidative stress are similarly perturbed across different youth mental health conditions, including FEP (309), SZ (310), bipolar disorder (311) and major depressive disorder (312). Thus, at baseline, before any exercise bout is undertaken, the redox status of a person

with mental ill health will likely be altered in comparison to a healthy individual. In addition, the physiological stress of a bout of exercise, which can be monitored using heart rate (HR), and the characteristics of the person undertaking the exercise are likely to be affected by an individuals' illness.

Glutathione (GSH) is the most abundant antioxidant molecule in the brain and periphery. It acts as a scavenger of ROS, with the primary role of maintaining intracellular redox balance (313) (described in detail on pages 43-46). In a comparison of different acute exercise bouts on redox balance, Rai et al (314) invited sedentary young men to complete a bout of HIIT (high-intensity interval training) or moderate intensity continuous exercise. The response of blood GSH to the two different intensity bouts was different. HIIT resulted in a significant increase in circulating GSH, whereas no change was observed after moderate intensity continuous exercise. Increased synthesis of endogenous GSH in response to HIIT training is known to activate the GSH redox cycle (199), increasing the activity of other antioxidant enzymes associated with this cycle (202). This occurs alongside a transient increase in ROS, which can act as a signalling mechanism to stimulate adaptation (315). Resting blood GSH has been shown to be depleted in FEP and SZ (71, 134, 154), indicating a reduced capacity of the antioxidant system to quench damaging free radicals that may be elevated as a result of disease pathogenesis.

Markers of free radical-mediated damage can be present on any biomolecule, e.g. protein, lipid or DNA, and can give an indication of the source of the radical. For example, 8-isoprostane is a specific marker of ROS induced lipid damage (316). These prostaglandin-like compounds are formed via the

peroxidation of the free-fatty arachidonic acid (317). 8-isoprostane production in response to acute exercise in healthy sedentary populations has been measured, with exercise intensity proving an important determinant of oxidative stress, resultant cell injury and response. One study compared a high intensity interval-style bout with a continuous moderate intensity bout (318) and detected a significant increase in plasma 8-isoprostane concentration following the high intensity bout only. Other studies have observed significantly increased isoprostane concentrations after a 50km ultramarathon (319), and conversely no change after 2 hours of exhaustive weight lifting exercise (320).

It is clear that different exercise mode, duration and intensity elicit a different redox response, either via stimulation of antioxidant production, or via accumulation of adducts that have resulted from ROS interaction with biomolecules. By assessing redox markers following different bouts of exercise, it may be possible to ‘tariff’ or equate the amount of physiological stress, or stimulus of adaptation, that has been caused by that exercise bout, alongside a more traditional measure of exercise intensity such as heart rate. With this information, it may therefore be possible to design training sessions that stimulate key adaptive processes relevant for populations with altered redox status.

2.1.5 Aims

The aim of this study was to assess the redox perturbation caused by different exercise modes, undertaken at differing intensities and durations to create a series of standardised exercise sessions. These exercise sessions were created using a range of different exercise modes to offer variety and appeal to

individuals who were not normally motivated to exercise. A healthy, but sedentary group was targeted to replicate the activity levels of a clinical population of people with psychosis.

By assessing acute changes in a marker of oxidative damage, alongside the protective antioxidant capacity of the blood, it was hypothesized that it is possible to observe the physiological demand of a particular exercise session, and therefore equate sessions for use in a clinical intervention. Since this study design is destined to be implemented in a youth mental health population, promoting attendance and enjoyment was salient considering the high rates of attrition reported in similar studies, and therefore variety in exercise mode to offer broader choice was a primary aim.

2.2 Methods

2.2.1 Participants

Ten healthy male and female participants were recruited from the University of Birmingham and surrounding area. The sample size was calculated prior to recruitment, using G*Power software (321), based on exercise stimulating change in blood GSH concentration. Eligibility criteria were assessed using a general health questionnaire (supplementary section, page 285), to ensure that participants were free of medical conditions that prevented them from taking part in moderate intensity exercise bouts. Volunteers were aged between 18-30 years and were sedentary (<150 minutes moderate to vigorous physical activity per week), as assessed by International Physical Activity questionnaire (IPAQ)

(supplementary section, page 288). Baseline characteristics are summarized in table 2.1. Recruitment commenced following approval from the University of Birmingham STEM research ethics committee (ERN_17-0249).

2.2.2 Baseline measurements

Participant height and weight were measured, as well as habitual activity (Garmin activity monitor) and physical activity enjoyment (PACES questionnaire) (supplementary section, page 287). These questionnaires were repeated at the end of the study. Before exercise sessions commenced, each participant wore a Garmin VivoActive HR™ activity monitoring band for one week, in an assessment of habitual activity. The Garmin device has been validated for use in healthy and clinical populations (322-324).

2.2.3 Determination of target heart rate zone

Participants attended one exercise session in the laboratory, in the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham, to assess physical fitness and heart rate zones. After familiarization with the cycle ergometer (Lode Excalibur Sport®) and gas exchange equipment (Vyntus CPX, CareFusion; UK), participants cycled for 40 minutes at a chosen resistance, maintaining a heart rate within a target zone, based on 50-70% VO_2max (60-80% HR max (age-related number of beats per minute of the heart when working at its maximum) from Tanaka's 208-(0.7*age) equation) (325). Heart rate was monitored using a Polar T-31™ (Polar T-31, Polar; Finland) coded

transmitter and with the Garmin VivoSmart HR® wristwatch, in order to compare inter-device accuracy.

2.2.4 Acute exercise bouts

Participants were given a choice of the order in which each exercise bout was completed, with each bout taking place at least one week apart to prevent adaptation and improvement in fitness. Garmin VivoSmart HR™ activity monitors were used to record HR, with participant height/ weight/ age pre-programmed onto the device. Each bout had a main aim of maintaining a target HR, based on 50-70% VO_2max, individual to the participant. Sessions (run, cycle, interval running, swimming, tennis and aerobics) concluded at 40-minutes. All exercise sessions were accompanied and supervised with a researcher from the School of Sport, Exercise and Rehabilitation Studies at the University of Birmingham.

2.2.5 Enjoyment

Enjoyment of each activity was assessed via the Physical Activity Enjoyment Scale (supplementary section). This questionnaire was completed following each bout of exercise.

2.2.6 Blood sampling and assays

Finger-prick blood samples were taken immediately before and after each exercise bout, including the laboratory test, in BD Microtainer® MAP K_2EDTA

1.0mg tubes (BD, USA), in order to measure plasma 8-isoprostane and whole-blood GSH concentration.

2.2.7 Blood glutathione

Whole blood total glutathione was determined using a commercially available luminescence-based assay (GSH-Glo™ Glutathione Assay, Promega, Wisconsin, USA). The luciferin formed in the presence of GSH is detected in a coupled-reaction using Ultra-Glo™ Recombinant Luciferase that generates a glow-type luminescence proportional to GSH concentration in the cells present. Standards were prepared by serial dilution from a stock solution (5mM) of glutathione (1:100), concentrations ranging from 0-5μM. Whole blood lysate is prepared from frozen whole blood, by gentle inversion with an anticoagulant. Cells are diluted 1:5 in GSH-Glo™ Reaction buffer (50mM Tricine pH 7.9), incubated, and centrifuged (10000 x g for 15 minutes at 4 degrees). Supernatant (lysed whole blood sample was collected), and further diluted 1:15 in dH_2O. Diluted lysate (10μl) was transferred to a 96-well plate. 1X GSH-Glo™ reagent (100μl) (luciferin-NT substrate and glutathione-S-transferase diluted 1:100 in GSH-Glo™ reaction buffer) was added to each sample/ standard, and incubated (RT for 30 minutes). Luciferin detection reagent (100μl) (reconstitution buffer, esterase and lyophilized luciferin detection reagent) was added to each well, incubated for a further 15 minutes, and luminescence was measured.

2.2.8 8-Isoprostane determination

The concentration of 8-isoprostane in plasma samples was determined by a competitive enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (516351 Cayman Chemical, Michigan, USA). In order to assess lipid peroxidation in plasma samples from each participant, pre- and post each exercise session. The methodological process had been described in detail previously (Pradelles et al, 1985). In brief, plasma (30µL) was added to 8-isoprostane affinity sorbent (401113, Cayman Chemical) and incubated for 60 minutes with gentle mixing. Samples were then centrifuged (1500xg for 30 seconds) to sediment the sorbent, the resulting supernatant was removed and discarded, this was repeated twice following addition of Eicosanoid Column Affinity buffer (100µL) (400220, Cayman Chemical) and ultrapure water (100µL). Elution solution (95% ethanol) was added to the sediment and evaporated to dryness under nitrogen. Samples were suspended in the ELISA buffer. Next, standards were prepared from the assay stock solution to create an 8-point standard curve ranging from 0.8 to 500pg.ml, a blank well was used as the 0pg.ml standard. Standard or sample (30µL) was added to the appropriate well with 8-isoprostane tracer (50µL) and antiserum (50µL), and incubated (18hours at $4^{O}C$). Ellman's reagent was added to each well (200µL) and incubated (2 hours with gentle agitation). The plate was read at 405nm.

2.2.9 Data analysis

Data analysis was performed using GraphPad Prism 8 software (version 8.0.1, 2018). At baseline, relationships between markers were determined using linear regression, and to assess any difference at baseline between the two groups, two-sample t-tests were used. To assess changes between different time points in the study, paired t-tests were used. To compare relationships between marker pre-exercise bout/ post-exercise bout, Pearson's correlation coefficient was employed. Outlying values were identified using the ROUT method (Q=1%). Shapiro-Wilk normality, testing Gaussian distribution were used, with alpha significance level set at 0.05. Cohen's D test was also used to assess effect size between time points using the standard deviation of paired differences. GoldenCheetah software was used for heart rate analysis of data obtained by the Garmin watch.

2.3 Results

2.3.1 Baseline

Baseline characteristics are expressed in table 2.1. Ten healthy sedentary participants (7 females and 3 males) were recruited from the University of Birmingham. Retention rate was 100%.

	Mean ± SD
Gender	Female n=7, male n=3
Age (years)	23.50 ± 3.66
Height (cm)	167.84 ± 11.00
Weight (kg)	66.35 ± 11.79
BMI (kg/m2)	23.62 ± 3.83

Table 2.1. Baseline characteristics of the cohort. BMI: body mass index, SD: standard deviation.

2.3.2 Enjoyment

Based on PACES data, participants displayed no preference to one individual activity, although the most popular were tennis and outdoor run, whereas the lab cycle and interval running sessions were least popular. There was also a positive correlation (*r=0.58, p=0.079, df=9*) between IPAQ scores and PACES, implying that those who enjoyed physical activity more were more likely to be more habitually active.

2.3.3 Compliance

There was an 90% completion of the study activities. Five out of the ten participants completed all 7 exercise sessions. Since participants were allowed to choose in which order the exercise sessions were completed, it is likely that those that did not complete all sessions of the study simply did not want to take part in the remaining activities. 50% of the sessions missed were due to a lack of essential skills for completion e.g. unable to swim, or ride a bicycle.

2.3.4 Heart rate data

The Garmin HR data for each activity was split into five zones, representing the percentage of time during the bout that participants were in a particular HR range based on their individual laboratory bout test results (Figure 2.1 (A)). Zones are described as 1: active recovery, 2: endurance, 3: tempo, 4: threshold and 5: VO_2max. Zone 3 represented the target HR range for each bout of acute exercise. For the average distribution of HR zones, the Shapiro-Wilk test of normality demonstrated a normal distribution of results (*p=0.63*), meaning that for each session, participants were within their target 'moderate intensity' zone for more time (34%) than the other zones. Since HR was an easy factor to control within the lab, participants were within zone 3 for 69% of the bout, whereas the run session was 30%, intervals 19%, tennis 25%, aerobics 19%, cycle 34% and swim 25% zone 3 distribution. The run and aerobics sessions both favoured majority HR zone 4 (42% and 30% respectively), whereas intervals, tennis and swimming favoured zone 2 (23%, 27% and 45% respectively).

Each individual's resting heart rate was compared, derived from the 1-week assessment of habitual activity from the Garmin, with heart rate during exercise bouts. Participants were asked to maintain a specific, individual HR range for the duration of each bout, to the best of their ability. A significant positive correlation was observed (*r=0.62, p=0.05, df=9*) between average bout heart rate and resting heart rate.

Figure 2.1 (B) presents the average HR for each bout. The target HR range (70-80% HRmax) is represented by the grey region. In this untrained population, running posed more of a metabolic demand to participants, since the average HR (158.90 bpm ± 6.17) was higher than the target range (134.40-153.40 bpm average). Swimming and tennis were below the range (130.8 ± 16.86 and 134.10 ± 25.25 bmp respectively), possibly due to the skilled nature of these activities, and lack of familiarity for these activities among participants. 52% of all bouts were inside the target HR zone.

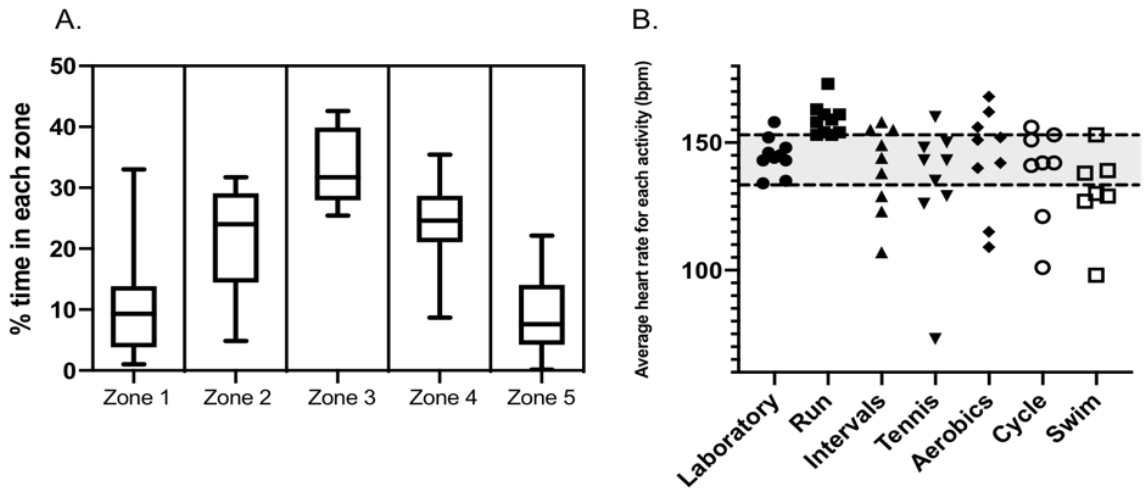


Figure 2.1 (A) The average heart rate zone distribution for all acute bouts of exercise. Median values are expressed, along with upper and lower quartiles, and maximum and minimum values. (B) The heart rate during each activity type, including grey band representing the 50-70% VO_2max target zone.

2.3.5 Erythrocyte glutathione

Whole blood GSH concentration, prior to and post each acute exercise bout, is shown in figure 2.2. Overall, there was a 12.09% increase in circulating GSH between pre-and post-bout. The greatest change between pre-and post-bout measures was caused by the interval running session, where a 71.4% increase in GSH concentration from 0.36 µM (± 0.18) to 0.62 µM (± 0.11) (*p=0.0071, d=2.27, df=4*) was detected. There was a general trend for GSH to increase

across the different activity types, including the lab bout (8.87%, from 0.26 µM (± 0.21) to 0.28 µM (± 0.18), *p=0.48 d=0.74, df=3*), run (8.74% from 0.35 µM (± 0.11) to 0.38 µM (± 0.15), *p=0.66, d=0.21, df=4*), aerobics (0.99% from 0.31 µM (± 0.23) to 0.31 µM (± 0.14*), p=0.96, d=0.02, df=5*) and swim (37.57%, from 0.23 µM (±0.13) to 0.32 µM (± 0.06) *p=0.41, d=0.48, df=3*). For both the tennis and cycle bouts, circulating GSH was decreased following the exercise bouts, 14.26%, from 0.46 µM (± 0.15) to 0.39 µM (± 0.13) *(p=0.30, d=-0.43, df= 6*), and 6.48%, from 0.43 µM (± 0.2) to 0.40 µM (± 0.27) (*p=0.86, d=-0.08, df=4*) respectively. These two bouts demonstrated the lowest average heart rate during the exercise period. GSH response and average HR for the exercise bouts were negatively correlated (*r=-0.45, p=0.26*). The Pearson's r correlation coefficients for average bout heart rate and change in GSH concentration are *r=0.66 (p=0.22)* for run, *r=0.21 (p=0.74)* for cycle, *r= 0.97* (*p=0.026*) for swim*, r=-0.6 (p=0.21)* for aerobics, *r=-0.11 (p=0.82)* for tennis and *r=-0.02 (p=0.98)* for intervals. Due to failure of one of the GSH assay kits, only two pre-post values were attainable for the lab bout.

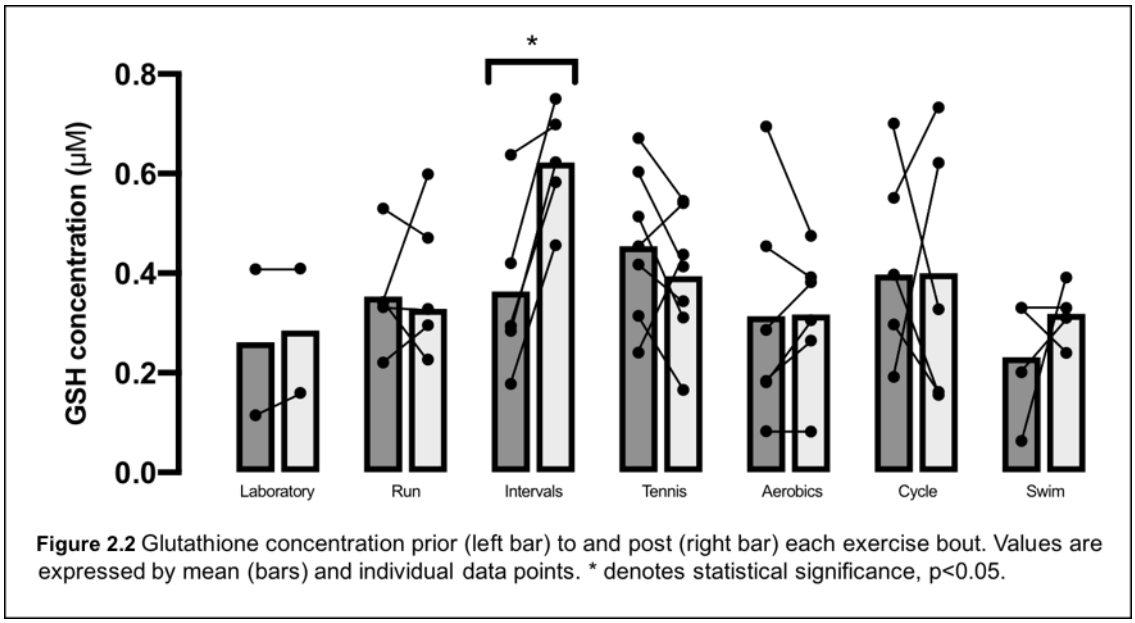


Figure 2.2 Glutathione concentration prior (left bar) to and post (right bar) each exercise bout. Values are expressed by mean (bars) and individual data points. * denotes statistical significance, p<0.05.

2.3.6 8-isoprostane concentration

Figure 2.3 shows the isoprostane concentration across each exercise session: pre-and post. Increases in 8-isoprostane concentration were present for the run (0.39%, from 8.71 pg.ml (± 4.84) to 9.18 pg.ml (± 5.71), *p=0.59, d=0.18, df=8*), aerobics (18.71%, from 9.68 pg.ml (±5.03) to 10.82 pg.ml (±7.43), *p=0.43, d=0.23, df=8*), tennis (12.50%, from 8.39 pg.ml (±3.81) to 9.2 pg.ml (±8.02), *p=0.79, d=0.11, df=6*), intervals (5.82%, from 12.72 pg.ml (±6.09) to 13.48 pg.ml (±9.82*), p=0.80, d=0.08, df=7*), cycle (44.8%, from 8.64 pg.ml (±3.27) to 11.96 pg.ml (±5.57), *p=0.21, d=0.11, df=5*), and swim bouts (59.31%, from 15.51 pg.ml (±16.76) to 20.23 pg.ml (±21.09), *p=0.13, d=0.86, df=4*). Decreases were observed for the lab bout (-12.77%, from 13.3 pg.ml (±8.98) to 11.26 pg.ml (±7.92), *p=0.11, d=-0.5, df=8*) only. One-way ANOVA showed no significant difference between resting plasma 8-isoprostane concentration and post-exercise bout concentration, for any of the acute bouts. 8-isoprostane response and average HR for the exercise bouts were positively correlated (*r=0.21, p=57*). At rest, 8-isoprostane concentration was positively correlated with GSH (*r=0.13, p=0.78, df=6*), and post-bout (*r=0.13, p=0.77, df=6*). The Pearson's r correlation coefficients for average bout heart rate and change in 8-isoprostane concentration are *r=0.36 (p=0.35, df=8)* for the lab bout, *r=-0.23 (p=0.54, df=8)* for run, *r=-0.82 (p=0.046, df=5)* for cycle, *r= 0.19 (p=0.76, df=4)* for swim*, r=0.34 (p=0.41, df=8)* for aerobics, *r=0.46 (p=0.30, df=6)* for tennis and *r=-0.43 (p=0.29, df=7)* for intervals. Outlier analysis (ROUT method) did not identify any outlying values.

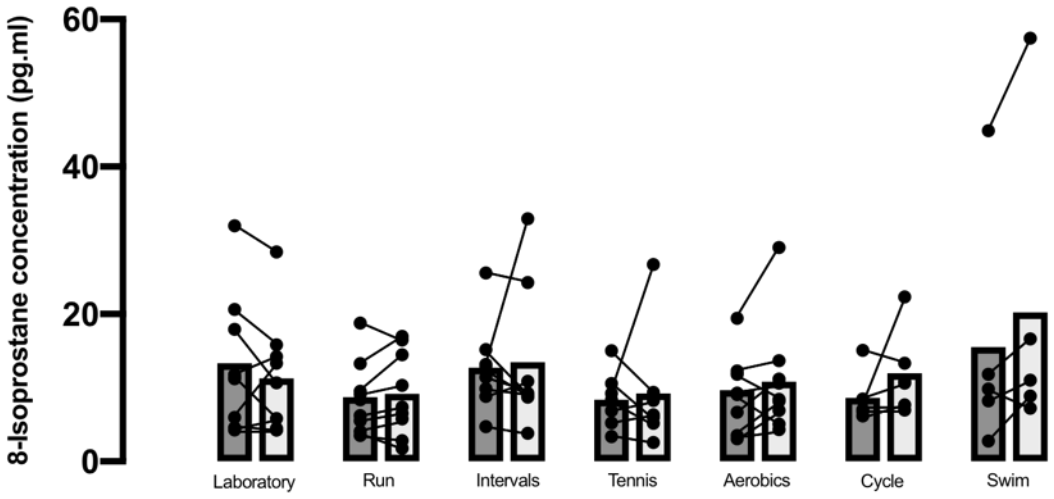


Figure 2.3 8-isoprostane concentration prior (left bar) to and post (right bar) each exercise bout. Values are expressed as mean (bars) and individual data points.

2.4 Discussion

This study demonstrated that it is possible to engage sedentary people in exercise sessions that are able to stimulate a redox response without the onset of free-radical mediated cell damage. The redox system was challenged, as can be observed by the change in GSH post-exercise, but there was no resultant increase in 8-isoprostane concentration between bouts. Using HR as a measure of exercise intensity, it was possible to achieve a target range that is known to elicit change; exercise at this intensity has been shown to provoke ROS release and stimulate adaptation (307, 326, 327). The different exercise bouts incited a similar response in both HR and GSH and thus suggests that these different exercise modes, undertaken at a standardised intensity and duration, can cause a similar physiological stress response in individuals unaccustomed to regular exercise. This model of exercise standardization is particularly relevant in a clinical group where it is important to provide choice of exercise type to engage participants.

2.4.1 Variable HR during exercise

HR results suggest that maintaining a target exercise intensity through some of the exercise modalities was difficult. This may be due to the participants in this study being sedentary (328). Although provision of HR data in real-time was useful to the participant, poor respiratory fitness among the individuals may have made maintaining a consistent HR difficult. Other than the baseline cycle ergometer laboratory bout, the only activity for which the participants were able to maintain HR at the target zone of 3, was cycling. Both indoor and outdoor cycling demonstrate steady-state modes of activity that are easy to monitor, and control in situ (329).

In approximately half (48%) of the sessions, participants were unable maintain target HR for the majority of the exercise sessions. For some participants this may have been due to a higher perceived intensity by the participant, or a reduced intensity due to skill acquisition e.g. tennis or swimming (330). Conversely, in activities such as the outdoor run or aerobics, where sustained HR was more often reported above the target HR range, low fitness may account for the difficulty in maintaining the target. Equally, the metabolic demand of these types of activity is greater. Ainsworth (331) classified the energy costs of human activities, including occupational and household movement. Of the exercise modalities provided by the current study, running and aerobics are reported to have the highest energy demand, measured in MET minutes.

2.4.2 Intensity and duration of exercise intervention needed to elicit a stress response

This study aimed to investigate redox status in response to the different activities. Exercise is a physiological stressor, and as such will stimulate adaptation. The results presented here suggest that the exercise bouts were not a sufficient stressor to elicit lipid peroxidation as assessed by 8-isoprostane concentration. However, this does not mean that the exercise sessions did not provoke ROS. Redox status is a balance between ROS production and antioxidant defense, and where antioxidants are able to balance or quench ROS sufficiently then redox homeostasis is maintained, and adducts or markers of oxidation may not be detected (332). Exercise did however, provoke a change in GSH concentration in some of the bouts, suggesting that the exercise resulted in ROS release. Moderate and strong effect sizes of change are useful indicators of the magnitude of change observed, despite most bouts failing to reach statistical significance. Bloomer et al (333) observed a transient decrease in blood GSH after acute bouts of both aerobic cycling and anaerobic squatting exercises. This is in contrast to the results of the present study, where an average increase of 12.09% across the activities was detected. Only tennis and cycling provoked a similar decrease in GSH to that reported by Bloomer. In a review of acute exercise and oxidative stress, Fisher-Wellman et al (334) concluded that bouts resulted in either no change or a decrease in GSH, but this was dependent on training status, duration of exercise, intensity and mode of exercise. The measure of GSH used in the present study is representative of total GSH; rather than constituent reduced GSH and oxidized

GSH (GSSG) separately. It is difficult to draw accurate comparisons with other studies as the post-exercise reductions of GSH reported in much of the literature may represent reduced GSH only, and since most bouts of exercise often result in a transient increase in GSSG (198, 335, 336), any decrease in reduced GSH may not take this into account.

Increased total GSH may occur as a result of hepatic GSH efflux (337) which occurs to balance the increased production of ROS during exercise. The different exercise modes in the present study were able to provoke slightly different responses in GSH concentration. For example, running, which was the exercise mode where participants consistently achieved highest HR caused a significant increase in GSH post-exercise. Whereas GSH was unchanged or slightly decreased during the cycle and tennis bouts. These sessions also provoked lowest average heart rate, therefore representing the lowest physiological stress. It appears that cycling and tennis were of sufficient intensity to use a greater amount of GSH than the resting state, but not intense enough, shown by the other bouts, to mount a hyper-compensatory response to the decreased pool of local GSH (338, 339). The overall elevation of GSH post-acute bout of exercise demonstrates a response to increased free-radical generation as a result of exercise. Continuous replenishment (recovery) and stimulation of new GSH production and release may account for this transient increase post-bout (340).

The absence of significant change in 8-isoprostane concentration in the current study is consistent with some other studies of acute exercise bouts (314, 341).

However, studies that have employed exhaustive bouts of acute exercise often report increases in plasma 8-isoprostane concentration (342-344). Two of the studies that used exhaustive bouts also used a sub-maximal effort for comparison, where no change in 8-isoprostane plasma concentration was reported (343, 344). In a review by Nikolaidis et al (345), an evaluation of 30 human and animal studies assessing 8-isoprostane changes after acute exercise bouts found that 20 of the 30 studies showed increases between pre- and post-bout. The remaining 10 studies reported no change. Unfortunately, the intensity of each bout was not recorded and therefore no conclusions on the relationship between exercise intensity and lipid peroxidation were drawn. However, it is accepted that exercise intensity is a factor in adduct formation (346, 347). The results presented in the current study do suggest that exercise caused a redox response, but the stimulus was not intense enough to result in lipid peroxidation.

2.4.3 Designing a standardized exercise intervention for a clinical population

The study achieved 100% retention and 90% completion. In an inactive cohort of participants, supporting autonomy i.e. freedom of choice and presentation of alternatives promoted engagement, and therefore it could be suggested will ultimately reduce study attrition (348). Supervision and accompaniment were also key characteristics of this study design. There are a number of studies that have assessed the benefits of a structured, supervised programme of exercise across various clinical groups, compared with less-formal exercise prescription (349, 350). All reports show greater changes to outcome measures, and improved compliance with an intervention if supervision is provided. Alongside

supervision of bouts, exercise accompaniment appears to be a novel approach to intervention design. A meta-analysis by Firth et al (295) that described motivating factors and barriers towards exercise in severe mental illness, a number of the most well-reported factors preventing regular exercise could be negated by accompaniment during an exercise bout. These included lack of motivation, being too shy or embarrassed, feeling unsafe outdoors, lack of support, lack of information and no-one to exercise with.

This study also demonstrated that activity types that have a greater emphasis on learned skills, such as tennis and swimming, resulted in the lowest average heart rates across the range of activities on offer. Therefore, for future trial design, it should be noted that the activities that rely heavily on skill may result in a lower physiological stimulus, until a participant is sufficiently familiar with the required skills to be able to perform the activity at a higher intensity. Additionally, the current study has informed the design of a clinical intervention from a monitoring perspective. The target zone 3 (130-150 bpm) described in this study represented a range slightly above the 50-70% VO_2max (115-150 bpm), and was demonstrably difficult to attain by this cohort of sedentary participants. Moving into a randomised trial in FEP, zone 2 (110-130 bmp) will be the target for participants, a range that can be monitored during each exercise bout by individual participants.

2.4.4 Strengths and Limitations

This study demonstrated novel approaches to providing and assessing exercise bout quality, including the measurement of HR and accompaniment of

participants for the duration of each session. Each of these factors contributed to the successful engagement and monitoring of acute bouts, and thus are examples of the strengths of this study.

This study has several limitations. The swimming and tennis bouts did not reach the target HR range for the study, compared to the laboratory bout, in which all participants (n=10) average HR was above the minimum target. For a dichotomous end-point (either reached the target zone or not) (α=0.05, power =0.8), the swim bout was underpowered by n=2, whereas the underachievement of a target HR can be attributable to participant number (n=8). Conversely, the tennis bout was sufficiently powered (n=9), implying that the lowed average HR was due to a lack of sufficient intensity.

Also, there was not an equal distribution of male and female participants. This may be important when assessing antioxidants since gender can affect GSH metabolism; when estrogen is elevated so is GSH, in a dose-response manner (351). There were also issues with the Garmin devices, such as dropped heart rate signal for periods of time, which can be mitigated in the future by recommending that the watch is worn tighter to the wrist, as suggested by Garmin.

2.4.5 Conclusions

This study demonstrated that it is possible to stimulate change in peripheral GSH, after an acute bout of exercise of differing modalities. This implies that bouts of different modes of exercise can elicit a similar effect when undertaken at an intensity referenced to a pre-determined heart rate zone. These exercise

bouts were of sufficient intensity to cause change a change in GSH, but not a high enough stressor to cause markers of cell damage. This standardisation approach is essential in the design of an exercise intervention in a first-episode psychosis population, where exercise quality and choice of mode is of primary importance but is rarely the priority of a programme.

CHAPTER 3: DESIGNING A FEASIBLE EXERCISE INTERVENTION IN FIRST-EPISODE PSYCHOSIS: EXERCISE QUALITY, ENGAGEMENT AND EFFECT

Abstract

First-episode psychosis (FEP) is the first presentation of a psychotic disorder that usually propagates during early adulthood. FEP represents an important early intervention point to attenuate the negative metabolic risks seen in psychosis and side-effects of antipsychotic medication. Exercise has the potential to improve measures of metabolic health and psychological symptoms, but engaging an FEP population in regular exercise is typically difficult. Interventions that promote enjoyment and attendance may improve participation.

22 men with FEP were randomised to a 12-week intervention of exercise training, or treatment as usual. Exercise bouts were pre-standardised based on measures of heart rate to assess intensity. Positive and negative symptoms of psychosis were assessed via Positive and Negative Syndrome Scale (PANSS), alongside measures of quality of life, perceived disability and habitual activity. The study observed 83% attendance at exercise sessions with target intensity attained throughout the intervention (50-70% VO_2max). There were clinically meaningful decreases in PANSS positive (17.31%) and general psychopathology (10.98%) scores, whereas exercise was protective of negative symptom score increase observed in the control group (13.89%). The

assessment of disability declined as a result of exercise (12.65%) compared with a 20.78% increase in the control group.

This study was able to demonstrate that engagement of FEP patients in a 12-week intervention of moderate intensity exercise was possible, whilst maintaining exercise quality. Positive changes in psychopathology scores and disability show that the benefits of regular exercise are achievable and have a potential positive impact on clinical presentation. Further larger scale definitive trials are needed.

Keywords

First-Episode Psychosis, Youth Mental Health, Exercise, Positive and Negative Symptoms

3.1 Introduction

First-episode psychosis (FEP) is a psychiatric illness characterised by positive and negative symptoms, cognitive difficulties and, for some, the onset of a poor physical health trajectory. FEP represents a crucial early intervention point to target symptoms of psychopathology, reduce the negative metabolic side-effects of antipsychotic medication and encourage a habitually active lifestyle. The transition from FEP to a functional psychosis like schizophrenia (SZ) is accompanied by a plethora of cardio-metabolic diseases.

3.1.1 Sedentary behaviour and metabolic perturbations in severe mental illness

Premature mortality is one of the principal issues associated with severe mental illness, including SZ or related disorders. This is a result of co-morbid illness that is common across chronic mental ill health (31). Sedentariness and physical inactivity are independent but modifiable risk factors that can contribute to all-cause mortality. In a meta-analysis comparing physical activity levels and sedentary lifestyles in SZ, bipolar disorder and major depression, the average amount of sedentary time per day was 476 minutes during waking hours; significantly more than the age and gender matched healthy controls (296). Compounding this, many psychotropic drugs, including antidepressants, mood stabilisers and antipsychotics are associated with long-term metabolic issues, such as significant weight gain (352), insulin resistance and an atherogenic lipid profile (353). NICE recommend that FEP be treated with an antipsychotic medication, alongside cognitive behavioural therapy (354), but do

not address the cardiometabolic risk factors, or subsequent weight gain as a result of antipsychotic medication. Antipsychotic medications exert their influence via influence on dopaminergic, serotonergic, histamatergic and muscarinic receptors (355). The differential effects on weight gain has been attributed to differing affinity for antipsychotic drugs at these receptors (356). Antipsychotics affect the expression of neuropeptides that have been associated with appetite control and energy metabolism. Elevated blood concentrations of leptin (inhibits hunger) (357), ghrelin (enhances food intake ad adipose deposition) (358) and reduced adiponectin adipokines (regulate glucose) (359) are all characteristics of peripheral alterations following antipsychotic prescription. The reduced capacity for glucose control further contributes to dysregulated triglyceride control, insulin resistance and elevated release of low density lipoproteins from adipocytes.

Wetterling et al (360) demonstrated that at first-psychotic hospital admission, drug naive patients had an average BMI less than that of the general population. However, after 5 years of neuroleptic treatment, the proportion of overweight and obese patients outweighed that of the general population. Aside from medication, there are a number of metabolic alterations that have been implicated in FEP, as a predisposition to illness. Dysglycaemia, a term that encapsulates insulin resistance, impaired glucose tolerance and fasting insulin, has been associated with FEP pathology independent of medication, and unbiased by sociological factors (361). In a meta-analysis assessing lipid and adipocytokine status in FEP (362), hypertriglyceridemia and resultant impaired glucose regulation were found to be intrinsic to psychosis, not a side-effect of antipsychotic medication. These underlying perturbed mechanisms are

also cogent targets for exercise training, an enhancer of glucose control (363). Thus, the combination of a sedentary lifestyle with the poor metabolic profile associated with FEP pathogenesis and antipsychotic medication can lead to an exacerbated risk of type 2 diabetes, hypertension and cardiovascular disease (364, 365) in FEP. Exercise is a key modulator of metabolic health, regulating chronic adaptions that not only improve physical fitness but also affect energy metabolism. Prescribing exercise as an adjunct treatment, and encouraging habitual activity in FEP is therefore important to counter the negative metabolic effects both potentially inherent in psychosis and precipitated by antipsychotic medication.

3.1.2 Exercise as an adjunctive therapy

It is well-established that exercise is beneficial to metabolic health measures, as well as the many known positive effects on psychological outcomes (228-230, 232, 233) in FEP and SZ. However, most studies experience difficulties with drop-out or attendance, with many reporting less than 50% compliance with the intervention (233) (366). Supervised exercise training appears to be more successful in engaging participants. In a comparison of FEP and SZ studies that employed supervised training (233, 241, 245, 248-250, 252, 255, 256, 271, 276, 279) over non-supervised (243, 246, 251, 270), the drop-out rates were 21.3% (0-62%) and 51% (26-90%) respectively. Average attendance for supervised sessions was 74.6%. The best attended interventions were those that included group sessions and structured activities, like football (245) or interval training (255), rather than a more general provision of gym access (249), or jogging (246).

All of these approaches to design must be considered when developing an exercise intervention to enable effective exercise. Allowing choice of mode of exercise for the intervention is important in this population, together with supervised exercise training has the potential to promote attendance and reduce drop-out rates that are normally very high in this clinical group.

The cost-benefit of exercise provision in early intervention care may potentially outweigh the increasing cost of treatment of symptoms and long-term cardio-metabolic comorbidities. The 2014 NICE costing statement for psychosis and SZ (367) reported an annual cost of £2.2 billion predicted to rise to £3.7 billion by 2026. The guidelines also stated that "Savings are likely to arise in the future from avoiding adverse health events". It was also reported that 60% of SZ patients have at least one cardiac co-morbidity, costing between $10606 and $15355 per year (368).

3.1.3 Targeting motivations and barriers to exercise participation

A meta-analysis assessed the motivations and barriers to exercise in severe mental illness (295), the most common barriers to participation being stress/ depression, lack of support, tiredness, disinterest and physical illness. These self-reported factors closely link to many of the negative symptoms that could be causally linked to the very high rates of exercise-study attrition seen in this clinical group (301). However, a review by Firth et al (31) reported motivating factors for taking part in exercise, which were to improve physical health, reduce weight, manage mood, mental health benefits, reducing stress and tension and appearance. Stress was reported as both an important motivating

factor as well as a barrier to exercise.

3.1.4 Standardising exercise bouts for use in an intervention

In order to successfully design an intervention of exercise that is replicable in the future, it is crucial to control as many components of each training session as possible. It is necessary to employ a particular training intensity that will have a therapeutic benefit, whilst catering to the preferences of each individual patient, in terms of environment and activity type. The minimum training intensity for improvement in cardiovascular fitness, or VO_2max, is 55-65% heart rate (HR) max (369), so it would be logical to exceed this level in order to observe a meaningful and significant effect of exercise. A review by Roy et al (370) described the most accurate equation for estimating HRmax, (*208-(0.7 x age)),* first proposed by Tanaka (325). The use of this equation, alongside 50-70% VO_2max, which corresponds to 60-80% HR max has the potential to elicit improvements in measures of cardiorespiratory fitness, a method that may be standardized for use during an intervention by setting a personalized 'heart-rate target zone' that corresponds to this range. Standardising exercise bouts for the purpose of research is salient for controlling variables in order to maintain scientific integrity of a study. Translating these techniques into 'real-life', or clinical practice is also useful for progressing with an exercise programme or goal setting to encourage participation and habitual adoption of regular exercise training.

Alongside these intensity measures, the World Health Organisation (WHO) recommend that people aged 18-64 should engage in 150 moderate intensity or 75 vigorous intensity minutes of physical activity per week; simple measures

that can be accrued in variety of ways, and represent a motivating target for sedentary individuals initiating a more active lifestyle.

3.1.5 Aims

This study aimed to assess the feasibility of engaging people with FEP with a 12-week exercise training programme. The exercise intervention was designed to maximize participation and engagement by allowing choice of exercise mode and choice of environment in which to exercise.

3.2 Methods

The data presented in chapters 3 and 4 are collected from the same randomised trial, and therefore represent data collected from the same participants. Chapter 3 presents data on the feasibility of the trial and Chapter 4 focusses on biomarker analysis.

3.2.1 Participants

Male patients, aged 16-35, with a diagnosis of first-episode psychosis (as identified by a psychiatrist in keeping with ICD-10 F 20-29, F31.2, 32.3), were recruited from the community-based Birmingham Early Intervention service. Male participants only were recruited, to negate the need to control for the menstrual cycle in female participants. The oestrogen cycle has a significant effect on antioxidant concentration, which was one of the primary outcomes in

the other arm of this study (371). Patients were within 3-years of first presentation of illness. Eligibility criteria were assessed initially by the primary care coordinators for each patient, followed by assessment of habitual activity, via Garmin activity monitoring device, to ensure a sedentary lifestyle. A general health questionnaire (page 285) was provided to ensure the patient was free from medical conditions that would have prevented participation in moderate intensity aerobic exercise. Exclusion criteria included failure to adhere to pre-testing requirements e.g. refusal to be scanned, provide a blood sample, or significant risk to self or others as identified by the clinical team. Height and weight were assessed at baseline, mid and post-intervention and BMI was calculated at each stage. This study was commenced following approval from the NIHR HRA ethics committee (West Midlands- Edgbaston REC 17/WM/04120).

3.2.2 Randomisation

Following consent, participants were randomised to either the exercise intervention arm or the control arm (treatment as usual; in terms of clinical care, medication, psychotherapy) of the study. A block randomisation method (http://www.randomization.com) was utilised to allow for equal group distribution in the event of poor or slow recruitment.

3.2.3 Exercise intervention

Exercise sessions were delivered by an exercise scientist from the School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham.

Exercise was undertaken 2-3 times per week, for 40-60 minutes, for 12 weeks (figure 3.1).

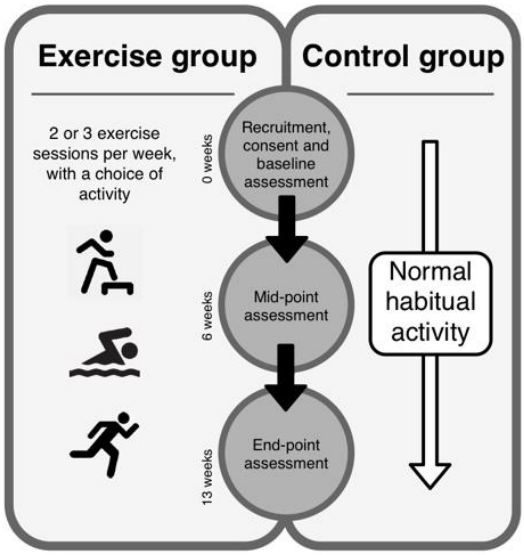


Figure 3.1 Study schematic, including assessment time-points and general outline of the trial period for both exercise and control groups.

To maximise attendance and participation, participants were given a choice of different activities. Modes of exercise included were running; cycling; swimming; tennis; squash; badminton; circuit training and football. Each training session was supervised and accompanied by a researcher in a 1-to-1 manner. Researchers participated in each activity alongside subjects. Each training session was standardised by use of a heart rate target zone, based on 60-80% HRmax for each participant. Attendance, drop-out and tracking of heart rate was measured throughout the intervention period.

3.2.4 Activity tracking

Habitual activity was assessed for one week at baseline, mid-intervention and post-intervention for both exercise and control groups via a Garmin

VivoSmart® HR (Garmin Ltd., Kansas, USA) activity monitoring watch. Measures of steps taken, number of floors climbed, active calories burned and intensity minutes were taken. 'Intensity minutes' refers to the number of minutes per week that a participant achieves a heart rate above a threshold for 'moderate intensity', for periods of 10 minutes or more. Garmin watches were also worn by each participant during every exercise session of the intervention. Average heart rate during the bout, maximum heart rate, number of calories burned, time participating in exercise, and distance covered if applicable were measured during exercise bouts. Exercising participants were also asked to fill in the International Physical Activity Questionnaire (IPAQ) (page 288) at each time-point. This questionnaire was employed as a subjective measure to collect information on habitual activity, including non-leisure time activities (372).

3.2.5 Analysis of activity data

The quality of the exercise undertaken in training interventions was explored based on heart rate during the activity and by activity type. Each heart rate zone is defined by a predicted lactate threshold (LT). Zone 1 <68% LT or 'active recovery', zone 2 69-82% LT or 'endurance', zone 3 83-92% LT or 'tempo', zone 4 94-104% LT or 'threshold and zone 5 105 to max LT or 'VO2max'. Zone 2 represented the average activity distribution (43%), which was equivalent to 110-130 bpm. The aim of the present study was to exercise participants at 50-70% VO_2max, which would be represented by 115-150 bpm average heart rate during a session.

3.2.6 Psychiatric outcomes and functional disability

At each assessment time-point, participants completed the PANSS (373) (page 302), via a structured clinical interview designed to monitor symptoms of psychosis. The PANSS interview assesses positive and negative symptoms and is widely considered the 'gold-standard' method of scoring psychotic behaviour. Interviewers were trained in the completion of PANSS. Quality of life and functional disability were also assessed via the WHOQOL-BREF (374) (page 298) and WHODAS (375) (page 294) questionnaires. The PANSS interviews and questionnaires alike were delivered by the same researcher delivering the exercise intervention (EF).

3.2.7 Data analysis

Data analysis was performed using GraphPad Prism 8 software (version 8.0.1, 2018). For baseline and post-intervention measures, relationships between markers were determined using linear regression, and to assess any difference at baseline between the two groups, two-sample t-tests were used. To assess changes between different time points in the study, paired t-tests were used. To compare relationships between marker pre-intervention/ control period vs mid or post, Pearson's correlation coefficient was employed. Outlying values were identified using the ROUT method (Q=1%). Shapiro-Wilk tests for normality were also performed. Fisher's exact test of qualitative measures

(smoking status) was employed. An alpha significance value of 0.05 was used throughout.

3.3 Results

3.3.1 Baseline

Twenty-two early intervention service users with psychosis were recruited and randomised into the study, aged between 17 and 34 (24.77 years ± 4.92 years) (average length of service use was 19 months). The exercise group (n=11) experienced 4 cases of drop-out, one immediately after baseline assessment, two during the first week of exercise, and one after 10 weeks of intervention duration. In the control group 3 participants dropped out immediately before the 13-week post assessment. At baseline, there were no significant differences between participants in the two groups with respect to physical health markers, symptomology and habitual activity (table 3.1). Antipsychotic, anti-depressant and anti-anxiety medication prescription are expressed in the table.

	Exercise		Control			
	Average	SD	Average	SD	p	Effect size
Age (years)	23.45	3.75	26.10	5.74	0.22	-0.54
Duration of illness (months)	20.45	9.99	18.20	11.64	0.61	0.24
Body composition						
Weight (kg)	82.51	14.69	84.1	16.76	0.82	-0.10
BMI (kg/m2)	25.45	3.71	25.49	4.65	0.98	-0.01
Smoking status						
Tobacco consumption	73%		64%		NA	
Cannabis consumption	50%		91%		NA	
Habitual activity						
Garmin steps (steps/day)	6508.38	1712.15	4735.42	2051.16	0.05	0.86
Garmin floors (floors/day)	7.27	4.62	5.52	3.84	0.39	0.42
Garmin intensity minutes (min/day)	29.47	68.61	30.52	32.33	0.94	-0.04
Garmin active calories (kcal/day)	861.35	475.44	1041.79	914.55	0.58	-0.24
IPAQ MET minutes (min/day)	884.18	711.26	1231.91	1547.86	0.51	-0.29
IPAQ sitting minutes (min/day)	1601.0	1031.0	2121.0	974.5	0.98	0.01
Symptoms and functional measures						
PANSS positive	18.14	5.55	17.63	5.88	0.63	-0.21
PANSS negative	19.43	2.07	18.88	6.66	0.97	0.01
PANSS general	40.43	9.81	39.88	11.37	0.85	0.13
WHOQOL overall (%)	45.41	16.18	48.13	18.77	0.94	-0.27
WHODAS overall (%)	45.93	19.80	27.48	20.29	0.08	0.74

	Exercise		***Control***	
	n	%	n	%
Medication status				
Olanzapine	2	29	2	25
Amisulpride	3	43	0	0
Risperidone	1	14	2	25
Paliperidol	2	29	0	0
Depixol	1	14	1	13
Quetiapine	1	14	2	25
Aripiprazole	1	14	0	0
Cloxipol	0	0	1	13
Haloperidol	0	0	1	13
Anti-depressant prescription	1	14	4	50
Anti-anxiety prescription	1	14	2	25

Table 3.1. Baseline characteristics for both exercise and control groups. Group mean values, standard deviation, P values and effect size (Cohen's D) are expressed. BMI: body mass index, RHR: resting heart rate, PANSS: Positive and negative symptom scale, IPAQ: International physical activity questionnaire, WHOQOL: World Health Organisation quality of life scale, WHODAS: World Health Organisation disability assessment scale.

Recruitment and drop-out patterns are detailed in the consort diagram below (figure 3.2).

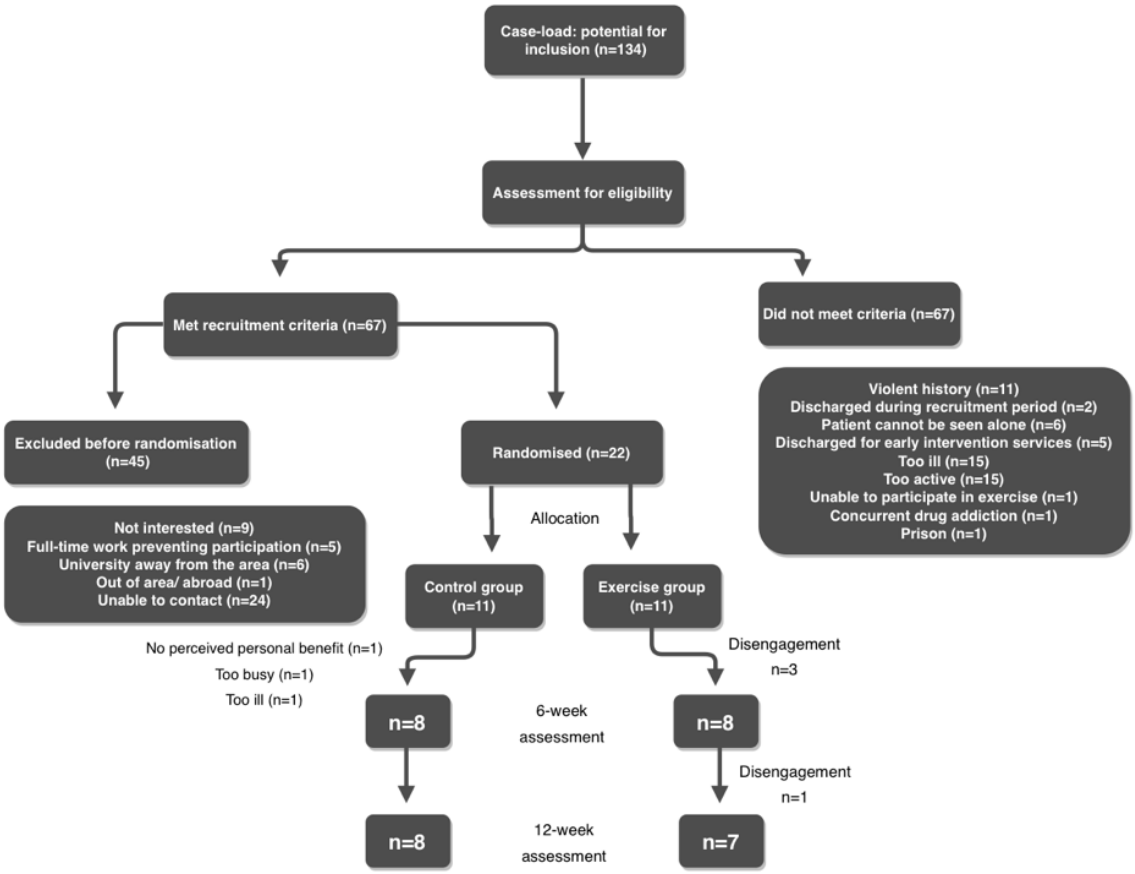


Figure 3.2 Consort diagram detailing the recruitment and retention process of the study. Of the potential 134 participants identified from the Early Intervention caseload, only 67 met recruitment criteria, and of those, 22 participants were randomised to enter either the control group or the exercise group. 8 participants completed the control arm of the study, and 7 the exercise arm.

3.3.2 Attendance and retention

Following consent, retention was 76% overall, 64% in the exercise group and 88% in the control group. For exercise group compliance, there was 83% attendance of 2 out of 3 sessions per week, and 41% attendance of 3 out of 3 sessions per week.

3.3.3 Quality of exercise sessions

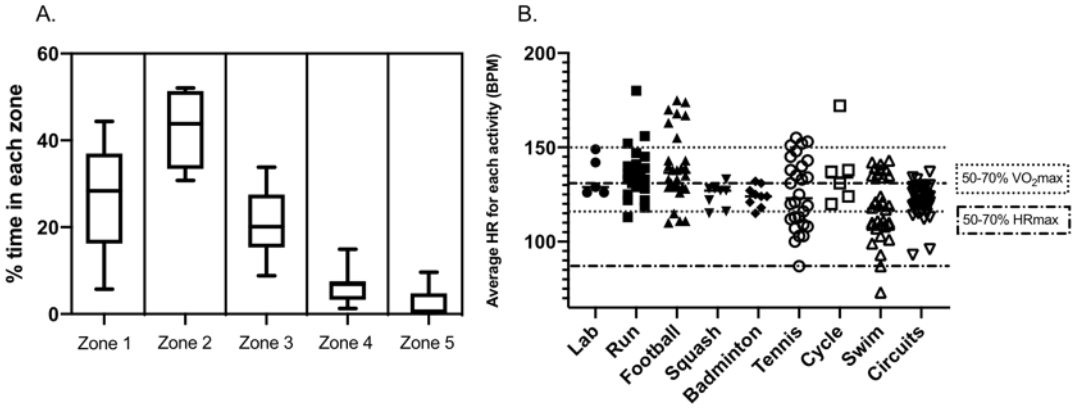


Figure 3.3 (A) The average heart rate zone distribution for each exercise session during the intervention. (B) The average heart rate during each activity type, including bands representing 50-70% HRmax- the most commonly defined 'moderate intensity' and this studies' own target for the intervention of 50-70% VO_2max.

As shown by figure 3.3, participants in the exercise group did not achieve the same intensity measure as the target HR, on average. Each activity type resulted in a different average HR, with running and football achieving the highest average HR. All activities except for circuit training (which required greater rest periods) were within the target HR zone (50-70% VO_2max), and the distribution of heart rate zones for the average session was 43% for the target zone 2.

3.3.4 Concordance with WHO activity recommendations

Using data collected from the Garmin devices at baseline, mid- and end-intervention it was possible to compare the activity levels of the participants with the WHO recommended level (figure 3.4). In the exercise group, intensity minutes per week increased by 73.6% between pre-intervention and mid-intervention, from 214.97 to 358.96 minutes (±44.29) (*p=0.31, df=5*). However,

at the post-intervention assessment the intensity minutes per week had reduced to 150.99 (±158.3) (*p=0.05, df=5*) minutes. The activity in the control group declined between pre-and post-intervention, from 178.92 to 124.10 minutes (±18.59, *p=0.24, df=6*).

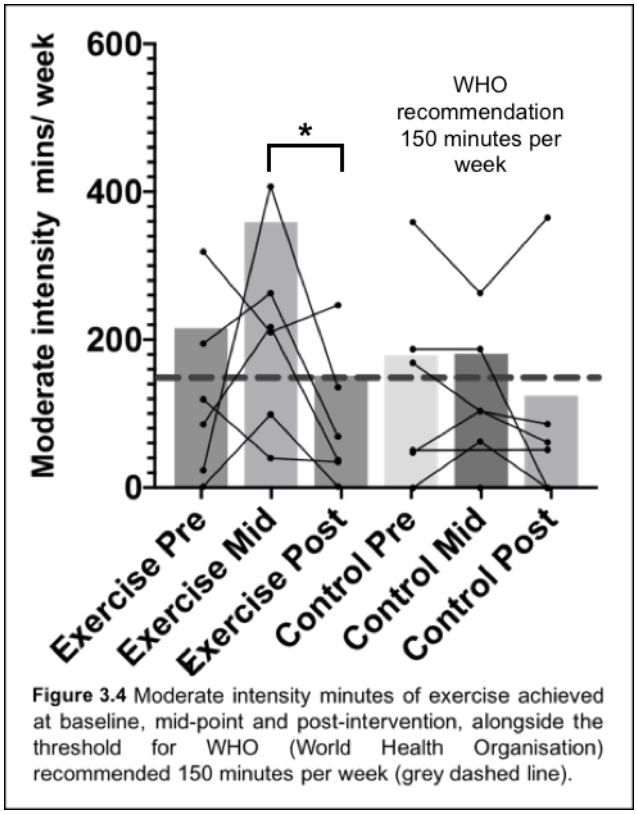


Figure 3.4 Moderate intensity minutes of exercise achieved at baseline, mid-point and post-intervention, alongside the threshold for WHO (World Health Organisation) recommended 150 minutes per week (grey dashed line).

3.3.5 Comparison of self-report activity versus Garmin-measured activity

At baseline, the control group self-reported (via IPAQ) activity was 744 MET minutes per week, which was similar to the number of minutes measured objectively (via Garmin device). Step-count (*r=0.77 p=0.024, df=6*) and intensity minutes (*r=0.86, p=0.006, df=6*) recorded by the Garmin activity monitors were significantly correlated with the self-reported IPAQ MET minutes. The exercise group over-reported their own volume of physical activity. Figure 3.5 shows

Garmin measured moderate intensity exercise per week (bars) alongside self-reported IPAQ measured MET minutes per week (dots).

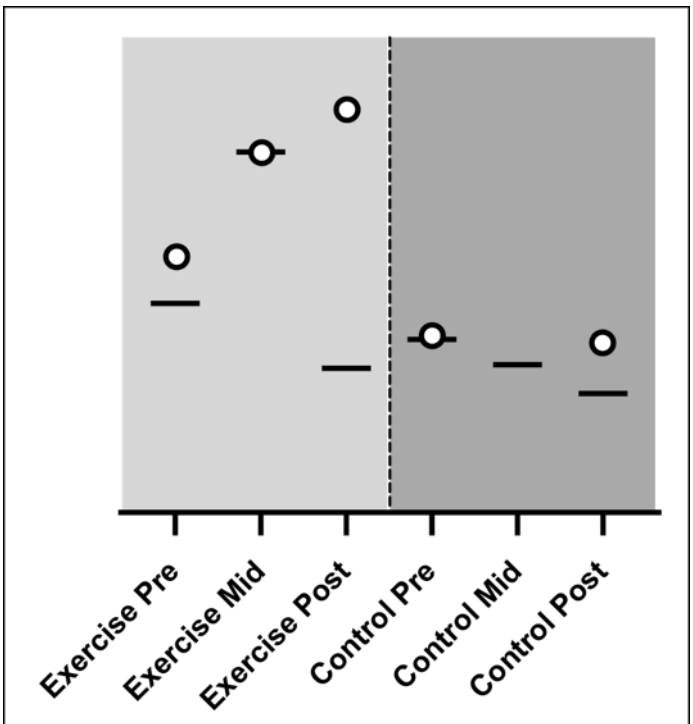


Figure 3.5 A comparison between Garmin measured 'intensity minutes' (bars), with self-reported IPAQ habitual activity score, expressed as MET minutes (dots). Both represent the same measure, but participant perception of actual activity achieved was different to the direct measure.

There was no mid-point measure of IPAQ score for the control group. At mid-intervention assessment (6-weeks), self-reported activity versus Garmin-measured step count (*r=0.53, p=61, df=4*) and intensity minutes (*r=0.20, p=0.74, df=4*) were concordant, however, by end-point assessment the self-reported activity was far greater than actual recorded activity by the Garmin devices; IPAQ versus steps (*r=-0.93, p=0.0021, df=6*) and intensity minutes (*r=-0.011, p=0.9, df=7*). At post-intervention, the control group self-reported activity versus Garmin measures was significantly correlated for intensity minutes (*r=0.94, p=0.0005, df=7*) and positively correlated for steps (*r=0.48, p=0.22, df=7*). There was a significant decline in Garmin-recorded intensity

minutes between mid and post intervention for the exercise group (*p=0.05, df=5*), which was not reflected by the self-reported measure of activity.

3.3.6 PANSS scores and symptomology

In each of the three scores exercise either conferred protection against declining scores observed in the control group or resulted in an improvement between baseline and post-intervention measurement, expressed as mean (±SD). There was a 1.44% increase in negative score in participants in the exercise group (a score of 19.43 (±2.10) to 19.71 (±4.11), *p=0.85, df=6*), and a 13.89% increase in participants in the control group (18.88 (±6.66) to 21.50 (±4.45) *p=0.07, df=7*) (*SMD=-0.33*). There was a 17.31% decrease in positive symptoms of PANSS in participants in the exercise group (18.14 (±5.55) to 15 (±4.97), *p=0.19, df=6*), and a 7.83% decrease in the non-exercising controls (17.63 (±5.88) to 16.25 (±4.68), *p=0.40, df=7*) (*SMD=-0.18*). Participants in the exercise group reported a 10.98% decrease in PANSS general score (40.43 (±9.81) to 36.43 (±5.86), *p=0.17, df=6*), compared to a 2.82% decrease

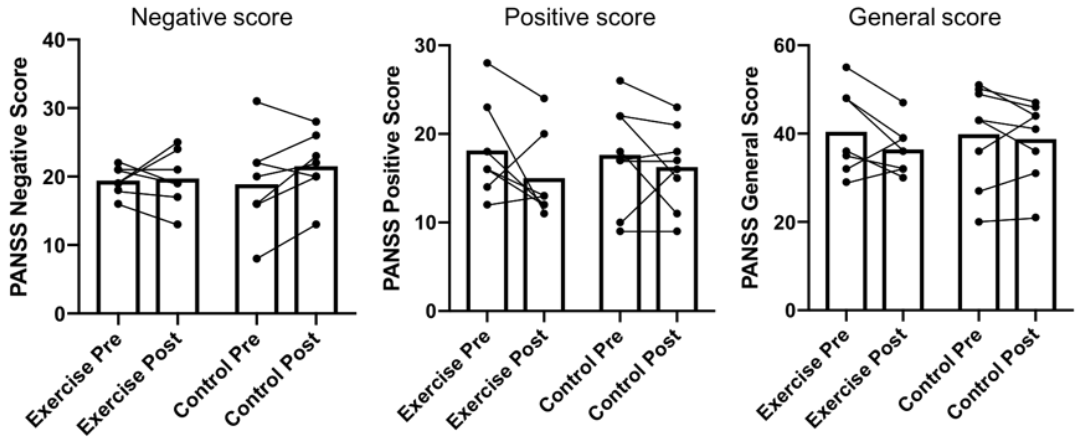


Figure 3.6 PANSS (Positive and Negative Symptom Score) psychotic symptom interview results at baseline and post intervention for exercise and control arms of the study.

observed in participants in the control group (39.88 (±11.37) to 38.75 (±8.97), *p=0.56, df=7*) (*SMD=-0.24*) (figure 3.6).

3.3.7 Patient measures of quality of life and disability

In an assessment of the WHODAS questionnaire, there was an overall decrease of 12.65%, from a score of 45.93 to 40.12 ((± 28.39), *p=0.61, df=6*) in the exercise group, and an increase of 20.78%, from a score of 27.48 to 33.19 ((±19.34), *p=0.43, df=7*) in the control group (*SMD=-0.24*). For the WHOQOL, score increased in both groups with an 11.21% increase, from 45.93 to 50.5 ((±10.60) *p=0.25, df=6*) observed in participants in the exercise group and a 5.32% increase observed in the control group, from 48.13 to 50.69 ((±17.28) *p=0.69, df=7*) (*SMD=0.09*) (figure 3.7).

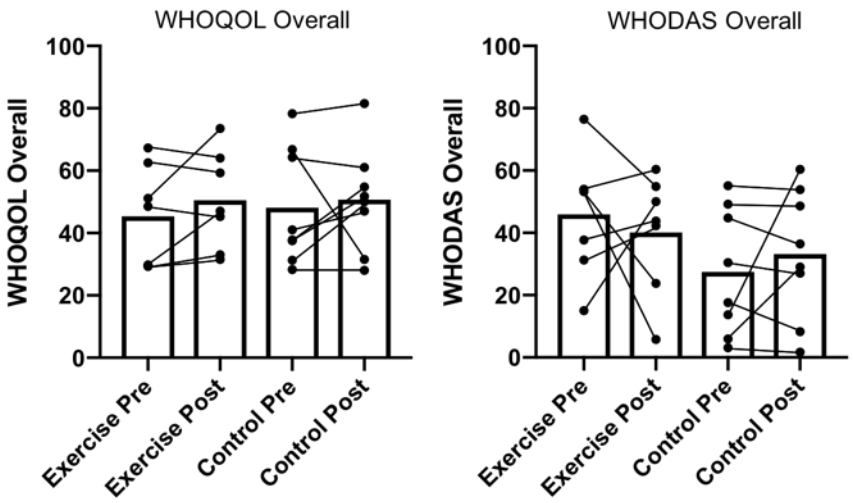


Figure 3.7 WHOQOL (World Health Organisation Quality of Life scale) and WHODAS (World Health Organisation Disability Assessment scale) questionnaire results.

3.3.8 Metabolic changes

No significant change in weight or BMI was observed in either group. A 2.27% increase in body weight was observed in participants in the exercise group (from 86.82 to 88.78kg (±22.24), *p=0.90, df=6)*, but this was not statistically significant. The control demonstrated a 1.40% change (from 85.50 to 86.64kg (±3.91), *p=0.40, df=7*) (*SMD=0.11*). A 7% weight gain is defined as clinically meaningful, particularly in the context of antipsychotic medication prescription (376).

3.4 Discussion

This study has shown that it is feasible to engage an FEP population in a 12-week intervention of exercise training. The present study achieved 83% compliance in exercise twice per week, and 41% compliance in exercise three times per week. This level of engagement in regular exercise resulted in reductions in positive and general psychopathology scores, and the exercising patient group reported a reduced disability score (as can be seen by figure 3.7). Only 13% of eligible cases for this intervention refused as a result of disinterest, suggesting that study design promoted recruitment and participation. The choice of exercise modality offered to patients did not have an effect on reaching the pre-standardised target intensity for exercise bouts. Designing an intervention to maximise participation and retention prioritised exercise session quality, alongside a choice element for the participant. This

choice provided the intervention cohort with novel experiences, and the opportunity for skill acquisition whilst maintaining intensity.

3.4.1 Facilitating a lifestyle change

As well as the barriers to participation and motivating factors described previously, there are a number of common co-morbid conditions and symptoms associated with psychosis that may also impact the ability of FEP patients to initiate exercise. Anergia, anhedonia, tardive dyskinesia, depression, anxiety and poor concentration are all characteristic of FEP, and are contributing factors to the sedentary lifestyle that is often reported in this population. Facilitating behaviour change in a healthy group is challenging, and when transferred to an FEP group the feasibility of exercise alone is difficult, but to encourage a lifestyle change is especially challenging. This study demonstrated the sustainability of regular exercise in an FEP population, when accompanied by another leading the session.

Participants in the current study recorded physical activity via the IPAQ questionnaire, a well-validated self-report measure, in addition to wearing a Garmin physical activity monitor, which measured habitual activity objectively. The results from the Garmin activity device suggest that both the exercise and control groups met the WHO recommendations for moderate intensity physical activity per week, at baseline. This was unexpected, but this observation is likely to be an effect of being enrolled into an ‘exercise study’, without knowledge of which group they will be part of (377). In the UK biobank study (378), people with SZ were significantly less active than the general population,

however self-report measures failed to identify this. In that study, SZ patients and healthy controls both reported the same activity levels, but accelerometer data showed that 95% of SZ patients fell within the bottom 15-35% of the general population. In the current study, the post-intervention measure of habitual activity was decreased compared to that of the mid-intervention measure (figure 3.5), in spite of the perceived high level of activity (IPAQ measure). These data indicate that in order for activity levels to be sustained, the support provided throughout the intervention also needs to be sustained, providing an argument for provision of regular exercise as part of day-to-day care. Alongside the positive psychological and functional outcomes demonstrated by this study, exercise provides a low cost, low side-effect adjunctive therapy to the traditional antipsychotic medications prescribed in FEP.

3.4.2 Functional measures, body weight and psychopathology

Exercise has the potential to protect against emerging functional decline in the early phases of psychosis. This study observed clinically meaningful changes in the PANSS positive and general psychopathology subscales, as well as the self-assessment of disability in those participants engaged in exercise training. The control group demonstrated an increase in the negative subscale, indicating that exercise was protective of this disease-related worsening of symptoms.

Antipsychotic medications are reported to be most effective in reducing the positive symptoms of psychosis (379), whilst having minimal effect on negative

symptoms and cognitive function. Positive symptoms represent a therapeutic target of exercise training, shown by this study, which may ultimately reduce the reliance on psychotropic medication. Conversely, since all participants in this study were treated with at least one antipsychotic, exercise may enhance the effect of medication, although there is no evidence to support this theory.

Physiologically, one of the greatest problems associated with psychosis and subsequent antipsychotic prescription is weight gain and related metabolic illnesses. Adaption to exercise training, as an adjunctive therapy, has a number of metabolic benefits alongside the psychological measures described, including cardiovascular fitness, reduced blood pressure and BMI and improved lean muscle mass (222). Exercise is often used as a means to lose weight in a healthy population. However, body weight was maintained in the participants of the present study, with neither a reduction nor an increase in weight observed, which was expected in this FEP population (241, 244, 380). An absence of reduction in body weight could be a result of continued poor diet alongside the intervention, or may be attributable to an increase in muscle mass often seen alongside a reduction in fat mass (381), which is a positive metabolic change, but often not reflected in body weight assessment. However, as antipsychotic medication often causes weight gain, exercise as an adjunct alongside this treatment alongside antipsychotic treatment has the potential to negate this weight-gain, before it arises (382).

The quality of life score improved in both the exercise and control groups across the 12-weeks of intervention, but a greater increase was recorded in the

exercise group. Although the current study was not powered to detect this, further trials should focus on the physical health and functional outcomes measured here. Measures of quality of life and disability are informative markers of a patients' well-being, with 'recovery-orientated' outcomes being the most important measure from the point of view of a care team, and the patient themselves (383). Other research has described exercise as a "robust add-on treatment in psychosis that improves quality of life, global functioning and depressive symptoms" (384). The WHODAS questionnaire represents a subjective measure of disability and questions the patients' own perception of incapacity in many daily tasks. This acuity is one of the salient barriers to recovery. Exercise may affect this notion as even the process of regular physical activity may interact with the self-perceived notion of inability and disability. The observed reduction in WHODAS score post-exercise versus the increased score in the non-exercising group may therefore represent a change in this perception.

3.4.3 Limitations and strengths

The strength of this study was that it demonstrated the feasibility of undertaking supervised exercise in a FEP group, at least twice per week. However, if considering this study as a trial based on symptom improvement then the main limitation of this study was participant number. The sample size was small, rendering many of the time-point and inter-marker comparisons statistically insignificant, despite the argued clinical importance of patient-orientated changes observed in the exercise group. However, as a proof-of-concept trial, it was successful in terms of intervention design and compliance. Football, the

only team-based activity offered, was very popular, and was an easy way to facilitate target energy expenditure. Based on this experience, provision of group-sports is an aspect of the intervention that's should be increased, across a variety of exercise modes.

Despite the provision of many indoor-based activities, poor and unpredictable weather in the UK was certainly a factor for participant engagement. There was a greater incidence of 'no-shows' during wet and cold days. This effect was particularly salient during the winter months of the year-long trial period.

For the exercise group, the effects of interaction with an engaged 'therapist' throughout the 12-week intervention period may, in comparison with the treatment as usual group, indicate that changes in symptom score could be a consequence of engagement with the therapist. In addition, there is the possibility of bias in symptom assessment, due to the fact that the PANSS clinical interview was performed by the same therapist providing the exercise sessions.

3.4.4 Conclusions

This study demonstrated the feasibility of a supervised exercise intervention in a cohort of males with a diagnosis of first-episode psychosis. Exercise was able to elicit a change in symptomology, quality of life and disability. By employing a study design that prioritised variety in exercise, but ensured the quality and intensity of each training bout this study recorded compliance to the

intervention that was much greater than most other exercise studies in this clinical group.

CHAPTER 4: EXERCISE AS A PROTECTIVE MECHANISM AGAINST THE NEGATIVE EFFECTS OF OXIDATIVE STRESS IN FIRST-EPISODE PSYCHOSIS: A BIOMARKER LED STUDY.

Abstract

First-episode psychosis (FEP) is a psychiatric disorder, characterised by positive and negative symptoms, usually emerging during adolescence and early adulthood. FEP represents an early intervention opportunity for intervention in psychosis. Redox disturbance and subsequent oxidative stress have been linked to the pathophysiology of FEP. Exercise training can perturb oxidative stress and rebalance the antioxidant system and thus represents an intervention with the potential to interact with a mechanism of disease. The aim of this study was to assess the effect of exercise on markers of redox status in FEP.

Twenty-two young men were recruited from Birmingham Early Intervention services and randomized to either a 12-week exercise programme, or treatment as usual (control). Measures of blood and brain glutathione (GSH), markers of oxidative damage, inflammation, neuronal health, symptomology and habitual physical activity were assessed.

Exercise training was protective against changes related to continued psychosis. Symptomatically, those in the exercise group showed reductions in positive and general psychopathology scores, and stable negative symptoms

(compared to increased negative symptoms in the control group). Peripheral GSH was increased by 5.60% in the exercise group, compared to a significant decrease (24.37%) (*p=0.04*) in the control group. Exercise attenuated negative changes in markers of neuronal function (BDNF), lipid damage (TBARS) and total antioxidant capacity. CRP and TNF-α also decreased in the exercise group, although protein and DNA oxidation were unchanged.

Moderate intensity exercise training has the ability to elicit changes in markers of oxidative stress and antioxidant concentration, with subsequent improvements in symptoms of psychosis.

Keywords

First-Episode Psychosis, Youth Mental Health, Exercise, Oxidative Stress, Glutathione, Inflammation

4.1 Introduction

First-episode psychosis (FEP) is a multifaceted psychiatric disorder, characterised by positive and negative symptoms, usually emerging during adolescence and early adulthood (385) (pages 26-30). It is described that a delay in FEP treatment can lead to cognitive alterations and worsened functional outcome (386). FEP patients are highly heterogeneous in symptom profile and treatment response, highlighting the need for improved characterisation of biomarkers related to disease pathophysiology in order to better understand pathology of disease. Many of the biochemical perturbations observed in this population are associated with the psychosis phenotype and thus represent targets for treatment.

4.1.1 Altered redox state in FEP

Several aspects of (neuro) physiology are altered in FEP and schizophrenia patients, including dysfunctional neurotransmitter systems (86, 387), decreased synaptic plasticity (388) and reduced hippocampal volume (389). In addition, inflammation and cellular redox status is also altered. This is often termed oxidative stress, and represents an imbalance between reactive radicals or oxidants, which are naturally produced in aerobic metabolism, and anti-oxidants, which balance and quench oxidants to allow essential signalling processes but prevent damage to biomolecules leading to cell injury or death (390) (pages 37-46). Brain-derived neurotrophic factor (BDNF), which has an essential role in neural signal transmission and synaptic plasticity, is depleted in FEP (391). It has also been suggested that BDNF is responsible for

protecting against oxidative stress by upregulating the expression of antioxidant enzymes (392), highlighting the value of BDNF as a candidate biomarker of psychosis and functional recovery. Redox homeostasis is described in chapter 1 of this thesis (pages 37-43).

One of the principal perturbations in the context of FEP is glutathione (GSH) dysregulation. Briefly, GSH acts as a donor of electrons to potentially damaging free-radical molecules, and scavenges products of oxidation for removal out of the cell. The role of GSH has been described in detail in pages 43-45 of this thesis. When GSH is depleted, as described in FEP, the intracellular redox balance is no longer maintained, and cellular components are more vulnerable to free radical-mediated damage.

There have been reports of decreased total GSH (136, 154, 156), increased oxidized GSH (GSSG) (136), increased lipid (131, 134), protein (394, 395) and DNA damage (396, 397). However, conflicting data by Reddy et al (164) also showed no impairment of antioxidant defences in comparison with a healthy control group, hypothesising that disease stage is related to progression of oxidative stress accumulation. These biomarkers of damage and perturbed oxidative state in FEP may underlie many of the symptoms observed in this clinical group, and represent a target for intervention.

When high brain oxygen concentration is coupled with a modest endogenous antioxidant content (398), and inability of GSH to cross the blood brain barrier, particularly from exogenous supplementation or circulating pools, the antioxidant defence system is stretched. Assessment of brain redox state *in vivo* is difficult, and peripheral markers are usually employed as an estimate of

whole body oxidative environment and antioxidant concentration. However, recent methodological advances in magnetic resonance spectroscopy (MRS) have allowed the quantification of GSH in cerebral tissue (176). This is particularly important in the context of FEP, since brain redox status is perturbed.

4.1.2 Inflammation in FEP

Alongside perturbed mechanisms of redox homeostasis, there is robust evidence to also link a systemic pro-inflammatory state to FEP pathogenesis. Many of the deleterious effects of oxidative stress are mediated by inflammation and vice versa. Inflammation is modulated by activation of nuclear factor κβ (NFκβ), a transcriptional activator of the inflammatory response, via radical signalling (399). The immune system is also a potent source of free radical mediated damage. Activated microglia use NADPH oxidase enzyme to generate superoxide to destroy pathogens (400). Systematic review (401) associates elevated pro-inflammatory cytokines in drug-naïve FEP, including IL-6, TNF-α and IL-1β, as well as evidence of elevated IL-6 in childhood, predicting risk for both psychosis and metabolic dysfunction.

4.1.3 The potential of exercise to normalise redox homeostasis

Exercise is a modifiable lifestyle intervention that has been identified as a potential treatment for psychosis as it has the ability to improve clinical symptoms and restore redox homeostasis in psychosis and schizophrenia.

However, no study has proven the efficacy of exercise in altering the underlying pathology of disease.

Mechanisms underpinning exercise adaptation are well established in studies of healthy participants and are characterized by an antioxidant response to radical signals (315). Elokda (199) demonstrated adaptations to either aerobic training, or combined aerobic and circuit weight training (standardized workload). Both training programmes led to a significant increase in GSH, and decreased oxidised glutathione (GSSG). Regular exercise training enhances antioxidant expression (402) and repair enzyme capacity in healthy humans (403), as well as reducing damage to cellular components through the process of adaptation, such as DNA oxidation (404), lipid peroxidation products (405) and protein carbonyl concentration (201). Additionally, exercise training has the potential to modify other indices of redox status. Studies in healthy volunteers has shown that exercise training may also elevate BDNF (406).

Furthermore, exercise training is beneficial in reducing circulating pro-inflammatory cytokines (407). Inflammation and oxidative stress are strongly linked in a number of pathologies. Systematic review (401) demonstrates elevated pro-inflammatory cytokines in drug-naïve FEP including interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), Il-1β and sIL-2R, and there is clear evidence of elevated IL-6 in childhood, predicting risk for both psychosis and metabolic dysfunction. Regular physical activity can lead to reductions in circulating IL-6 (408, 409), TNF-α (410) and C-rreactive protein (CRP) (411, 412).

4.1.4 Aims

The aim of this exploratory pilot study was to assess the effect of 12-weeks of exercise training on the GSH system in FEP, via *in vivo* brain and peripheral blood measures. To add context around changes in GSH, the effect of exercise on psychotic symptoms, biomarkers of redox state and inflammation were secondary aims, assessed to provide context. No study has yet assessed the effects of exercise training on markers of antioxidant capacity, free-radical mediated damage or chronic inflammation in this clinical FEP group. Exercise as a potential therapy in FEP may partially act through reducing oxidative stress.

4.2 Methods

The data presented in chapters 3 and 4 are collected from the same randomised trial, and therefore represent data collected from the same participants. Chapter 3 presents data on the feasibility of the trial and Chapter 4 focusses on biomarker analysis.

4.2.1 Participants

Male patients, aged 16-35, with a diagnosis of first-episode psychosis (as identified by a psychiatrist in keeping with ICD-10 F 20-29, F31.2, 32.3 diagnostic criteria), were recruited from the community based Birmingham Early Intervention service. Patients were within 3-years of first presentation of illness. Male patients only were recruited, since the oestrogen cycle has a

significant effect on antioxidant concentration, in particular GSH (351). Eligibility criteria were assessed initially by the primary care coordinators for each patient, followed by assessment of habitual activity to ensure a sedentary lifestyle, as well as the use of a general health questionnaire to assess cannabis use and confirm the patient was free from medical conditions that prevent participation in moderate intensity aerobic exercise. Exclusion criteria included failure to adhere to pre-testing requirements e.g. provide a blood sample, or significant risk to self of others as identified by the clinical team. Study assessments took place at baseline, mid-point (for the exercise group only), and post-intervention. This study was commenced following approval from the NIHR HRA ethics committee (West Midlands- Edgbaston REC 17/WM/0412). Intervention design, quality and patient-orientated outcomes were assessed and are summarised by Fisher et al (413).

4.2.2 Randomization

Following consent, participants were randomized to either the exercise intervention group or the control arm (treatment as usual) of the study. A block randomization method (http://www.randomization.com) (414, 415) was used to allow for equal group distribution in the event of poor recruitment.

4.2.3 Exercise intervention

Exercise sessions were designed and supervised by a trained researcher at the School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham. The intervention was 12 weeks long, with each participant

required to exercise at least 2 times per week, for 40-60 minutes per session. In an effort to maximise attendance and compliance, which has historically been difficult in this group (301), participants were given a choice of different activities to undertake at each session (Available exercise: running; cycling; swimming; tennis; squash; badminton; circuit training and football). Each training session was standardised by heart rate target zone, based on 70-80% HRmax. The minimum training intensity for improvement in aerobic fitness is 55-65% HR max (369), therefore in order to observe a meaningful and significant effect of exercise, intensity was set above this. The ACSM recommends 50% VO_2max as a minimum intensity for exercise training (416), which corresponds to 65% HR max. The relationship between HR and VO_2 reflect energy expenditure in a linear fashion, up to 85% max HR (417). Energy consumption, resting heart rate, active calories and intensity minutes (equivalent to moderate-intensity exercise) was tracked at three time-points in the intervention period (baseline, mid-intervention and post-intervention), and for the duration of each exercise bout by Garmin VivoSmart™ HR activity monitor (Garmin, USA).

4.2.4 Blood sampling

Cephalic/ cubital venous blood samples were taken at pre (0 weeks), mid (6 weeks) and post (13 weeks) intervention time-points, into BD Vacutainer® MAP K_2EDTA 1.0mg tubes (BD, USA). Following removal of whole blood (2 x 1ml) for comet assay and glutathione analysis, samples were centrifuged (2000rpm, 15 min, 10^{o}C) and the resultant plasma was aliquoted for subsequent determination of FRAP, TBARS, 8-isoprostane, BDNF, IL-6, CRP, TNF-α and

protein carbonyl. All samples were stored at -80ºC for a maximum of 9 months until analysis.

4.2.5 MRS glutathione measurement

Scans were conducted at the Birmingham University Imaging centre (BUIC), using a 3 Tesla Phillips Achieva MRI scanner, with a 32-channel head coil. The ^{1}H single-voxel Mescher-Garwood point-resolved spectroscopy (MEGA-PRESS) method of acquisition was employed, with TR=2s; TE=131ms; 55 Hz bandwidth editing pulse at 4.56ppm; and 1024 complex data points acquired at a sampling frequency of 2000 Hz, followed by water suppression. The MRS protocol included a T1-weighted structural MRI for MRS planning (5 minutes). The volume of interest (VOI) was located in the anterior cingulate cortex (30 x 30 x 20mm) (figure 4.1). Total MRS scan time for GSH measurement was 18 minutes. Spectral alignment was completed using the RATS method (Robust Alignment to a Target Spectrum) (418), implemented in R (v3.5.0) (Vienna, Austria), and integrated into the SPANT (SPectroscopy ANalysis Tools) package (v0.12.0) for MRS analysis. GSH was then fitted using TARQUIN (Totally Automatic Robust QUantitation IN nuclear MR) (assessment tool for

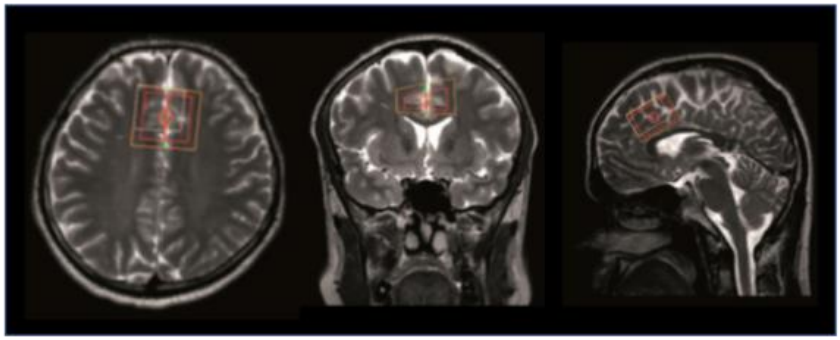


Figure 4.1 T1 weighted MRI image demonstrating the volume of interest (VOI) pictured. 30 x 30 x 20 mm. Placed in the anterior cingulate cortex. Image above represents axial, frontal and sagittal views from left to right.

molecule quantity determination). An example spectrum is detailed in chapter 1 of this thesis (page 55), following post-processing, with the GSH peak highlighted.

4.2.6 Blood analyses

4.2.6.1 Blood glutathione

Whole blood glutathione was determined using a commercially available luminescence-based assay (GSH-Glo™ Glutathione Assay, Promega, Wisconsin, USA). This protocol has already been described in detail in chapter 2 (page 82).

4.2.6.2 Comet assay

Validation of using thawed versus fresh blood from analysis of comet tail intensity was first conducted (supplement 1). Finding no statistical difference in single-strand break intensity, analysis of freeze-thawed whole-blood samples was commenced. From whole blood collected in K_2EDTA tubes, samples were frozen for up to 9 months at -80 degrees. Cells were then diluted in low melting point agarose (LMA) (1:10) (LMA agarose 0.7% w/v in PBS) and suspended onto pre-coated 1% NMA coated slides. After incubation at 4 degrees more LMA was added to the slide then allowed to set at 4 degrees. Slides were submerged in lysis solution (2.5M NaCl, 0.1M Na_2EDTA-2H_2O, 8mM Tris HCl with 10% DMSO and 1% Triton-X for working solution) overnight, at 4 degrees. Slides were placed into an electrophoretic chamber and submerged in electrophoretic buffer (300mM NaOH, 1mM Na_2EDTA into a final volume of

2.5L dH_2O) to allow the unwinding step to occur. The current was run, ensuring that the voltage was constantly maintained (0.78V/cm and 300mA for 20 minutes). Slides were transferred to neutralizing buffer (0.4M Tris HCl), followed by fixing with -20 degrees EtOH, followed by drying at room temperature. Active SYBR-GOLD staining solution (Invitrogen, S11494) (90μl) (1:1000 dilution of stock solution in neutralization buffer) was distributed across the slides and incubated at room temperature before scoring, using a fluorescent microscope (figure 4.2). The slides were examined at 320 × magnification (32×/0.40 dry objective) using a fluorescence microscope (Zeiss Axiovert 10, Germany), fitted with a 515–560 nm excitation filter and a barrier filter of 590 nm. A USB digital camera (Merlin, Allied VisionTechnologies) captured the images, which were analysed using a personal computer-based image analysis system (Comet assay IV, Perceptive Instruments). Images of one hundred randomly selected nuclei were analysed per slide. Measurement of median percent tail DNA was chosen to assess the extent of DNA damage as it exhibits far less inter-run variability than other comet parameters due to its independence from electrophoresis voltage and run time (419).

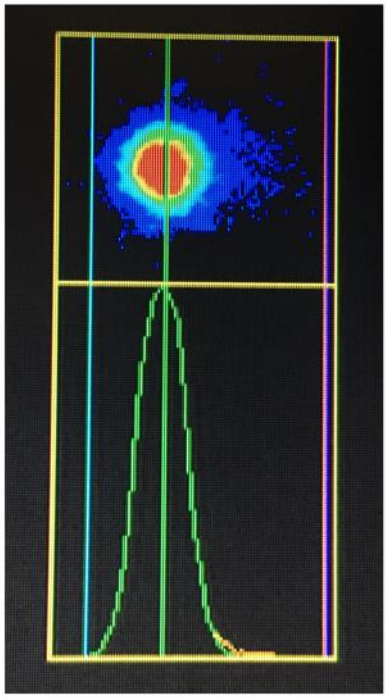


Figure 4.2 Cell pictured via the fluorescent microscope. The outer blue ring highlights the single-strand breaks of DNA, forming a 'comet' tail drawn towards the anode end of the slide.

4.2.6.3 Lipid peroxidation

8-Isoprostane determination

The concentration of 8-isoprostane in plasma samples was determined by a competitive enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (516351 Cayman Chemical, Michigan, USA). This protocol has already been described in detail in chapter 2 (page 83).

Thiobarbituric acid reactive substances

As a measure of lipid peroxidation via MDA (malondialdehyde) production, TBARS (thiobarbituric acid reactive substances) method is a non-specific measure of lipid damage in plasma. Standards were prepared by addition of 125μM 1,1,3,3-tetramethoxypropane stock to ddH_2O, with a standard curve being generated from concentrations between 0 and 50μM. Sample and standard (100μl) was added to TCA solution (100μl) (15ml trichloroacetic acid (1.23M) with 30ml H_2SO_4 (0.05M)) and mixed. Colour reagent (800μl of 30ml thiobarbituric acid (0.67g/100ml ddH_2O diluted 1:1 in glacial acetic acid), ddH_2O (120ml) and BHT (500μl of 200μM butylated hydroxytoluene; 44.07mg/100ml 100% ethanol, diluted 1:10 in 100% ethanol) was added to each tube and vortexed. Tubes were placed in boiling water (100 degrees) for 1 hour, and immediately placed in an ice bath to stop reaction. After 10 minutes, tubes were centrifuged (10 minutes at 1600 x g). 200μl was removed from each tube, without disturbing the pellet, and transferred to wells of a 96-well plate. Absorbance was read at 540nm.

4.2.6.4 Protein determination

Plasma protein concentration was determined using the BCA (bicinchoninic acid) method characterised by Smith et al (420). Standards were prepared by dilution of bovine serum albumin (BSA), in a concentration range of 0-1mg/ml (supplement 2). A working solution was prepared with copper sulphate (4% w/v) and BCA solution in a 1:50 ratio. Sample were diluted 1:20 were loaded into wells of a 96-well microplate. Working solution was added to each well (200µl), and the plate incubated (30 minutes at 37 degrees), protected from light. Absorbance was read at 540nm.

4.2.6.5 Carbonyl concentration

Based on the methods described by Buss et al (421) and Alamdari et al (422), protein carbonyl concentration was determined in plasma. Samples/ standards were diluted in coating buffer (0.05mg/ml) (sodium carbonate 50mM, pH 9.2), and standard or sample (50µl) were loaded into wells of a 96-well microplate. The plate was incubated (1 hour at RT). The plate was the rinsed with wash buffer (TBST 0.05%) four times. 1mM DNPH (50µl) (2,4-dinitrophenylhydrazine) (in 2M HCl) was added to each well, and incubated (1 hour at RT). The wash step was repeated. Blocking buffer (200µl) (TBST 0.1%) was added to each well, and incubated overnight at 4 degrees. The wash step was repeated, and anti-DNP antiserum (50µl, 1:1000) (Sigma Aldrich, D9656) was added to each well and incubated (RT for 2 hours). Wash step was repeated, and peroxidase labelled secondary antibody (50µl) (rat anti mouse IgE in blocking buffer 1:5000) was added to each well, and incubated (2 hours at RT). The wash step was repeated, and substrate (50µl) (citrate phosphate

buffer 0.15M, pH5) was added, and incubated (15 minutes at RT), away from light. Stop solution (50μl) (2M H_2SO_4) was added to each well to arrest the colour reaction, and absorbance read at 490nm.

4.2.6.6 Inflammatory markers

Plasma IL-6, CRP and TNF-α concentrations were determined using commercially available immunoassay kits (Human IL-6 Quantikine ELISA kit (D6050), Human TNF-alpha Quantikine ELISA kit (DTA00C) and Human C-Reactive protein/ CRP Quantikine ELISA kit (DCRP00)), from R&D systems, Minnesota, USA. Concentrations were determined according to the manufacturer's instructions.

Interleukin-6

IL-6 cytokine concentration was determined using a commercially available immunoassay kit (Human IL-6 Quantikine ELISA kit (D6050), R&D systems Inc. Minnesota, USA). Standards were prepared in a serial dilution from a 300pg/mL stock solution of calibrator diluent RD6F (animal serum with preservatives) and human IL-6 standard (recombinant human IL-6 in buffered protein base, lyophilized). Standard concentrations ranged from 100pg/mL to 3.13 pg/mL. Assay diluent RD1 (100μl) (buffered protein base with preservatives) was added to each well of a 96-well microplate. Plasma sample or standard was added to each well (100μl), and incubated (2 hours at RT). Each well was aspirated, and washed (wash buffer: diluted solution of buffered surfactant with preservative), the process repeated four times. Human IL-6 conjugate (200μl) (polyclonal antibody specific to human IL-6 conjugated to

horseradish peroxidase) was added to each well, and incubated (RT, 2 hours). Aspiration/ wash step was repeated, and substrate solution (200μl) (stabilized hydrogen peroxide and stabilized chromogen TMB (tetramethylbenzidine)) was added to each well, and incubated (RT for 20 minutes) in the dark. Stop solution (50μl) (2M sulphuric acid) was then added to each well. A colour change from blue to yellow was observed, and the optical density of each well was determined at 450nm, and 540nm. The 540nm plate was subtracted from the 450nm plate to correct for optical imperfections in the plate.

C-reactive protein

C-reactive protein concentration was determined using a commercially available immunoassay kit (Human C-Reactive protein/ CRP Quantikine ELISA kit (DCRP00), R&D systems Inc. Minnesota, USA). Standards were prepared in a serial dilution from a 50 ng/mL stock solution of calibrator diluent RD5P (concentrated buffered protein base) and human CRP standard (50ng/vial of recombinant human CRP in buffered protein base). Standard concentrations ranged from 25ng/mL to 0.78ng/mL. Assay diluent RD1F (100μl) (buffered protein base with preservatives) was added to each well of a 96-well microplate. Plasma sample or standard was added to each well (50μl), and incubated (2 hours at RT). Each well was aspirated, and washed (wash buffer: diluted solution of buffered surfactant with preservative), the process repeated four times. Human CRP conjugate (200μl) (monoclonal antibody specific for human CRP conjugated to horseradish peroxidase) was added to each well, and incubated (2 hours at RT). Aspiration/ wash step was repeated, and substrate solution (200μl) (stabilized hydrogen peroxide and stabilized

chromogen TMB was added to each well, and incubated (RT for 30 minutes) in the dark. Stop solution (50μl) (2M sulphuric acid) was then added to each well. A colour change from blue to yellow was observed, and the optical density of each well was determined at 450nm, and 540nm. The 540nm plate was subtracted from the 450nm plate to correct for optical imperfections in the plate.

TNF-alpha

Tumour necrosis factor alpha (TNF-α) concentration was determined using a commercially available immunoassay kit (Human TNF-alpha Quantikine ELISA kit (DTA00C), R&D systems, Minnesota, USA). Standards were prepared in a serial dilution from a stock solution (10000 pg/mL) of calibrator diluent RD6-12 (buffered protein base) and human TNF-α standard (recombinant human TNF-α in a buffered protein base). Standard concentrations ranged from 1000pg/mL to 15.6pg/mL. Assay diluent RD1F (50μl) (buffered protein base with preservatives) was added to each well of a 96-well microplate. Plasma sample or standard was added to each well (50μl), and incubated (2 hours on a plate shaker at RT). Each well was aspirated, and washed (wash buffer: diluted solution of buffered surfactant with preservative), the process repeated four times. Human TNF-α conjugate (200μl) (polyclonal antibody specific for human TNF-α conjugates to horseradish peroxidase) was added to each well, and incubated (2 hours at RT on a plate shaker). Aspiration/ wash step was repeated, and substrate solution (200μl) (stabilized hydrogen peroxide and stabilized chromogen TMB was added to each well. The plate was incubated (30 minutes at RT in the dark). Stop solution (50μl) (2M sulphuric acid) was then added to each well. A colour change from blue to yellow was observed,

and the optical density of each well was determined at 450nm, and 540nm. The 540nm plate was subtracted from the 450nm plate to correct for optical imperfections in the plate.

4.2.6.7 Total antioxidant capacity using the FRAP method

The FRAP method (ferric reducing ability of plasma) was developed by Benzie and Strain (423). Standards were prepared, using a 0-1000μM concentration range of ascorbic acid. FRAP reagent- consisting of acetate buffer (300mM sodium acetate at pH 3.6 (3.1g) into 16ml glacial acetic acid per litre of buffer solution), TPTZ (2, 4, 6-Tris (2-pyridyl)-S-triazine) solution (160mM: 0.05g/ml-0.1g TPTZ in 2ml methanol, then 2ml into 30ml 40mM HCl) and $FeCl_3.6H_2O$ solution (0.332g ferric chloride in 100ml ddH_2O) was added to the samples/ standards on the plate, incubated for 8 minutes at room temperature, and read at 650nm. FRAP concentrations were determined by linear regression relative to ascorbic acid.

4.2.6.8 BDNF

Brain derived neurotrophic factor (BDNF) concentration was determined using a commercially available immunoassay kit (Human BDNF ELISA kit (ab99978), Abcam, Cambridge, UK). Standards were prepared using a serial dilution. A 400ng/mL stock solution of BDNF standard (assay diluent A (0.01% sodium azide), and BDNF stock standard) was used to prepare a set of standards ranging in concentration from 0.006ng/mL to 16ng/mL. Plasma sample or standard were loaded into the wells of a 96-well microplate (100μL). Each well

of the plate contained immobilized BDNF antibody. The plate was covered, and incubated (4 degrees overnight with gentle shaking). Solution was discarded and the plate was washed 4 times with a 1 X wash solution. 1 X biotinylated anti-human BDNF detector antibody (100µL) was added to each well. The plate was incubated (RT for 1 hour). The wash step was repeated, before addition of 1 X HRP-streptavidin solution (100µL) to each well. The plate was incubated (RT for 45 minutes on a plate shaker). Wash step was repeated. TMB one-step substrate reagent, a chromogenic substrate for horseradish peroxidase, was added to each well (100µL), and the plate was incubated (30 minutes at RT), protected from light. Stop solution (50µL) (1.96% sulphuric acid) was added to each well, and the optical density of each well was read at 450nm.

4.2.7 Psychiatric outcomes

At each assessment time-point, participants completed the Positive and Negative Symptom Scale (PANSS) (373), via a structured clinical interview designed to monitor symptoms of psychosis. The PANSS interview assesses positive and negative symptoms, and is widely considered the 'gold-standard' method of quantifying psychotic behaviour. Interviewers were trained in the completion of PANSS.

4.2.8 Data analysis

Data analysis was performed using GraphPad Prism 8 software (version 8.0.1, 2018). At baseline, relationships between markers were determined using linear regression, and to assess any difference at baseline between the two groups, two-sample t-tests were used. To assess changes between different

time points in the study, paired t-tests were used. To compare relationships between marker pre-intervention/ control period vs mid or post, Pearson's correlation coefficient was employed. Outlying values were identified using the ROUT method (Q=1%). Shapiro-Wilk normality, testing Gaussian distribution were used, with alpha significance level set at 0.05. Standardised mean difference (SMD) test was also used to assess effect size between time points, and intervention groups, using the standard deviation of paired differences.

4.3 Results

4.3.1 Baseline

Twenty-two early intervention service users were recruited and randomised into the study, aged between 17 and 34 (average length of service use, at recruitment, was 19 months). Baseline characteristics are presented below (table 4.1). From a potential case-load of 134, 67 patients did not meet eligibility criteria. Of the 67 remaining, with potential for inclusion into the study, 22 were randomised. Reasons for eligible patients not being randomised included discharge from the early intervention services, work/ university time commitments, no interest in taking part in the study, and an inability to make initial contact with the patient. 15 participants completed the trial, across the exercise (n=7) and control (n=8) groups. Peripheral biomarker analysis and symptom assessment was completed for all participants, whereas only 4 members of each intervention group were able to complete the MRS brain scan.

	Exercise		*Control*		
	Average	SD	Average	SD	p
Age (years)	23.45	3.75	26.10	5.74	0.82
Weight (kg)	82.51	14.69	84.1	16.76	0.98
BMI (kg/m^2)	25.45	3.71	25.49	4.65	0.61
Duration of illness (months)	20.45	9.99	18.20	11.64	
Tobacco consumption	73.0%		64%		NA
Cannabis consumption	50.0%		91%		NA
Brain GSH (mM/kg) (tNAA)	0.29	0.05	0.34	0.05	0.25
GSH (µM)	0.59	0.85	0.72	0.24	0.08
TNF-α (pg/mL)	84.82	13.28	87.44	2.89	0.75
CRP (ng/mL)	2.43	1.63	3.07	0.67	0.54
IL-6 (pg/mL)	1.27	0.40	1.94	1.51	0.31
BDNF (ng/mL)	47.20	23.52	41.06	26.64	0.73
Protein carbonyls (nmol/mg protein)	138.2	46.53	241.01	161.54	0.16
TBARS (µM)	12.94	3.86	10.43	2.45	0.21
Comet (%)	3.67	1.53	2.76	1.42	0.31

Table 4.1. Summary of baseline characteristics, for both exercise and control groups. BMI: body mass index, GSH: glutathione, TNF-α: tumor necrosis factor alpha, BDNF: brain-derived neurotrophic factor, CRP: C-reactive protein, IL-6: interleukin 6, TBARS: thiobarbituric acid reactive substances.

4.3.2 Brain and blood glutathione

Peripheral whole-blood GSH increased by 6.13%, from 0.72 to 0.76 µM (±0.20) (*p=0.59, df=6*) in the exercise group, but significantly decreased by 24.37%, from 0.92 to 0.70 µM (±0.25) in the control group (*p=0.04, df=7*), in a group-time interaction (*SMD=0.47*).

Ineligibility for scanning included foreign metal in the body (n=2), anxiety/ fear of enclosure in the scanner (n=6) and injury preventing prolonged periods of time in the supine position (n=1). Brain GSH concentration increased by 8.50%, from 0.30 to 0.32 mM/kg (±0.07) (*p=0.55, df=3*) in the exercise group, and 8.80%, from 0.34 to 0.37 mM/kg (±0.06) (*p=0.34, df=3*) in the control group (*SMD=-0.06*). This data is limited by the small sample of the cohort that were

able to be scanned (exercise group n=4 and control group n=4 for pre-post scans).

The r value for the exercise group pre-intervention was 0.91 (*p=0.09*), and post-intervention 0.06 (*p=0.93*). These changes indicate that, in this exercising group of participants, exercise affected peripheral and brain GSH regulation differently. The difference between these two correlation coefficients is *z=1.04* (*p=0.15*). This indicates that the relationships at baseline and post-intervention are not significantly different. Both blood and brain GSH changes are presented in figure 4.3. Additionally, in the exercise group, the relationship between GSH change and intensity minutes increase, as a result of being in the intervention, was strongly positively correlated, for both blood (*r=0.61, p=0.14*) and brain (*r=0.68, p=0.32*) measures.

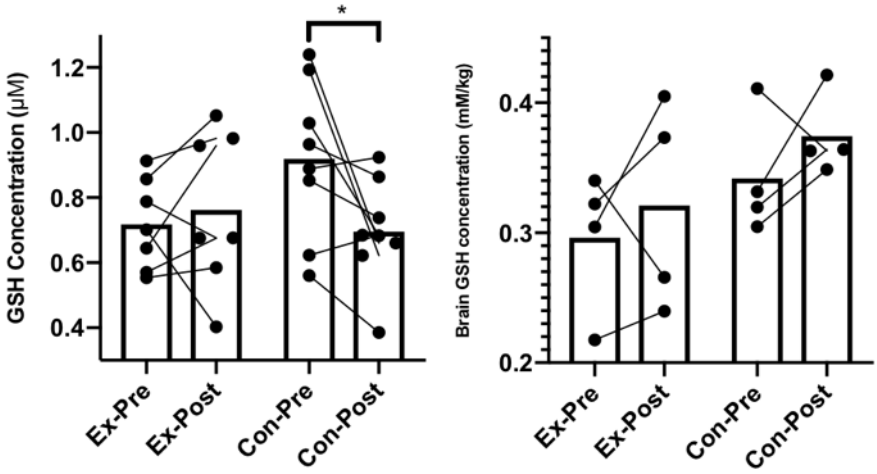


Figure 4.3 A comparison of whole blood GSH (left) versus cerebral GSH (right), pre-intervention versus post-intervention. Both measures are expressed a mean and standard deviation. * indicates a significant difference between pre- and post measures.

In a comparison of whole blood GSH change (over the 12-week study period), and change in symptoms, correlation analysis showed that for the exercise

group, PANSS general score (*r=-0.16, p=0.74, df=6*), positive symptom score (*r=0.30, p=0.51, df=6*) and negative symptom score (*r=-0.50, p=0.25, df=6*) were differentially correlated in response to exercise training. In the control group, PANSS general score (*r=-0.68, p=0.065, df=7*), positive symptom score (*r=-0.35, p=0.39, df=7*) and negative symptom score (*r=-0.50, p=0.20, df=7*) demonstrated negative correlations between changes in peripheral GSH and symptomology.

4.3.3 Markers of oxidative damage

Lipid peroxidation (assessed by TBARS and 8-isoprostane concentration), protein oxidation (assessed by protein carbonylation) and DNA oxidation (assessed by single-strand breaks) are presented in figure 6.

TBARS concentration increased by 5.6%, from 12.94 to 13.69 μM (± 2.37) (*p=0.47, df=5*) in the exercise group, and significantly increased by 32.5%, from 10.43 to 13.92 μM ((± 1.09) (*p=0.0005, df=5*) in the control group (*SMD=-0.67*). 8-isoprostane concentration increased by 11.74%, from 5.17 to 5.71 pg/mL ((± 1.48) (*p=0.26, df=5*) in the exercise group, and decreased by 28.23% from 5.71 to 5.41 pg/mL (± 0.97) (*p=0.48, df=5*) in the control group (*SMD= 0.41*). Protein carbonyl concentration decreased by 15.20%, from 138.20 to 117.20 nmol/mg protein (± 44.77) (*p=0.30, df=5*) in the exercise group, and decreased by 37.85%, from 241.22 to 147.14 nmol/mg protein (± 174.0) (*p=0.24, df=5*) in the control group (*SMD=0.31*). DNA oxidation comet percentage increased by 11.72%, from 3.67 to 4.10% (± 3.25) (*p=0.76, df=5*) in the exercise group, and decreased by 11.10%, from 2.76 to 2.57% (± 0.70) (*p=0.54, df=5*) in the control group (*SMD=0.18*).

Exercise did not cause any significant changes in markers of oxidative stress. Repeated measures ANOVA showed no difference between time points for each marker of oxidative damage.

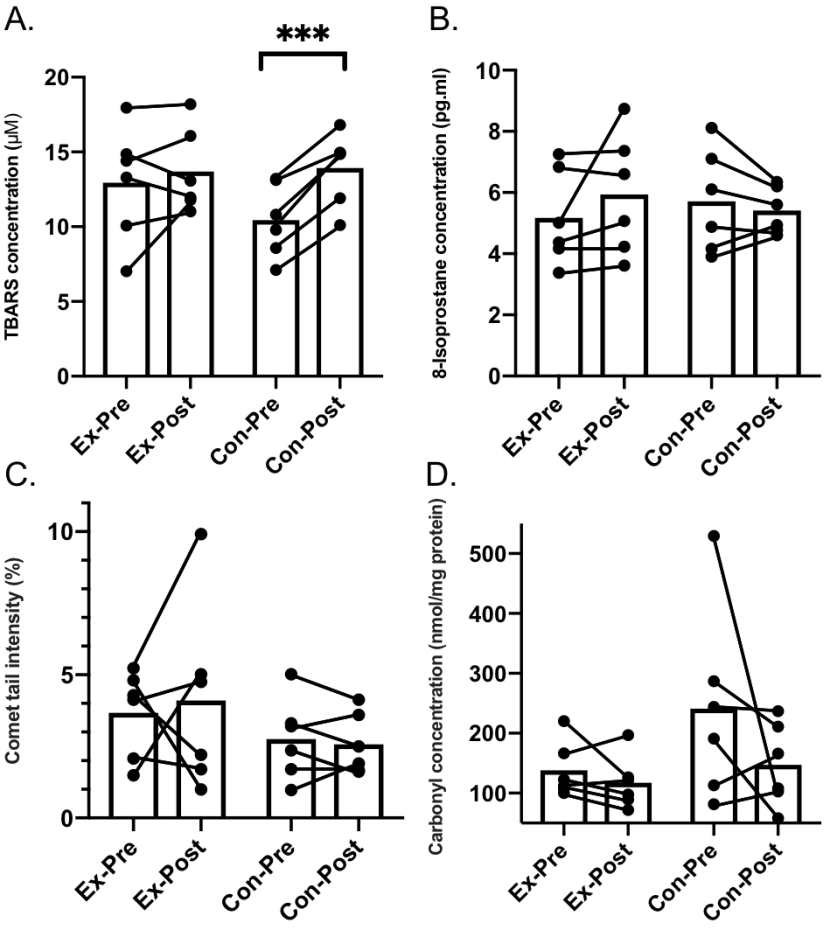


Figure 4.4 (A) Plasma thiobarbituric acid reactive substances concentration, a measure of lipid peroxidation (μM) (B) Plasma 8-isoprostane concentration, a measure of lipid peroxidation (pg/mL) (C) Single-stranded DNA damage quantification from whole blood (%) (D) Plasma protein carbonyl concentration (nmol/mg protein). Damage marker results are expressed as mean and individual values. ***: p<0.001.

4.3.4 Inflammatory markers

The markers of inflammation CRP, IL-6 and TNF-α each changed differently in response to the exercise intervention. IL-6 concentration decreased between pre-and post-time-points in both groups; 25.98%, from 1.27 to 0.94 pg/mL (± 0.49 (*p=0.16, df=5*) in the exercise group, and 43.30%, from 1.94 to 1.10 pg/mL

(±1.55) (*p=0.45, df=5*) in the control group (*SMD=0.25*). CRP decreased by 10.29%, from 2.43 to 2.18 ng/mL (±1.02) (*p=0.58, df=5*) in the exercise group, and increased by 12.05%, from 3.07 to 3.44 ng/mL (±0.64) (*p=0.43, df=5*) in the control group (*SMD=-0.37*). TNF-α decreased by 4.28%, from 84.82 to 81.19 pg/mL (±12.69) (*p=0.51, df=5*) in the exercise group, and 6.80%, from 87.44 to 81.49 pg/mL (±5.81) (*p=0.2, df=52*) in the control group (*SMD=0.13*). Repeated measures ANOVA showed no difference between time points for each inflammatory marker.

4.3.5 Brain-derived neurotrophic factor concentration

Plasma BDNF content increased by 10.38% in the exercise group, from 47.20 to 52.09 ng/mL (±29.57) (*p=0.70, df=5*), and declined by 17.52% in the non-exercising controls, from 41.01 to 33.91 ng/mL (±22.98) (*p=0.64, df=5*) (*SMD=0.23*). Despite the negligible increase in BDNF content in the exercise group over the full 12-weeks of the intervention period, between mid- and post-time-points in the exercise group, plasma BDNF concentration increased by a much greater margin of 41.58%, from 36.77 to 52.77 ng/mL (±0.92) (*p=0.12, df=5*).

4.3.6 Cannabis and tobacco consumption

Sub-group analysis was undertaken in the exercise intervention group in participants who self-identified as regular cannabis smokers (n=4), compared with non-smokers (n=3). TBARS concentration decreased 1.30% in non-smokers but increased 37.76% in smokers, and isoprostane concentration

increased 5.22% in non-smokers compared to 17.72% in smokers. Protein carbonylation decreased 31.90% in non-smokers and decreased 5.84% in smokers. These effects may have been fortified by tobacco consumption, since all cannabis smokers were also tobacco smokers, and only one non-smoker consumed tobacco only.

4.3.7 Intensity minutes and markers of inflammation

The potential effect of quantity of exercise (intensity minutes) on markers of inflammation was assessed (figure 4.5). 'Intensity minutes', measured by the Garmin devices, are representative of the number of minutes of moderate intensity physical activity that contribute to continuous bouts of activity lasting 10 minutes or more. CRP, IL-6 and TNF-α concentrations at pre-intervention, mid-point and post-intervention were compared with intensity minutes/day. It can be seen in figure 4.5 that as intensity minutes increased from baseline to mid-intervention, resting IL-6 concentration also increased (38.58%, *p=0.57, df=4*). Levels then fell as activity levels decreased post-intervention (45.49%, *p=0.38, df=4*). TNF-α plasma concentration decreased in response to increased activity (10.78%, *p=0.51, df=4*), and increased once again post intervention (7.28%, *p=0.17, df=4*). CRP decreased throughout the intervention period (5.76%, *p=0.43, df=4*) and was still decreased post-intervention (4.80%, *p=0.73, df=4*).

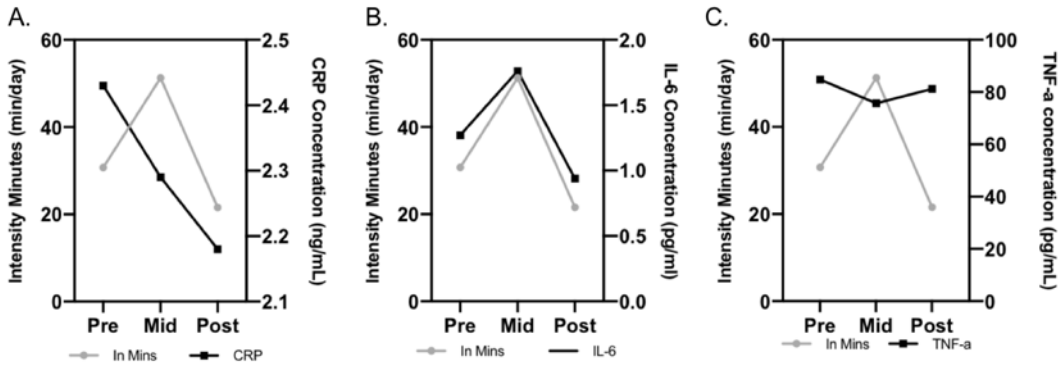


Figure 4.5 Garmin device measured 'intensity minutes', alongside markers of chronic inflammation CRP (A), Il-6 (B) and TNF- α (C).

4.3.8 Retention, exercise quality and psychopathology

Overall retention to the intervention was 68%, with attrition rates of 18% and 45% for the exercise and control groups respectively. Four members of the exercise group dropped out, one immediately after baseline assessment, two during the first week of exercise, and one after 10 weeks of intervention duration. Three members of the control group dropped out just before the 13-week post assessment was due. Attendance to the exercise sessions was 83% for the target two training sessions per week, and 41% for three sessions per week. The average duration of each exercise session was 37.74 min (±2.19, df=7), and the mean number of sessions attended per week was 2.31 (±0.58, df=7), above the minimum target for the intervention. Exercise session data is summarised in table 4.2. In the assessment of habitual activity at baseline, mid-

	Bout modality							
	Swim	Run	Cycle	Tennis	Football	Squash	Badminton	Circuits
% total sessions	18.32	15.35	3.47	14.85	16.83	4.46	5.94	20.79
Average HR (BPM)	117.50	136.80	137.0	125.50	139.40	124.50	124.10	121.30
SD	17.37	14.11	18.55	18.79	19.03	6.30	5.58	8.24

Table 4.2. Summary of bout distribution and heart rate for each separate activity type. BPM: beats per minute, HR: heart rate, SD: standard deviation.

intervention and post-intervention, the exercise group demonstrated an increase from 30.71 (±29.56, df=5) minutes of activity per day to 51.28 (±32.26, df=5) during the intervention. This fell significantly to 21.57 (±22.61, df=5) min/day in the week following the intervention (*p=0.05*). The control group were similarly active at baseline, with an average of 25.56 (±32.13, df=7) min/day, which fell to 7.16 (±9.30, df=6) min/day.

There were strong negative correlations between exercise intervention adherence and TBARS (*r=-0.75, p=0.08, df=5*) and protein carbonyl concentration (*r=-0.73, p=0.09, df=5*). The PANSS interview assessed symptomology across positive, negative and general psychopathology domains. In each of the three domains exercise either conferred protection against declining scores observed in the control group, or resulted in a much greater change between baseline and post-intervention measurement. There was a 1.44% increase in negative score in participants in the exercise group, and a 13.89% increase in participants in the control group (*SMD=-0.33*). There was a 17.31% decrease in positive symptoms of PANSS in participants in the exercise group, and a 7.83% decrease in the non-exercising controls (*SMD=-0.18*). Participants in the exercise group reported a 10.98% decrease in PANSS general score, compared to a 2.82% decrease observed in participants in the control group (*SMD=-0.24*). These data have been assessed alongside other functional measures that better shape the psychotic state, and the effects of the exercise intervention in Fisher et al (413).

4.4 Discussion

This exploratory study demonstrated that 12-weeks of regular exercise training may be sufficient to elicit positive changes in biomarkers of redox status and inflammation in a cohort of young men with FEP. The study highlighted potential differences in the response of GSH to training in blood and brain. Although some results were not significant when analysing individual outcomes (due to small participant numbers), when taken together, the battery of outcomes suggest a shift in redox and inflammatory status. In many cases, exercise appeared to be protective of the negative changes that were observed in the control group, which are likely a result of disease status; either redox-linked disease progression, treatment, or the impact the disease has on lifestyle factors. These are all representative indicators of poorer long-term outcomes for the patient.

4.4.1 Brain GSH versus peripheral GSH

The data presented suggest that blood and brain GSH responded differently to exercise. Exercise caused increased blood GSH, which is a common response (315), however, no detectable change was seen in brain GSH in this study. There was no correlation between changes in blood and brain GSH, for either study group. It should be noted that the number of participants able to be scanned was less than those that were able to give a blood sample, and as such assessment of brain GSH was only possible in a sub-set of an already small sample of FEP patients. However, Rai et al (2018) also reported

differences in blood and brain GSH response to exercise (314). The blood brain barrier is impermeable to peripheral GSH and as such it is probable that the two pools of GSH are regulated separately, protecting the brain from peripheral chemical changes that could produce toxic effects to brain function and integrity (424). The present study is the first to assess brain and blood GSH in response to exercise in a psychosis population.

In addition to the discordant regulation of GSH in brain and blood, there are a number of methodological considerations that are worthy of note. GSH determination by MRS does not discriminate between glial, neuronal or extracellular sources of GSH (425), and since GSH concentration in astrocytes is 10 times that of neurons, differential neuronal regulation may be masked. The complexity of FEP, as with most mental illnesses, means that brain GSH has not been identified as having a causal role in disease pathogenesis, but rather perturbed GSH metabolism (176) alongside other stress-related markers and antioxidants are believed to create the FEP phenotype. In the present study, there was most variation in brain GSH in the exercise group and given that exercise training can cause changes in GSH, it may be appropriate to suggest that exercise may have indeed perturbed brain GSH, but participant number and confounding factors such as diet, smoking status and medication made it impossible to draw any conclusions from this data. From an exploratory standpoint, the data presented around GSH regulation warrants further investigation, given its critical role in health, informing the design of future studies.

4.4.2 Inflammation and redox status

No exercise intervention studies have yet assessed the effects of training on markers of inflammation in an FEP population. Exercise resulted in a positive change in many of the markers of redox status and inflammation. For example, CRP concentration reduced throughout the study in response to exercise (-10.29%) compared to an increase (12.05%) in the control group. This response is highlighted in figure 4, where exercise intensity minutes increased across the study, CRP concentration fell. In a schizophrenia population, a 10-week intervention of high intensity exercise resulted in a 66% decrease in plasma CRP (255), matching the trend shown in the present study. Other studies that have assessed CRP in psychotic disorders reported no change after exercise training (241, 426). IL-6 has been described as a myokine, which can act as a regulator of exercise-induced metabolic changes. IL-6 release is elevated during dynamic exercise (218) and thus explains the observed increased plasma IL-6 concentration observed at mid-point during this intervention. A review paper by Gomez-Rubio et al (2018) summarised that in SZ, regular exercise leads to a reduction in disease-associated IL-6 elevation, as was observed at the post-intervention measure in the present study.

Exercise training conferred a protective effect for a number of blood biomarkers; including GSH, BDNF and TBARS. All of these measures demonstrated greater detrimental change in the control group, which may be associated with disease progression (142, 427, 428). Increased TBARS, as a measure of lipid peroxidation is thought to predate presentation of psychotic symptoms, in a comparison of un-medicated FEP patients, chronic

schizophrenia patients and healthy controls (131). In that study, the concentration of TBARS was greater in both patient groups, and greatest in the chronic schizophrenic population. This is in line with the results of the present study, which suggest that there is a disease course-dependent increase in lipid markers of free radical-mediated damage, over time. BDNF, a regulator of neuronal health and synaptic plasticity, can be depleted in FEP, via an inflammatory-mediated pathway (429). Increased circulating pro-inflammatory cytokine concentration, demonstrated in this clinical group, downregulates BDNF expression (430). The current study observed a variety of responses to exercise in different inflammatory markers, which partially act to influence BDNF expression downstream. Most importantly, the protective effect of exercise was demonstrated as preventing the BDNF decline observed in the control group. BDNF concentration increased between the mid-intervention and post-intervention time-points, with a 22.03% decrease between baseline and mid-intervention, suggesting a lag-period between exercise initiation and the beginning of an adaptive response to training in this marker. Peripheral BDNF measures are used as a proxy for brain BDNF, since it is able to cross the highly restrictive blood brain barrier (BBB). Measurement in the plasma is representative of free BDNF that crosses the BBB, and a strong reflection of the influence of an intervention in the brain, rather than a serum measure that takes into account platelet secreted BDNF upon activation (431). This study brings together many of the linked biochemical perturbations observed in FEP, and highlights the potential of exercise training to normalise indices of antioxidant depletion, oxidative damage, inflammation and neuronal function.

4.4.3 Adaption time for inflammatory markers

There is evidence of chronic low-grade inflammation in long-term SZ (432), and emerging evidence has recently described this state in early psychosis (361, 427). Thompson et al (211) recruited sedentary middle-aged men to a 24-week exercise intervention, with a 2-week de-training assessment post-exercise. The authors observed that IL-6 was decreased at 24 weeks, but within the two weeks de-training period the cytokine had returned to baseline level. IL-6 increased after each acute bout to exercise, which is the natural response to exercise (218), and may explain the observed spike in IL-6 concentration at mid-intervention in this study.

Aerobic endurance training can increase basal IL-6 receptor expression in skeletal muscle (410), which in time can result in reduced upregulation of IL-6 in response to an acute bout of exercise. Therefore, regular exercise training improves sensitivity to IL-6 which may account for the reduction observed in IL-6 concentration between mid and post-intervention time points. IL-6 in principally defined as a pro-inflammatory cytokine, but also exhibits autocrine, paracrine and endocrine effects when released by monocytes during muscular contraction (216). Many cytokines, including IL-6, and other small proteins can be described as myokines, which act as regulators of exercise-induced metabolic changes. Contrary to the belief that increases in IL-6 during exercise represent an acute-phase response initiated by local damage to the muscle (217), IL-6 is released and elevated in the circulation during dynamic exercise, without muscle damage (212). This effect means that Il-6 is involved with

adaption to exercise, as a signalling molecule, and accounts for the increased plasma IL-6 concentration observed at mid-point during this intervention.

Interestingly, this study found no significant change in resting CRP concentration, despite it being modulated in response to IL-6 (433). CRP is a stable marker of inflammation (434), and as such, the magnitude of change in IL-6 in response to exercise, or perhaps the frequency or intensity of exercise may have not been sufficient to alter CRP concentration. However, CRP did decline non-significantly (by 13%) as a result of exercise in the present study, which may be indicative of the positive effects of exercise in circulating inflammatory cytokines.

4.4.4 Cannabis interaction with redox markers

Regular use of cannabis in mental illness is far greater than the general population (6.6%) (435), particularly in psychosis populations (23%) (436). When data was assessed by sub-group in the current study, the positive effects of exercise that were seen in non-smokers were not observed in the sub-group of smokers. In a study assessing the redox effects of regular cannabis consumption in healthy young people, there was no difference in markers of lipid peroxidation or protein carbonylation between smokers and non-smokers (437). However, there are many studies that show the negative effects of smoking on makers of redox status (438). It is possible that changes in redox status as a result of cannabis smoking, followed by increases in markers of oxidative damage do contribute to the pathology of psychotic disease, however more investigation of the specific effects of cannabis use on redox status in

FEP is needed to draw any conclusions. Exercise did not confer complete protection for markers of free-radical medicated damage in the current FEP group.

4.4.5 The impact of exercise on symptoms of psychosis

The changes in biomarker described herein were accompanied by a positive change in symptom profile as a result of exercise. The observed changes in symptoms of FEP indicate that the current intervention design was sufficient to elicit beneficial changes in functional status (PANSS symptomology), in addition to changes in biomarkers of redox status and inflammation as a result of exercise training. This is in concordance with the current literature; exercise has been shown to be superior to control conditions in improving indices of symptomology, quality of life and depressive symptoms (384). Additionally, another meta-analysis showed the value of 90 minutes of moderate intensity exercise per week (below the World Health Organisation recommendation of 150 minutes moderate-vigorous intensity exercise per week) in improving psychiatric symptoms (439).

4.4.6 Limitations

The principle limitation in this study was a small sample size, rendering many of our time-point and inter-marker comparisons statistically insignificant. However, the current study did observe a measurable change in redox status and clinical outcome measures. Not all participants were able to be assessed for brain GSH due to ineligibility or inability to enter the brain scanner. The main barrier

to scanning was anxiety of participants to be scanned, but there were some incidences of metal compounds in the body, or injury preventing prolonged time in the supine position. Lastly, despite the opportunity to undertake a variety of indoor-based activities, poor and unpredictable weather in the UK was a factor in participants' willingness to engaging in the intervention. This effect was particularly salient during the winter months. Confounding factors such as adherence to the intervention, BMI and cannabis consumption may have affected the outcomes of this exercise intervention, together with potential contributions from antipsychotic medication and duration of illness.

4.4.7 Conclusions

This study, in male patients with first-episode psychosis, highlighted the beneficial effects of exercise training on markers of redox homeostasis. The study showed that 12-weeks of moderate-intensity exercise training was sufficient to reduce markers of lipid oxidation and increase antioxidant status. In many of the markers assessed in this study, exercise was able to arrest the changes that were seen in the control group, that would usually be associated with FEP. Future studies should use these data to explore more definitive outcomes, given that the feasibility of such an intervention, and the observed change in markers that describe several aspects of disease pathology.

CHAPTER 5: ASSESSING THE NEURODEVELOPMENTAL HYPOTHESIS OF SCHIZOPHRENIA, FROM A REDOX PERSPECTIVE, IN IPSC-DERIVED ASTROCYTES AND NEURONS

Abstract

Induced pluripotent stem cell (iPSC) technology offers a promising opportunity to assess disease in patient-derived cerebral cells. The pathogenesis of schizophrenia (SZ) is thought to initiate during neurodevelopment and may be related to the observed perturbations in antioxidant capacity and oxidative stress that have been described in symptomatic SZ patients. This study aimed to compare iPSC-derived neuron and astrocyte co-cultures, and pure astrocyte cultures from SZ and healthy control donors. Cultured cells were characterised during growth, which is representative of human neurodevelopment, and the glutathione (GSH) cycle with constituent enzymes; glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST) were assessed.

Both SZ and healthy derived neural progenitor cells (NPCs) were generated following iPSC neural induction. NPCs were further differentiated into mature astrocytes or co-cultures. One SZ and one control cell line were lost to infection and failure of MEF feeder cells to support iPSC growth. This rendered the final analysis strong in terms of biological repeats (n=4), but the data presented is only representative of one clinical phenotype. Cell development was characterised by immunocytochemistry at iPSC, NPC, neuron and astrocyte

stages. Following maturation (35 days), analysis of GPx, GR and GST activity was undertaken, and GSH concentration was assessed. Protein carbonylation and total 8-isoprostanes were measured as markers of oxidative stress.
GST activity was significantly decreased in SZ astrocytes (*p=0.022*) and co-cultures (*p=0.008*), and total GSH concentration was decreased in SZ astrocytes (*p=0.044*) when compared with controls. Oxidation products, 8-isoprostane and protein carbonyls were also perturbed in SZ iPSCs.
The GSH cycle is perturbed in iPSC-derived co-culture and astrocyte models from a person with SZ, supporting the hypothesis that there is altered neurodevelopment in SZ, which includes an altered redox state. These data provide an important novel viewpoint in the process of SZ pathogenesis, and may highlight potential targets for early detection of disease and intervention.

Key words

iPSC, schizophrenia, glutathione, oxidative stress, neurodevelopment

5.1 Introduction

Development of disease-relevant cell models that resemble human physiology is critical to enabling the investigation of disease mechanisms. Not only do such models enable characterisation of disease and more precise monitoring of cell function, but they allow manipulation of cell conditions and exogenous stress in the pursuit of solutions to treat mechanisms of disease.

Human clinical studies are inherently heterogeneous, and biomarkers of interest are often influenced by lifestyle factors, such as diet and drug use (440). Research in clinical population can also be invasive, especially in the context of neurological disorders. Typically, brain ill health is studied by assessment of peripheral markers that act as surrogates for the central nervous system e.g. in the blood or cerebrospinal fluid, or via brain imaging methodology such as MRS scanning or via post-mortem tissue. The limitations of these methods can be mitigated by the use of a cerebral cell model. Induced pluripotent stem cells (iPSCs) offer the opportunity to monitor and assess cortical cell development in a system that can recapitulate donor gene profiles. Markers of brain health which are commonly measured indirectly in peripheral blood analysis can also be quantified in cell models, and neural induction models allow the investigation of single cell types and co-cultures respectively. The final point of introduction worth noting here is that many studies investigating brain health assess human disease tissue post-mortem, which, particularly in the context of redox biology, is not an accurate reflection of the living-state.

5.1.1 What is an induced pluripotent stem cell?

Induced pluripotent stem cells (iPSCs) were initially developed by Takahashi and Yamanaka in 2006 (441). They first demonstrated the ability to induce pluripotency of a mature somatic cell by treatment of defined factors. They were able to eliminate the ethical concern of using embryonic stem cells in research, since the donor cells have great ease of access. This study represented a turning-point in characterising disease processes *in vitro*, since previous models involved arduous ethical dilemma or the use of a non-human substitution. iPSCs provide the capability to take a somatic cell from a patient donor and re-programme it to a pluripotent state before it can then be differentiated and cultured as a cell type of choice whilst recapturing the genetic profile of the donor.

This reprogramming of somatic cells into a pluripotent state is reliant on retroviral delivery of four genes; Sox1, Oct4, klf4 and c-Myc (442). These are known as the Yamanka factors. These genes are highly expressed in embryonic stem cells, and over-expression induced pluripotency. Early attempts to integrate these transcription factors into the genome of the donor cell proved highly inefficient; although more recent developments have altered this method to minimise chromosomal disruption by inserting genetic material non-permanently (e.g. via retroviral transduction) (443).

Once the pluripotent cell has been generated from a donor somatic cell, it is then possible to induce differentiation to the desired cell type. The process of neural differentiation has been well-described (444, 445). iPSC-derived mature

cerebral cells can be cultured as single cell types, or as co-cultures (446). Maturation of astrocytes as a mono-culture is perhaps more straightforward as these cells appear more self-sufficient, whereas neuronal growth and maturation requires support from astrocytes, and therefore the most successful method of culture for neurons is achieved by allowing 'spontaneous differentiation' (447). This results in a mature co-culture with astroglial cells and better represents the metabolic processes of a mature brain than neurons alone.

5.1.2 Glutathione metabolism in the brain

The brain consumes 20% of the oxygen the body uses, despite comprising only 2% total mass (448), and this high oxygen metabolism makes the brain much more susceptible to the production and accumulation of ROS. Lipids are a key target of ROS, and the brain is highly lipid-rich, rendering this organ even more susceptible to oxidative damage (449). As introduced in chapter 1, GSH is the primary antioxidant in the brain (313), and although concentrations vary across the brain, it still represents the most important defence mechanism against oxidative insult. Other molecules with antioxidant capacity, such as catalase and superoxide dismutase enzymes are only present in comparatively low concentrations (450).

In the brain, there appears to be a co-operative process of GSH production and provision by astroglial cells and neurons (figure 5.1). Astrocytes are the most abundant glial cells in the human brain. They are closely associated with neuronal synapses, and support neuronal metabolic function and

communication. In fact, the principal functions of astrocytes are often concerned with maintaining or regulating homeostasis in the brain. Astrocytes maintain synaptic function, clear extracellular ions and transmitters, regulate neuronal metabolism and aid in the migration, secretion and absorption of neural transmitters (451) and astrocytes also maintain the integrity of the blood brain barrier (452). Neurons cannot use cysteine from the extracellular matrix, so must therefore rely on provision from astrocytes (453). The process of cooperative GSH synthesis in astrocytes and neurons is summarised in figure 5.1, adapted from Dringen et al, (454).

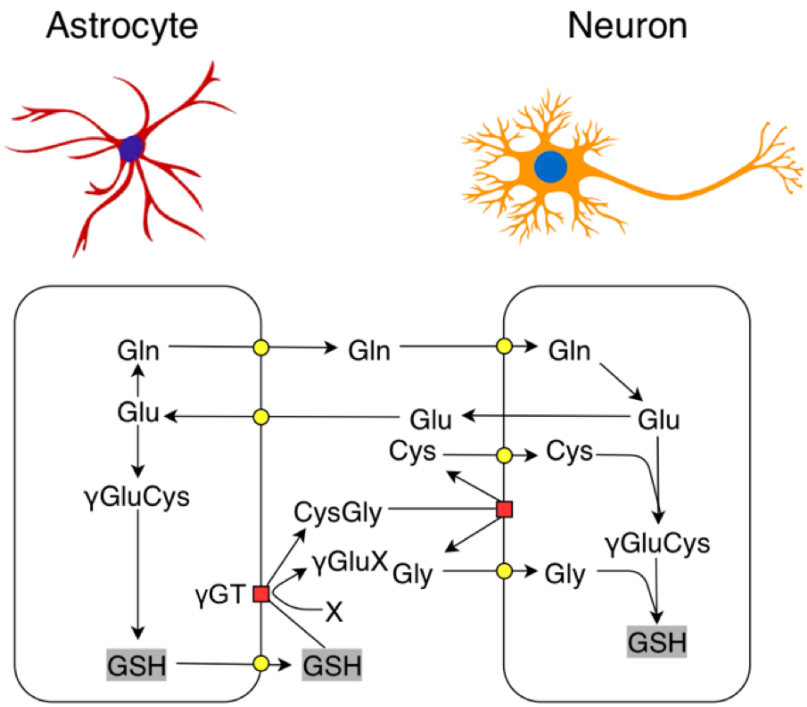


Figure 5.1. Interaction between astrocytes and neurons in glutathione metabolism. Yellow dots: transporter proteins, red boxes: enzymes. Gln: glutamine, Glu: glutamate, Gly: glycine, Cys: cysteine, GSH: glutathione, γGT: gamma glutamyl transpeptidase, γGluCys: gamma glutamyl cysteine synthetase. Free to use software draw.io was employed for the generation of this figure.

In normal cell function GSH release is primarily modulated by astrocytes (455). Only marginal amounts of GSH have neuronal, microglial or oligodendrocyte origin (456). Astrocytes and neurons synthesise GSH through different

extracellular precursors (453), and the balance of cellular synthesis and consumption of GSH is regulated by feedback inhibition of the γGluCys synthetase reaction by the end product GSH (457). GSH is consumed by all cells through conjugation with compounds to be removed from the cell, via glutathione-S-transferase, or by release of GSH from cells, to downstream targets (458). Neurons are able to use the cysteine donors, CysGly, γGluCys and NAC as precursors for GSH formation (459, 460), rather than direct transport of GSH from astrocytes. Extracellular GSH and GSH conjugates are substrates for the ectoenzyme γ-glutamyl transpeptidase (γGT), which catalyses transfer of a γ-glutamyl moiety from GSH or a conjugate onto an acceptor molecule. This results in the formation of a γ-glutamyl compound and dipeptide cysteinyl-glycine (CycGly) or CysGly conjugate (461). Peptidases hydrolyse CysGly to cysteine and glycine- therefore regenerate substrates for GSH synthesis.

Astrocytes play a crucial role in the protection of neurons against the damaging effects of ROS. This is made possible by the significantly higher GSH concentration in astrocytes compared to neurons (454). Exogenous stress to neurons, inflicted by incubation of cell models with hydrogen peroxide, causes rapid oxidation of GSH (462). GSH synthesis is restored upon removal of hydrogen peroxide. A crucial metabolite for this process is the cysteine residue. Depletion of GSH in culture, followed by re-feeding with cysteine restored GSH content (463). This may also highlight cysteine as an important target for supplementation.

Astroglial cultures have a higher capacity to detoxify H_2O_2 than neurons (464), and are in fact protected in co-culture at a cellular ratio of 1 astroglial cell to 20 neurons. When astrocytes are co-cultured with neurons, the neuronal GSH content increases substantially. One potential mechanism is that the cysteine or cysteine precursor component necessary for GSH synthesis is shuttled to neurons by astroglial cells (465).

Astrocytes are also key in the protection of neurons against nitric oxide (•NO) toxicity. •NO diffuses freely across cell membranes (466) and concentrations are elevated under stress conditions, another feature of SZ neuropathology (467). Astrocytes possess high GSH activity and can trap •NO to reduce neuronal exposure. Modelling this interaction *in vitro* by culturing a layer of astrocytes over neurons, and thus, partially shielding neurons from direct contact with extracellular •NO content has shown that astrocytes can have neuroprotective action in a GSH dependant manner (468). Further, when GSH formation was inhibited by buthionine sulphoxamine (an inhibitor of GSH synthesis) the cells were less effective in preventing NO-induced neuronal cell death.

5.1.3 Modelling schizophrenia with iPSCs

There are many benefits to using an iPSC cell line to replicate aspects of the SZ disease phenotype. Somatic cell samples are easy to obtain and they retain the unique genetic profile of the donor; providing an insight into the relationship between the donor's genotype and an *in vitro* endophenotype. These cells

have the potential to be differentiated into relevant neurons and astroglia in order to re-enact pathways of brain development in an individual with the condition (469).

iPSCs are ideal models for neuropsychiatric disorders, that are hypothesised to originate, at least in part, from neurodevelopmental defects (470-473) (described in chapter 1). Referring to the Allen BrainSpan Atlas, which compares gene expression of human PSC derived neurons, Brennand et al (294) proposed that iPSCs in a SZ context are better suited to modelling disease predisposition (e.g. FEP or prodrome) rather than late features of SZ. Since this early stage of illness can only be retrospectively identified, after onset of symptoms in adolescence or early adulthood, iPSC-derived cortical cells recapitulate this developmental state (474). Pre-natal insults, as early as first or second trimester have been linked to psychosis and SZ onset, caused by both environmental and genetic factors (475). The most well-documented causes of embryologic cortical mal-development are obstetric complications such as periventricular haemorrhages, hypoxia or ischemic injury, and viral infection e.g. influenza (476). In two studies by Keshavan et al (477, 478), the '2-hit' model of SZ is proposed, suggesting that there are two critical points of neurodevelopment; namely, early brain development and adolescence. Perturbations in either of these stages of development can contribute to dysfunction of specific neural networks that propagate symptoms. The relatively short maturation period of iPSC-derived neurons means it is not possible to capture the environmental and epigenetic changes throughout childhood and adolescence. However, by reprogramming somatic cells into

iPSCs from a donor with a diagnosis of SZ we can observe the early developmental phenotype. iPSCs from SZ patients provide a platform for assessing the neurodevelopmental changes caused by risk genes or exogenous stressors, or both (479). Despite the complex inheritance patterns of SZ and lack of any dominant genes that confer pathogenesis, individuals with risk genes are more susceptible to the environmental stressors that have the potential to contribute to onset of psychosis.

A number of SZ iPSC lines have been assessed, particularly in the context of redox balance and ROS. Reports of impaired dopaminergic differentiation and glutamatergic maturation (480), increased basal and activity dependent cortical secretion of dopamine (481), increased extra-mitochondrial oxygen consumption and ROS production (482) and increased oxidative stress and subsequent ROS (294) have been reported. An elevation in extra-mitochondrial oxygen consumption in iPSC neurons from SZ patients compared with healthy control cells increased oxygen flow in neurons, and resulted in increased electron leakage and accumulation of ROS (483).

Neural progenitor cells (NPCs) from SZ patients also consumed more oxygen and generated more ROS compared with an age and gender-matched healthy control cell line, which highlights mitochondrial deficits associated with defective neurogenesis in SZ (482). Brennand et al (294) also assessed SZ NPCs using microarray and mass spectrometry, showing aberrant expression of genes related to oxidative stress and cytoskeletal remodelling. One could hypothesise from these two studies that defects in oxidative homeostasis in SZ

originate in NPCs. Since SZ has been characterised as a neurodevelopmental disease, the iPSC model fits with *in vivo* data- highlighting the value of using an iPSC model of SZ, and its relevance to human physiology.

There are however, challenges to utilising SZ iPSC lines (484). One of the most critical steps in successfully utilising iPSC-derived neural tissues with consistent and reproducible results is to overcome batch heterogeneity and stochasticity (randomly determined) of differentiation, which can result in unreliable assay results. This is overcome by the development of efficient differentiation protocols and/or isolation strategies for specific cell populations e.g. GABAergic, dopaminergic cells. However, utilising pure populations of specific cell types removes the advantage of a mixed cell model which more closely resembles the brain. A recent genome-wide association study of the risk for SZ identified 108 genomic loci with genome-wide significance, opening up the potential to identify such SZ –selective risk gene networks (47). However, the functional effects of many of these loci have not yet been identified. More than 99% of genetic variants lie outside coding regions, presumably in gene-regulatory sites (485). Because of the huge variation in genetic profiles of SZ patients, alongside the environmental risk factors (urbanicity, prenatal infection, immigration, drug-abuse), studies suggest that it is important to focus on early-onset mechanistic perturbations that may or may not have been conferred by genetic predisposition, such as redox imbalance. This is a well-characterised effect seen in people with psychosis, and use of an iPSC model to measure, probe and test treatments for redox abnormalities would be extremely valuable.

There are a number of perturbations in SZ astroglial-neuronal interactions in the SZ brain that could ultimately contribute to the altered redox state and antioxidant capacity of the brain. What is unknown, however, is the specific role of the glutathione cycle in detoxifying these elevated oxidative species.

5.1.4 Aims

The aims of this study were to assess the glutathione cycle and redox status in iPSC neuronal and astrocytic cultures from SZ and healthy donors. The GSH cycle is a novel research objective for cell-type specific studies in SZ. Perturbations in the GSH cycle have been well documented in peripheral blood measures and MRS studies, but never at this early stage of neurodevelopment. Since neuronal and astroglial cells work in tandem, it is salient to compare both cell types separately to elucidate any specific disease-related differences.

5.2 Methods

All cell lines utilised by this study were sourced from the Coriell institute repository (New Jersey, USA). Details of generation methods from somatic cell to iPSC are described in supplementary section 3. In brief, the SZ iPSC cells were derived from symptomatic patient donors, both with a family history of mental illness and SZ. GM23760 iPSCs were derived from a 26-year old male, with a diagnosis of SZ. Their symptoms included episodes of agitation, delusions of persecution and fear of assassination. GM23762 iPSCs were derived from a 23-year old male, with a diagnosis of SZ. Symptoms included paralogical thinking, affective shielding, slitting of affect from consciousness

and suspiciousness. The first control cell line, GM25256, was derived from fibroblast cells from a clinically normal 30-year old male donor. The second control cell line, GM23450 iPSC cell line was derived from healthy male 20-week fetal tissue.

5.2.1 Matrigel plate preparation

One aliquot of Corning Matrigel® (FisherScientific, 354277) was thawed on ice. Dilution of stock solution was dependent upon lot concentration. All plate coating for this study required 12.48μL Corning Matrigel® solution per mL of DMEM/F12, to make up a final protein concentration of 8-12mg/mL. Thawed Corning Matrigel® was added to the cold DMEM/F-12 and mixed well. The diluted Corning Matrigel® solution was used to coat the tissue culture-treated cultureware (6 well plate (1mL per well). Corning Matrigel® solution was evenly distributed across the surface, and incubated at room temperature (RT) for at least 1 hour. Immediately prior to seeding the cells, the cultureware was tilted

Growth factor	Range of GF concentration in Corning Matrigel Matrix	Average GF concentration in Corning Matrigel Matrix
EGF	0.5-1.3 mg/mL	0.7 ng/mL
bFGF	<0.1-0.2 pg/mL	NA
NGF	<0.2 mg/mL	NA
PDGF	5-48 pg/mL	12 pg/mL
IGF-1	11-24 ng/mL	16 ng/mL
TGF-beta	1.7-4.7 ng/mL	2.3 ng/mL

Table 5.1. Growth factor (GF) concentrations present in Corning Matrigel Matrix. EGF: epidermal growth factor, bFGF: bovine fibroblast growth factor, NGF: nerve growth factor, PDGF: platelet-derived growth factor, IGF-1: insulin-like growth factor, TGF-beta: transforming growth factor beta.

to allow excess Corning Matrigel® solution to collect at the edge. This was removed by aspiration. Alongside the extracellular matrix proteins that maintain pluripotency, Matrigel® contains growth factors that prevent differentiation to off-target cells, and promote proliferation of iPSCs, NPCs, neurons and astrocytes. Growth factor concentration is detailed in table 5.1.

5.2.2 Cell counting

To measure the proportion of viable cells and to quantify the number of cells retrieved from culture, cells were counted prior to each passage using a haemocytometer. Sample (20μl) was taken from the cell suspension and diluted into media (200μl). Trypan blue solution (20μl) was added to generate a 1:1 solution. Trypan Blue is a selective colour stain for dead cells. Live cells with an intact membrane are not stained. Solution (10μl) was loaded onto both sides of the haemocytometer, and cells counted at the mid-point grid. The average value was take, and multiplied by the Trypan Blue dilution factor, media dilution factor and by 10,000 (since the gridded area of the haemocytometer consists of nine $1mm^2$ squares which correspond to give a value of 1 counted cell- 10,000 in 1ml media).

5.2.3 iPSC removal from cryopreservation, plating and expansion

Four iPSC cell lines (GM25256, GM235450, GM23760, GM23762 (Coriell Institute, NJ)) were removed from cryopreservation onto either Matrigel® or mouse embryonic fibroblast (MEF) (Merck, PMEF-CFX) coated plates, followed by maintenance and expansion with mTeSR™(Stem Cell Technologies, 85850)

media. In the thawing process, cryovials were removed from liquid nitrogen, and immersed in a 37°C water bath until a small piece of frozen material remained. The thawed cell suspension was added dropwise to warm culture medium (10mL) in a sterile 15mL falcon tube and mixed gently by swirling. Cells were centrifuged (1100rpm for 3 minutes at RT). Supernatant was aspirated, and cells gently re-suspended in warm culture medium (2mL), supplemented with Y-27632 Rho kinase (ROCK) inhibitor (Y-compound) (Sigma-Aldrich, SCM075) (10μM) (1μL/mL media, dissolved in DMSO)). Cells were plated in 1 well of a 6-well plate, coated with either Matrigel® (GM25256) or MEFs (GM23760, GM23762 and GM23450).

5.2.3.1 iPSC culture on mouse embryonic fibroblasts (MEFs)

For the GM25256 control line, GM23760 and GM23762 SZ cell lines, the cell repository recommended culture on MEF-coated cultureware (Merck, PMEF-CFX).

5.2.3.2 Plating mouse embryonic fibroblasts

Culture wells were coated with 0.1% gelatin in water (Stem Cell Technologies, 07903), and incubated for 20 minutes (37°C). A cryovial of MEFs was thawed by swirling in a water bath (37°C), followed by dropwise addition of the cell suspension into 10mL warm MEF medium (table 5.2), and centrifugation (1100RPM) for 5 minutes. Medium was aspirated and the pellet re-suspended in MEF medium (10mL). The gelatin-coated plate was removed from the

incubator, aspirated, and replaced with MEF media (2mL/well) (6-well plate). After a cell count, irradiated MEFs were seeded at a density of 42,000/cm^2, and incubated overnight to allow adherence and flattening prior to iPSC plating.

MEF medium	human iPSC growth medium
DMEM (Gibco, 14965039) 10% FBS (ThermoFisher, A3160401) 1mM sodium pyruvate (Gibco, 11360070) 1 x 10-4M non-essential amino acids (Gibco, 11140050) 2mM L-glutamine (Gibco, 25030149)	DMEM/F12 (Gibco, 21331020) 20% KOSR (Gibco, 10828010) 2mM L-glutamine 1 x 10-4M non-essential amino acids 55uM 2-mercaptoethanol (Sigma Aldrich, M3148) bFGF (100ng/mL) (Peprotech, 100-18B)

Table 5.2 MEF medium and human iPSC growth medium composition. DMEM: Dulbecco's Modified Eagle's Medium, FBS: Fetal Bovine Serum, KOSR: knock-out serum replacement, bFGF: bovine fetal growth factor.

5.2.3.3 iPSC passage

When iPSC colonies approached the borders of an adjacent colony, cells were passaged, in ratios recommended by the provided Certificates of Analysis (GM25256 1:8, GM23450 1:6, GM23762 1:6 and GM23762 1:6). Spent medium was removed from each well, and cells rinsed with D-PBS (Dulbecco's phosphate-buffered saline) (Sigma-Aldrich, D8537). ReLeSR™ (1mL/well) (Stem Cell Technologies, 05872) was added for 1 minute, then aspirated, followed by incubation of the culture platform for 5 minutes at 37°C, or until the edges of the colonies began to peel. Culture medium (1mL) was added to each well, and iPSC colonies dislodged. Detached cell aggregates were transferred to a 15mL falcon tube, re-suspended in growth medium and seeded at the appropriate ratio onto a pre-prepared plate, in a 'no-spin' method of passage.

5.2.3.4 Cryopreservation of iPSCs

Spent medium was removed from each well, and cells were rinsed with D-PBS without magnesium or calcium. EDTA (1mL/well 0.5mM) was added to each well (for a 6-well plate), and incubated (5 minutes at 37°C). EDTA was aspirated, and cells detached from the culture platform with gentle addition of growth medium (1mL). Detached cell aggregates were suspended in growth medium (0.5mL), and 20% DMSO solution (0.5mL). Cryovials were placed in an isopropanol freezing container (24 hours at -80°C for 24 hours), before transfer to liquid nitrogen for long-term storage.

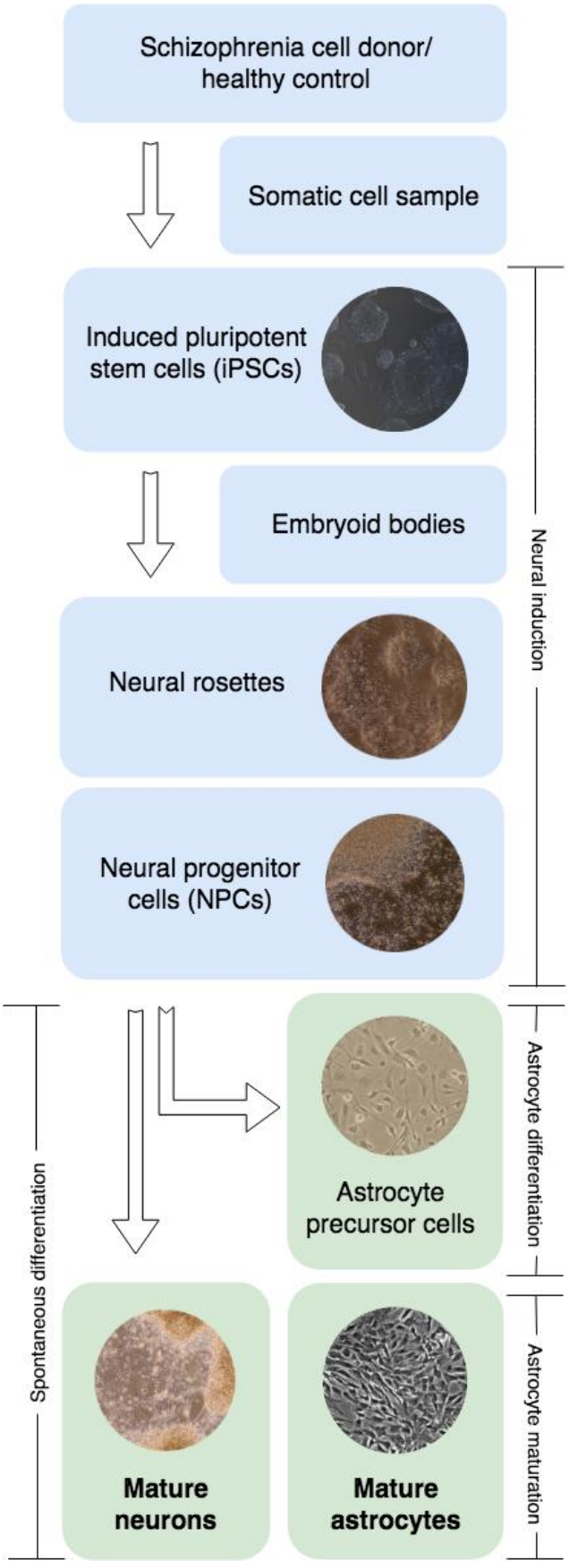


Figure 5.2. Schematic diagram of the process of donor cell collection through neural induction and differentiation of mature neurons and astrocytes.

5.2.4 Neural induction of iPSCs

The process of neural induction, astrocyte induction and spontaneous differentiation are summarised in figures 5.2 and 5.3 In the case of neural cells, figure 5.2 describes the pathway of differentiation; from iPSC to embryoid body, rosettes, neural progenitor cells and finally, co-culture or astroglial cells. In order to generate high purity NPCs from iPSCs, a high quality of starting culture was ensured; requiring low levels of differentiation (>10%) and a high confluency indicated by colony formation (100%).

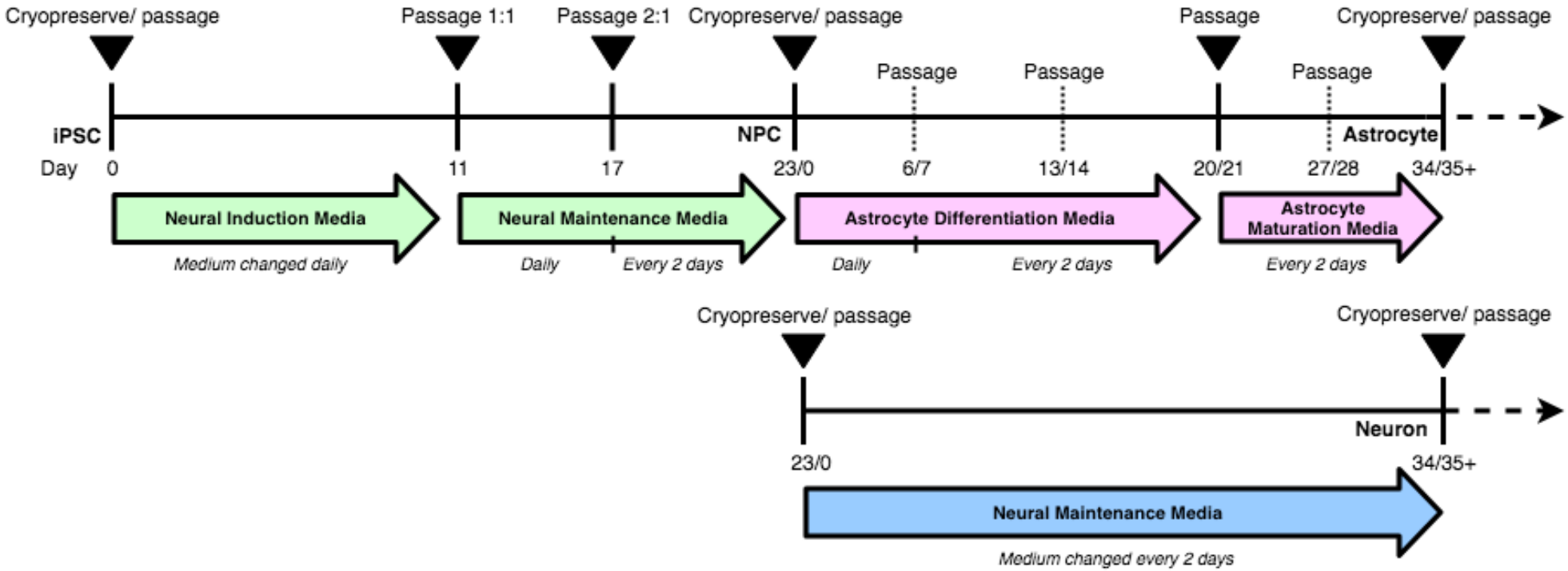


Figure 5.3. Schematic outline of the neurogenesis process, from induced pluripotent stem cell to neural progenitor cell and either spontaneous differentiation or astrocyte induction.

Day 0 (re-plating for monolayer)

iPSCs were ready for passage at 80% confluency, which is characterised by a merging of large, dense colonies towards the centre of the culture platform. Multiple layers of cells were avoided, to prevent aggregate formation. Spent media was removed from the iPSC culture platform, and the vessel rinsed twice with RT D-PBS. Cell detachment solution (1ml/10 cm^2 RT Accutase™, Stem Cell technologies, 07922) was added to the culture platform and incubated (5 minutes at 37°C), until cells began to detach. The cell suspension was pipetted from the platform surface, going from side-to-side and top-to-bottom to ensure complete re-collection, avoiding excessive titration. The cell suspension was collected into a conical tube with two volumes of DMEM: F12 (Dulbecco's Modified Eagle Medium/ Nutrient Mixture F-12) media. Cell suspension was centrifuged (200g for 5 minutes at RT). After successful pellet formation, supernatant was removed and the pellet re-suspended in medium (1ml mTeSR, Stem Cell Technologies, 85850) with Rho kinase inhibitor (10μM) until single cell suspension was achieved. Cell suspension was added to the prepared culture platform, and the concentration of media was adjusted to reach a final volume of 1:1 for re-plating (3ml per 6-well plate, 7.5ml per T25 flask and 20ml per T75 flask). The platform was then rocked to ensure maximum suspension homogeneity, and incubated overnight.

Day 1 (starting to pattern neuroepithelium)

When cultures reached 100% confluency as a continuous monolayer, without breaks, spent media was discarded and one volume of Neural Induction Medium was added (table 5.3), and cells were returned to the incubator overnight. Each subsequent day for a further 9 days a full media change was executed. During days 4-5, cells rounded, shrunk and became phase bright. During days 7-10, cells rapidly multiplied, became multi-layered and phase dark.

Gibco™ Essential 6™ Medium (ThermoFisher Scientific, A1516401)	**50mL**
XAV939 (XAV) (Stem Cell Technologies, 72672)	**10 uL**
LDN193189 (LDN) (Stem Cell Technologies, 72147)	**0.5 uL**
SB431542 (SB) (Stem Cell Technologies, 72232)	**50uL**

Table 5.3. Constituents of Neural Induction Medium, including E6 base media and small molecule pathway i hibitors XAV, LDN and SB for induction of neurogenesis.

Day 11 (re-plating to clonally expand neural progenitor cells)

All spent media was removed from the culture platform, and rinsed twice with D-PBS (2ml per $10cm^{2)}$. The D-PBS was then discarded, and Accutase™(4°C) added to the platform (1ml per $10cm^2$). The culture was incubated (5 minutes at 37°C), until detachment. Three volumes of DMEM: F12 were added, with gentle pipetting of the supernatant over the platform surface to dislodge remaining cells. Two further volumes of DMEM: F12 were used to rinse the culture platform and collect into a falcon tube. Cells were centrifuged (RT for 5 minutes at 200g). Supernatant was removed from the pellet, and resuspended in 2ml Axol Neural Maintenance Media (Axol Biosciences, AX0031) plus Rho kinase inhibitor (10μM). Cells were plated 1:1 in freshly prepared culture medium, and adjusted to reach maximum working volume, as on day 0. Cells

were rocked to ensure maximum homogeneity, and incubated (48 hours at 37°C), limiting disturbances to ensure high recovery. Fresh media was fully exchanged every 2 days until day 17. By days 12-14 neural rosettes were obvious, suggesting successful neural induction.

Day 17 (expansion of purified neural progenitor population)

Neural progenitor cells were expanded and passaged as described above, in a ratio of 2:1, followed by Neural Maintenance Media exchanges every two days.

Day 23 (cryopreservation of neural progenitor cells)

On day 23, culture platforms were confluent and ready to passage. On average, every 10cm^2 of culture platform provided 10-20 million cells at high confluency. Spent culture medium was removed and platforms washed with D-PBS. Cells were incubated for 5 minutes with RT Accutase™ (37°C), until detachment. A serological pipette was used to break the tissue into clusters. Cell suspension was collected to a falcon tube and two volumes of cold (4°C) DMEM:F12 added. The culture platform was rinsed with two further volumes of DMEM:F12. Cells were centrifuged (RT for 8 minutes and 200g). Supernatant was removed and Axol Neural Maintenance Media added (2-3ml). A sample was taken to perform a cell count to determine total cell number. Media was adjusted to get 4-6 million viable cells per ml, and 0.5ml per cryovial to get 2-3 million viable cells per vial. Over-packing cells decreases viability. 20% DMSO (0.5mL) made up in medium was added to each cryovial, to give an overall

concentration of 10% DMSO. Cryovials were inverted to mix cell suspension, and rapidly placed into a 4°C cryo-cooler, then -80°C freezer for 24 hours. After 24 hours, vials were transferred to liquid nitrogen for long term storage.

5.2.4.1 Thawing and plating of NPCs

Cryovials containing iPSC-derived NPCs were removed from liquid nitrogen, and rapidly transferred to 37°C water for 1-2 minutes, followed by rapid transfer to a cell culture hood. After spraying with 70% EtOH, cells were transferred to a 15ml centrifuge tube. Neural maintenance media was added to the tube drop-by-drop (8mL), whilst gently swirling the tube to reduce osmotic shock. Media was used to wash any residual cells from the cryovial (1mL). The cellular suspension was centrifuged (200g for 5 minutes at RT). The cell pellet was re-suspended in neural plating media (1ml), removing large clumps by gentle repeated pipetting until a single suspension was formed. Suspension was removed to perform a cell count (20μl), and cells re-suspended in media to reach an optimal cell density per ml of media. Cells were inverted to re-suspended the solution, and added drop-by-drop to the culture dish (100μl per cm^2). To avoid premature differentiation and loss of cortical identity, cells were seeded at a density higher than 70,000 cells per cm^2, and to prevent aggregation, less than 200,000 cells per cm^2. Newly plated cells were incubated for 24 hours in a 37°C, 5% CO_2, 95% atmospheric air incubator. After 24 hours, all media was exchanged with neural maintenance media, (200μl per cm^2) then a change of media every 2-3 days with full exchanges.

Once cells reached 70-80% confluency the passaging protocol for expansion or terminal plating was followed.

5.2.4.2 Passaging of NPCs for expansion or terminal plating

For continued expansion, cells were passaged, and plated on to Matrigel-coated plates. Media was removed from plates and the surfaces rinsed for 30 seconds with RT D-PBS (200μl per cm^2). D-PBS was removed and RT Accutase (cell detachment solution) was added (100μl per $cm^{2)}$, followed by incubation (3-5 mins at 37°C). Culture was deemed ready at detachment of cells. Accutase was passed across the cells twice to break up the monolayer, and transferred to a 15ml centrifuge tube. Three volumes of 37°C neural maintenance media was added, with a 4^{th} volume wash of the surface of the well and collection into the centrifuge tube. The tube was centrifuged (200g for 5 minutes), and supernatant removed, followed by addition of neural maintenance media. A cell count was performed (described previously), and cells suspension diluted to 1ml per desired cell density (recommended 100,000 cells per cm^2 for continued passage, and 70,000 cells per cm^2 for terminal plating). Diluted cells were then plated, using a phase contrast microscope to ensure seeding, and incubation for 24 hours. Each cell culture stayed below four passages, as prolonged passaging increased the amount of glia and off-target cells.

5.2.5 Astrocyte differentiation and maturation

Preparation of complete astrocyte induction medium

Astrocyte induction medium (XCell Science, AI-001-M100) was prepared from constituent provided reagents. Per 50mL of complete medium, 4mL of supplement A, 0.5mL of supplement B, and 25μL of supplement C was added to 100mL basal medium and mixed.

Preparation of complete astrocyte maturation medium

Astrocyte maturation medium (AMM) was prepared according to table 5.4. Growth factors were pre-diluted in HSA-PBS and stored at -80^{o}C until addition with the remaining reagents listed. Astrocyte pre-cursors were passaged and treated with AMM on day 20/21.

Components	Volume	Stock concentration	Final concentration
Neuralbasal-A medium (ThermoFisher, 1234905)	95mL	NA	NA
N21-Max (R&D Systems, AR008)	2mL	50X	1X
FBS, One Shot (Thermo Fisher, A3160401)	2mL	NA	NA
GlutaMAX (ThermoFisher, 35050061)	1mL	100X	1X
Heregulin-beta1 (Peprotech, 100-03)	100uL	100ug/mL	10ng/mL
FGF2 (Axol Biosciences, ax0047)	80uL	10ug/mL	8ng/mL

Table 5.4. Components of astrocyte maturation medium. Growth factor stock solution preparation: 0.05% stock of HSA (Sigma-Aldrich, A97131), D-PBS (ThermoFisher, 14190-250), was made by dilution of 50ug HSA in 100mL D-PBS. Working stocks of heregulin beta-1 and FGF2 were prepared by addition of 10ug growth factor to 1mL HSA D-PBS (10ug/mL final). FBS: fetal bovine serum, FGF: fibroblast growth factor, D-PBS: Dulbecco's phosphate-buffered saline.

5.2.5.1 Differentiation of NPCs to astrocyte precursors

Astrocyte differentiation

On day 0, (day 11/12 after embryoid body (EB) formation), selected neural rosettes were placed into a Corning® Matrigel®-coated well of a 6-well plate in neural maintenance media (2mL), and incubated (37°C and 5% CO_2 for 24 hours). On day 1 (day 12/13 after EB formation) medium was aspirated and replaced with 2mL complete astrocyte induction medium, incubated (37°C and 5% CO_2), with full daily medium changes with astrocyte induction medium. On day 6/7 (day 17-19 after EB formation), cells reached 90-95% confluence and were ready for passaging.

Passaging astrocyte precursors

Medium was aspirated and cells washed with sterile D-PBS (1mL) to remove cell debris. Accutase™ (1mL) was added and cells incubated (37°C and 5% CO_2 for 5-10 minutes). DMEM/F-12 (5mL) was added to wash the cell off the well, and cell suspension centrifuged (400g for 5 minutes). Supernatant was removed and discarded. Cells were re-suspended in complete astrocyte induction medium, and a cell count was performed using Trypan Blue and a haemocytometer. Cells were seeded onto Corning® Matrigel®-coated culture ware at a density of 1 x 10^5 cells/cm^2. Seeded cells were incubated (37°C and for 5% CO_2 for 7 days), with a full medium change being performed every other day with astrocyte induction medium. On day 13/14 (24-26 days after EB formation) cells were approximately 90-95% confluent, and passaged according to the protocol described above. At day 20/21 (31-33 days after EB

formation), cells were approximately 90-95% confluent, and ready for maturation.

Astrocyte maturation

The following protocol describes the maturation process for a single well of a 6-well plate- volumes were adjusted accordingly for different cell-culture environments. Medium was aspirated and 1mL Accutase™ was added, followed by incubation (37°C and 5% CO_2 for 5-10 minutes). DMEM/F-12 (5mL) was added to wash cell off the well surface, and the cell suspension was centrifuged (400g for 5 minutes). The supernatant was removed and discarded. Cells were re-suspended in AMM, and a cell count performed using Trypan Blue and a haemocytometer. Cells were seeded onto Corning® Matrigel®-coated culture ware at a density of 1.5 x 10^5 cells/cm^2. Cells were incubated at 37°C and for 5% CO_2 for 6-7 days, with a full medium change every other day with complete AMM. On day 27/28, cells were approximately 90-95% confluent, and a passage was completed according to the protocol described previously. After two passages in AMM, mature astrocytes (GFAP+) were observed. For immunocytochemistry, cells were seeded at a lower density of 5 x 10^4 cells/cm^2.

5.2.6 Preparation of cell lysates

After maturation of the cell condition, cells were washed with D-PBS without magnesium or calcium. Ice-cold lysis buffer (200 mM NaCl, 10 mM EDTA, 10 mM Na_2HPO_4, 0.5% NP40, 0.1% SDS, 1x protease inhibitors) was added to

the washed cells and incubated for 10 minutes at room temperature. Cells were dissociated from the plate using a cell scraper. Cell lysates were then frozen at -80°C until further use.

5.2.7 Immunocytochemistry for characterisation of iPSCs, NPC, neurons and astrocytes

5.2.7.1 Cell fixing

4% PFA (paraformaldehyde) (Sigma-Aldrich, 252549, 37wt% in H_2O) was prepared by dilution in D-PBS. An equal volume of 4% PFA was added to each well, and left for 5 minutes at room temperature (RT). PFA was aspirated and the process repeated twice more. 1ml D-PBS was added to each well, and placed on rocker for 5 minutes. D-PBS was aspirated and re-added to keep the sample hydrated, with storage at 4°C.

5.2.7.2 Antibody staining

All primary and secondary antibodies used in this study are summarised in table 5.5. Blocking buffer (1mL) (1% BSA, 0.05% Tween and D-PBS) was added to each well, and incubated at RT for 1 hour. Buffer was removed, and primary antibody/blocking buffer (1mL) (dilutions in table 5.5) solution was added to each well, followed by 1 hour RT incubation. Cells were then washed three times in blocking buffer, and secondary antibody/ blocking buffer solution (1mL) added to each well for 1 hour at RT. Three more washes, and addition of

DAPI (Invitrogen, P36935) (4'.6-diamidino-2-phenylindole, a blue fluorescent DNA stain). Slides were stored overnight, away from light.

Antibody	Expression	Dilution	Species	Secondary	Dilution
Sox2 (R&D Systems, MAB2018)	Pluripotency marker (iPSC)	1:100	Mouse	Goat anti-mouse IgG (ThermoFisher, A21052)	1:2000
Oct4 (Abcam, ab19857)	Pluripotency marker (iPSC)	1:100	Rabbit	Goat anti-rabbit IgG (Jackson Scientific, 111-545-144)	1:2000
Nestin (Millipore, MAB5326)	Neural progenitor marker	1:100	Mouse	Goat anti-mouse IgG (ThermoFisher, A21052)	1:2000
Pax6 (Biolegend, 901301)	Neural progenitor marker	1:100	Rabbit	Goat anti-rabbit IgG (Jackson Scientific, 111-545-144)	1:2000
Map2 (Abcam, ab5392)	Mature neurons	1:1000	Chicken	Goat anti-chicken IgG (Invitrogen, A110040))	1:1000
Tuj1 (Abcam, ab7751)	Neuron-specific tubulin	1:500	Mouse	Goat anti-mouse IgG (ThermoFisher, A21052)	1:1000
GFAP (Millipore, MAB360)	Mature astrocytes	1:100	Mouse	Goat anti-mouse IgG (ThermoFisher, A21052)	1:2000
S100-beta (Sigma, SAB5500172)	Mature astrocytes	1:100	Rabbit	Goat anti-rabbit IgG (Jackson Scientific, 111-545-144)	1:2000

Table 5.5. Primary and secondary antibodies used for ICC (immunocytochemistry) experiments. Species, source, catalogue number, cellular target and secondary antibody are listed. Primary antibodies were co-stained as described in the table.

5.2.7.3 Imaging

Cells were imaged using a fluorescent microscope with a 20x objective (Nikon A1R Inverted Confocal/TIRF microscope) and analysed using NIS-Elements software (Nikon).

5.2.8 Glutathione cycle and redox assessment assays

The protein concentration of cell lysates was determined prior to each assay, using the BCA assay (described in chapter 4, page 137). All antioxidant, oxidative adducts and enzyme activities were adjusted for protein content of each sample.

5.2.8.1 Glutathione concentration

Glutathione concentration in cell lysates was determined using a commercially available kit (Cayman Chemical, 703002). This assay uses an optimised enzymatic recycling method, using glutathione reductase. The sulfhydryl group of GSH reacts with DNTB (5.5'-dithio-*bis*-2(nitrobenzoic acid), Ellman's reagent) to produce 5-thio-2-nitrobenzoic acid (TNB). The resultant mixed disulphide (GSTNB) is reduced by glutathione reductase to recycle the GSH and produce more TNB. The rate of TNB production is directly proportional to the amount of GSH present in the sample. Cells were collected by centrifugation (1500 x g for 10 minutes at 4^{o}C), followed by homogenisation of the pellet in cold buffer (2mL/well) (50mM MES buffer (Cayman Chemical, 703010), 0.4mM 2-(N-morpholino) ethanesulphonic acid, 0.1M phosphate and 2mM EDTA, pH 6.0). Homogenised cells were centrifuged (10,000 x g for 15 minutes at 4^{o}C). The supernatant was removed, and de-proteinated; an equal volume of MPA reagent (5g metaphosphoric acid (Sigma-Aldrich, 239275) in 50mL water) was added to the sample before it was vortexed. The mixture was left to stand at RT for 5 minutes followed by centrifugation (>2000 x g for 2

minutes). Supernatant was collected without disturbing the precipitate. Potassium hydroxide reagent ((pH10); 531μL added to 469μL water) 50μL per mL of supernatant was added and vortexed immediately. The supernatant was then ready for the GSH assay. Standards were prepared according to the protocol provided, using the GSSG standard (Cayman Chemical, 703014) and MES buffer.

Standard and sample (50μL) were added to each designated well. The assay cocktail was prepared (11.25mL MES buffer, 0.45mL reconstituted cofactor mixture (lyophilised powder of $NADP^+$ and glucose-6-phosphate, Cayman Chemical (703016)), 2.1mL reconstituted enzyme mixture (glutathione reductase and glucose-6-phosphate dehydrogenase in 0.2mL buffer (Cayman Chemical, 703018)), 2.3mL water and 0.45mL reconstituted DTNB). The plate cover was removed and assay cocktail added (150μL) to each of the wells containing standards and samples. The cover was replaced, and the plate incubated in the dark on an orbital shaker between absorbance readings. GSH concentration of the samples was determined using the kinetic method; the plate was read at 405 nm at five minute intervals for 30 minutes. The average absorbance was calculated, and the absorbance value of standard 1 subtracted from itself and all other values. Corrected absorbance values for standards were plotted and each concentration determined, taking into account the sample dilution factor and a multiplication by two to account for the addition of MPA reagent during the de-proteination step.

5.2.8.2 Glutathione reductase fluorescence activity

Cell glutathione reductase activity in cell lysates was determined using a commercially available fluorescence-based assay kit (Invitrogen, EIAGRF). The kit uses a proprietary non-fluorescent molecule to covalently bind to the thiol product of the reaction between the substrate (GSSG) and GR to produce a fluorescent product. Mature co-culture neurons astrocytes and NPCs were centrifuged, and pellets washed and re-suspended in 1X assay buffer. Cells were then lysed by vigorous vortexing, then centrifuged (14,000rpm for 10 minutes at 4^{o}C), with supernatant collected for analysis. Assay standards were prepared by addition of glutathione reductase standard (10μL) to one tube containing 1X assay buffer (390μL), and labelled as 5mU/mL glutathione reductase. 1X assay buffer (200μL) was then added to 6 tubes labelled as follows: 2.5, 1.25, 0.625, 0.3125, 0.156 and 0mU/mL glutathione reductase. A serial dilution was performed by adding the 5mU/mL (200μL) to standard tube 2 etc. NADPH and detection reagents were reconstituted. Samples and standards (25μL) were added to appropriate wells, followed by detection reagent (15μL). The plate was incubated for 5 minutes at room temperature. Fluorescence emission was read at 510nm, with excitation at 390nm, the subtraction of emission from excitation controlling for background thiol signal. Oxidised glutathione (25μL) was added to each well, and NADPH (25μL), followed by incubation at room temperature for 15 minutes. The background thiol signal was subtracted for each well. A standard curve was generated, and glutathione reductase activity deduced after adjustment for dilution.

5.2.8.3 Glutathione peroxidase activity

Cell glutathione peroxidase activity in cell lysates was determined by commercially available assay kit (Cayman Chemical, 703102), measured indirectly by a coupled reaction with glutathione reductase. Oxidised glutathione (GSSG) produced upon reaction of hydroperoxide by GPx is recycled to its reduced state by GR and NADPH.

$$R\text{-}O\text{-}O\text{-}H + 2GSH \rightarrow GPx \rightarrow R\text{-}O\text{-}H + GSSG + H_2O$$

$$GSSG + NADPH + H^+ \rightarrow GR \rightarrow 2GSH + NADP^+$$

Cells were collected by centrifugation (2000 x g for 10 minutes at 4^{o}C). The cell pellet was homogenised in cold buffer (50mM Tris-HCl, pH 7.5, 5mM EDTA and 1mM DTT) (Cayman Chemical, 703110), then centrifuged at 10,000 x g for 15 minutes at 4^{o}C. Supernatant was removed for the assay. Background wells were prepared by addition of assay buffer (70μL) and co-substrate mixture (50μL) (lyophilised glutathione and glutathione reductase). Contents of vial re-constituted in 6mL diluted GPx assay buffer (Cayman Chemical, 703111) and NADPH (50μL) (lyophilised NADPH, reconstituted in 6mL diluted GPx assay buffer) (Cayman Chemical, 703119) to three wells. These wells provided a non-enzymatic control, of which the absorbance value was subtracted from the absorbance rate measured in the GPx samples and control wells. Positive control wells were prepared by addition of assay buffer (50μL), co-substrate mixture (50μL), NADPH (50μL) and diluted GPx control (20μL) (bovine

erythrocyte GPx, diluted 1:20 in diluted sample buffer) (Cayman Chemical, 703114) to three wells. Sample wells were prepared by addition of assay buffer (50μL), co-substrate mixture (50μL), NADPH (50μL) and sample (20μL) to each well. Reactions were initiated quickly by addition of Cumene Hydroperoxide (20μL) (Cayman Chemical, 703118) to each well, followed by mixing. Absorbance was read once every minute at 340nm for 5 time points.

5.2.8.4 Glutathione-S-transferase activity

Glutathione-S-transferase (GST) activity in cell lysates was determined by commercially available assay kit (Cayman Chemical, 703302). Total GST activity (cytosolic and microsomal) was quantified by measurement of the conjugation of 1-chloro-2.4-dinitrobenzene (CDNB) with reduced glutathione. The accompanied increased in absorbance at 340nm is directly proportional to the GST activity in the sample. Cells were collected by centrifugation (2000 x g for 10 minutes at 4^{o}C). The pellet was homogenised in cold buffer (2mL) (100mM potassium phosphate, pH 7.0, containing 2mM EDTA), then centrifuged (10,000 x g for 15 minutes at 4 ^{o}C). The supernatant was removed for the assay. Background (non-enzymatic control) wells were prepared by addition of assay buffer (170μL) to glutathione (20μL) in three wells. Positive control wells (equine liver GST) were prepared by addition of assay buffer (150μL), glutathione (20μL) and reconstituted GST (20μL) (enzyme re-constituted with 0.5mL Milli-Q water) (Cayman Chemical, 703314) to three wells. Sample wells were prepared by addition of assay buffer (150μL), glutathione (20μL) and sample (20μL) to each well. Reactions were initiated by

addition of CDNB (10μL) (Cayman Chemical, 703318) to all of the experimental wells, and mixed. Absorbance was read once every minute at 340nm for 5 time-points.

5.2.8.5 8-isoprostane, protein carbonyl and total antioxidant capacity measurements

Quantification of 8-isoprostane concentration, protein carbonyl concentration and antioxidant capacity (using the FRAP method) from cell lysates, rather than plasma, methods are detailed in chapters 2 (page 83) and 4 (pages 138 and 142).

5.2.9 Data analysis

Data analysis was performed using GraphPad Prism 8 software (version 8.0.1, 2018). To assess differences between astrocyte and co-culture biomarkers, for control and SZ iPSCs, unpaired t-tests were used. To compare relationships between GSH-cycle components and oxidative stress indices, Pearson's correlation coefficient was employed. Outliers were identified using the ROUT method (Q=1%).

5.3 Results

5.3.1 Cell characterisation

To track cell development and confirmation of desired cell-type attainment, both SZ and healthy control cell lines were characterised at each stage of cell development for specific cell-type marker expression; iPSCs, NPCs, neurons and astrocytes (figure 5.4). Full details of donor characteristics, the iPSC generation process, cell viability, and immunocytochemistry are presented in appendix; ‘Characterisation of iPSC cell lines modelling SZ and healthy control comparisons’. Cell lines GM23760 and GM23450 were lost before the attainment of mature neurons and astrocytes. This has been described in the characterisation appendix.

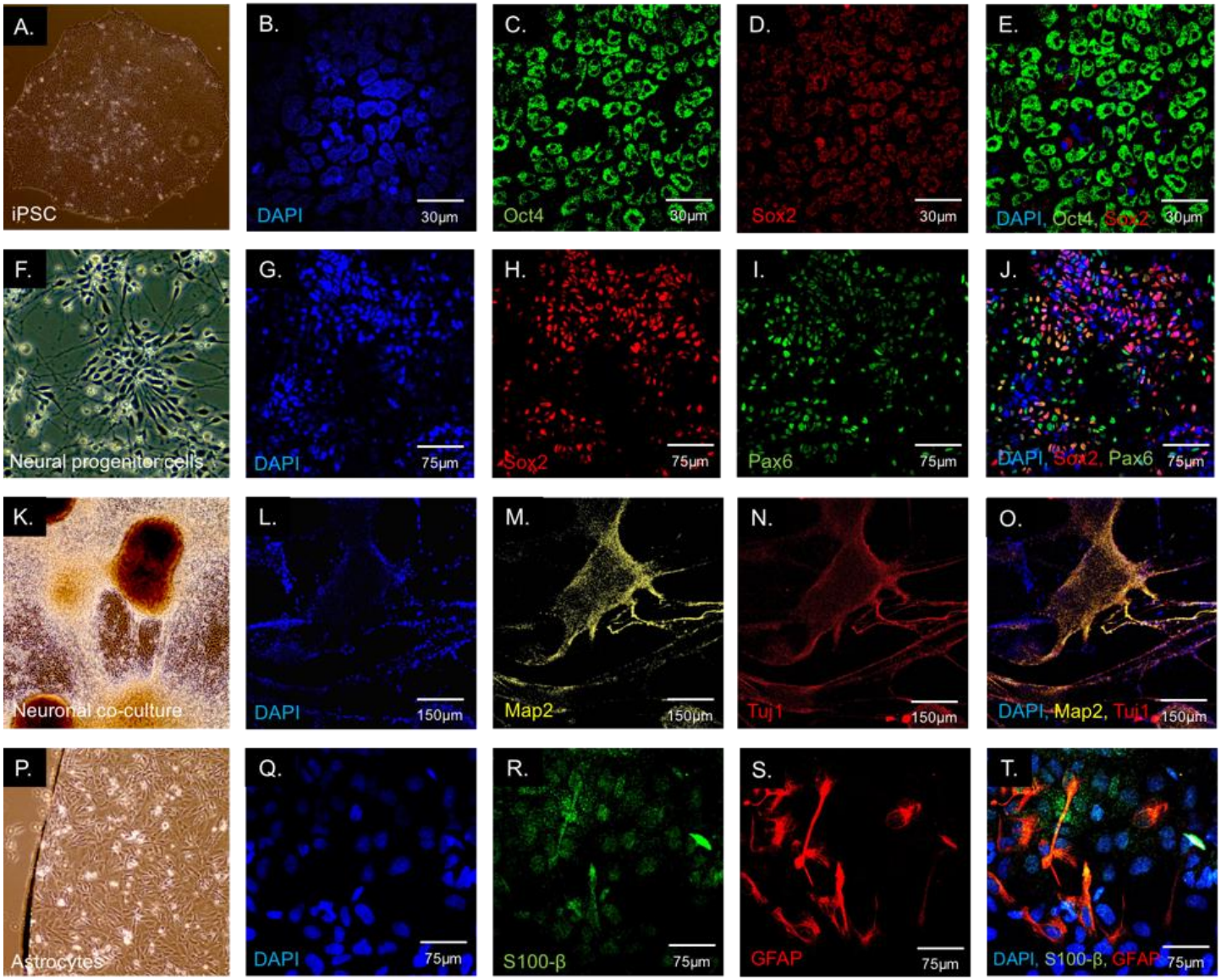


Figure 5.4. Immunocytochemistry of *in vivo* neurodevelopmental models of iPSCs (A-E), neural progenitor cells (F-J), spontaneous differentiation co-cultures (K-O) and astrocytic mono-cultures (P-T). All images shown are the control cell line GM25256, but both lines were characterised at each stage of neural induction. (A) iPSC cell colony (100X), (B) DAPI nuclear stain, (C) Oct4, (D) Sox2, (E) DAPI, Oct4 and Sox2, (F) Neural progenitor cell rosette (200X), (G) DAPI nuclear stain, (H) Sox2, (I) Pax6, (J) DAPI, Sox2 and Pax6, (K) Spontaneous differentiation co-culture (100X), (L) DAPI nuclear stain, (M) Map2, (N) Tij1, (O) DAPI, Map2 and Tuj1, (P) Astrocyte culture (100X), (Q) DAPI nuclear stain, (R) S100-β, (S) GFAP, (T) DAPI, S100-β and GFAP.
Oct4 and Sox2 are both transcription factors critical for maintaining pluripotency of a cell, and maintaining self-renewal. Pax6 is an informative marker of NPC formation, responsible for regulating differentiation and proliferation during neurogenesis. Map2, a cytoskeletal protein, and Tuj1 are neuron-specific proteins expressed in mature cells. GFAP, a filament protein, and s100-β, a regulator of cell cycle progression, are proteins expressed exclusively in mature astrocyte cells.

5.3.2 Glutathione concentration

GSH concentration was significantly depleted in SZ astrocytes (*p=0.044*) compared to controls. However, there was no observed difference for co-cultures (*p=0.82*) (figure 5.5). The co-culture GSH concentration was 0.13% greater in control compared to SZ cells (22.84 ± 3.41 versus 22.23 ±3.73 μM), and astrocyte GSH was 5.87% greater in control compared to SZ cells (27.58 ±1.18 versus 25.96 ± 0.46 μM). The difference between co-culture and astrocyte GSH concentration was significant for control cells (*p=0.039*) and non-significant for SZ cells (*p=0.094*).

5.3.3 Glutathione peroxidase activity

There was no significant difference in GPx activity observed between control cell and SZ cell for co-culture or astrocyte cultures (figure 5.5). However, GPx activity in co-cultures was 41.85% greater in control compared to SZ cells (53.67 ±12.43 versus 31.21 ±20.69 nmol/min/mL), and astrocyte GPx activity was 7.11% greater in SZ cells compared to control (45.28 ±9.89 versus 48.50 ±6.41 nmol/min/mL). The difference between co-culture and astrocyte GPx activity was non-significant for control cells (*p=0.33*) and SZ cells (*p=0.16*). Correlation analysis demonstrated significant negative correlation between GPx activity and GSH concentration in SZ astrocytes (r=-0.98).

5.3.4 Glutathione reductase activity

GR activity was not significantly different between control and SZ cell GR activity for co-culture or astrocyte cultures (figure 5.5). The co-culture GR content was 81.25% greater in SZ compared to control cells (0.16 ± 0.02 versus 0.29 ± 0.33 nmol/min/mL), and astrocyte GR activity 14.63% greater in control cells compared to SZ (0.82 ±0.16 versus 0.70 ± 0.07 nmol/min/mL). The difference between co-culture and astrocyte GR activity was significant for control cells (*p=0.0002*), but not SZ cells (*p=0.051*).

5.3.5 Glutathione-S-transferase activity

GST demonstrated a significant difference between control and SZ cell activity for co-cultures (*p=0.008*) and astrocytes (*p=0.022*) (figure 5.5). The co-culture GST activity was 47.98% greater in control compared to SZ cells (28.43 ± 5.38 versus 14.79 ± 4.79 nmol/min/mL), and astrocyte GST was 29.22% greater in control compared to SZ cells (28.37 ± 3.96 versus 20.08 ± 3.70 nmol/min/mL). The difference between co-culture and astrocyte GST activity was non-significant for control cells (*p=0.99*) and SZ cells (*p=0.12*).

5.3.6 8-isoprostane concentration

8-isoprostane concentration was no different observed between control and SZ co-culture and astrocyte cultures (figure 5.5). The co-culture 8-isoprostane concentration was 17.9% greater in SZ compared to control cells (127.1 ± 18.77 versus 149.8 ±115.5 pg/mL), and astrocyte 8-isoprostane concentration

3.36% greater in control compared to SZ cells (323.8 ± 50.88 versus 312.9 ± 41.52 pg/ml). The difference between co-culture and astrocyte 8-isoprostane concentration was significant for control cells (*p=0.0003*) and SZ cells (*p=0.037*).

5.3.7 Protein carbonyl concentration

Protein carbonyl concentration was no different between control and SZ co-cultures and astrocytes (figure 5.5). The co-culture carbonyl concentration was 87.5% greater in SZ compared to control cells (127.1 ± 18.77 versus 149.8 ± 115.5 nmol/mg protein), and astrocyte carbonyl concentration 5.78% greater in SZ compared to control cells (323.8 ± 50.88 versus 312.9 ±41.52 nmol/mg protein). The difference between co-culture and astrocyte protein carbonyl concentration was significant for control cells (*p=0.007*) and SZ cells (*p=0.032*). ROUT outlier analysis (Q=1%) identified one outlying value in the control line co-culture group.

5.3.8 Total antioxidant capacity

There was no significant difference observed between control and SZ antioxidant capacity concentration for co-culture and astrocyte cultures (figure 5.5). The co-culture antioxidant capacity concentration was 20.89% greater in SZ compared to control cells (361.5 ± 43.64 versus 437.0 ± 57.56 μM), and astrocyte antioxidant capacity concentration 3.71% greater in control cells compared to SZ (648.7 ± 11.62 versus 624.7 ± 25.85 μM), although these data were non-significant. The difference between co-culture and astrocyte

antioxidant capacity concentration was significant for control cells ($p<0.0001$) and SZ cells ($p=0.001$).

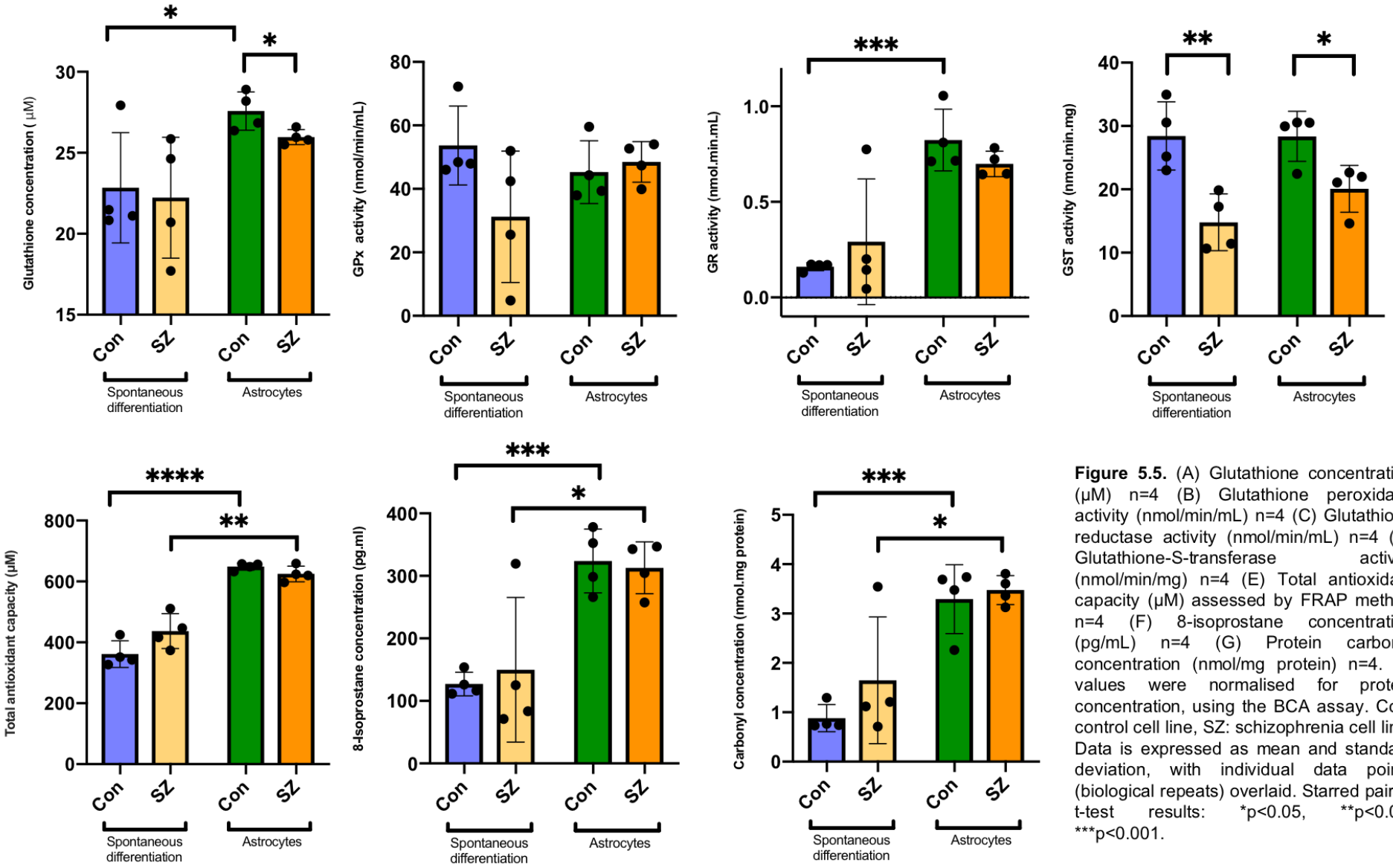


Figure 5.5. (A) Glutathione concentration (µM) n=4 (B) Glutathione peroxidase activity (nmol/min/mL) n=4 (C) Glutathione reductase activity (nmol/min/mL) n=4 (D) Glutathione-S-transferase activity (nmol/min/mg) n=4 (E) Total antioxidant capacity (µM) assessed by FRAP method n=4 (F) 8-isoprostane concentration (pg/mL) n=4 (G) Protein carbonyl concentration (nmol/mg protein) n=4. All values were normalised for protein concentration, using the BCA assay. Con: control cell line, SZ: schizophrenia cell line. Data is expressed as mean and standard deviation, with individual data points (biological repeats) overlaid. Starred paired t-test results: *p<0.05, **p<0.01, ***p<0.001.

5.4 Discussion

Antioxidant perturbation, ROS accumulation and subsequent oxidative damage are cogent pathophysiological factors in SZ and thus investigation of the molecular mechanisms underlying these events is crucial. This was the first study of its kind to assess the glutathione cycle in an iPSC model of SZ. The data presented show significantly altered GSH and GST in SZ in comparison with healthy control co-culture and astrocyte cells. Elevated co-culture lipid peroxidation and protein oxidation in SZ iPSCs demonstrate, alongside the GSH-cycle data, that the redox balance in these cells is perturbed. This supports the neurodevelopmental onset of psychosis and SZ, redox homeostasis is perturbed and represents an important target for intervention. These data suggest that redox balance is likely disrupted prior to symptom presentation.

5.4.1 The glutathione cycle in neurons and astrocytes

The brain's antioxidant defence and bioenergetic system is coupled between neurons and astrocytes. Whilst neurons are most vulnerable to ROS, due to increased energy demand, their survival relies on the antioxidant protection provided by astrocytes (486). Neurons express antioxidants at very low concentrations (462, 487), relying on co-operation with astrocytes for ROS detoxification and neuroprotection. The data from the current study showed that GSH concentration was approximately half in neurons compared with astrocytes. This is in agreement with other studies (487, 488). The abundance

of antioxidants in astrocytes is orchestrated transcriptionally by a high basal level of Nrf2 (nuclear factor-erythroid 2-related factor 2) (489), with its release activated by oxidative stress. Nrf2 is translocated to the nucleus, where it binds ARE (antioxidant response element) to induce the production of antioxidants (490, 491), including enzymes related to GSH metabolism. These upregulated antioxidant molecules can then be shuttled to neurons. This elevated transport mechanism may account for the observed depletion of GSH in astrocytes, but no difference between co-culture concentration. Neurons exhibit a hyper-compensatory response to GSH production in the presence of elevated ROS in response to neurodevelopmental elevation in ROS concentration.

From a bioenergetic perspective, neurons and astrocytes are very different, which may account for some of the differences observed between the two cell types in this study. Astrocytes are the only brain cell that store glycogen, and in response to neuronal activity provide the energy precursors for oxidative phosphorylation (492), despite the dominant energy generation mechanism in astrocytes being glycolysis. This metabolic coupling of neurons and astrocytes provides the energy to function, as well as maintain antioxidant defences. Mitochondrial dysfunction has been implicated as a key aspect of SZ; in particular defects in neuronal oxidative phosphorylation (493, 494). Increased cerebral mitochondrial oxidative phosphorylation has been associated with increased oxygen flux, and elevated ROS production (495). Oxidative phosphorylation is a potent source of ROS, and may partially explain the perturbations in the GSH cycle observed by this study. Elevated ROS put the protective response under stress, and may subsequently be overwhelmed. This

study observed a difference between GSH is SZ and control astrocytes, which may be related to metabolic dysfunction in the coupling of neurons and astrocytes.

Disturbances in metabolic processes have been reported in both astrocytes and neurons in SZ; glial cell impairment has been characterised in SZ; high extracellular concentrations of glutamate in the synaptic cleft, and additionally poor uptake by astrocytes has been described (496). Glutamate toxicity, as a result of NMDAR hypofunction in astrocytes and neurons leads to downregulation of antioxidant genes, including GSH. Synaptic NMDAR activity enhances the capacity of GSH through transcriptional control (497). This may account for the depleted GSH observed in the SZ astrocytes in this study. Depleted antioxidant function and reduced supply of energy appears to be a hallmark of SZ but has only been demonstrated up to now in post-mortem brain or animal models. This study shows similar perturbation of antioxidant capacity in SZ compared to healthy control iPSCs, demonstrating the ability of iPSCs to represent some of the perturbations associated with the pathology of disease effectively. This evidence also further supports the neurodevelopmental hypothesis of SZ.

Total GSH was depleted in SZ astrocytes when compared to co-cultures of neurons and astrocytes. Given that astrocytes are the main producers and suppliers of GSH to neurons in healthy cells, and the shuttling mechanisms of precursors and capacity of neurons to receive GSH both appear to be unaltered (there was no difference between SZ and control co-culture GSH).

These results suggest that SZ astrocytes have an altered ability to produce GSH. It has also been shown that SZ patients have a decreased glial cell density (498, 499), which would likely contribute to an overall depletion in GSH in both neurons and astrocytes.

GR and GPx did not differ significantly when comparing control cells with SZ cells. GR activity, however, was 81.25% greater in SZ co-cultures compared with control counterparts, although the biological repeats showed high variability. GPx enzyme activity was 41.85% lower in SZ co-cultures that controls. Despite no significant difference in these measures, the results suggest that there is a decreased capacity to oxidise GSH in SZ, and thus a downregulation GSH cycle may result in an inability of the cell to meet the demands of hydrogen peroxide quenching, and thus lead to increased radical formation. Additionally, reduced activity of GPx may be a result of decreased GSH as both impaired GPx activity (500, 501) and decreased GSH concentration (136, 155, 162) have been well-described in human SZ peripheral and brain measures. Figure 5.6 summarises the results of the current study and proposes a model for how the GSH cycle is affected by SZ state in neurons and astrocytes.

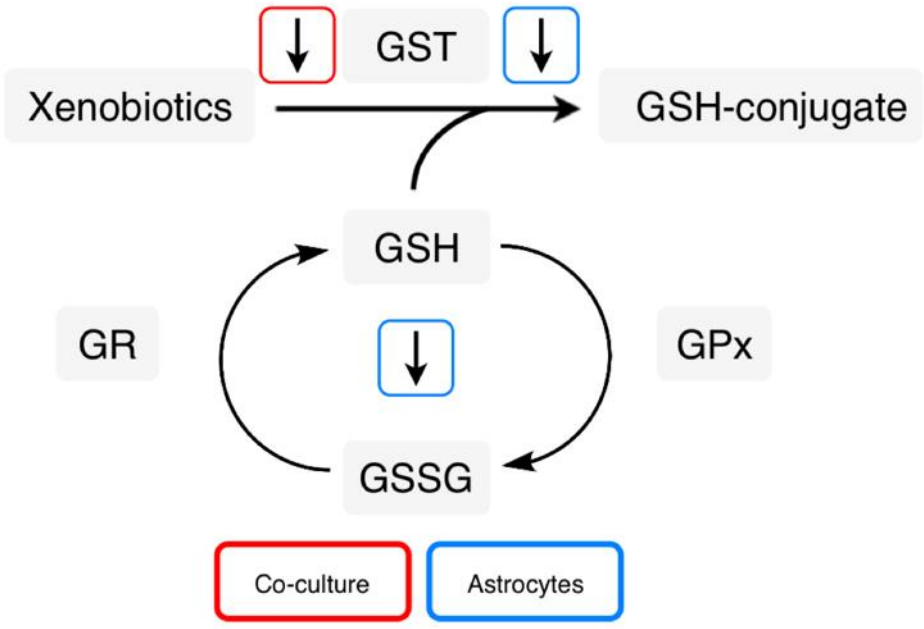


Figure 5.6. Glutathione cycle, including all components assessed in this study. Changes are compared to the control line (GM25256), including disease pathology. Red box: spontaneous differentiation co-cultures, blue box: astrocyte mono-cultures. GSH: glutathione, GST: glutathione-S-transferase, GPx: glutathione peroxidase, GR: glutathione reductase.

The catalytic enzyme GST has a key role in the conjugation of GSH with potentially damaging agents such as xenobiotics and lipid peroxidation products. This occurs via neutralisation of electrophilic sites and renders the products more water soluble. The current study observed significant depletions in GST activity in both SZ cell lines, in both co-cultures and astrocytes. Pejovic-Milovancevic et al (502) identified deletion polymorphisms of the *GSTM1* gene in early-onset SZ, which codes for GST. These authors found that the *GSTM1-null* candidate genotype conferred a 3.36-fold higher risk of developing severe mental disorders, including SZ. GST has also been identified as an important factor in SZ development in post-mortem brain (503), as evidence has shown that one of the GST isoforms was depleted in SZ brains compared with healthy control samples. The current study is the first

study to assess GST in a human SZ iPSC model, and therefore the first to show perturbations in the enzymes that enable GSH to prevent metabolite oxidation.

5.4.2 Oxidative damage in different cerebral cell types

In this study, astrocytes carried greater protein and lipid oxidative damage than the co-culture, which was primarily neuronal. Total antioxidant capacity was also significantly greater in astrocytes than co-culture. It has been well-described that the major pool of antioxidants, especially GSH, is produced and stored in glial cells, to be transported to neurons when needed (486, 504, 505).

There is significant cross-talk between astrocytes and neurons, since glial cells are present to support the function of surrounding neurons. In SZ, hyper-dopaminergic neurotransmission can produce more ROS, which leads to the peroxidation of lipids in the cell. The oxidised lipids can be shuttled to nearby astrocytes which can then act as a buffer for lipid peroxidation toxicity (506). Neighbouring astrocytes endocytose peroxidised lipids and either store them, or use them in the mitochondria to facilitate oxidative phosphorylation (507). The current study observed significantly greater 8-isoprostane concentration in astrocytes compared with co-culture, in both control and SZ samples. These data correspond with other studies that also show that astrocytes can quench the toxic peroxidised lipids produced by nearby neuronal cells. Astrocytes are more lipid-rich than neurons (508), and represent a greater target for peroxidation by ROS, but still appear able to absorb peroxidation products for neurons.

Protein carbonyl concentration was 87.5% greater in SZ co-culture neurons than the control cultures. This may be crucial as carbonylation and increased protein oxidation end products during neurodevelopment could predispose disease manifestation. Studies have noted that offspring from diabetic mothers, who display elevated protein carbonyl concentration, carried a 7-fold increased risk of SZ (509).

Similarly, the lipid peroxidation was 17.9% greater in SZ cells compared with controls. These measures reflect the elevated markers of oxidative damage that have been previously demonstrated in assessment of peripheral tissue of symptomatic SZ patient groups (310, 510, 511). These data support the hypothesis of SZ being a result of neurodevelopmental insult *in utero*, which may later be exacerbated during adolescence to propagate a psychotic phenotype.

5.4.3 Biomarker relationships across different cell types

The relationships between each of the components of the GSH cycle, and markers of stress are important when comparing how disease process may have an effect on overall function.

Although there was no difference in GPx activity between control and SZ astrocytes, there was a significant negative correlation between GPx activity and GSH concentration in SZ astrocytes. This suggests that although enzyme activity is normal, protein content is depleted, downstream of GPx gene expression. This is demonstrated by Xin et al (512), who assessed GSH in the medial prefrontal cortex of FEP patients, using MRS. They observed a positive

correlation between GSH and GPx in healthy control subjects, and a negative correlation in FEP, reflecting the observation in astrocytes in the current study. They also noted that low GSH was related to low oxidative damage in controls, but high oxidation status in patients. This was also observed in the current study, highlighting the relevance of using iPSCs as a model for disease process but also empowering the neurodevelopmental hypothesis posed.

5.4.4 Strengths and limitations

The novelty of assessing the GSH cycle, in the context of diseased human-derived cells is profound. Characterisation of cells at each stage of neural induction gave confidence that neural induction was successful, and is clearly feasible in SZ cells as well as healthy control lines. The attainment of four biological repeats for each cell type allows reasonable conclusions to be drawn with regard to outcomes that are relevant for disease. However, it should be acknowledged that these cells, and therefore the reported data, does not represent all clinical SZ phenotypes as the number of genetic and epigenetic variants that contribute to SZ pathogenesis is large.

There are methodological limitations: principally, allowing the spontaneous development of neural cells creates a very heterogeneous culture model. Specific cell type, including mature and immature cells, as well as the dominance of a neurotransmitter type e.g. dopaminergic/ GABAergic may be different in each experimental ‘n’. Also, the co-culture cells used in this study were cultured for 35 days after 7 neural progenitor passages. Typically, the longer these cells are left in culture, the more astrocytic the culture platform becomes. In an attempt to mitigate this, and make each culture comparable all

cells used in this study were lysed at day 35, but if cultures were to be left for longer periods of differentiation, the ratio of neurons to astrocytes would be different. Therefore, comparison with other studies using iPSCs should be undertaken with caution. The SZ cell line used for analysis in this study provided a snapshot of one affected genotype that conferred the SZ phenotype, but since inheritance of SZ and psychosis is a complex combination of aberrant gene expression and environmental influence it is impossible to say whether these data are truly representative of a disease phenotype in a mature human.

Cells in culture rely on the constant manual removal and replacement of growth media, which is not entirely reflective of the *in vivo* physiological environment, as cells in the human brain receive a flow of fresh growth factors and removal of metabolites continually, without the gradual build-up of waste products over one or two days followed by total replacement.

The loss of two cell lines; control (GM23450) and SZ (GM23760), reduced the statistical power of the study to make conclusions.

5.4.5 Conclusions

This study offered valuable insight into a number of the research questions posed; principally, the study shows that it was possible to neurally induce iPSCs and characterise mature neurons and astrocytes from patient donors. In addition, the GSH-cycle and redox status perturbations that have been previously observed in symptomatic SZ patients were also demonstrated in this neurodevelopmental model of SZ, indicating that the pathological changes associated with disease may occur *in utero*.

Future research should investigate the role of potential therapeutic agents that may increase cellular GSH, taking into account the limited ability of many molecules to pass through the blood brain barrier. This iPSC model of SZ has demonstrated the research potential of using a human model of disease that recapitulates the peripheral deficits observed in symptomatic humans.

CHAPTER 6. THESIS SUMMARY AND FUTURE DIRECTIONS

The work presented in this thesis has investigated the role of exercise in altering some of the redox-related perturbations associated with first-episode psychosis (FEP) pathogenesis. The principal theme is the antioxidant glutathione (GSH), its role in response to exercise training, peripherally and in the brain, and a characterisation of the neurodevelopmental role it may have as a precursor to psychosis, and subsequent therapeutic target for exercise.

Studies have shown that redox homeostasis and the GSH antioxidant cycle are altered in FEP. Given that a number of interventional strategies can ameliorate these perturbations, FEP can be considered a clear target for early intervention. Persuasive evidence suggested that exercise training represents a positive therapeutic intervention to target these mechanistic alterations, based on previous work in healthy populations. Using exercise as an intervention in FEP has highlighted limitations however. It is difficult to retain this population in an exercise intervention, and engaging participants in worthwhile exercise bouts that are known to elicit an adaptive response has proved problematic. Evidence suggests that FEP and SZ are conditions that propagate during neurodevelopment, and thus, the alterations in cell metabolism, antioxidant status and redox balance described during illness may precede symptom presentation.

The overall aim of this thesis was to investigate exercise as a strategy to perturb the oxidative stress which is characteristic in first-episode psychosis. A

collection of exploratory studies were conducted, incorporating healthy sedentary participants to inform study design, FEP patients to take part in an intervention of exercise, and a cell model of SZ to allowed specific evaluation of the glutathione cycle in a model controlled environment, designed to represent neurodevelopment. This body of work employed a novel combination of methodological approaches to address the aims of each study.

6.1 Summary of work

Chapter 2, *"Standardising bouts of exercise for use in a clinical population",* aimed to assess the ability of different modes of exercise to perturb markers of redox status, in order to standardise exercise for use in a clinical intervention. The study investigated if, by allowing more choice of exercise modalities for the FEP participants, engagement and retention of participants would be better than had previously been seen. A healthy but sedentary cohort was recruited to complete acute bouts of exercise, standardised by heart rate, across a variety of exercise modalities. Blood GSH and a marker of lipid peroxidation, 8-isoprostane, were determined pre-and post-bout, in order to tariff the bouts based on redox response. In standardising activity types for use in the randomised trial, we found that the provided exercise bouts stimulated a change in GSH, signifying the activation of adaptive processes, but exercise did not cause an increase in oxidative adduct formation, precursors to a state of oxidative stress. It was possible to observe a similar effect of exercise when undertaken in a personalised, pre-determined heart rate range. High completion rates of this study demonstrated that compliance can be achieved through choice and variety of exercise modalities provided.

Having designed an intervention that would better encourage participation and exercise quality, as well as prioritise bout quality, we invited a cohort of FEP patients to take part in a randomised trial, presented in chapters 3 and 4. In chapter 3: "*Designing a feasible exercise intervention in first-episode psychosis: exercise quality, engagement and effect",* the feasibility of exercise as an intervention was assessed. Using the pre-standardised design, this randomised controlled trial assessed the feasibility of engaging young FEP patients from an Early Intervention service in regular supervised exercise. The study was designed to maintain bout quality, as assessed by heart rate, as well as engage participants in order to promote compliance. Functional outcomes including quality of life, disability and symptoms of psychosis were compared before and after exercise training to explore the potential for exercise in this particular study design to elicit any benefit. From a patient's perspective, improvements in functional measures and facilitation of 'normal' everyday life is a priority. This project has demonstrated that it is possible to engage an FEP group in regular exercise, meeting WHO recommendations, as long as it is provided; supervised and accompanied. Indices of quality of life, perceived disability and symptom profile were all augmented with exercise provision; factors that therefore contribute to a better-functioning patient cohort. Ultimately the cost burden to local health services can be reduced.

Chapter 4 then presented the outcomes of the biomarker arm of this randomised trial: *"Exercise as a protective mechanism against the negative effects of oxidative stress in first-episode psychosis: a biomarker led study".*

This aspect of the randomised trial focused away from patient-orientated outcomes, toward the effect of the intervention on markers of antioxidant capacity (blood and brain), free-radical mediated damage and chronic inflammation in FEP. In order to assess how GSH was regulated in the brain and peripherally, whole blood GSH was quantified using an enzyme-based assay, and measurement of brain GSH employed magnetic resonance spectroscopy. In psychosis research, this is the only study to have measured both pools of GSH before and after an intervention. Further, blood samples prior to and post-intervention provided a descriptive picture of redox status, antioxidant capacity and inflammation in FEP, and the adaptive effects of exercise on mitigating redox status in symptomatic patients. Exercise training in a group of young men with a FEP diagnosis was sufficient to arrest negative disease-related changes observed in the control group of care-as-usual participants, including markers of lipid peroxidation and total antioxidant capacity. Peripheral blood and brain GSH appear to be regulated differently, since exercise was protective of significant decline in peripheral GSH in the control group. We also observed reductions in CRP and TNF-α markers of chronic inflammation as a result of exercise, linking inflammation and oxidative stress as targets for exercise therapy.

Overall, the intervention study presented in chapters 3 and 4 suggested that prescription of regular exercise in this clinical group is a feasible and well-received adjunctive therapy alongside usual care in FEP. A number of difficulties that have been well-reported in FEP exercise intervention design were addressed during this study. Vancampfort et al (301) identified the main

predictors of retention as delivery of exercise by a professional, supervision and being an inpatient. All of the participants of this study were outpatients, and the lack of motivation to travel for exercise, or anxiety related to travel were limited by provision of taxis to and from each session. Additionally, a meta-analysis suggested that outcomes in FEP can only be improved in programmes that maximise adherence (439). Each of these important factors were prioritised throughout the delivery of the intervention, hence the high retention rate, session attendance and achievement of target activity levels.

From a biomarker perspective, this exercise intervention informs the design of future studies; exercise training has the potential to normalise or protect against disease-linked deficits in redox homeostasis- a novel direction of mechanistic study that requires further investigation.

These two-distinct series of outcome measures offer a more coherent picture of disease pathogenesis, symptomology and the effects of this training intervention on mechanistic features as well as functional outcome.

Finally, the experimental work in this thesis moved towards a using a controlled model system to investigate redox status as a potential cellular change that might occur during neurodevelopment in SZ. Chapter 5 presents the study *"Neurodevelopmental hypothesis of SZ assessment of iPSC-derived neurons and astrocytes".* It was felt that having assessed glutathione in the periphery and in the brain of symptomatic patients, a cell model of SZ would provide an excellent environment to study the antioxidant cycle in detail. In human populations, FEP in particular, achieving adherence to a lifestyle change is difficult, making description of mechanisms that may underpin an intervention

challenging. This presents a need for models of a population to investigate mechanistic processes in a controlled environment. The main aim of this study was to compare the different elements of the GSH system in control and disease human-derived cerebral cells; neurons and astrocytes. The rationale for using this design was that it would provide a neurodevelopmental model of disease pathogenesis, and offer the opportunity to understand one potential aspect of the emergence of psychoses during adolescence. To our knowledge, this study was the first time these patient-derived SZ iPSC lines have been successfully neurally induced to produce mature astrocytes and neurons. Cells were characterised at each step of neural induction, alongside healthy control cells. The iPSC section of this research has highlighted novel targets for the identification and emergence of psychotic illness in adolescence, since there appears to be a neurodevelopmental role of the GSH cycle and oxidative stress. In particular, the ability of GSH to remove toxic xenobiotics was impaired in SZ rather than control cerebral cells, and total GSH was significantly depleted in astrocytes, the ‘care-takers’ of neuronal cells. iPSCs as a model of human disease, and assessment of the neurodevelopmental perturbations in SZ cells warrants further investigation.

6.2 Insights to inform future studies

The data collected during this collection of pilot studies provides the rational for much larger scale, refined research around the role of exercise as a therapy for early psychosis, and the potential of iPSCs to offer mechanistic insight into the neurodevelopmental aspects of psychosis pathogenesis. Given the opportunity to develop this research, I would first of all begin a randomised interventional

trial, similar to the one described in this thesis. This new, multi-site project would be slightly different in a number of ways; principally, the patient-focused aspect would prioritise long-term lifestyle change, encouraging regular physical activity outside of the study setting, and dis-associated from the 'care' environment. Longitudinal monitoring of exercise habits, post-intervention period, as well as a collaboration with local leisure providers is a key strategy to encourage success. The assessment of brain GSH described in this thesis was an important measure of antioxidant capacity, especially when compared with the peripheral blood sample. We determined that both GSH pools were regulated differently in response to exercise, but a greater number of successful scans would suggest potential targets for treatment, as well as reduce the methodological heterogeneity of using MRS. Positive and negative symptoms were well characterised in the current project, but the third aspect of symptomology, cognition, was neglected. A future study should take this into account, especially given the beneficial potential of exercise to improve indices of cognition (513). Alongside exercise training, with its cornucopia of physiological and psychological benefits, research on cognitive remediation holds therapeutic potential. Cognitive remediation is a rehabilitation treatment aimed at improving attention, memory, language and/or executive functions (514), with indirect positive effects on daily functioning (515-517). There is also some literature describing some important targets of cognitive training with neurobiological correlates in SZ. Cognitive performance has been linked to neuro-inflammation in SZ, as well as prolactin protein concentration and neurotrophin growth factor depletion, in particular BDNF (518).

An exercise trail with three arms; control (cross-over design, treatment as usual), exercise and exercise with cognitive remediation can also be mirrored using iPSCs. This would provide detailed insight into the effects of each intervention type, in different cerebral cell types, in disease versus healthy control donor-derived cells. Treating a culture with low concentrations of H_2O_2 replicates the low-level radical production synonymous with moderate intensity exercise training, and stimulates the adaptive response (519). Additionally, treatment of cells with factors associated with elevated cognition e.g. BDNF can, to an extent, model the cognitive remediation aspect of the proposed human trial.

6.3 Conclusions

The research in this thesis supports evidence that moderate-intensity exercise training has the capacity to stimulate change in markers of antioxidant capacity (200), oxidative stress (199) and inflammation (208). We also observed positive changes in measures of psychopathology (233), quality of life (232) and disability (263) as reported previously. The cell work described mirrors previous work characterising aberrant oxygen metabolism in SZ iPSCs (294).
Work undertaken during this PhD also adds to the knowledge base with the following novel findings:

1. It is possible to implement a trial design in FEP that promotes autonomy, enjoyment and compliance whilst achieving an intensity known to elicit redox adaptation.
2. Exercise training improves indices of psychopathology and functional outcome, including symptomology, quality of life and disability.

3. Sustained exercise training is feasible in an FEP group as long as it is provided. It is possible to achieve a target intensity of individual bouts through a prioritisation of autonomy of exercise modality and provision of personalised heart rate zones.
4. Moderate-intensity exercise training has the capacity to arrest disease-related changes in redox biomarkers.
5. It is possible to generate mature astrocytes and neurons from a SZ model of iPSCs.
6. SZ astrocytes have a decreased ability to generate GSH as a protective mechanism in the brain.
7. SZ astrocytes and neurons demonstrate a decreased capacity to remove oxidative products and xenobiotics via glutathione-S-transferase.
8. The perturbed GSH cycle and redox status that has been well-characterised in symptomatic patients demonstrates aberrant regulation during neurodevelopment.
9. There is a decreased capacity of SZ cerebral cells to oxidise GSH, and subsequently quench H_2O_2, rendering the cell more susceptible to oxidative damage.
10. Oxidative damage to cell components (lipids and protein) is elevated in SZ cerebral cells compared with healthy control counterparts, implicating the initiation of redox balance *in utero*.

SUPPLEMENT 1: USE OF FREEZE-THAWED WHOLE BLOOD FOR DNA DAMAGE ANALYSIS

In order to validate the use of freeze-thawed whole blood samples for Comet assay in this study, a preparation method that is not the norm in DNA damage analysis, fresh versus frozen samples were compared for single strand break quantification using samples from a single healthy participant. It was important to assess the difference in comet tail intensity, between immediate analysis and freezing at -80 degrees. The freeze-thaw process has potential to cause cell damage and lysis (520).

Whole blood was collected at two time-points in an EDTA vacutainer, from the same participant. The sample take at time point 1 was immediately

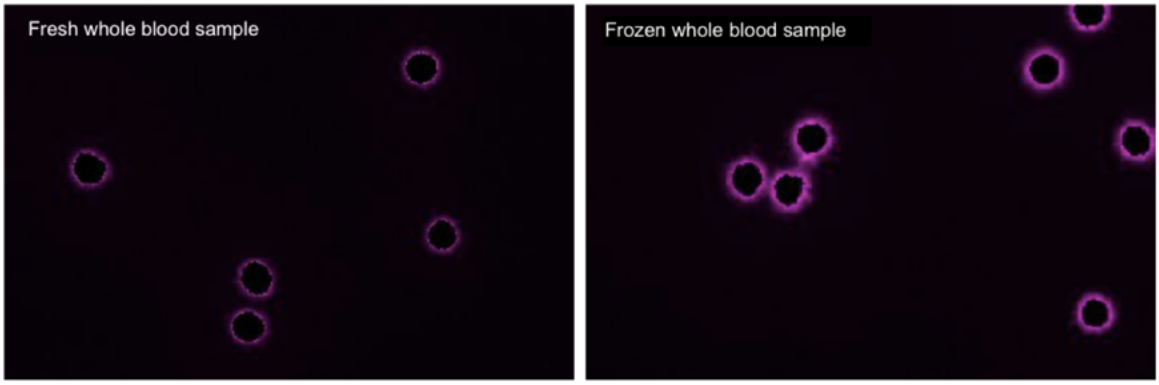


Figure S1.1. Cells from fresh whole blood sample (left) versus cryopreserved whole blood sample (right). Frozen cells appeared brighter, since the quantity of single strand DNA breaks increased as a result of the freeze-thaw process.

stored at -80 degrees, for 1 week. The second sample was taken and prepared for comet assay immediately. The samples were taken at a similar time of day, with no change in habitual exercise or diet between the time points. Both sample types (fresh and frozen) were assayed in triplicate, at the same time (figure S1.1).

Normally, storage, extraction and assay workup of blood samples associated with a risk of artefactual formation of damage. Al-Samani et al

(521) successfully stored whole blood in 250μl aliquots, without cryopreservative, for up to 1 month without artefactual formation of DNA damage. Conventional comet analysis involves the isolation of mononuclear cells by centrifugation, suspension in freezing medium and slow freezing to -80 degrees, and percentage DNA fluorescence in the comet tail indicates frequency of strand breaks (522). For this comparison, and the main intervention study, blood cells were not isolated, or suspended in freezing medium before cryopreservation.

It was found that the freeze-thaw process resulted in a significant increase in comet tail intensity (*p=0.05*), and therefore single strand DNA breaks (figure S1.2).

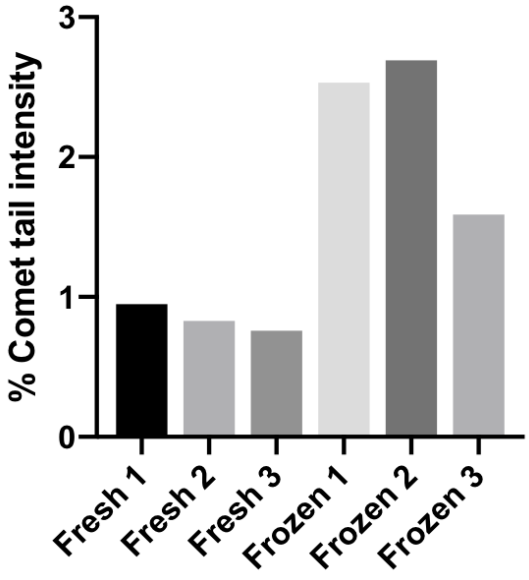


Figure S1.2. Comparison of fresh whole blood versus freeze-thawed whole blood for Comet-tail intensity.

Because every sample take for the intervention study was stored and thawed in the same way, our data is comparable between individuals and

groups, but it is important to note that the freeze-thaw process as opposed to analysing fresh blood samples may show a false-increase in comet tail intensity percentage.

SUPPLEMENT 2: PREPARATION OF PROTEIN CARBONYL STANDARDS

The protein carbonyl standard curve was generated using the following protocol. Dialysis tubing was prepared firstly by boiling in 2% w/v sodium bicarbonate and 1mM EDTA (pH8), followed by rinsing in ddH_2O. The tubing was boiled for a further 10 minutes in 1mM EDTA (pH8), followed by storage in EtOH at 4 degrees.

Oxidising BSA

BSA (10mg/mL) (50mL) was oxidised with AAPH (dihydrochloride) (500mM) for 1h at 37 degrees.

Reducing BSA

BSA (10mg/mL) was added to sodium borohydride (1g) in TBS (50mL) and allowed to reduce overnight at 4 degrees. The soltion was then dialysed against TBS at pH7, with frequent buffer changes. This served to sualyse the pH to 7 without the need to use acid. Dialysis was complete after approximately 24h, and was checked with pH.

Oxidised BSA was also dialysed for 24h with frequent buffer changes (TBS pH7).

Protein content was checked after dialysis (usually 6-8mg/mL) (some loss expected after dialysis).

Assessing carbonylation of standards

Protein concentration of the oxidised and reduced BSA was adjusted to the same value, and solutions were mixed in ratios to give a range e.g. 1mL oxidised and 9mL reduced. Standards (500μL) were added to DNPH (10mM, 500μL) and left for 1h with gentle agitation. Controls were made with HCl (2N, 500μL), and added to DNPH (10mM, 500μL). TCA (20%, 500μL) was then added to each standard and mixed before centrifugation (3min at 13000 x g). The supernatant was discarded and the pellet washed three times with ethanol:ethyl acetate (1:1). Following washing, pellets were re-suspended in guarnidine HCl (1mL), vortexed, and left for 30 mins at 37 degrees. Solutions were vortexed once more before centrifugation (1min at 13000 x g). The supernatant was then removed and absorbance read at 360nm. The molarity of the solution was determined by the following equation:

(absorbance-blanks)/22000

Protein content was checked, to give carbonyl content, M/mg or nmol/mg.

SUPPLEMENT 3: CHARACTERISATION OF iPSC CELL LINES MODELLING SCHIZOPHRENIA AND HEALTHY CONTROL COMPARISONS

There is a perpetual requirement for novel cell lines in disease modelling, and the iPSC lines presented in this study are limited in publication record, most likely due to the relatively recent emergence of iPSC technology. Using observations from the Coriell repository from donor fibroblast to iPSC, as well

Table S3.1. Characteristics of iPSC generation process for each donor

	GM23760	GM23762	GM23450	GM25256
Diagnosis	Schizophrenia	Schizophrenia	Healthy control	Healthy control
Passage of iPSC at submission	14	15	21	P37 total
No. passages at Coriell	11	12	7	
Media	KO DMEM + 20% KOSR + 50ng/ml FGF	KO DMEM + 20% KOSR + 50ng/ml FGF	DMEM/F12 + 20% KOSR + 100ng/ml bFGF	mTeSR1
Feeder	CF1 MEFs on 0.1% Gelatin	CF1 MEFs on 0.1% Gelatin	CF1 MEFs on 0.1% Gelatin	Matrigel
Passage method	TrypLE Express	TrypLE Express	Collagenase	Versene
Split ratio	1:6 every 5-7 days	1:6 every 5-7 days	1:6 every 4-6 days	1:8 every 5 days
Pluripotency score	Pluripotency: 38.54 Novelty: 1.478	Pluripotency: 32.37 Novelty: 1.546	NA	NA
SSEA4	82%	87%	80%	NA

KO DMEM: Knock-out Dulbecco's Modified Eagle's Medium, KOSR: Knock-out Serum Replacement, FGF: Fibroblast Growth Factor, bFGF: Fibroblast Growth Factor- basic, MEFs: Mouse Embryonic Fibroblasts, TrypLE Express/ Collagenase/ Versene: different reagents used to lift cells from culture vessel, SSEA4: Surface Antigen Expression of Stem Cell Markers.

as our own establishment of mature cortical cells from pluripotent iPSCs, it has been possible to describe the immunophenotypic, morphological and growth characteristics of these four cell models. Details of the iPSC generation processes for each cell line are described in table S3.1.

GM23760 (schizophrenia)

Human fibroblasts were reprogrammed with five factors (Oct4, Sox2, KLF4, MYC, Lin28), using a lentiviral vector. The donor was a 26-year old male, with a diagnosis of SZ. Symptom profile consisted of episodes of agitation, delusions of persecution and fear of assassination. The father was also affected, suggesting a strong genetic factor for pathogenesis.

This cell line has been used in SZ research to describe impaired angiogenesis and migration in neural progenitor cells (523), suggesting defective crosstalk between neural stem cells and endothelial cells during the formation of the neuro-angiogenic niche. Brennand at al (524) also used these cells to produce neurons, showing diminished neural connectivity in conjunction with neurite number, and reduced glutamate receptor expression.

Post-thaw viability (figure S3.1) (as reported by Coriell Institute):

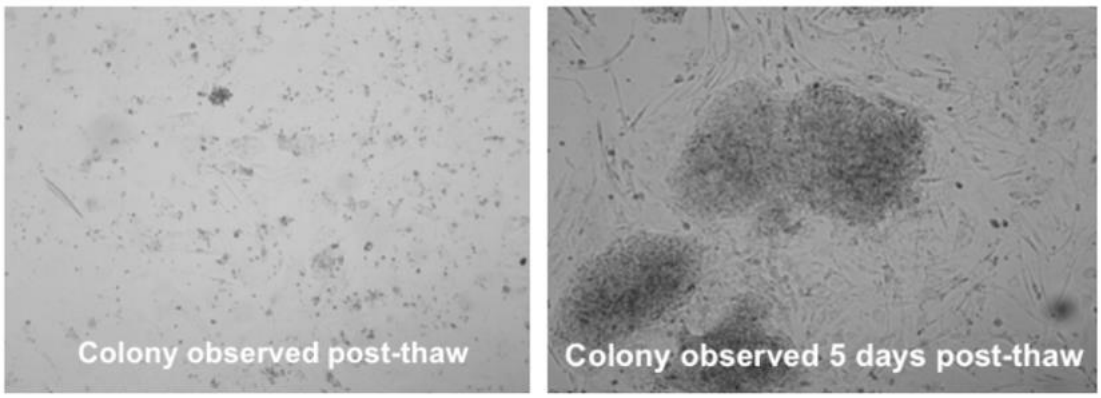


Figure S3.1 Post-thaw viability of GM23760 iPSC, using the 'colony doubling' method. Coriell Institute passed this cell line.

GM23762 (schizophrenia)

Human fibroblasts were reprogrammed with five factors (OCT4, SOX2, KLF4, MYC, LIN28), using a lentiviral vector. The donor was a 23-old male, with a diagnosis of SZ, symptoms included paralogical thinking, affective shielding, slitting of affect from consciousness and suspiciousness. Symptom onset began at age 15, leading to hospitalisation. There is a positive family history of psychosis and SZ.

This cell line was also used in the Brennand study, utilising a neural model of SZ (524).

Post-thaw viability (figure S3.2) (as reported by Coriell Institute):

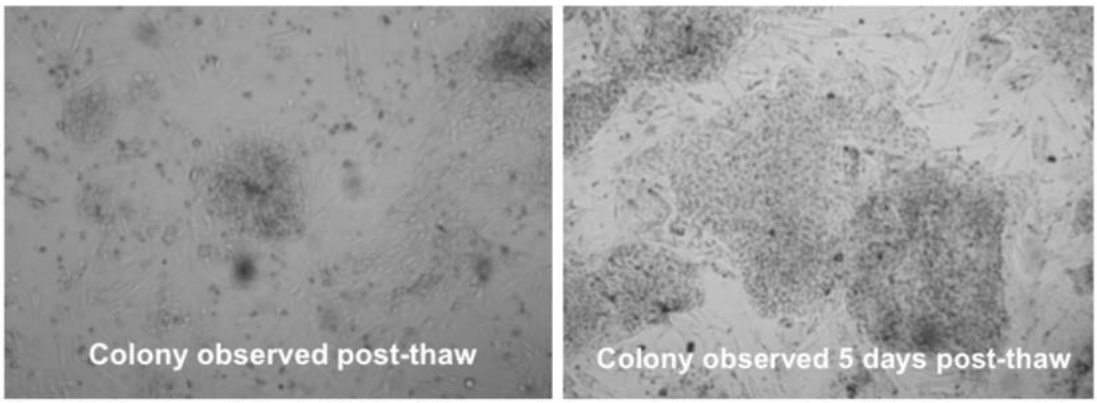


Figure S3.2 Post-thaw viability of GM23762 iPSC, using the 'colony doubling' method. Coriell Institute passed this cell line.

GM23450 (healthy control)

Human fibroblasts were reprogrammed with seven factors (Oct4, Sox2, Klf, c-Myc, Nanog, Lin-28 and T-antigen) using a retroviral transformant. The donor cells were derived from apparently healthy fetal tissue, 20 weeks.

This cell line has been used in the protocol development of novel human-iPSC-derived neuron seeding methods (525).

Post-thaw viability (figure S3.3) (as reported by Coriell)

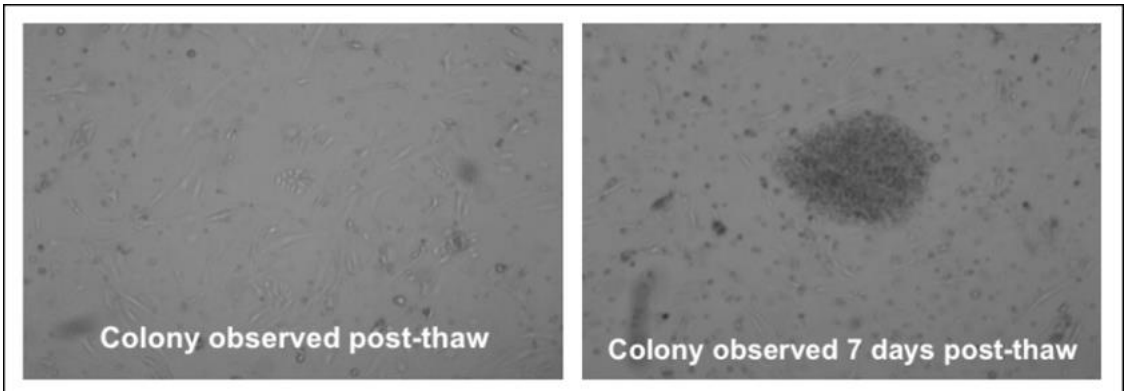


Figure S3.3 Post-thaw viability of GM23450 iPSC, using the 'colony doubling' method. Coriell Institute passed this cell line.

GM25256 (healthy control)

Human fibroblasts were reprogrammed with episomal vectors containing OCT3/4 with shp53, SOX2, KLF4, LMYC and LIN28A. The donor was a clinically-normal 30-year old male.

This cell line has successfully been used in studies assessing a variety of cell types; neurons (526), cardiac organoids (527), cardiomyocytes (528) and genetic and cell-cycle studies of iPSCs (529) (530) (531).

Post-thaw viability (figure S3.4) (as reported by Coriell)

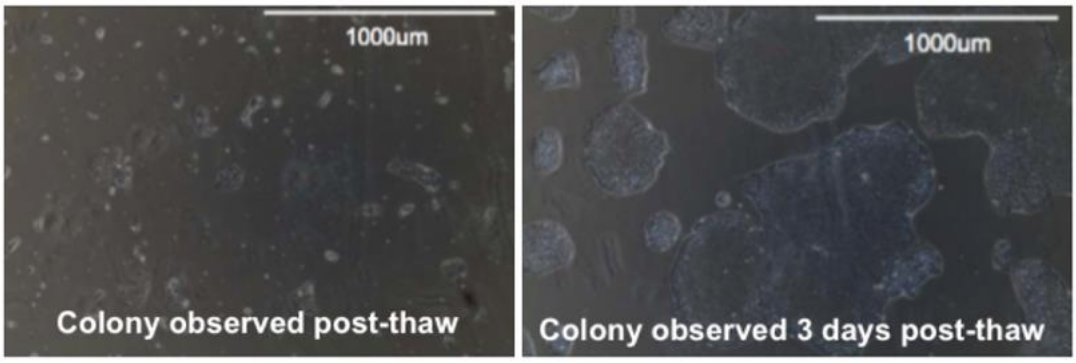
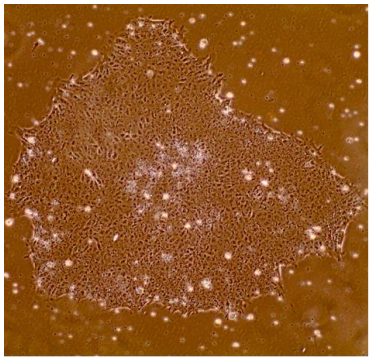


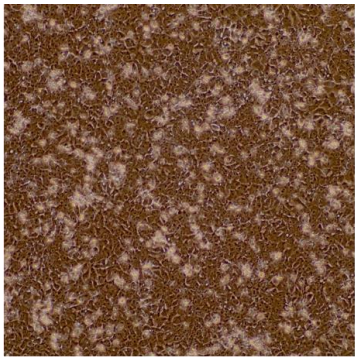
Figure S3.4 Post-thaw viability of GM25256 iPSC, using the 'colony doubling' method. Coriell Institute passed this cell line.

Morphology of iPSC, NPC, astrocytes and co-culture cells

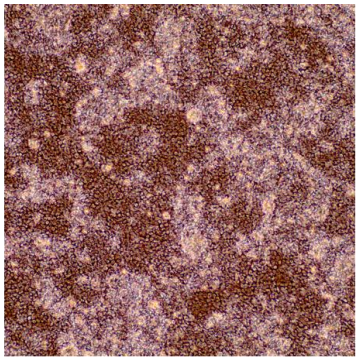
Figure S3.5 GM25256



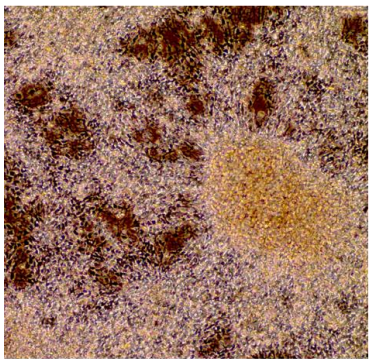
iPSC colony, 2 days post-thaw. P38



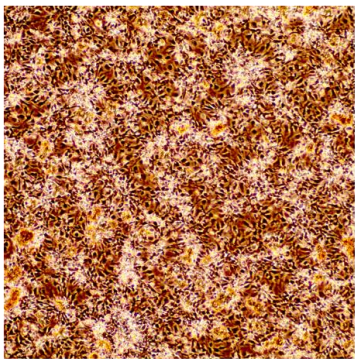
iPSCs, 100% confluency, neural induction day 1. p40



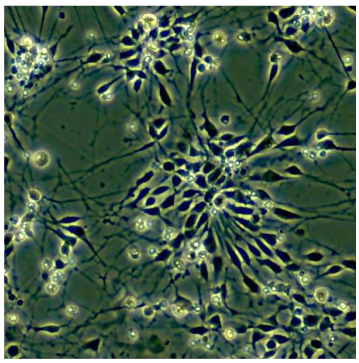
iPSCs, neural induction day 11



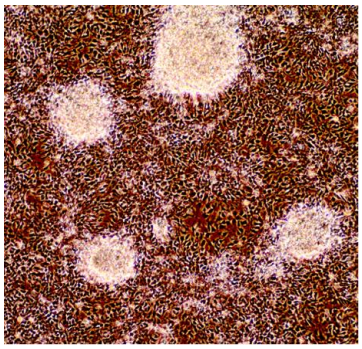
iPSCs, neural induction day 17



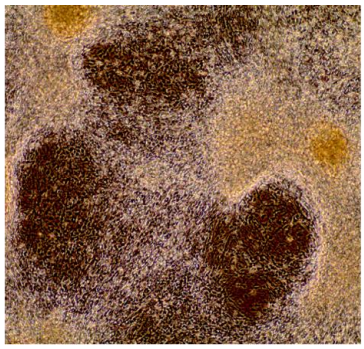
Neural progenitor cells, neural induction day 23. n1



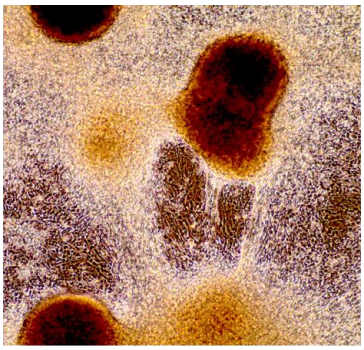
Expanded neural progenitor cells. n5.



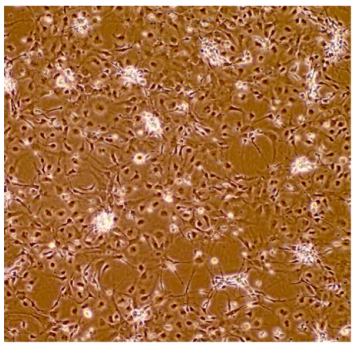
Spontaneous differentiation day 5.



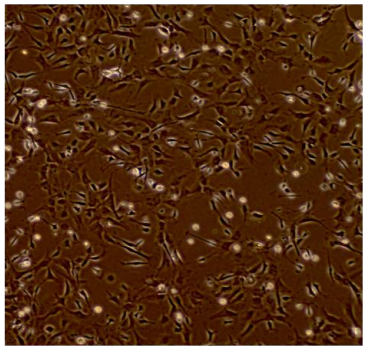
Spontaneous differentiation day 20.



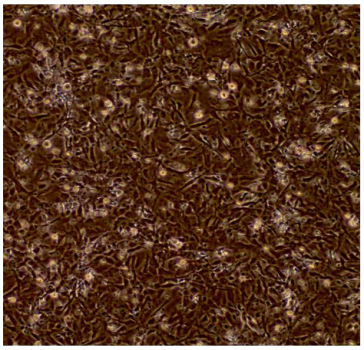
Spontaneous differentiation day 35.



Astrocyte induction day 3.

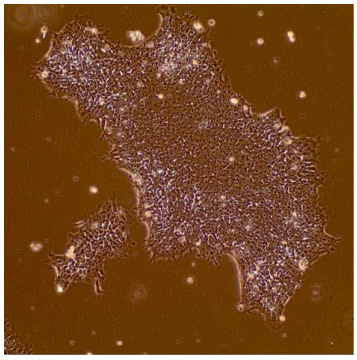


Astrocyte maturation day 8.

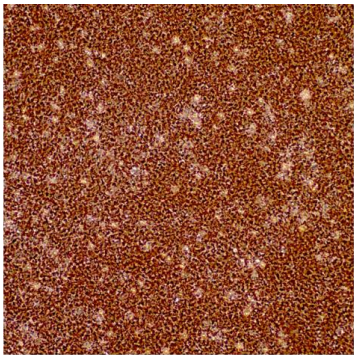


Mature astrocytes day 35.

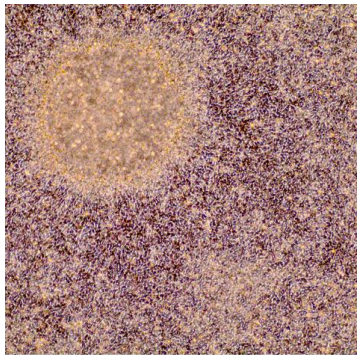
Figure S3.6 GM23762



iPSC colony, expanded and passaged three times post-thaw. p18

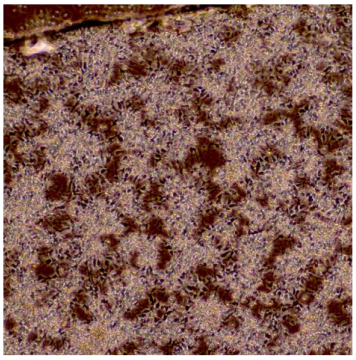


iPSCs, 100% confluency, neural induction day 1. p20

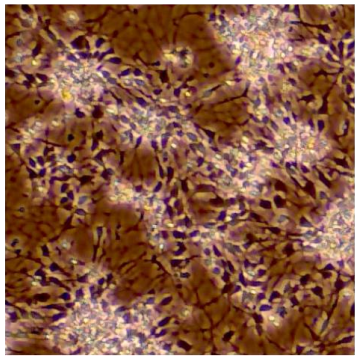


iPSCs, neural induction day 11

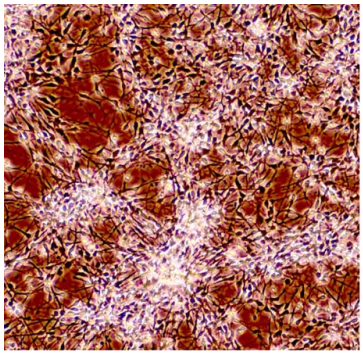
iPSCs, neural induction day 17



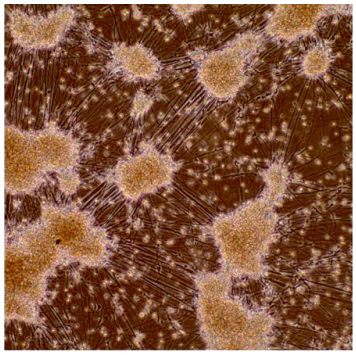
Neural progenitor cells, neural induction day 23. n1



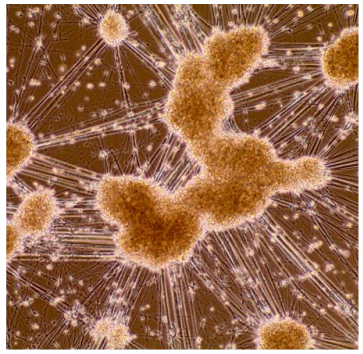
Expanded neural progenitor cells. n4



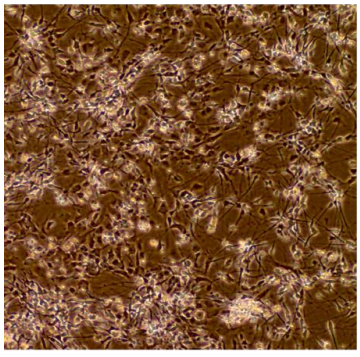
Spontaneous differentiation day 3.



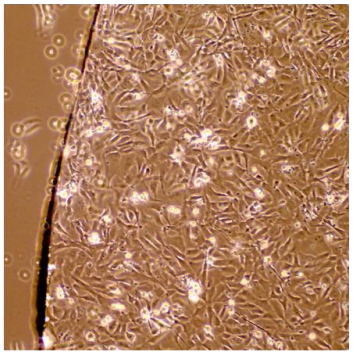
Spontaneous differentiation day 20.



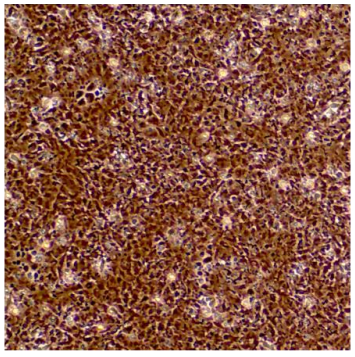
Spontaneous differentiation day 35.



Astrocyte induction day 8.

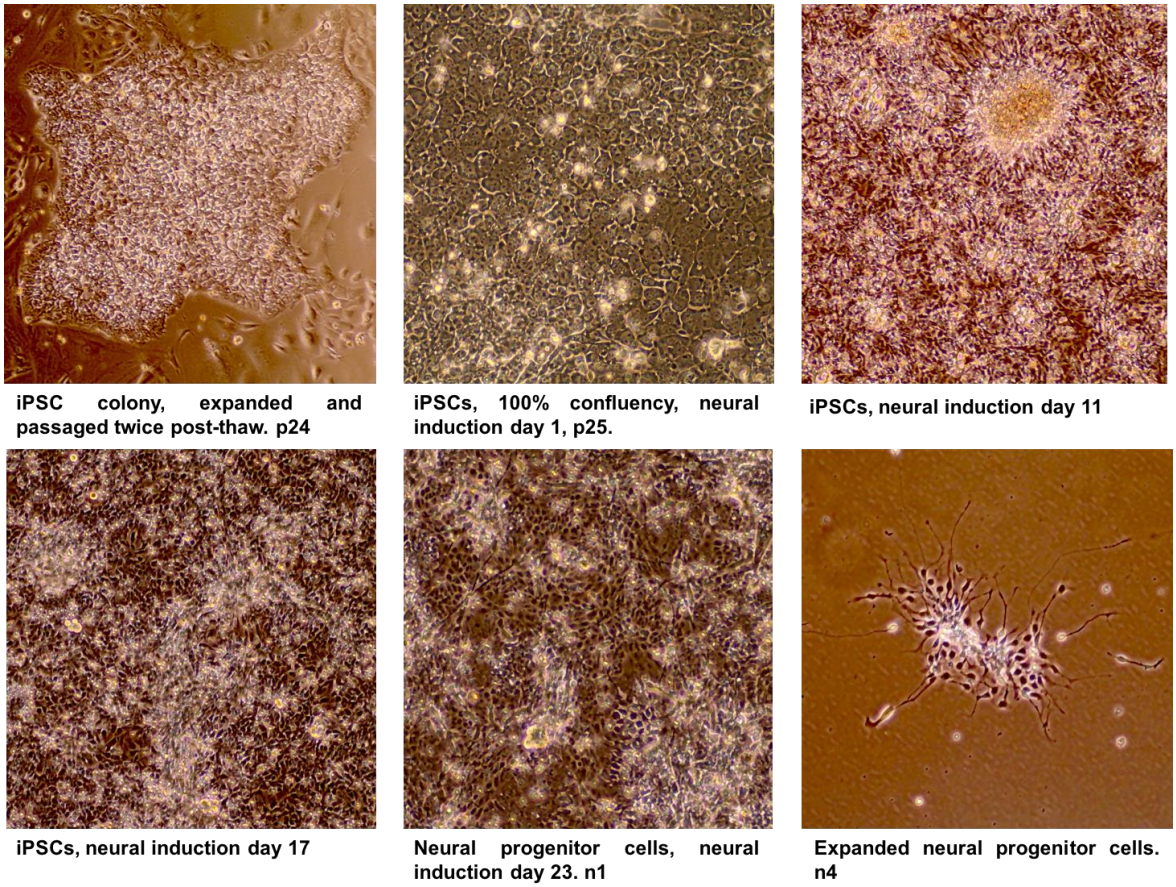


Astrocyte maturation day 9.



Mature astrocytes day 35.

Figure S3.7 GM23450



iPSC colony, expanded and passaged twice post-thaw. p24

iPSCs, 100% confluency, neural induction day 1, p25.

iPSCs, neural induction day 11

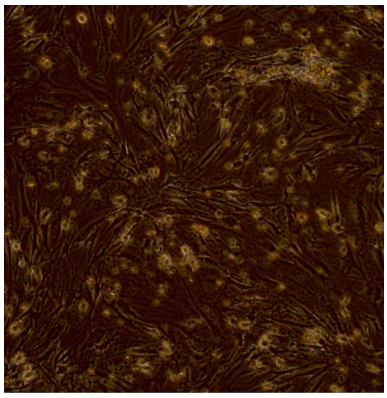
iPSCs, neural induction day 17

Neural progenitor cells, neural induction day 23. n1

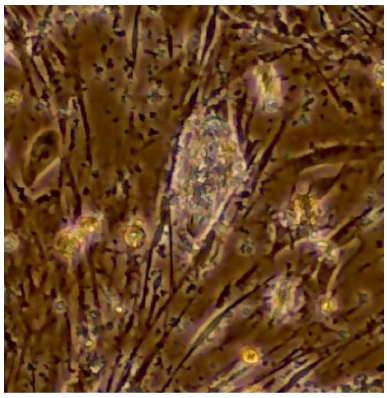
Expanded neural progenitor cells. n4

This cell line was successfully neurally induced, despite a larger-than-normal morphology of iPSCs and NPCs. The day 23 neural induction cells were cryopreserved, and upon thawing developed a bacterial infection and subsequently were disposed of before spontaneous differentiation and astrocyte induction could begin.

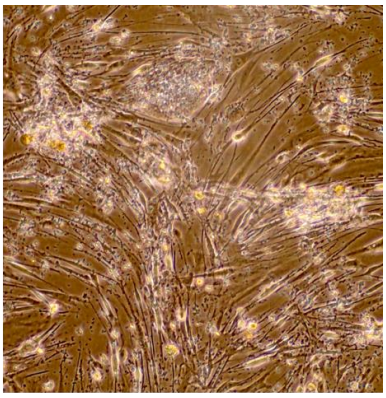
Figure S3.8 GM23760



Mouse embryonic fibroblasts, plated on gelatin ready for iPSC seeding



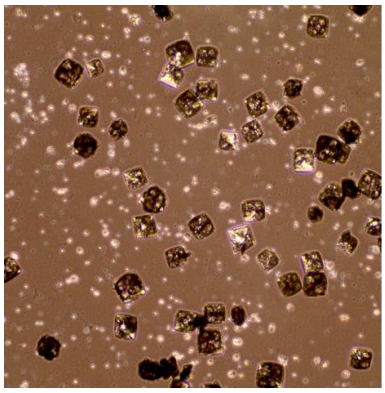
iPSC colony supported by MEF cells



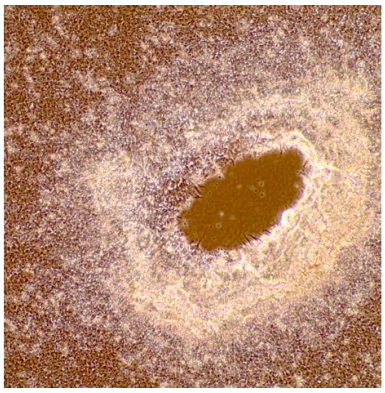
iPSC colonies supported by MEF cells

The GM23760 cell line was seeded onto a MEF cell layer, designed to support the growth of iPSC colonies. At day 7 of expansion, the gelatin coating on the plate surface began to degrade, resulting in lifting of the MEF cells and subsequent expiration of the iPSCs.

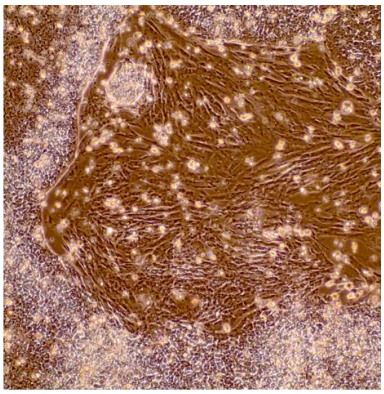
Figure S3.9 Specific problems faced during expansion and neural induction



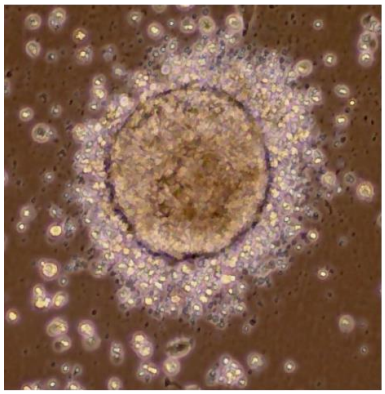
GM25256. Crystal formation as a result of rapid thawing of mTeSR media.



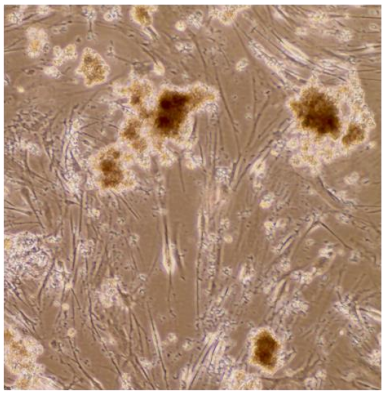
GM23762. Neural induction day 4. spontaneous differentiation of off-target cells due to hole formation in Matrigel-coated plate.



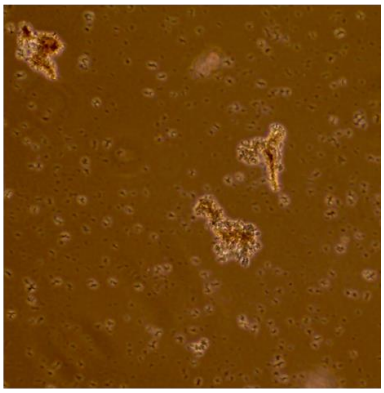
GM23762. Neural induction day 15. Spontaneous differentiation of off-target cells in Matrigel-coated plate.



GM23450. iPSC colony folding on Matrigel coated plate



GM23450. MEF de-attachment due to gelatin degradation



GM23760. Failed recovery of cell line onto Matrigel coated plate following MEF failure

Below are examples of co-stained cells, characterised by immunocytochemistry. In order to recapitulate the tissue of interest (cerebral tissue), it is important to identify the cell-specific antigenic properties that define a particular cell type e.g. iPSC, NPC, neuron or astrocyte. Below is a list of the specific marker employed in this study, the cell-type they are expressed in, and their function.

Oct4 (Octamer-binding transcription factor 4): critically involved in the self-renewal of undifferentiated stem cells, a marker of pluripotency in iPSCs.

Sox2 ((sex-determining region Y)-box 2): a transcription factor essential for maintaining self-renewal, and has a critical role in neural stem cells.

Pax6 (paired box protein Pax-6): transcription factor responsible for regulating coordination and pattern formation, and differentiation and proliferation during neurogenesis. Expressed in NPCs.

Nestin (neuroectodermal stem cell marker): an intermediate filament protein, replaced by GFAP during neurogenesis and gliogenesis. Expressed in NPCs.

Map2 (microtubule-associated protein 2): a neuron specific cytoskeletal protein involved in microtubule assembly.

Tuj1 (class III β tubulin): a neuronal marker present in differentiated neurons

GFAP (glial fibrillary acidic protein): intermediate filament protein, responsible for cytoskeletal structure and function, expressed by mature astrocytes.

S100- β (S100 calcium-binding protein B): Protein regulator of cell cycle progression and differentiation, expressed by astrocyte

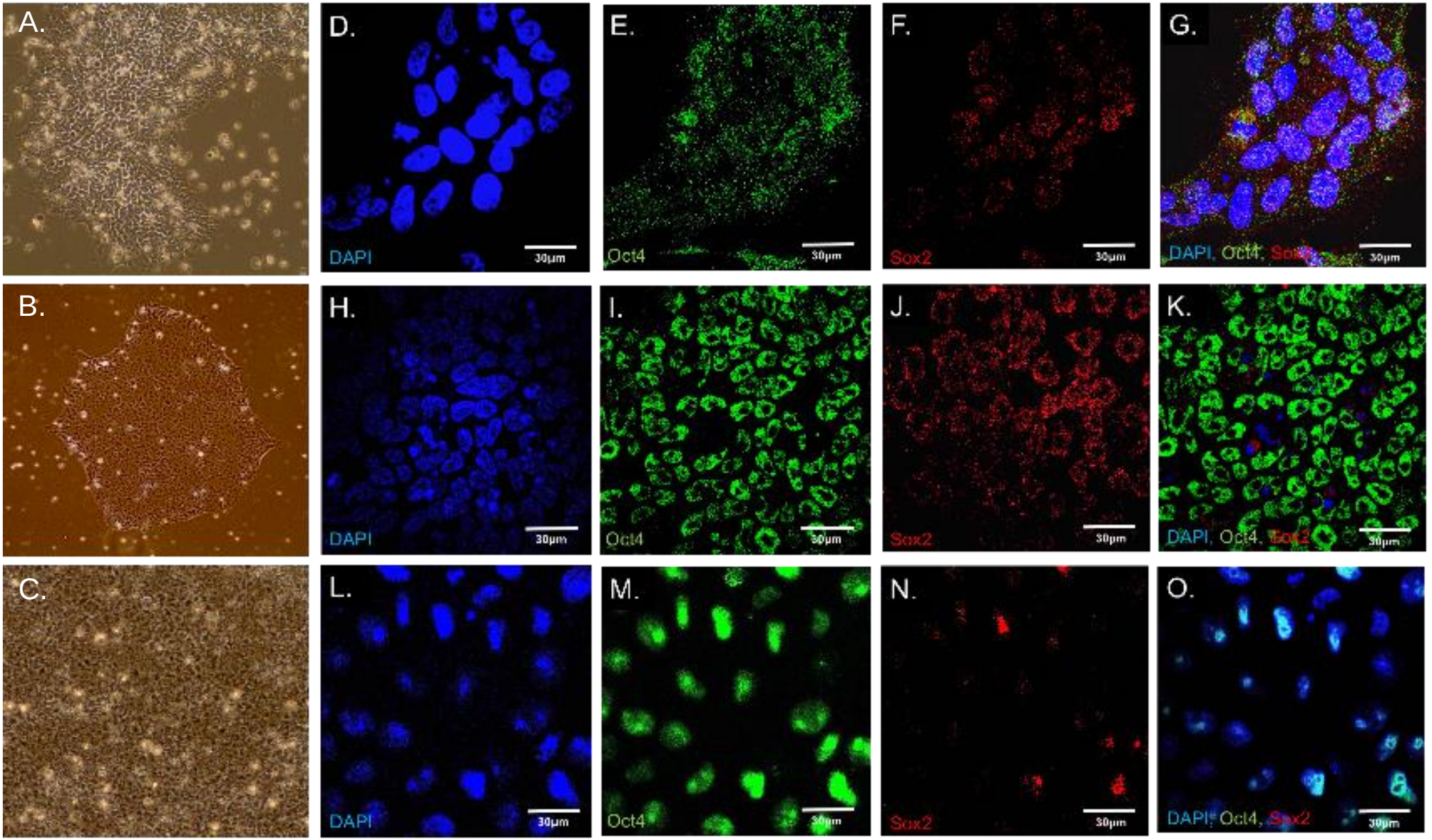


Figure S3.11. *In vitro* neurodevelopmental model of induced pluripotent stem cells (iPSCs), stained for pluripotency markers Oct4 and Sox2. (A, B) GM25256 iPSC colonies, 100X (C) GM23762 iPSC colonies, 100X (D) GM23450 DAPI nuclear stain (E) Oct4 (F) Sox2 (G) DAPI, Oxt4 and Sox2 (H) GM23762 DAPI nuclear stain (I) Oxt4 (J) Sox2 (K) DAPI, Oxt4 and Sox2 (L) GM23450 DAPI nuclear stain (M) Oct4 (N) Sox2 (O) DAPI, Oxt4 and Sox2.

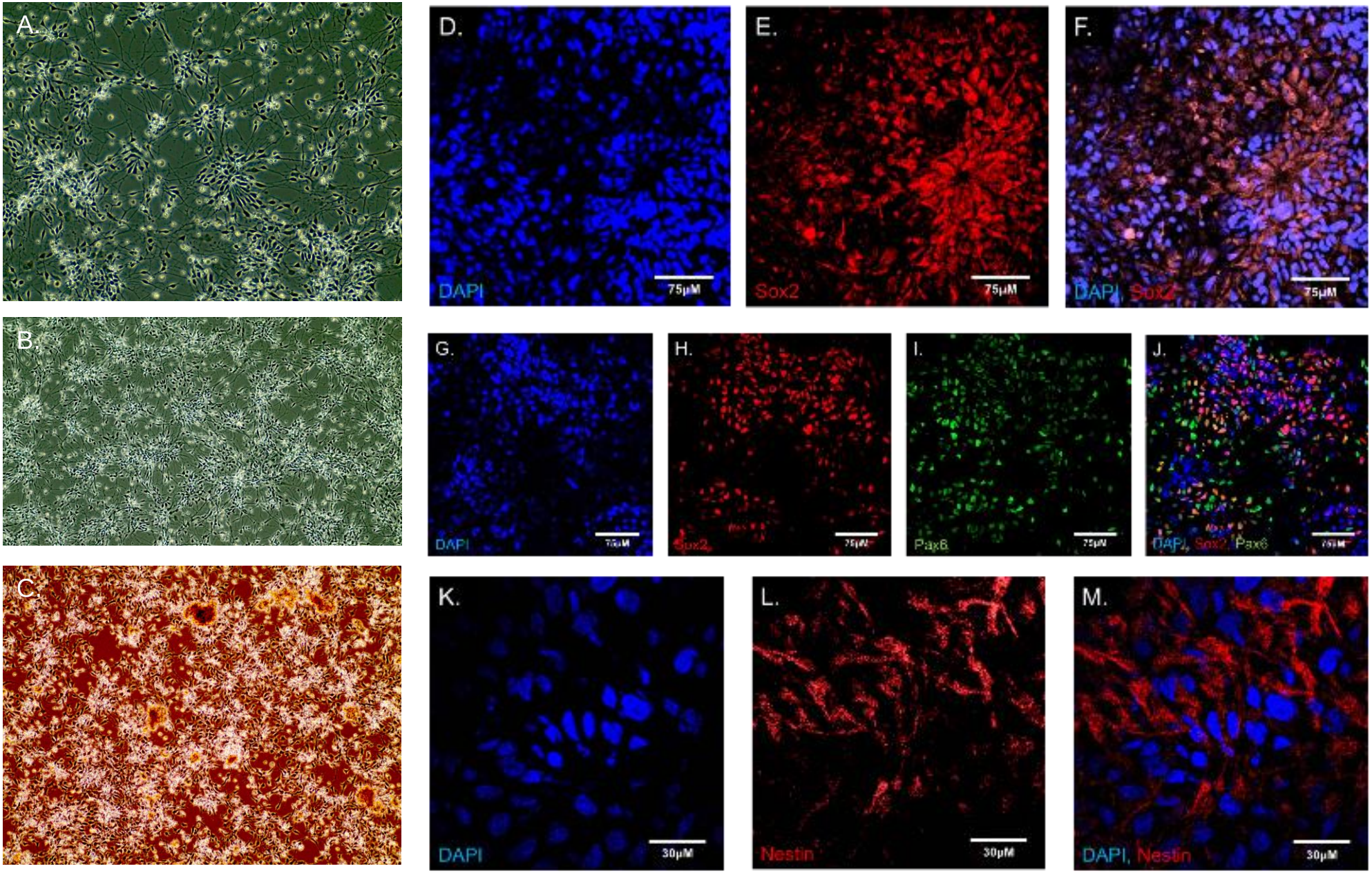


Figure S3.12. *In vitro* neurodevelopmental model of neural progenitor cells (NPCs), differentiated from induced pluripotent stem cells (iPSCs), and stained for Pax6, Sox2 and Nestin pluripotency markers. All images shown are the control cell line GM25256. (A, B, C) NPCs, 100X magnification. (D-M: Immunocytochemistry of NPC cells). (D) DAPI nuclear stain 40X (E) Pax6 (F) DAPI and Pax6 (G) DAPI nuclear stain 40X (H) Pax6 (I) Sox2 (J) DAPI, Pax6 and Sox2 (K) DAPI nuclear stain 100X (L) Nestin (M) DAPI and Nestin.

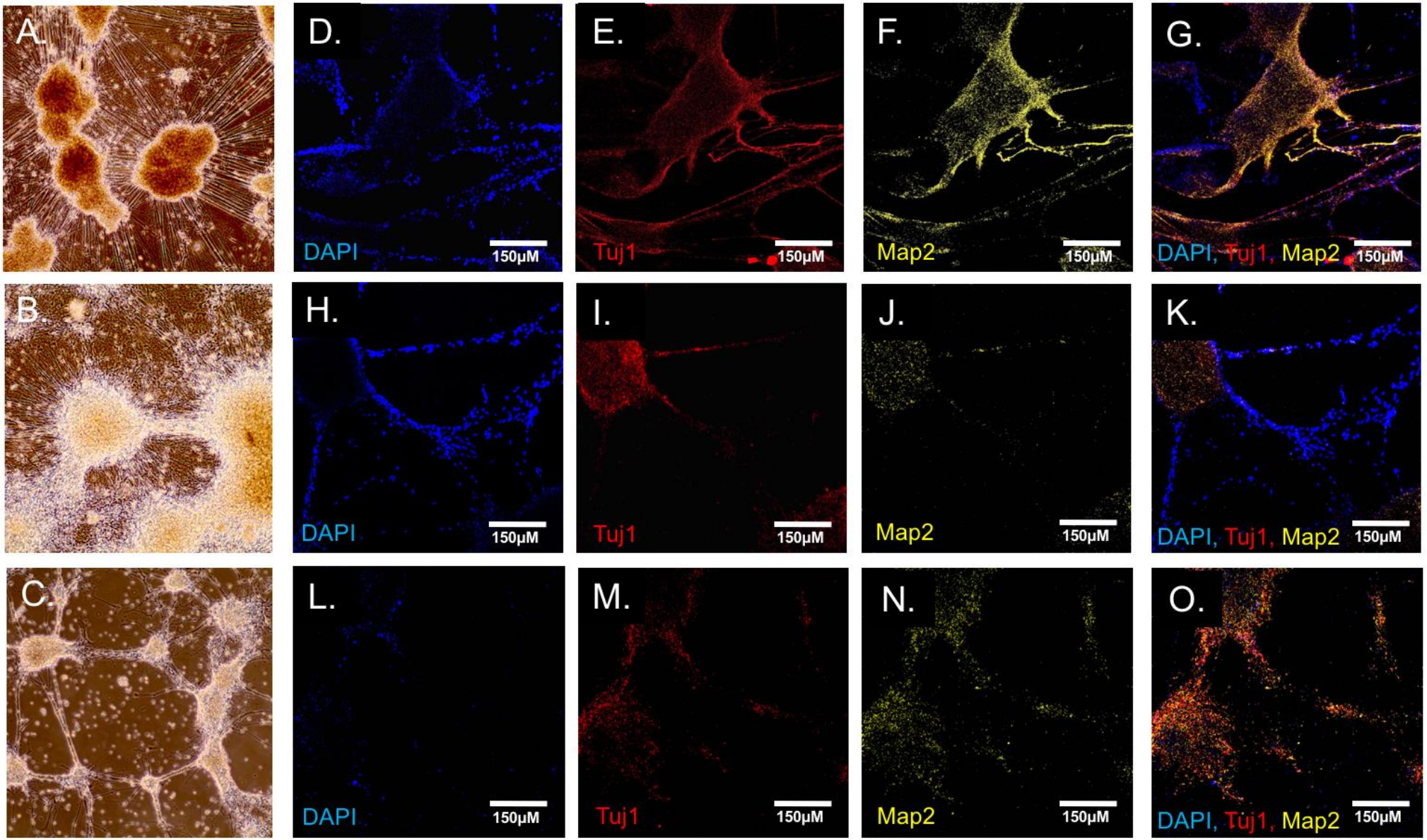


Figure S3.13. *In vitro* neurodevelopmental model of neurons, differentiated from induced pluripotent stem cells (iPSCs), and stained for Map2 and Tuj1 neuronal markers. All images shown are the control cell line GM25256. (A, B, C) Neurons, 100X magnification. (D-O: Immunocytochemistry of neuronal cells). (D) DAPI nuclear stain 20X (E) Map2 (F) Tuj1 (G) DAPI, SOX2 and Tuj1 (H) DAPI nuclear stain 20X (I) Map2 (J) Tuj1 (K) DAPI, Map2 and Tuj1 (L) DAPI nuclear stain 20X (M) Map2 (N) Tuj1 (O) DAPI, Map2 and Tuj1.

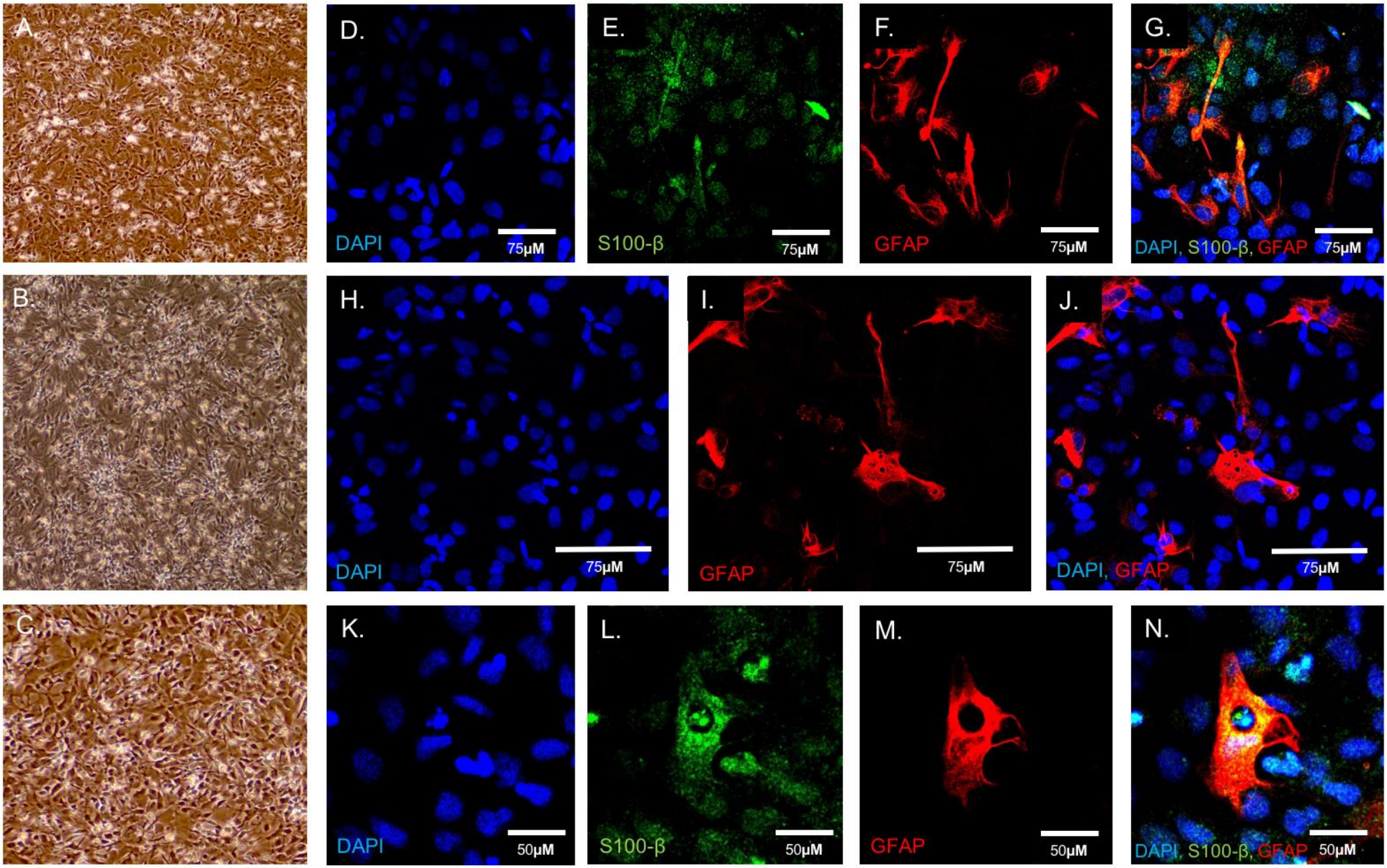


Figure S3.14. *In vitro* neurodevelopmental model of astrocytes, differentiated from induced pluripotent stem cells (iPSCs), and stained for GFAP and S-100ß neuronal markers. All images shown are the control cell line GM25256. (A, B, C) Astrocytes, 100X magnification. (D-O: Immunocytochemistry of astroglial cells). (D) DAPI nuclear stain 40X (E) S-100ß (F) GFAP (G) DAPI, S-100ß and GFAP (H) DAPI nuclear stain 40X (I) GFAP (J) DAPI and GFAP (K) DAPI nuclear stain (100X) (L) S-100ß (M) GFAP (N) DAIP, S-100ß and GFAP.

Cell growth rates

Figure S3.15 shows the rate of cell replication for each of the three lines that were passaged. At the iPSC expansion stage, cells were passaged in ratios, based on density on the plate, and the recommended ratio suggested by Coriell institute. During neural induction, cells were only passaged 1:1, and NPCs were passaged at 70-80% confluency. The astrocyte induction protocol is very specific on passage days so cells were counted according to day of the protocol rather that density in the plate.

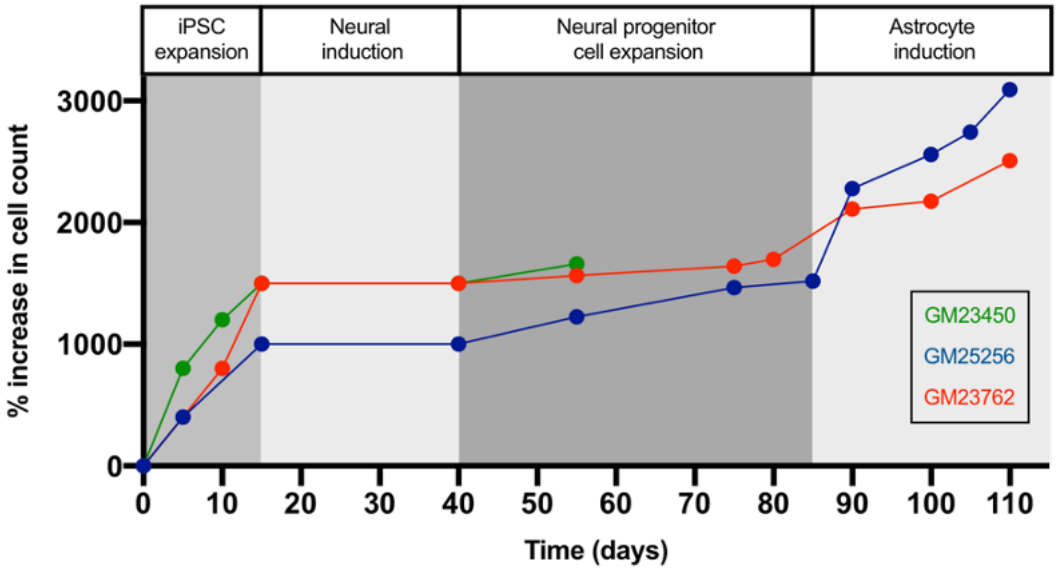


Figure S3.15 Cell growth rates during each stage of neurogenesis; iPSC expansion, neural induction, NPC expansion and astrocyte induction and maturation.

Glossary

MEFs: mouse embryonic fibroblasts; feeder cells ideal for supporting healthy undifferentiated human and mouse embryonic stem cells, and iPSCs in culture.

bFGF: Fibroblast growth factor-basic; cytokines in the FGF family possesses broad mitogenic and cell survival activities (532). It supports maintenance of undifferentiated stem cells (533), and stimulates stem cell neural rosette formation (534).

FGF: Fibroblast growth factor, maintains stem cells in an undifferentiated state.

KO DMEM: Knock-Out Dulbecco's Modified Eagle Medium, optimised for growth of iPSCs. The osmolarity is optimised to approximate that of mouse embryonic tissue.

KOSR: Knock-out serum replacement, a serum-free formulation for growth and maintainance of iPSCs, most effective when used in combination with KO DMEM (535).

MTeSR1: A standardised medium for feeder-independent maintenance of hiPSCs.

SSEA4: Surface antigen expression of stem cell markers. Undifferentiated cells are stained for the surface antigen, SSEA4, a stage-specific embryonic antigen 4, is expressed in undifferentiated human stem cells. Using immunostaining, a score of >80% expression of SSEA-4 is sufficient to confirm iPSC generation.

SUPPLEMENT 4: MRC 'INTERNSHIP' AT ORYGEN CENTRE OF EXCELLENCE FOR YOUTH MENTAL HEALTH, MELBOURNE

The MRC offered the opportunity to travel to a different research environment to pursue a new experience, linked to my PhD project. With an MRC 'flexible fund' grant, and a Universitas21 travel award, I spent time at Orygen, the National Centre of Excellence for Youth Mental Health, in Melbourne, Australia. This was in collaboration with the University of Melbourne.

Orygen is a translational research institute for preventative clinical care in young people. They work directly with young people, and their families and friends to pioneer new, positive approaches to the prevention and treatment of mental disorders, combining research, policy, education, training and clinical care.

My main objective in Melbourne was to meet peers with similar research interests, enhancing my own knowledge of the mechanisms of psychosis, approaches to exercise as an adjunctive treatment in youth mental health, with additional potential for future collaborations in research. I was also very keen to experience the unique set-up at Orygen, especially with exercise in mind. Usual treatment in youth mental health services in the UK does not normally integrate structured physical health and nutrition programmes; a formal aspect of early intervention treatment that could be incorporated by the NHS. Orygen has an exercise physiologist and a dietician, whose roles combine metabolic monitoring, health coaching, gym visits, home exercises, community links and individual/ group training. All service users have the opportunity for referral to

receive lifestyle interventions and physical health initiatives as part of standard care (536, 537), an approach that may be recapitulated across health service providers across the globe.

In a collaboration between Orygen and the University of Melbourne, the main achievement of my time in Melbourne was the organisation and participation in a public forum, entitled: “Special symposium: Exercise and Youth Mental Health- moving in the right direction”. I decided to invite speakers whose work fell into one of the following categories; mechanisms and feasibility. I also wanted a representation of the larger picture of exercise in youth mental health, allowing the attendees to engage in the different aspects of the theme of the day. I invited academics and clinicians who are involved in primary research (Professor Alison Yung, Professor Phillip Ward, Dr Michaela Pascoe and Melanie Ashton), as well as an exercise physiologist currently working with clients at Orygen (Lauren Foote) and a former service-user who detailed his experience of exercise as a therapy during his illness.

Programme:

1. **Professor Alison Yung**, Orygen: *“Exercise as an intervention in early psychosis- benefits and challenges”.*
2. **Emily Fisher**, University of Birmingham*: “The therapeutic benefits of exercise in first-episode psychosis: The FEPEX study”*
3. **Dr Michaela Pascoe**, Victoria University: *“How yoga can improve mental health in young people- possible mechanisms”*

4. **Melanie Ashton**, Deakin University*: "Physical activity as a predictor of clinical trial outcomes for bipolar depression"*
5. **Lauren Foote**, Orygen Youth Health: "*Establishing an exercise physiology program in a youth mental health service"*
6. **Professor Phillip Ward**, University of New South Wales: *"Keep on keeping on- taking exercise in youth mental health from short-term research project to core element on ongoing care*"

In addition to the symposium, I delivered my research to two other research groups in the Melbourne area; the Institute for Mental and Physical Health and Clinical Translation (IMPACT) at Deakin University (with Dr Olivia Dean), and the Institute for Health and Sport at Victoria University (with Professor Alex Parker). The IMPACT group have a broad focus on chronic disease patterns, risk factors and novel therapies in psychiatric disorders that span clinical trials, population-based epidemiological studies and laboratory-based research. The group at Victoria University have a more general focus on physical activity and lifestyle interventions in mental health. Both visits provided discussion around implementing exercise as an adjunctive treatment in psychosis with academic peers, and how the impact of exercise has been studied and will be studied in two clinical environments across the world.

This academic internship supported my development as a researcher, and facilitated sharing of important issues among a network of research-intensive universities and institutes. I was also able to identify a number of key

differences between the UK and Australia in approach to research in FEP, and employment of exercise in clinical settings.

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Designing a feasible exercise intervention in first-episode psychosis: Exercise quality, engagement and effect

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ABSTRACT

First-episode psychosis (FEP) is the first presentation of a psychotic disorder that usually propagates during early adulthood. FEP represents an important early intervention point to attenuate the metabolic risks associated with psychosis and its treatment. Exercise has potential to improve metabolic and functional outcome, but engaging this population in regular exercise is typically difficult. Promoting enjoyment and attendance may improve participation.

22 men with FEP were randomised to a 12-week intervention of exercise training, or treatment as usual. Exercise was pre-standardised based on measures of heart rate to assess intensity. Symptoms of psychosis were assessed, alongside measures of quality of life, disability and habitual activity.

The study observed 83% attendance at exercise sessions, with target intensity attained. There were clinically meaningful decreases in PANSS positive (17.31%) and general psychopathology (10.98%) scores and exercise was protective of negative score increase observed in the control group (13.89%). Assessment of disability declined after training (12.65%) compared with a 20.78% increase in controls.

This study demonstrated that engagement of FEP patients in an intervention of high quality exercise was possible. Positive changes in psychopathology scores and disability show that the benefits of regular exercise are achievable with a potential positive impact on clinical presentation.

1. Introduction

First-episode psychosis (FEP) is a psychiatric illness characterised by positive and negative symptoms, cognitive difficulties and, for some, the onset of a poor physical health trajectory. FEP represents a crucial early intervention point to target symptoms of psychopathology, reduce the negative metabolic side-effects of antipsychotic medication and encourage a habitually active lifestyle. The transition from FEP to a functional psychosis like schizophrenia is often accompanied by a plethora of cardio-metabolic diseases.

1.1. Sedentary behaviour and metabolic perturbations in severe mental illness

Premature mortality is one of the principal issues associated with severe mental illness, including schizophrenia or related disorders. This is a result of co-morbid illness that is common across chronic mental ill health (Laursen et al., 2014). Sedentariness and physical inactivity are independent but modifiable risk factors that can contribute to all-cause mortality. In a meta-analysis comparing physical activity levels and sedentary lifestyles in schizophrenia, bipolar disorder and major depression, the average amount of sedentary time per day was 476 min during waking hours; significantly more than the age and gender matched healthy controls (Vancampfort et al., 2017). Compounding this, many psychotropic drugs, including antidepressants, mood stabilisers and antipsychotics are associated with long-term metabolic issues, such as significant weight gain (Allison et al., 1999), insulin resistance and an atherogenic lipid profile (Tschoner et al., 2007). NICE recommend that FEP be treated with an antipsychotic medication, alongside cognitive behavioural therapy (NICE, 2014), but do not address the cardiometabolic risk factors, or subsequent weight gain as a result of antipsychotic medication. One study demonstrated that at first-

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psychotic hospital admission, drug naive patients had an average BMI less than that of the general population (Wetterling et al., 2004). However, after 5 years of neuroleptic treatment, the proportion of overweight and obese patients greater than that of the general population. Aside from medication, there are a number of metabolic alterations that have been implicated in FEP, as a predisposition to illness. Dysglycaemia, a term that encapsulates insulin resistance, impaired glucose tolerance and fasting insulin, has been associated with FEP pathology independent of medication, and unbiased by sociological factors (Perry et al., 2019). In a meta-analysis assessing lipid and adipocytokine status in FEP (Pillinger et al., 2017), hypertriglyceridemia and resultant impaired glucose regulation were found to be intrinsic to psychosis, not a side-effect of antipsychotic medication. These underlying perturbed mechanisms are also cogent targets for exercise training, an enhancer of glucose control (Hawley and Lessard, 2008). Thus, the combination of a sedentary lifestyle with the poor metabolic profile associated with FEP pathogenesis and antipsychotic medication can lead to an exacerbated risk of type 2 diabetes, hypertension and cardiovascular disease (Glassman, 2005; Haslam and James, 2005) in FEP. Importantly, the recent Lancet Psychiatry Commission emphasized the importance of protecting physical health from the first presentation of mental illness, with the rationale of aiming to prevent physical comorbidities from arising, particularly in young people receiving antipsychotic treatment (Firth et al., 2019). Within this, exercise was recommended as a fundamental aspect of lifestyle interventions for improving health outcomes. Exercise is a key modulator of metabolic health, regulating chronic adaptions that not only improve physical fitness but also affect energy metabolism. Prescribing exercise as an adjunct treatment, and encouraging habitual activity in FEP is therefore important to counter the negative metabolic effects inherent in psychosis and precipitated by antipsychotic medication.

1.2. Exercise as an adjunctive therapy

It is well-established that exercise is beneficial to metabolic health measures, as well as the many known positive effects on psychological outcomes (Acil et al., 2008; Beebe et al., 2005; Carek et al., 2011; Dunn et al., 2005; Galper et al., 2006) in FEP and schizophrenia. However, most studies experience difficulties with drop-out or attendance, with many reporting less than 50% compliance with the intervention (Beebe et al., 2005) (Beebe et al., 2012). Supervised exercise training appears to be more successful in engaging participants. In our own comparison of FEP and schizophrenia studies that employed supervised training (Abdel-Baki et al., 2013; Battaglia et al., 2013; Beebe et al., 2005; Bredin et al., 2013; Curtis et al., 2016; Dodd et al., 2011; Firth et al., 2018a; Heggelund et al., 2012, 2011; Marzolini and J.B., 2009; Pajonk et al., 2010; Scheewe et al., 2013) over non-supervised (Archie et al., 2003; Behere et al., 2011; Duraiswamy et al., 2007; Manjunath et al., 2013), the drop-out rates were 21.3% and 51% respectively. Average attendance for supervised sessions was 74.6%. The best attended interventions were those that included group sessions and structured activities, like football (Battaglia et al., 2013) or interval training (Heggelund et al., 2011), rather than a more general provision of gym access (Curtis et al., 2016), or jogging (Behere et al., 2011).

All of these approaches to design must be considered when developing an exercise intervention to enable effective exercise. Allowing choice of mode of exercise for the intervention is important in this population, together with supervised exercise training has the potential to promote attendance and reduce drop-out rates that are normally very high in this clinical group.

The cost-benefit of exercise provision in early intervention care may potentially outweigh the increasing cost of treatment of symptoms and long-term cardio-metabolic comorbidities. The 2014 NICE costing statement for psychosis and schizophrenia ((NICE), N.I.f.H.a.C.E. 2014) reported an annual cost of £2.2 billion predicted to rise to £3.7 billion by 2026. The guidelines also stated that "Savings are likely to arise in the future from avoiding adverse health events". It was also reported that 60% of schizophrenia patients have at least one cardiac co-morbidity, costing between $10,606 and $15,355 per year (Correll et al., 2017).

1.3. Targeting motivations and barriers to exercise participation

A meta-analysis assessed the motivations and barriers to exercise in severe mental illness (Firth et al., 2016), the most common barriers to participation being stress/ depression, lack of support, tiredness, disinterest and physical illness. These self-reported factors closely link to many of the negative symptoms that could be causally linked to the very high rates of exercise-study attrition seen in this clinical group (Vancampfort et al., 2016). However, a review by Firth (Firth et al., 2016) reported motivating factors for taking part in exercise, which were to improve physical health, reduce weight, manage mood, mental health benefits, reducing stress and tension and appearance. Stress was reported as both an important motivating factor as well as a barrier to exercise.

1.4. Standardising exercise bouts for use in an intervention

In order to successfully design an intervention of exercise that is replicable in the future, it is crucial to control for as many components of each training session as possible. It is necessary to employ a particular training intensity that will have a therapeutic benefit, whilst catering to the preferences of each individual patient, in terms of environment and activity type. The minimum training intensity for improvement in cardiovascular fitness, or VO_2max, is 55–65% heart rate (HR) max (Karvonen and Vuorimaa, 1988), so it would be logical to exceed this level in order to observe a meaningful and significant effect of exercise. A review by Roy et al. (Roy and McCrory, 2015) described the most accurate equation for estimating HRmax, (*208-(0.7 x age)),* first proposed by Tanaka (Tanaka et al., 2001). The use of this equation, alongside 50–70% VO_2max, which corresponds to 60–80% HR max has the potential to elicit improvements in measures of cardiorespiratory fitness, a method that may be standardized for use during an intervention by setting a personalized 'HR target zone' that corresponds to this range. Standardising exercise bouts for the purpose of research is salient for controlling variables in order to maintain scientific integrity of a study. Translating these techniques into 'real-life', or clinical practice, is also useful for progressing with an exercise programme or habitual adoption of regular exercise training. Alongside these intensity measures, the World Health Organisation (WHO) recommend that people aged 18–64 should engage in 150 moderate intensity or 75 vigorous intensity minutes of physical activity per week; simple measures that can be accrued in variety of ways, and represent a motivating target for sedentary individuals initiating a more active lifestyle.

1.5. Aims

This study aimed to assess the feasibility of engaging people with FEP with a 12-week exercise training programme. The exercise intervention was designed to maximize participation and engagement by allowing choice of exercise mode and choice of environment in which to exercise.

2. Methods

2.1. Participants

Male patients, aged 16–35, with a diagnosis of first-episode psychosis (as identified by a psychiatrist in keeping with ICD-10 F 20–29, F31.2, 32.3), were recruited from the community-based Birmingham Early Intervention service. Male patients only were recruited, to negate

the need to control for the menstrual cycle in female participants. The oestrogen cycle has a significant effect on antioxidant concentration, which was one of the primary outcomes in another arm of this study (Fisher et al., 2020)(in submission). Patients were within 3-years of first presentation of illness. Eligibility criteria were assessed initially by the primary care coordinators for each patient, followed by assessment of habitual activity, via Garmin activity monitoring device, to ensure a sedentary lifestyle. A general health questionnaire was provided to ensure the patient was free from medical conditions that would have prevented participation in moderate intensity aerobic exercise. Exclusion criteria included failure to adhere to pre-testing requirements e.g. refusal to be scanned, provide a blood sample, or significant risk to self or others as identified by the clinical team. Height and weight were assessed at baseline, mid and post-intervention and BMI was calculated at each stage. This study was commenced following approval from the NIHR HRA ethics committee (West Midlands- Edgbaston REC 17/WM/04120).

2.2. Randomisation

Following consent, participants were randomised to either the exercise intervention arm or the control arm (treatment as usual; in terms of clinical care, medication, psychotherapy) of the study. A block randomisation method (http://www.randomization.com) was utilised to allow for equal group distribution in the event of poor or slow recruitment.

2.3. Exercise intervention

Exercise sessions were delivered by an exercise scientist from the School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham. Exercise was undertaken 2–3 times per week, for 40–60 min, for 12 weeks. To maximise attendance and participation, participants were given a choice of different activities. Modes of exercise included were running; cycling; swimming; tennis; squash; badminton; circuit training and football. Each training session was supervised and accompanied by a researcher in a 1-to-1 manner. Researchers participated in each activity alongside subjects. Each training session was standardised by use of a HR target zone, based on 60–80% HRmax for each participant. Attendance, drop-out and tracking of HR was measured throughout the intervention period.

2.4. Activity tracking

Habitual activity was assessed for one week at baseline, mid-intervention and post-intervention for both intervention groups via a Garmin VivoSmart® HR (Garmin Ltd., Kansas, USA) activity monitoring watch. Measures of steps, floors climbed, active calories and intensity minutes were taken. 'Intensity minutes' refers to the number of minutes per week that a participant achieves a HR above a threshold for 'moderate intensity', for periods of 10 min or more. Garmin watches were also worn by participants during every exercise session of the intervention. Average HR during the bout, calories burnt and time participating in exercise were measured during exercise bouts. Exercising participants were also asked to fill in the International Physical Activity Questionnaire (IPAQ) at each time-point. This questionnaire was employed as a subjective measure to assess habitual activity, including non-leisure time activities (Duncan et al., 2017).

2.5. Analysis of activity data

The quality of the exercise undertaken in training sessions was explored based on heart rate during the activity and by activity type. Each HR zone is defined by a predicted lactate threshold (LT). Zone 1 < 68% LT or 'active recovery', zone 2 69–82% LT or 'endurance', zone 3 83–92% LT or 'tempo', zone 4 94–104% LT or 'threshold and zone 5 105 to max LT or 'VO2max'. Zone 2 represented the average activity distribution (43%), which was equivalent to 110–130 bpm. The aim of the present study was to exercise participants at 50–70% VO_2max, which would be represented by 115–150 bpm average HR during a session.

2.6. Psychiatric outcomes and functional disability

At each assessment time-point, participants completed the Positive and Negative Symptom Score (PANSS) (Kay et al., 1987), via a structured clinical interview designed to monitor symptoms of psychosis. The PANSS interview assesses positive and negative symptoms and is widely considered the 'gold-standard' method of scoring psychotic behaviour. Interviewers were trained in the completion of PANSS. Quality of life and functional disability were also assessed via the World Health Organisation Quality of Life (WHOQOL) (Group, 1998) and World Health Organisation Disability Assessment (WHODAS) (Andrews et al., 2009) questionnaires. The PANSS interviews and questionnaires alike were delivered by the same researcher delivering the exercise intervention (EF).

2.7. Data analysis

Data analysis was performed using GraphPad Prism 8 software (version 8.0.1, 2018). For baseline and post-intervention measures, relationships between markers were determined using linear regression, and to assess any difference at baseline between the two groups, two-sample t-tests were used. To assess changes between different time points in the study, paired t-tests were used. To compare relationships between marker pre-intervention/ control period vs mid or post, Pearson's correlation coefficient was employed. Outlying values were identified using the ROUT method (Q = 1%). Shapiro–Wilk tests for normality were also performed. Fisher's exact test of qualitative measures (smoking status) was employed. An alpha significance value of 0.05 was used throughout.

3. Results

3.1. Baseline

Twenty-two service users with psychosis were recruited and randomised into the study, aged between 17 and 34 (24.8 years ± 4.8 years) (average length of service use was 19 months). The exercise group (n = 11) experienced 4 cases of drop-out, one immediately after baseline assessment, two during the first week of exercise, and one after 10 weeks of intervention duration. In the control group 3 participants dropped out immediately before the 13-week post assessment. At baseline, there were no significant differences between participants in the two groups with respect to physical health markers, symptomology and habitual activity (Table 1). Antipsychotic, anti-depressant and anti-anxiety medication prescription are expressed in the table. Recruitment and drop-out patterns are detailed in the consort diagram below (Fig. 1).

3.2. Attendance and retention

Following consent, retention was 76% overall, 64% in the exercise group and 88% in the control group. For exercise group compliance, there was 83% attendance of 2 out of 3 sessions per week, and 41% attendance of 3 out of 3 sessions per week.

3.3. Quality of exercise sessions

As shown by Fig. 2, participants in the exercise group did not achieve the same intensity measure as the target HR, on average. Each activity type resulted in a different average HR, with running and

Table 1
Baseline characteristic for both exercise and control groups.

	Exercise		*Control*		p	Effect size
	Average	SD	Average	SD		
Age (years)	23.5	3.8	26.1	5.7	0.22	−0.54
Duration of illness (months)	20.6	10.0	18.0	11.7	0.61	0.24
Body composition						
Weight (kg)	82.5	14.7	84.1	16.8	0.82	−0.10
BMI (kg/m²)	25.5	3.7	25.5	4.7	0.98	−0.01
Smoking status						
Tobacco consumption	73.0%		64.0%		NA	
Cannabis consumption	50.0%		91.0%		NA	
Habitual activity						
Garmin Steps (steps/day)	6508.4	1712.2	4735.4	2051.2	0.05	0.86
Garmin floors (floors climbed/day)	7.3	4.6	5.5	3.8	0.39	0.42
Garmin intensity minutes (min/day)	29.5	68.6	30.5	32.3	0.94	−0.04
Garmin active calories (kcal/day)	861.4	475.4	1041.8	914.6	0.58	−0.24
IPAQ MET mins (min/day)	884.2	711.3	1231.9	1547.9	0.51	−0.29
IPAQ Sitting mins (min/day)	1939.5	931.4	1491.4	1804.1	0.98	0.01
Symptoms and functional measures						
PANSS Positive	16.7	4.8	17.8	5.6	0.63	−0.21
PANSS Negative	19.6	3.6	19.5	6.1	0.97	0.01
PANSS general	40.2	9.3	39.0	10.0	0.85	0.13
WHOQOL Overall (%)	45.4	17.8	48.1	16.7	0.94	−0.27
WHODAS Overall (%)	40.8	24.3	24.6	16.5	0.08	0.74
	n	%	n	%		
Medication status						
Olanzapine	1	29	2	25		
Amisulpride	3	43	0	0		
Risperidone	1	14	2	25		
Paliperidone	2	29	0	0		
Depixol	1	14	1	13		
Quetiapine	1	14	2	25		
Aripiprazole	1	14	0	0		
Cloxipol	0	0	1	13		
Haloperidol	0	0	1	13		
Anti-depressant prescription	1	14	4	50		
Anti-anxiety prescription	1	14	2	25		

Group mean values, standard deviation, P values (unpaired *t*-test) and effect sizes (Cohen's D) are expressed. BMI: body mass index, RHR: resting heart rate, PANSS: positive and negative symptom scale, IPAQ: international physical activity questionnaire, WHOQOL: World Health Organisation quality of life scale, WHODAS: World Health Organisation disability assessment scale.

football the achieving the highest average HR. All activities except for circuit training (which required greater rest periods) were within the target HR zone (50–70% VO_2max), and the distribution of HR zones for the average session was 43% for the target zone 2.

3.4. Concordance with WHO activity recommendations

Using data collected from the Garmin devices at baseline, mid- and post- intervention, it was possible to compare the activity levels of the participants with the WHO recommended level, expressed below as median (Fig. 3). In the exercise group, intensity minutes per week increased by 73.6% between pre- and mid-intervention, from 215 (± 206.9) minutes to 358.9 (± 225.8) minutes. However, at the post-intervention assessment the intensity minutes per week had reduced to 151 (± 158.3) minutes. The activity in the control group declined between pre-and post-intervention, from 178.9 (± 224.9) minutes to 124.1 (± 217.7) minutes.

3.5. Comparison of self-report activity versus Garmin-measured activity

At baseline, the control group self-reported (via IPAQ) activity was 744 MET minutes per week, which was similar to the number of minutes measured objectively (via Garmin device). Step-count ($r = 0.77$ $p = 0.024$)) and intensity minutes ($r = 0.86$, $p = 0.006$) recorded by the Garmin activity monitors were significantly correlated with the self-reported IPAQ MET minutes. The exercise group over-reported their own volume of physical activity. Fig. 4 shows Garmin measured moderate intensity exercise per week (bars) alongside self-reported IPAQ measured MET minutes per week (dots). There was no mid-point measure of IPAQ score for the control group. At mid-intervention assessment (6 weeks), self-reported activity recorded as step count ($r = 0.53$) and intensity minutes ($r = 0.78$) were concordant, however, by end-point assessment the self-reported activity was far greater than actual recorded activity by the Garmin devices; IPAQ versus steps ($r = -0.93$, $p = 0.0021$) and intensity minutes ($r = -0.011$, $p = 0.98$). At post-intervention, the control group self-reported activity versus Garmin measures was significantly correlated for intensity minutes ($r = 0.94$, $p = 0.0005$) and positively correlated for steps ($r = 0.48$, $p = 0.22$). There was a significant decline in Garmin-recorded intensity minutes between mid and post intervention for the exercise group ($p = 0.05$), which was not reflected by the self-reported measure of activity.

3.6. PANSS scores and symptomology

In each of the three scores exercise either conferred protection against declining scores observed in the control group or resulted in an improvement between baseline and post-intervention measurement, expressed as mean (± s.d.). There was a 1.44% increase in negative score in participants in the exercise group (a score of 19.43 (± 2.1)–19.71 (± 4.11)), and a 13.89% increase in participants in the control group (18.88 (± 6.66)–21.5 (± 4.45)) ($d = 0.76$). There was a 17.31% decrease in positive symptoms of PANSS in participants in the exercise group (18.14 (± 5.55)–15 (± 4.97)), and a 7.83% decrease in the non-exercising controls (17.63 (± 5.88)–16.25 (± 4.68)). Participants in the exercise group reported a 10.98% decrease in PANSS general score (40.43 (± 9.81)–36.43 (± 5.86)) ($d = -0.59$), compared to a 2.82% decrease observed in participants in the control group (39.88 (± 11.37)–38.75 (± 8.97)) ($d = -0.21$) (Fig. 5).

3.7. Patient measures of quality of life and disability

In an assessment of the WHODAS questionnaire, expressed as mean (± s.d.), there was an observed decrease of 12.65% in the exercise group (45.93 (± 19.8)–40.12 (± 19.09)), and an increase of 20.78% in the control group (27.48 (± 20.29)–33.19 (± 20.99)). For the WHOQOL, score increased in both groups with an 11.21% increase observed in participants in the exercise group (45.41 (± 16.18)–50.5 (± 15.78)) and a 5.32% increase observed in the control group (48.13 (± 18.77)–50.69 (± 16.78)) (Fig. 6).

3.8. Metabolic changes

No significant change in weight or BMI was observed in either group. A 2.27% increase in body weight was observed in participants in the exercise group, but this was not statistically significant. The control group demonstrated a 1.45% increase in body weight. A 7% weight gain is defined as clinically meaningful, particularly in the context of

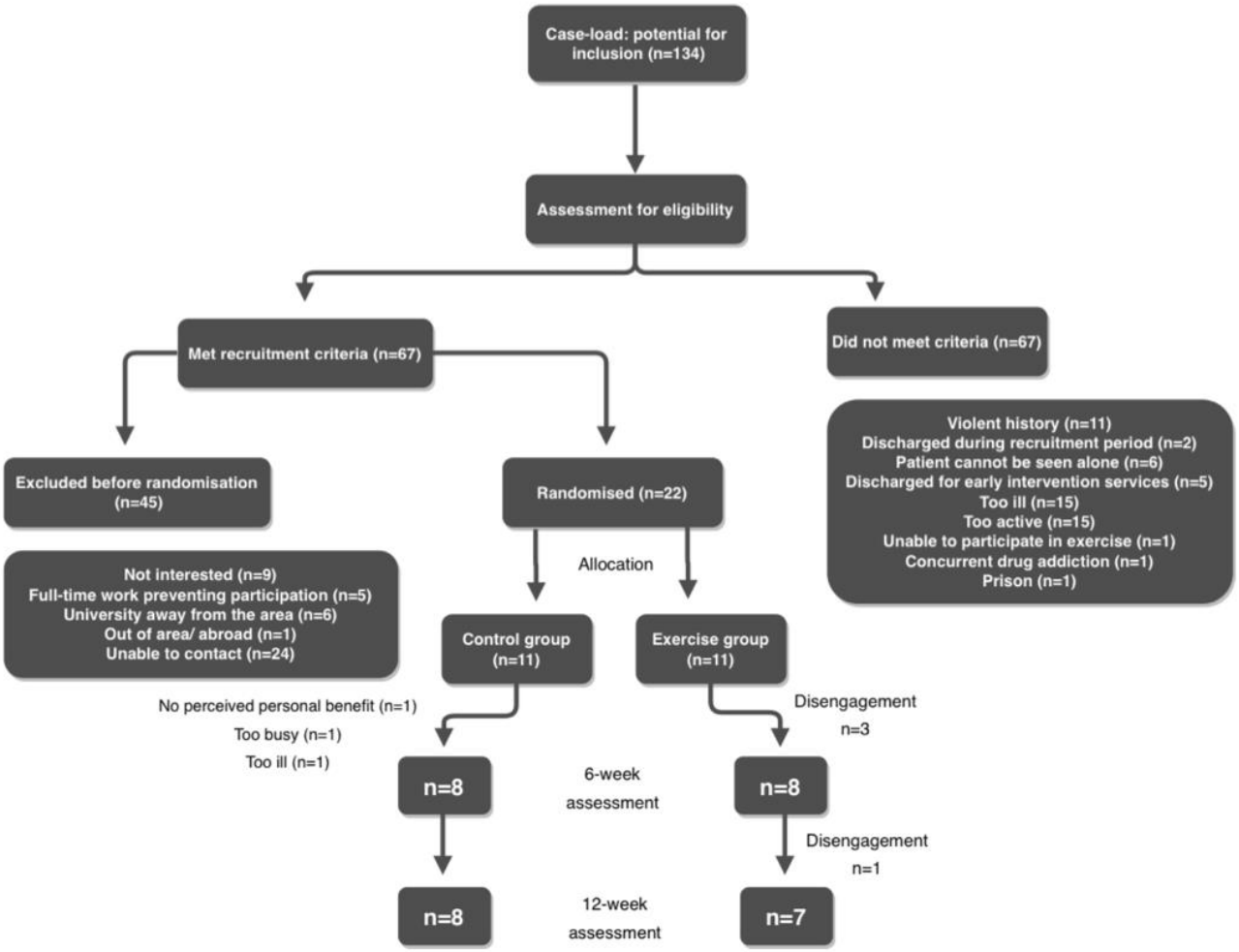

Fig. 1. Consort diagram detailing the recruitment and retention process of the study. Of the potential 134 participants identifed from the Early Intervention caseload, only 67 met recruitment criteria, and of those, 22 participants were randomised to enter either the control group or the exercise group. 8 participants completed the control arm of the study, and 7 the exercise arm.

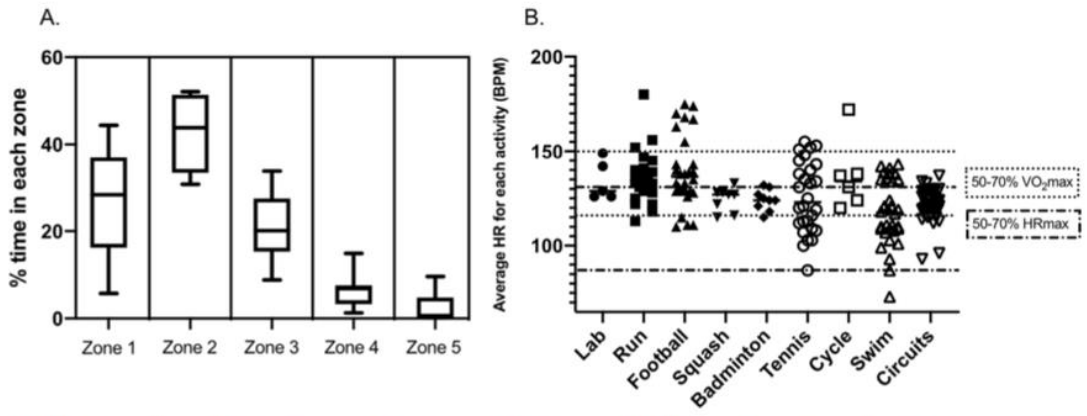

Fig. 2. (A) The heart rate zone distribution for each exercise session during the intervention. Median values are expressed, along with upper and lower quartiles, and minimum/ maximum values. (B) The heart rate during each activity type, including bands representing 50–70% HR max, the most commonly defined 'moderate intensity' and this studies' own target for the intervention of 50–70% VO_2max.

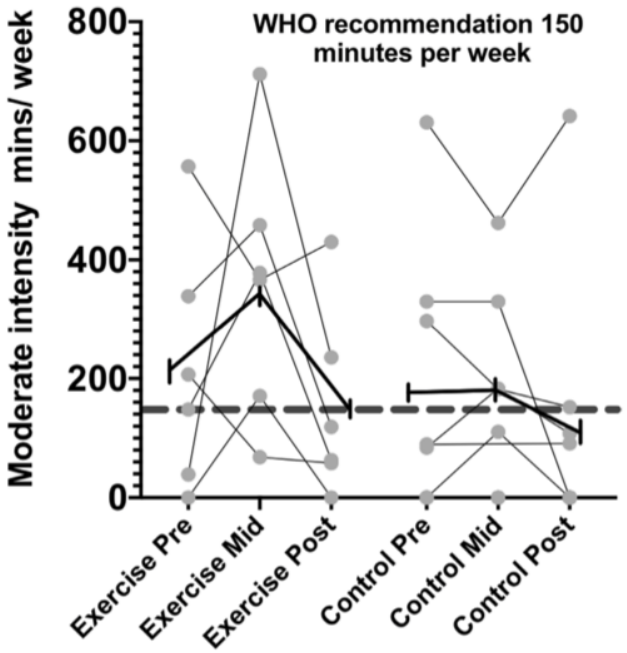

Fig. 3. Moderate intensity minutes of exercise achieved at baseline, mid-point and post-intervention, alongside the threshold foe WHO (World Health Organisation) recommended 150 min per week (grey dashed line). Data is expressed as individual values (grey dots) and mean (black bars).

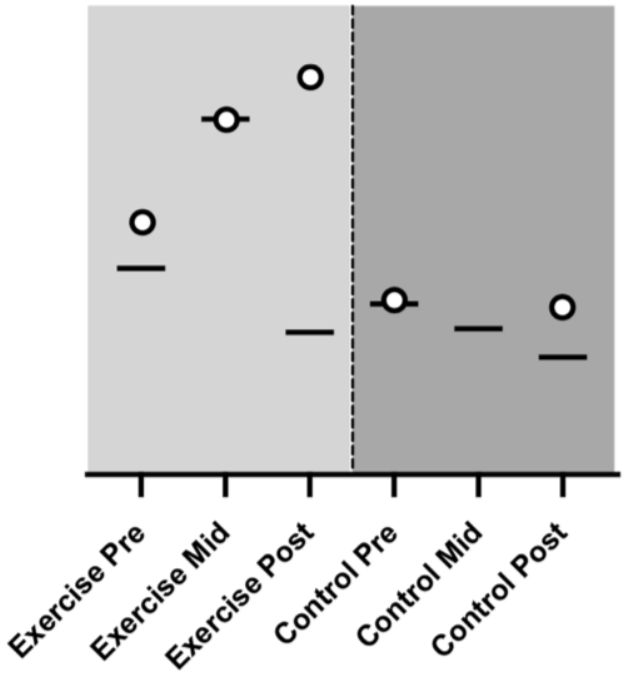

Fig. 4. A comparison between the average Garmin-measured 'intensity minutes' (horizontal bars), with self-reported IPAQ habitual activity score, expressed at MET minutes (white dots). Both represent the same measure, but participant perception of actual activity achieves was different to the direct measure.

antipsychotic medication prescription (Dayabandara et al., 2017).

4. Discussion

This study has shown that it is feasible to engage an FEP population in a 12-week intervention of exercise training. The present study achieved 83% compliance in exercise twice per week, and 41% compliance in exercise three times per week. This level of engagement in regular exercise resulted in reductions in positive and general psychopathology scores, and the patient group reported a reduced disability score. 13% of eligible cases for this intervention refused as a result of disinterest, suggesting that study design promoted recruitment and participation. The choice of exercise modality offered to patients did not have an effect on reaching the pre-standardised target intensity for exercise bouts. Designing an intervention to maximise participation and retention prioritised exercise session quality, alongside a choice element for the participant. This choice provided the intervention cohort with novel experiences, and the opportunity for skill acquisition whilst maintaining intensity.

4.1. Facilitating a lifestyle change

As well as the barriers to participation and motivating factors described above, there are a number of common co-morbid conditions and symptoms associated with psychosis that may also impact the ability of FEP patients to initiate exercise. Anergia, anhedonia, tardive dyskinesia, depression, anxiety and poor concentration are all characteristic of FEP, and are contributing factors to the sedentary lifestyle that is often reported in this population. Facilitating behaviour change in a healthy group is challenging, and when transferred to an FEP group the feasibility of exercise alone is difficult, but to encourage a lifestyle change is especially challenging. This study demonstrated the sustainability of regular exercise in an FEP population, when accompanied by another leading the session.

Participants in the current study recorded physical activity via the IPAQ questionnaire, a well-validated self-report measure, in addition to wearing a Garmin activity monitor, which measured habitual activity objectively. The results from the Garmin activity device suggest that both the exercise and control groups met the WHO recommendations for moderate intensity physical activity per week, at baseline. This was unexpected, but this observation is likely to be an effect of being enroled into an 'exercise study', without knowledge of which group they will be part of (Orne, 2002). In the UK biobank study (Firth et al., 2018b), people with schizophrenia were significantly less active than the general population, however self-report measures failed to identify this. In that study, schizophrenia patients and healthy controls both reported the same activity levels, but accelerometer data showed that 95% of schizophrenia patients fell within the bottom 15–35% of the general population. In the current study, the post-intervention measure of habitual activity was decreased compared to that of the mid-intervention measure (Fig. 4), in spite of the perceived high level of activity (IPAQ measure). These data indicate that in order for activity levels to be sustained, the support provided throughout the intervention also needs to be sustained, providing an argument for provision of regular exercise as part of day-to-day care. Alongside the positive psychological and functional outcomes demonstrated by this study, exercise provides a low cost, low side-effect adjunctive therapy to the traditional antipsychotic medications prescribed in FEP.

4.2. Functional measures, body weight and psychopathology

Exercise has the potential to protect against emerging functional decline in the early phases of psychosis. This study observed clinically meaningful changes in the PANSS positive and general psychopathology subscales, as well as the self-assessment of disability in those participants engaged in exercise training. The control group

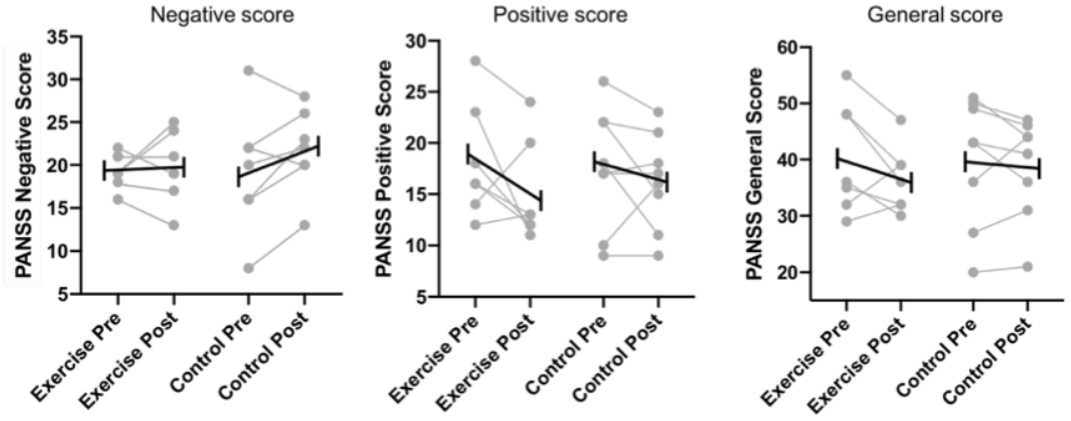

Fig. 5. PANSS (Positive and Negative Symptoms Score) psychotic symptoms interview results at baseline and post-intervention for exercise and control arms of the study. Individual values are expressed (grey) versus mean values (black).

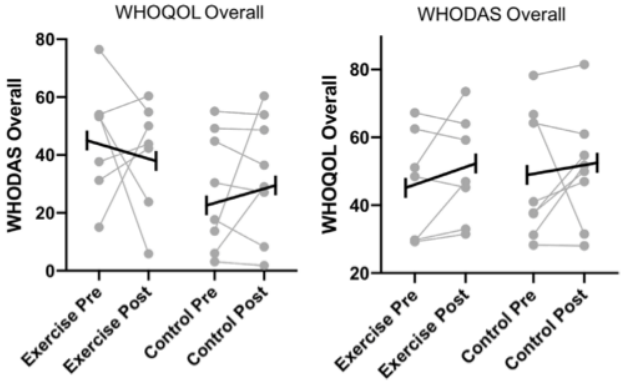

Fig. 6. WHOQOL (World Health Organisation Quality of Life scale) and WHODAS (World Health Organisation Disability Assessment Scale) questionnaire results. Individual results are expressed (grey) versus mean values (black).

demonstrated an increase in the negative subscale, indicating that exercise was protective of this disease-related worsening of symptoms.

Antipsychotic medications are reported to be most effective in reducing the positive symptoms of psychosis (Mueser et al., 2013), whilst having minimal effect on negative symptoms and cognitive function. Positive symptoms represent a therapeutic target of exercise training, shown by this study, which may ultimately reduce the reliance on psychotropic medication. Conversely, since all participants in this study were treated with at least one antipsychotic, exercise may enhance the effect of medication. A recent 18-month study in a severe long-term schizophrenia population compared treatment as usual and a multidisciplinary lifestyle treatment (MULTI) that focussed on decreasing sedentary behaviour, increasing physical activity levels and improving dietary habits (Deenik et al., 2018). The MULTI group displayed significant reductions in daily dose of medication, indicating a lesser need for drug treatment as a result of lifestyle changes.

Physiologically, one of the greatest problems associated with psychosis and subsequent antipsychotic prescription is weight gain and related metabolic illnesses. Adaption to exercise training, as an adjunctive therapy, has a number of metabolic benefits, including cardiovascular fitness, reduced blood pressure and BMI and improved lean muscle mass (Wong et al., 2008). Exercise is often used as a means to lose weight in a healthy population. However, body weight was maintained in the participants of the present study, with neither a reduction nor an increase in weight observed, which was expected in this FEP population (Abdel-Baki et al., 2013; Ball et al., 2001; Daumit et al., 2011). An absence of reduction in body weight could be a result of continued poor diet alongside the intervention, or may be attributable to an increase in muscle mass often seen alongside a reduction in fat mass (Egan and Zierath, 2013); a positive metabolic change, but often not reflected in body weight assessment. However, as antipsychotic medication often causes weight gain, exercise as an adjunct alongside this treatment alongside antipsychotic treatment has the potential to negate this weight-gain, before it arises (Maayan and Correll, 2010) (Firth et al., 2019).

The quality of life score improved in both intervention groups across the 12-weeks of intervention, but a greater increase was recorded in the exercise group. Although the current study was not powered to detect this, further trials should focus on the physical health and functional outcomes measured here. Measures of quality of life and disability are informative markers of a patients' well-being, with 'recovery-orientated' outcomes being the most important measure from the view of a care team, and the patient themselves (Dixon et al., 2016). Other research has described exercise as a "robust add-on treatment in psychosis that improves quality of life, global functioning and depressive symptoms" (Dauwan et al., 2016). The WHODAS questionnaire represents a subjective measure of disability and questions the patients' own perception of incapacity in many daily tasks. This acuity is one of the salient barriers to recovery. Exercise may affect this notion as even the process of regular physical activity may interact with the self-perceived notion of inability and disability. The observed reduction in WHODAS score post-exercise versus the increased score in the non-exercising group may therefore represent a change in this perception.

4.3. Limitations and strengths

The strength of this study was that it demonstrated the feasibility of undertaking supervised exercise in a FEP group, at least twice per week. However, if considering this study as a trial based on symptom improvement then the main limitation of this study was participant number. The sample size was small, rendering many of the time-point and inter-marker comparisons statistically insignificant, despite the argued clinical importance of patient-orientated changes observed in the exercise group. However, as a proof-of-concept trial, it was successful in terms of intervention design and compliance. Football, the only team-based activity offered, was very popular, and was an easy way to facilitate target energy expenditure. Based on this experience, provision of group-sports is an aspect of the intervention that could be increased, in a definitive study across a variety of exercise modes.

Despite the provision of many indoor-based activities, poor and unpredictable weather in the UK was certainly a factor for participant engagement. There was a greater incidence of 'no-shows' during wet

and cold days. This effect was particularly salient during the winter months of the year-long trial period. All time-point measures and activity accompaniment were conducted by the same researcher (EF). In the future, the processes of time-point data collection and exercise delivery should be separate, since interview answers may be somewhat different in the presence of a familiar member of the study team.

4.4. Conclusions

This study demonstrated the feasibility of a supervised exercise intervention in a cohort of males with a diagnosis of first-episode psychosis. Exercise was able to elicit a change in symptomology, quality of life and disability. By employing a study design that prioritised variety in exercise, but ensured the quality and intensity of each training bout this study recorded compliance to the intervention that was much greater than most other exercise studies in this clinical group.

CRediT authorship contribution statement

Emily Fisher: Data curation, Formal analysis, Conceptualization, Methodology. **Stephen J. Wood:** Conceptualization, Methodology. **Rachel Upthegrove:** Conceptualization, Methodology. **Sarah Aldred:** Formal analysis, Conceptualization, Methodology.

Declaration of Competing Interest

There is no conflict of interest that could inappropriately influence, or be perceived to influence this study.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2020.112840.

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REVIEW ARTICLE

WILEY

Role of magnetic resonance spectroscopy in cerebral glutathione quantification for youth mental health: A systematic review

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Abstract

Aim: Oxidative stress is strongly implicated in many psychiatric disorders, which has resulted in the development of new interventions to attempt to perturb this pathology. A great deal of attention has been paid to glutathione, which is the brain's dominant antioxidant and plays a fundamental role in removing free radicals and other reactive oxygen species. Measurement of glutathione concentration in the brain in vivo can provide information on redox status and potential for oxidative stress to develop. Glutathione might also represent a marker to assess treatment response.

Methods: This paper systematically reviews studies that assess glutathione concentration (measured using magnetic resonance spectroscopy) in various mental health conditions.

Results: There is limited evidence showing altered brain glutathione concentration in mental disorders; the best evidence suggests glutathione is decreased in depression, but is not altered in bipolar disorder. The review then outlines the various methodological options for acquiring glutathione data using spectroscopy.

Conclusions: Analysis of the minimum effect size measurable in existing studies indicates that increased number of participants is required to measure subtle but possibly important differences and move the field forward.

KEYWORDS
glutathione; magnetic resonance spectroscopy; youth mental health

1 | INTRODUCTION

There is now extensive evidence that oxidative stress, defined as a disturbance in the balance between the production of reactive oxygen species and antioxidant defences (Betteridge, 2000) plays a role in the pathophysiology of many psychiatric disorders, including depression, bipolar disorder, anxiety and schizophrenia (Smaga et al., 2015). For example, depressed patients display increased markers of oxidative stress in plasma with an associated decrease in total antioxidant capacity (Gałecki, Szemraj, Bieńkiewicz, Florkowski, & Gałecka, 2009) (Yumru et al., 2009), and similar findings have been reported in anxiety (Atmaca, Kuloglu, Tezcan, & Ustundag, 2008) and schizophrenia (Akiibinu, Ogundahunsi, & Ogunyemi, 2012; Dietrich-Muszalska, Olas, Głowacki, & Bald, 2009). The result of this oxidative stress is cellular damage, with consequent impacts on cell function or even cell death. The presence of markers of oxidative stress across diagnostic groups

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suggests some common pathophysiological process that might be amenable to universal treatment, an approach that would be particularly helpful in the early stages of mental illness when specific diagnoses have not yet crystalized (McGorry, Hickie, Yung, Pantelis, & Jackson, 2006).

The primary focus of much research in this area has been glutathione (GSH). GSH is the brain's dominant antioxidant, and plays a fundamental role in removing free radicals and other reactive oxygen species (Wood, Yücel, Pantelis, & Berk, 2009). Both reduced (GSH) and oxidized (GSSG) GSH are present in cerebral tissue. Reduced GSH is converted to GSSG either directly or through catalysis by glutathione peroxidase (GPx) (Xin et al., 2016), upon interaction with a radical species. This process is protective for cells and intracellular cellular components against damage. GSSG is subsequently reduced back to GSH, a reaction catalysed by glutathione reductase (GR), and in this way GSH contributes to regulation and maintenance of cellular redox status (Lushchak, 2012). The ratio of the redox active couple (GSH-GSSG) can be used directly to measure oxidative stress, but precise quantification of this ratio is challenging, even in blood measures, so that GPx and GR assessment are frequently used instead (Xin et al., 2016). In post-mortem studies of the prefrontal cortex (PFC) of patients and non-psychiatric controls, GSH, GPx and GR alterations were associated with bipolar disorder, major depressive disorder and schizophrenia (Gawryluk, Wang, Andreazza, Shao, Yatham et al., 2011; Gawryluk, Wang, Andreazza, Shao, & Young, 2011). While direct measurement of these metabolites can be conducted in brain post-mortem, in vivo quantification generally remains extremely challenging; however, GSH concentration in the brain can be assessed using magnetic resonance spectroscopy (MRS).

This review therefore aims to integrate the results of published studies employing MRS measurement of GSH in the brains of those presenting with mental illness, particularly young people. Two main questions will be addressed in this review:

1. Is perturbed GSH reliably associated with indicators of mental illness in young people, such as diagnosis, symptoms or functioning?
2. What are the strengths and weaknesses of the various approaches to the in vivo quantification of GSH concentration using MRS?

These two questions are of importance to ongoing and planned investigations regarding aetiology and prognosis in youth mental health. Simultaneously, the optimal GSH measurement protocols that utilize MRS are paramount. In order to address these questions, a systematic review was conducted selecting those publications that contained human in vivo neurological MRS studies in which cerebral GSH concentration was a target metabolite considered as a possible marker of mental health conditions.

2 | METHODS

This systematic review followed PRISMA guidelines (Moher et al., 2009) (Figure 1). However, due to the recent and emerging nature of the outcome measures, no quantitative meta-analysis was conducted on the resulting data.

2.1 | Databases and search terms

The review aims to provide information that will inform the design and parameters of an investigation into the role of GSH in the quantification of neurological markers associated with conditions relating to youth mental health. Such a study would require that optimal parameters to assess GSH concentration in small regions of interest within the brain, using standard, routine, clinical infrastructure currently available. Studies considered within the present review were limited to those published after 2000 in order to restrict results to technically relevant procedures.

Four databases were identified for search, three of which provided general coverage (Web of Science, ScienceDirect and Scopus) and one of which was medically focussed (PubMed). Records were requested from each database (as of July 16, 2018) using the same search terminology and using a breadth-first approach to ensure sufficient coverage. Three sub-strings were required, each arising from a range of alternatives: the population of interest ("youth mental health" OR "anxiety" OR "bipolar" OR "depression" OR "psychosis" OR "schizophrenia"), the measurement technique ("MRS" OR "magnetic resonance" OR "spectroscopy") and the comparative measure, in this case a target metabolite ("GSH" OR "Glutathione"), were required (AND) in the title or abstract (where possible) of the publications returned.

The database search returned 264 studies in total (WoS:82, Sd:35, Sc:98, PM:49) 103 of which were identified as duplicate studies (ie, the same paper was present within the studies returned from two or more data bases). The remaining studies were assessed for relevance. Inclusion criteria were reasonably broad, requiring human study-participants undergoing in vivo MRS of brain GSH with quantitative outcomes and accessible full-text material. From the considered studies, 108 were rejected as they did not meet these criteria, while nine were not assessed (as they were not appropriate for the analysis) leaving 49 studies of relevance to this review.

3 | RESULTS

Relevant features (deemed important variables for the review) extracted from studies are shown in Table 1.

Those studies that included quantitative comparisons (either tests for significant differences or regression analysis) were rated according to the minimum effect size required to achieve a power of 0.8, given a significance value of less than 0.05. Using a simplified simulation,[1] multiple realizations of participants were drawn from either:

- Two unit-variance normal distributions with mean separation $d_\mu = \mu_1 - \mu_1$, or
- A two-element, zero-mean, multivariate normal with covariance $\Sigma = \begin{bmatrix} 1 & d_\sigma \\ d_\sigma & 1 \end{bmatrix}$.

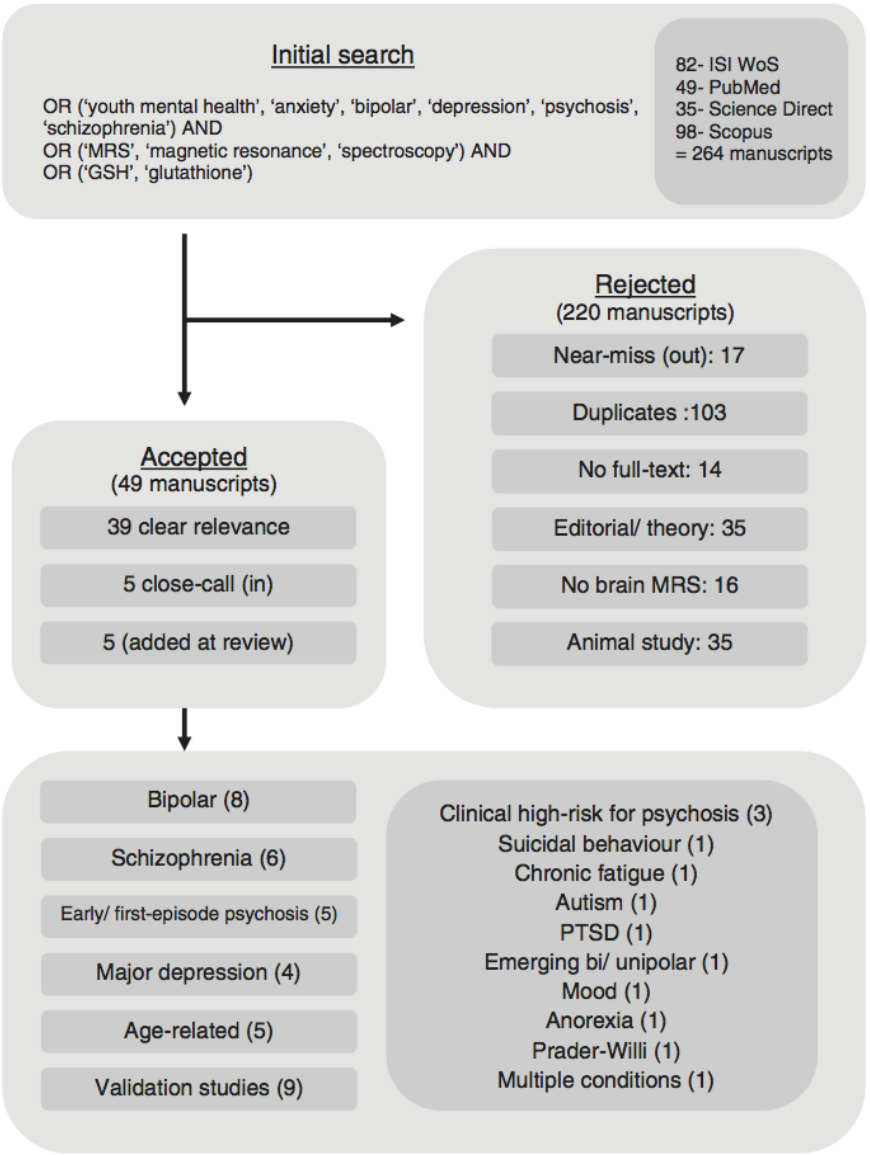

FIGURE 1 Flow diagram representing the database search with relevant accepted manuscripts considered in this investigation. PTSD, post traumatic stress disorder

The effect size (d_μ) or covariance (d_σ) was then increased from zero until at least 80% of 1×10^4 trials returned a result with a two-tailed significance of less than 0.05. Results are shown in Table 1 as $d_{\min}$ (where d_σ is only shown for single-group studies).

3.1 | Non-quantitative outcomes

3.1.1 | GSH and cerebral GSH Concentration

Given that GSH is the major free radical scavenger within the brain, that the GSH redox couple have been associated with psychiatric disorders, and that GSH may be directly measured using sophisticated MRS sequences in vivo within patient brains, a number of studies have been performed that probe this connection. However, due to the fact that there is considerable heterogeneity in methods for MRS data acquisition, most studies differ in voxel size and placement, parameters and post-processing, and also the quantitative measures used to assess study outcomes. Therefore, it is not feasible to perform quantitative analysis on acquired results. However, there are a number of conclusions that can be drawn from the data by summarizing the main findings of the studies conducted in this domain. Here attention is divided between discussion of the evidence regarding the role of GSH in the context of mental illness and the utility of MRS in the measurement of this metabolite in vivo.

3.1.2 | Findings of in vivo GSH and mental illness

The majority of the studies (37) were aimed at determining if GSH concentration significantly differed between healthy cohorts and those with diagnosed mental disorders, or if there were specific alterations by diagnostic sub-group. A second group of studies (7) were targeted at novel approaches to spectral measurement and

TABLE 1 In vivo MRS of GSH for mental health: summary of findings

Condition	Brain region	MR-Sq	Study and main finding	#Part.	d_{min}	Other GSH-specific correlations
BD	ACC	3 T PRESS	(Lagopoulos et al., 2013) • No difference in GSH between patients and controls: • ($t(115) = 1.253$, $P = .213$).	53 (BD) 51 (HC)	0.6	GSH vs YMRS: ($\rho = -0.198$; $P = .214$; N = 41) GSH vs HDRS: ($\rho = 0.127$; $P = .385$; N = 49) GSH vs age of onset: ($\rho = -0.09$; $P = .522$; N = 53) GSH vs duration ($\rho = -0.125$; $P = .374$; N = 53).
		3 T PRESS	(Chitty, Lagopoulos, et al., 2013) • Risky drinking in patients showed less GSH than non-risky: • ($t(48) = 2.44$, $P = .015$).	33 (BD) 17 (HC)	0.9	High alcohol use disorders identification test score negatively correlated with GSH in BD subjects ($r = -0.478$, $P = .005$).
		3 T PRESS	(Chitty, Lagopoulos, Hickie, & Hermens, 2015a, 2015b) • Change in alcohol use, smoking and age predict changes in GSH (at 15 months): • ($F(3, 26) = 3.69$, $P < .05$). • Not controlled as comorbid.	30 (BD)	0.5	GSH vs alcohol frequency: $r = -0.381$, $P < .05$ GSH vs smoking frequency: $r = -0.367$, $P < .05$
		3 T J-PRESS	(Soeiro-de-Souza et al., 2016) • No difference in GSH between euthymic BD and controls. • Lac $\propto$ GSH in BD patients	50 (BD) 38 (HC)	0.6	Lac vs GSH Patients: ($B = 0.20$, $t = 3.2$, $P = .003$ [0.07, 0.33]) Controls:($B = 0.17$, $t = 0.64$, $P = .11$ [0.04, 0.39])
	ACC + Hip	3 T PRESS	(Chitty, Lagopoulos, Hickie, & Hermens, 2014) • No difference in GSH in either region between BD and Controls. • Differences mediated by drinking and smoking.	64 (BD) 49 (HC)	0.5	GSH_{Hip} vs risky drinking (BD): ($r = 0.489$, $P < .021$) GSH_{ACC} vs smoking (BD): ($t(53) = 4.162$, $P < .001$)
	l-Hip	3 T PRESS	(Chitty et al., 2015b) • Controls: mismatch negativity correlated with GSH decrease in both temporal sites. • $r_{left} = -0.542$ [−0.8, −0.06] • $r_{right} = -0.374$ [−0.67, 0.07] • No association in BD patients.	28 (BD) 22 (HC)	0.8	GSH vs left-MMN ($r = 0.068$, $P = .74$, 95% [−0.36, 0.69]) GSH vs right-MMN ($r = -0.057$, $P = .78$, 95% [−0.52, 0.73]).
	OCC + mPFC	3 T SPECIAL	(Godlewska, Yip, Near, Goodwin, & Cowen, 2014) • No difference between BP and control in either region (for GSH or other metabolites).	13 (BD) 11 (HC)	1.2	
	ACC + OCC	7 T STEAM	(Masaki et al., 2016) After treatment: • No change in GSH_{OCC} • Decrease in GSH_{ACC} ($P = .033$) • $GSH_{ACC\ plc.}$ =1.31 ± 0.043 • $GSH_{ACC\ Ebselen}$ = 1.17±0.07	20 (HC)	0.6	
Schiz. (SZ)	ACC	4 T STEAM MEGA- PRESS	(Terpstra et al., 2005) • STEAM was within uncertainty of edited spectra in in vivo tests ($P = .4$). • GSH levels of patients not different from controls ($P = .4$, differences >10%).	13 (SZ) 9 (HC)	1.3	GSH_{pat} = 1.6±0.2 $GSH_{cont.}$ = 1.5±0.3
		7 T STEAM	(Brandt et al., 2016) • GSH differences between patients and controls under the age of 40:	24 (SZ) 24 (HC)	0.8	GSH not correlated with age Overall no GSH difference between patients and controls.

TABLE 1 (Continued)

			• [$t(25) = -2.47$, $P = .021$]			
	ACC + LI + VC	7 T STEAM	(Kumar et al., 2018) • GSH lower in patients vs healthy controls- only in ACC voxel • ACC $P = .008$ • LI $P = .784$ • VC $P = .464$	28 (SCH) 45 (HC)	0.7	GSH and glutamine correlated in all three voxels GSH vs ACC: $r = 0.56$ GSH vs LI: $r = 0.80$ GSH vs VC: $r = 0.65$
	mPFC	1.5 T PRESS	(Do, Trabesinger et al.) • Cerebrospinal fluid GSH sample showed 27% decrease in patients ($P < .05$). • MRS showed 52% decrease in patients ($P < .0012$).	14 (SZ) 14 (HC)	1.1	
	pMFC	3 T MEGA-PRESS	(Matsuzawa et al., 2008) • No significant difference between patients and controls.	20 (SZ) 16 (HC)	1.0	For patients GSH correlated to negative symptoms SANS and BPRS ($r = -0.68$, $P < .001$) and related to trail making test A ($P < .05$).
	Imag.	4 T proton echo planar spectroscopic imaging	(Bustillo et al., 2011) • No GSH-specific hypothesis tested.	30 (SZ) 28 (HC)	0.8	
Major Depression (MD)	OCC, bilat.	3 T MEGA-PRESS	(Lapidus et al., 2014) • GSH negatively correlated to anhedonia severity: • ($r = -0.55$, $P = .01$).	11 (MD) 10 (HC)	1.3	MDD sample in isolation showed associations between anhedonia and GSH: ($r = -0.53$, $P = .09$). No associations between fatigue severity and GSH
	OCC	3 T SPECIAL	(Godlewska, Near, & Cowen, 2015) • GSH was decreased in depressed patients • $F = 5.10$, $P = .028$ • $F = 4.28$, $P = .042$ (con. Age/sex)	39 (MD) 31 (HC)	0.7	
		3 T PRESS	(Freed et al., 2017) • GSH decreased in MD patients' vs HCs • $P = .04$	19 (MD) 8 (HC)	1.3	No correlation between GSH and anhedonia, MD severity, or onset
	Imag.	3 T MRSI	(Li et al., 2016) • In left putamen, GSH decreased in patients ($P = .044$) • Patient increase post therapy not significant.	16 (MD) 10 (HC)	1.2	$GSH/tCr_{pat.} = 0.23 \pm 0.06$ $GSH/tCr_{cont.} = 0.28 \pm 0.05$
Early Psych. (FEP/EP)	Temp	3 T PRESS	(Berger et al., 2008) • Bilateral GSH increase in treatment group response ($F_{1,12} = 6.1$, $P = .03$) • No longer significant when affective psychotic patients removed.	24 (FEP)	0.6	PANSS negative symptom change negatively correlated with GSH ($r = -0.57$, $P = .041$). Percent change in GSH and Glutamate/Glutamine correlated: ($r = 0.64$, $P = .01$)
		3 T PRESS	(Wood et al., 2009) • GSH 22% higher in patients than controls: • ($F_{1,46} = 4.7$, $P = .035$). • No difference in other tests: hemisphere ($P = .137$), group-by-hemisphere ($P = .513$).	30(FEP) 18(HC)	0.9	Patients not responding to topical niacin show 35% higher GSH than responders ($F_{1,28} = 5.1$, $P = .007$).
	mPFC	3 T SPECIAL	(Monin et al., 2015)	30 (EP) 40 (HC)	0.7	

TABLE 1 (Continued)

			• Potential dependence between GSH levels and white matter integrity during PFC developments.			Controls: GSH correlated to general FA (r = 0.34, P = .03) and functional connectivity (r = 0.40, P = .01). Patients controlled for medication and duration: GSH correlated to general FA (0.31, P = .01).
		3 T SPECIAL	(Xin et al., 2016) • GSH decrease (P = .006) in glutamate-cysteine ligase catalytic high-risk (1.15 ± 0.17) compared to low-risk (1.34±0.8).	25 (EP) 33 (HC)	0.8	GSH_{mPFC} correlated to GSH_{blood} in controls (P = .021) but not in patients (P = .39).
CHR for psychosis	mPFC	3 T PRESS	(Hafizi et al., 2018) • GSH and TSPO radioligand significant negative association in healthy volunteers, but not clinical high-risk group—indicative of an abnormal interaction TSPO expression and redox status in CHR group	27 (CHR) 21 (HC)	0.9	mPFC GSH and [^{18}F]FEPPA VT (radioligand of TSPO) not sig. Different between groups.
		3 T PRESS	(Da Silva, Hafizi et al. 2018a) • No sig difference between mPFC GSH in drug-naïve patients vs healthy controls	30 (CHR) 27 (HC)	0.8	No sig correlations between cerebral GSH and clinical and neuropsychological measures No sig difference between GPx activity and CHR vs HC (F = 0.15, P = .70) Significant effect lifetime cannabis use in GPx activity (F = 7.41, P = .01)
		3 T PRESS	(Da Silva et al., 2018) • No sig difference between mPFC GSH in drug-naïve patients vs healthy controls	27 (CHR) 16 (HC)	0.9	No differences between microglial activation and GSH between groups
	ACC + Striat.	3 T PRESS	(Demro et al., 2017) • GSH_{ACC} was shown to correlate significantly with P3 (Grandiosity, negative) and P5 (Disorganized Communication, positive) as measured by the SIPS • GSH_{STR} was shown to (negatively) correlate significantly only to Grandiosity (P3)	12 (CHR)	0.7	GSH correlation with SIPS: P1: r_{ACC} = −0.578 (0.062), r_{STR} = −0.566 (0.088) P2: r_{ACC} = −0.074 (0.828), r_{STR} = −0.474 (0.167) P3 r_{ACC} = −0.673 (0.023), r_{STR} = −0.775 (0.009) P4: r_{ACC} = −0.259 (0.441), r_{STR} = −0.409 (0.241) P5: r_{ACC} = 0.645 (0.032), r_{STR} = 0.138 (0.704) Positive symptom sum: r_{ACC} = −0.134 (0.695), r_{ACC} = −0.550 (0.099)
Age-related (AD, Deprs-at.risk, sleep-apnea, and MCI)	Th	3 T PRESS	(Duffy et al., 2015) • Older patients at risk of DEP taking placebo had larger GSH/Cr (t = 2.0, P = .049).	51 (DEP) (28 treat+ 23 plac.)	0.8	Increased GSH_{Th} associated with worsening symptoms (r = 0.43, P = .043)
	ACC	3 T PRESS	(Duffy et al., 2015) • Older patients at risk of DEP: increased GSH in the ACC (t = 2.7, P = .012)	58 (DEP) 12 (HC)	0.9	Depressed patients showed a correlation between HADS symptoms and GSH/Cr (r = 0.28, P = .035). Depressed patients showed a negative correlation between verbal

TABLE 1 (Continued)

						learning and GSH/Cr (r = −0.28, P = .04)
		3 T PRESS	(Duffy et al., 2016) • GSH/Cr correlated with decreased executive function.	24 (ARD)	0.6	GSH_{ACC} vs Oxygen desat: r = −0.54, P = .007 GSH_{ACC} vs apnea-hypopnea: r = .42, P = .050 GSH_{ACC} vs response inhib: r = −.49, P = .015. GSH_{ACC} vs set shifting: r = −0.43, P = .37.
	ACC + PCC	3 T PRESS	(Duffy et al., 2014) • MCI patients showed increased levels of GSH in the cingulate: • GSH_{ACC} (t = −2.2, P = .03) • GSH_{PCC} (t = −2.9, P = .05)	54 (MCI) 41 (HC)	0.6	MCI GSH_{ACC}: 0.47 ± 0.15 MCI GSH_{PCC}: 0.37 ± 0.07 Control GSH_{ACC}: 0.41 ± 0.10 Control GSH_{PCC}: 0.29 ± 0.05
	Vari.	3 T MEGA-PRESS	(Mandal, Tripathi, & Sugunan, 2012) • Female AD showed decreased GSH in RFC (P = .003) compared to young controls. • Male AD showed decreased GSH in the LFC	25 (ym) 20 (yf) 9 (om) 6 (of)	~1.3	GSH_{LFC} different from GSH_{RFC} in young female (P = .02) and male (P = .001) subjects. GSH_{LFC} vs GSH_{RFC}: young females (r = 0.641, P = .004)
			(P = .05) compared to young controls. • Gender differences in GSH distribution evident.	5 (mMCI) 6 (fMCI) 7 (mAD) 7 (fAD)		GSH_{LPC} vs GSH_{RpC}: young females (r = 0.797, P = .000) GSH_{LFC} vs GSH_{LPC}: young males (r = 0.481, P = .032) (Healthy young males/females (ym/yf); healthy older males/females (om/of); males/females with mild cognitive impairment and Alzheimer's disease.)
Mult.	ACC	3 T PRESS	(Hermens, Lagopoulos, Naismith, Tobias-Webb, & Hickie, 2012) Clustering of patients based on metabolites: 3 subgroups. GSH responsible for cluster 2.	37 (DD) 29 (BP) 22 (PD) 25 (HC)	N/A	Discriminant function 2 (40% variance) characterized by GSH/Cr (r = −0.753).
Suicidal behaviour	dPFC	3 T SPECIAL	(Jollant, Near, Turecki, & Richard-Devantoy, 2016) • No association was found between GSH and suicidal behaviour (F = 0.5, P = .6), but associations with other metabolites identified.	15 (SA) 10 (PC) 33 (HC)	~1.0	GSH_{dPFC} Suicide Attempters: 0.24 ± 0.03 GSH_{dPFC} Patient Controls: 0.23 ± 0.02 GSH_{dPFC} Healthy Controls: 0.23 ± 0.03
Chronic fatigue	OCC + Imag.	3 T MEGA-PRESS/ MRSI	(Shungu et al., 2012) • GSH deficits in patients with CFS and MDD relative to controls: • ($F_{2,40}$ = 15.93; P < .001)	15 (CFS) 15 (MD) 13 (HC)	1.0	GSH was inversely correlated with ventricular lactate (r = −0.545, P = .001) and a range of key indices of physical health.
Autism	Basal ganglia + dPFC	3 T PRESS	(Durieux et al., 2016) • No GSH difference observed between patients and controls.	21 (ASD) 29 (HC)	0.8	Correlation between GSH and Autism spectrum disorder was observed in either region.
PTSD	ACC + dLPFC	3 T MEGA-PRESS	(Michels et al., 2014) • Observed GSH levels 22.73% higher in patients than controls (F = 5.757, P = .025)	12 (PTS) 17 (HC)	1.1	GSH_{ACC}: PTSD = 0.15 ± 0.03, Non = 0.11 ± 0.03 (d = 1.33) GSH_{dLPFC}: = 0.14 ± 0.03, Non = 0.11 ± 0.03 (d = 1.00)
Emerging Unipolar/Bipolar	ACC	3 T PRESS	(Naismith et al., 2014) • GSH not associated with unipolar/bipolar differentiation (t = 1.15, P = .255).	53 (EBD)	0.4	GSH not associated with sleep midpoint (r = 0.211, P = .151)

TABLE 1 (Continued)

Mood	ACC + HIPP	3 T PRESS	(Hermens et al., 2018) • Positive correlations between Fractional Anisotropy in the stria terminalis and GSH_{ACC} were found across groups (r = 0.215, P < .01). • DEP or BD in combination with decreased GSH_{ACC} was associated with reduced FA.	94 (DEP) 76 (BD) 59 (HC)	0.2	Decreased white matter integrity was associated with decreased GSH_{HIPP}.
Anorexia Nervosa	ACC + OC + PUT	7 T STEAM	(Godlewska et al., 2017) • GSH levels unchanged between groups.	13 (AN) 12 (HC)	1.2	AN (SEM) HC (SEM) p-value GSH_{ACC} 1.19 (0.07) 1.27 (0.10) 0.38 CRLB 10.2 (2.9) 8.9 (2.8) GSH_{OCC} 0.95 (0.03) 0.94 (0.04) 0.85 CRLB 10.5 (3.0) 10.67 (3.08) GSH^{PUT} 1.51 (0.45) 1.10 (0.05) 0.43 CRLB 15.0 (4.5) 15.4 (4.6)
Prader-Willi Synd.	ACC + P-OCC	- MEGA- PRESS	(Rice, Lagopoulos, Brammer, & Einfeld, 2016) • No between-group differences were observed for GSH.	15(PWS) 15 (HC)	1.0	
.Validation studies	mPFC	7 T PRESS	(Choi et al., 2010)			Optimized PRESS with sub-TE pair showed improved selectability of coupled metabolites (eg, Glu, Gln, GSH).
	mPFC + rPFC	7 T J-PRESS	(An et al., 2015)			TE-optimized J-PRESS was shown to minimize NAA signals while retaining GSH peak resolution.
	Hipp	3 T semi-LASER	(Bednařík et al., 2015)			Using a short-echo sequence with 5 minutes averaging the GSH CRLB was kept below 30% in a 4 mL voxel at 3 T.
	ACC	7 T PRESS	(Lally et al., 2016)			Intra Class Correlation (ICC) using TE-optimized PRESS both within sessions (ICC > 0.7) and between sessions (ICC > 0.6) showed good repeatability. GSH negatively associated with age (r = –0.37, P < .05).
	ACC + PCC	3 T STEAM	(Wijtenburg et al., 2014)			Short-TE phase rotation STEAM at 3 T showed excellent reproducibility for GSH: absolute reliability: SEM < 9.9%, relative reliability: ICCs 0.42-0.51
	Midline parietal	3 T HERMES	(Saleh et al., 2016)			HERMES scanning protocol showed excellent separation of GABA and GSH. Results agree with MEGA-PRESS, achieving similar signal-to-noise ratio in half the time.
	mPFC	7 T PRESS	(Choi et al., 2010)			Optimized PRESS sequence showed lower CRLBs of Gln and GSH than with STEAM.
		3 T SPECIAL	(Schubert, Kühn, Gallinat, Mekle, & Ittermann, 2017)			Short-TE SPECIAL sequence was used to measure MRS spectra at 3 T in 21 healthy adults. GSH was detected with low uncertainty (CRLB < 30%) in only 16 cases.

TABLE 1 (Continued)

	3 T J-PRESS	(Jensen, Auerbach, Pisoni, & Pizzagalli, 2017)	Test–retest reliability of metabolite quantification was assessed in a 3 T shortened J-resolved MRS sequence in healthy adolecents. GSH demonstrated satisfactory reliability with a score of 8.8–4.1%.

Abbreviations: ACC, anterior cingulate cortex; AD, Alzheimer's disease; AN, anorexia nervosa; ARD, age-related disorder; ASD, autism spectrum disorder; BD, bipolar disorder; BP, bipolar disorder; BPRS, brief psychiatric rating scale; CAT, catalase; CFS, chronic fatigue syndrome; CRLB, cramer-rao lower bound; CHR, clinical high risk; CRLB, Cramer Rao lower bound; DD, depressive disorder; DEP, depression; dLPFC, dorsal left prefrontal cortex; dPFC, dorsolateral prefrontal cortex; EBD, emerging bipolar disorder; EP, early psychosis; EPI, echo-planar imaging; FA, fractional anisotropy; fAD, females with Alzheimer's disease; FEPPA, tracer; fMCI, females with mild cognitive impairment; FSL, FMRIB software library; GSH, glutathione; GCL, glutamate cysteine ligase; GABA, gamma-aminobutyric acid; GSSG, glutathione disulphide; HADS, hospital anxiety and depression scale; HC, healthy control; HDRS, hamilton depression rating scale; HERMES, Hadamard encoding and reconstruction of mega-edited spectroscopy; HIPP, hippocampus; ICC, inferior colliculus; J-PRESS, J-resolved spectroscopy; LFC, left frontal cortex; LI, left insular; mAD, males with Alzheimer's disease; MCI, mild cognitive impairment; MD, major depression; MDA, malondialdehyde; MDD, major depressive disorder; mMCI, males with mild cognitive impairment; MMN, mismatch negativity; MRS, magnetic resonance spectroscopy; MRSI, magnetic resonance spectroscopy imaging; mPFC, medial prefrontal cortex; NAA, n-acetyl aspartate; OCC, occipital cortex; OCD, obsessive compulsive disorder; PANSS, positive and negative symptom scale; PC, patient controls; PD, psychotic disorder; PFC, prefrontal cortex; pMFC, posterior medial frontal cortex; PTS, post traumatic stress; PTSD, post traumatic stress disorder; PRESS, point-resolved spectroscopy; PUT, putamen; PWS, prader-willi syndrome; RFC, right frontal cortex; SA, suicide attempters; SANS, scale for assessment of negative symptoms; SOD, superoxide dismutase; SEM, standard error of the mean; SIPS, structured interview for psychosis-risk syndromes; STEAM, stimulated echo acquisition model; SZ, schizophrenia; TBARS, thiobarbituric acid; TE, echo time; THC, tetrahydrocannabinol; TM, mixing time; TSPO, translocator protein; VC, visual cortex; VT, total distribution volume; YMRS, young mania rating scale.
Summary of findings, describing the clinical group, brain region of interest, magnetic resonance sequence used, study reference and main findings, number of control vs clinical participants, dmin (the minimum effect size that could be founds significant given the cohorts in each study), and other GSH-specific correlations that are not directly related to the outcomes of the paper, but important nonetheless.

quantification yet used in vivo measurements of GSH concentration both as a test-bed for novel techniques as well as a means of confirmation of earlier findings. Eighteen of the studies concentrated (at least in part) on the anterior cingulate cortex (ACC), while the remainder contained a diverse range of regions of interest from the medial (m), dorsal (d) and posterior (p) PFC (11 studies), hippocampus (Hip: three), occipital cortex (OCC: four), temporal lobes (Temp: two) and one study each from the basal ganglia (BG), the thalamus (Th), the midline parietal, the precuneus (Pre), the left insular LI) and the visual cortex (VI) with additional studies that performed spectroscopy over an array of voxels, or chemical shift imaging (CSI). With the exception of CSI, regions were studied using a single large voxel (of varying size, but generally around $2 \times 2 \times 2\ cm^3$). Voxels were placed and oriented by a researcher, guided by a scout scan over the region of interest, and multiple regions were studied with separate voxels acquired sequentially.

Any patterns of GSH perturbation are difficult to identify, since there are only a few relevant studies that met our criteria for this review.

Overall, evidence of altered GSH concentration was inconsistent. While there were some indications of a decrease in GSH for major depression, treatments did not alter GSH levels—even when those treatments were associated with decreased symptoms. However, these changes are consistent with the changes seen in blood GSH levels (Maes, Galecki, Chang, & Berk, 2011), providing some support for a true alteration of GSH systemically. While generally there was some evidence that GSH levels are perturbed in first-episode psychosis, there was no consistency in the direction of change, and no changes were observed in either chronic schizophrenia patients or individuals at clinical high risk for psychosis.

None of the studies of BD included in this review show a change in brain GSH compared with controls. This may reflect a medication effect, since lithium (a commonly prescribed mood-stabilizer) has antioxidant properties and most notably can increase GSH in rat cerebral cells (Cui, Shao, Young, & Wang, 2007). Another interesting point to consider is the difference between brain GSH and blood GSH in BD. Studies present conflicting blood and brain GSH data, suggesting that blood GSH levels are abnormal or perturbed (Gu, Chauhan, & Chauhan, 2015). This may represent a compensatory response of GSH production in the brain to balance the depleted peripheral GSH pool, or may suggest that the two systems are locally regulated via the GSH-GSSG cycle.

There were some correlational findings between GSH concentration and clinical variables. For example, in early psychosis, GSH concentration in the posterior medial frontal cortex was negatively correlated with negative symptoms of schizophrenia (Berger et al., 2008; Demro et al., 2017). Negative symptoms are typically more difficult to treat with traditional anti-psychotic medication, and this relationship suggests that an intervention to increase GSH may be a promising alternative treatment. Similarly, in MD, there was a negative relationship between GSH and anhedonia. While there were relationships in bipolar disorder between GSH and risky drinking (based on the alcohol use disorders identification test; Chitty, Kaur, Lagopoulos, Hickie, & Hermens, 2014; Chitty, Lagopoulos, , Hickie, & Hermens, 2014; Saunders et al., 1993) or smoking. Finally, as a function of age, a positive relationship was observed between GSH and AD, MCI and cognitive decline, yet these observations may simply be due to other age-related effects.

3.1.3 | Heterogeneity of studies and the strength of a brain GSH measurement

The complex nature of psychiatric illness, as well as the difficulty in GSH measurement, leaves a heterogeneous evidence base that makes comparison challenging. Contradictions between studies assessing brain GSH make it difficult to compare brain and blood GSH, where studies assessing blood GSH present consistent findings in perturbation in GSH metabolism for first-episode psychosis (Fraguas, Díaz-Caneja, Rodríguez-Quiroga, & Arango, 2017), schizophrenia (Ng, Berk, Dean, & Bush, 2008), bipolar disorder and autism (Gu et al., 2015).

Medication exposure

Medication status is important to consider as a confounder when assessing oxidative stress and GSH antioxidant action within youth mental health. In bipolar disorder, lithium and valproate (commonly prescribed mood stabilizing drugs) results in a dose-dependent GSH increase in rat cortical cells. After 1-week chronic treatment, the cells demonstrated reduced oxidative stress, including increased GSH and GCL expression (upstream GSH synthesis; Cui et al., 2007). This presents a new issue, here GSH is elevated as a result of drug administration, rather than as a result of illness. It is possible the cessation of the medication would result in a return to the baseline levels of GSH. Another study looking at first-episode mania in bipolar disorder (Machado-Vieira et al., 2007) assessed the effects of lithium treatment vs no-treatment. Antioxidants SOD and CAT were elevated in unmedicated patients, indicative of generation of reactive species intrinsic to the illness. Lithium reduced markers of oxidative stress (TBARS-lipid damage), as well as the SOD/CAT ratio. There is a small body of evidence associating anti-psychotic and mood-stabilizing medication with protective effects such as increased antioxidant enzyme activity or expression, and increased GSH (Cui et al., 2007; Wang, Xu, Dyck, & Li, 2005). This may have a profound effect on studies in early psychosis, since not all studies control for drug-naïve vs medicated patients. The use of a first generation dopamine antagonist like haloperidol may contribute to increased oxidative injury (Lohr, Kuczenski, Bracha, Moir, & Jeste, 1990). In patients with chronic schizophrenia treated with haloperidol, increased lipid peroxidation markers were observed (TBARS), as well as the antioxidant enzyme SOD, presumably in response to the peroxidative injury to membrane phospholipids (Gama, Salvador, Andreazza, Kapczinski, & Silva Belmonte-de-Abreu, 2006). There are some second-generation dopamine antagonists that demonstrate protective properties. Olanzapine, clozapine, quetiapine and risperidone all play a role in upregulating SOD1 gene expression (Bai et al., 2002), indicative of an antioxidant response against radical species. The most common treatment for depression, selective-serotonin reuptake inhibitor prescription, has resulted in significant reductions in lipid peroxidation as a marker of oxidative stress (Khanzode, Dakhale, Khanzode, Saoji, & Palasodkar, 2003). This study found that treatment with fluoxetine and citalopram decreased serum SOD (antioxidant) and MDA (lipid peroxidation marker).

Voxel placement

Because there are few studies analysing GSH levels in mental illness using MRS, there appears to be no "gold standard" voxel location. There is rationale for the use of the medial frontal cortex due to association of this region with schizophrenia (Pomarol-Clotet et al., 2010). It has been argued that the medial temporal lobe is a more appropriate location for spectroscopy since there are reported links with schizophrenia, as well as the regions vulnerability to insult, particularly in the context of oxidative stress (Wood, Yücel, et al., 2009). The concentration of GSH in different regions of the brain varies significantly. The studies that have considered brain GSH have focused on the cortex, which represents the greatest concentration of brain GSH, with the cerebellum, hippocampus and striatum following in descending order of GSH concentration (Kang et al., 1999). The location of GSH in the brain is highly tissue specific (Rae & Williams, 2017), with a report of 30% higher GSH in cortical white matter (WM) compared with grey matter dominated PFC (An et al., 2015). Grey matter (GM) demonstrates increased metabolite concentrations (Srinivasan, Ratiney, Hammond-Rosenbluth, Pelletier, & Nelson, 2010), and this tissue also consumes oxygen in a 4:1 ratio to WM, despite only comprising 40% of the total brain volume (Mintun et al., 2001), rendering the GM more susceptible to oxidative insult. When comparing the frontal cortex of young healthy participants, there was a significantly greater concentration of GSH in females compared with males (Mandal et al., 2012), which could provide a confounding factor when comparing both genders in youth mental health. These results indicate a significant gender bias towards GSH concentration in the brain, which may be important considering the greater incidence of schizophrenia and first-episode psychosis (Ochoa, Usall, Cobo, Labad, & Kulkarni, 2012) in males.

Tobacco and cannabis

The prevalence of smoking in people with mental illness far outweighs that in the general population. It is estimated in the United Kingdom that 16% of the general population smoke, whereas in psychosis incidence is 56%, major depression 40%, anxiety 37% and OCD 40% (Szatkowski & McNeill, 2013). Exogenous administration of nicotine to isolated cell lines in vivo reduces antioxidant constituents (Yildiz, Liu, Ercal, & Armstrong, 1999), including GSH. Within a chronic schizophrenia population, tobacco smoke induces the oxidation of lipids and proteins (Yao, Leonard, & Reddy, 2006). In bipolar disorder, GSH concentration is reduced in the ACC of smokers, but not the non-smoking patients (Chitty, Lagopoulos, et al., 2014), and in a longitudinal study assessing tobacco consumption, a reduction in smoking was a significant predictor of increased GSH (Chitty, Lagopoulos, Hickie, & Hermens, 2015a, 2015b).

In marijuana research, it has been reported that brief exposure of an endothelial cell line to marijuana (3.95%) stimulated increased oxidative stress by 80%, as well as an 81% reduction in GSH concentration (Sarafian, Magallanes, Shau, Tashkin, & Roth, 1999). In the same study, exposure to smoke containing no THC (psychoactive component of cannabis) resulted in no change in oxidative species, but a

decline in GSH of 70%. This is interesting since, as with tobacco, marijuana use in mental illness is far greater than in the general population (6.6% (UNDOC, World Drug Report, 2011) compared with 23% in psychosis (Green, Young, & Kavanagh, 2005), 9.5% in major depression (Chen, Wagner, & Anthony, 2002), 19% in bipolar disorder (Marken et al., 1992) and 17% in anxiety (Degenhardt, Hall, & Lynskey, 2001)).

3.2 | Strength of a brain GSH measure over periphery

A number of studies have tried to draw comparisons between GSH in the brain and peripheral measures such as blood components or cerebrospinal fluid (CSF). Due to the variable nature of study design in the tissue assessed and the brain region of interest, the concentration of GSH across differing compartments of the human body is not well characterized (Richie, Skowronski, Abraham, & Leutzinger, 1996; Samuelsson, Vainikka, & Ollinger, 2011; Sanaei Nezhad, Anton, Parkes, Deakin, & Williams, 2017)).

Many studies, when investigating GSH, do not differentiate between reduced, oxidized and total GSH, rendering the measure comparable only within its own intervention, and difficult to use in comparison to other studies. This makes the use of MRS as a tool for GSH quantification useful since the measure is direct, and informative of brain GSH status.

Blood measures of GSH are the most commonly reported methods, since assays are cheaper and more accessible than MRS. Plasma measures of GSH are commonly reported, but report a wide range of concentrations of GSH (0.5-759 μM) (Nuttall, Martin, Sinclair, & Kendall, 1998; Raffa et al., 2009). Plasma acts as a medium for metabolic waste products in the body, where cells excrete partial protein and lipid components for clearance. In addition, the role of GSH is to provide redox balance within a cellular system. Therefore, it is unlikely that plasma GSH offers a reliable and accurate representation of peripheral GSH, and even more unlikely that it would be a representation of brain GSH.

3.3 | MRS methods

MRS provides a means to explore novel chemical environments and analyse known molecular systems. Through the analysis of the free induction decay (FID) spectra of a target nucleus, the local chemical environment may be inferred. While exotic nuclei, such as ^{31}P and ^{13}C, are possible for targeting in clinical environments, ^{1}H is the most widely available (Blüml, 2013). However, accurate quantification of some metabolites is hampered by both the low spatial resolution, due to the low chemical concentrations and the decreased spectral resolution of clinical scanners with lower field strengths. Generally, in vivo MRS is conducted over a single voxel placed within a target brain region (identified using a preliminary scout scan); while imaging protocols that perform spectroscopy over an array of large voxels are possible they are less common.

3.4 | GSH measurement in vivo

In vivo quantification of GSH in humans is particularly challenging due to the low concentration (1.5-3 mmol/L), and the fact that all resonances overlap with stronger signals from alternate metabolites (De Graaf, 2013). However, it is possible to quantitatively isolate the spectral contribution from GSH through both refinement and calibration of standard approaches to MRS measurement as well as the development of novel sequences. Additionally, in order to accurately quantify the contribution of GSH to the measured spectra, a range of post-processing techniques, from spectral decomposition to partial volume correction, are required. While similarities and trends do exist, studies conducted within the past 10 years vary in voxel placement, measurement sequence, acceptance bounds on signal quality and post-processing methodologies. Here the predominant approaches are described.

3.5 | Acquisition modalities

In order to accurately estimate the concentration of GSH within a volume of interest, a range of different tradeoffs must be considered. The method of spatial localisation utilized is, perhaps, the first consideration, while voxel size and placement, which is affected by field and tissue homogeneity, are of related concern as are the methods used to isolate GSH from other contributors.

3.5.1 | Spectroscopy

The FID spectrum exhibits a range of peaks unique to the local electronic environment of contributing chemical systems. In order to quantify low concentration metabolites, the extracted spectra must be of high quality, predominantly assessed through the signal-to-noise ratio (SNR) and the spectral linewidth (usually expressed in terms of the full width at half maximum—FWHM). While quantification accuracy is usually dependant on the spectral SNR, small linewidths allow for good separation of distinct peaks. Averaging multiple signals can increase the SNR, yet patient motion means that shorter scan times are preferred (both in terms of field homogeneity and partial volume correction). Approaches to decomposition using basis vectors determined by contributing metabolites can decrease the importance of linewidth (removing any requirement of spectral separation), yet the accuracy to which each contribution can be estimated still relies on this parameter.

A high-quality shim can be used to provide field (B0) homogeneity over some region of interest, reducing the expected spectral linewidth sufficiently for GSH analysis. However, specific sequences are required in order to localize the induced FID to this region. A number of different approaches exist, each with competing properties in terms of measured spectral quality.

3.5.2 | Spatial selectivity

While a range of spatial localisation methods exist, the studies considered here used point -resolved spectroscopy (PRESS) or stimulated

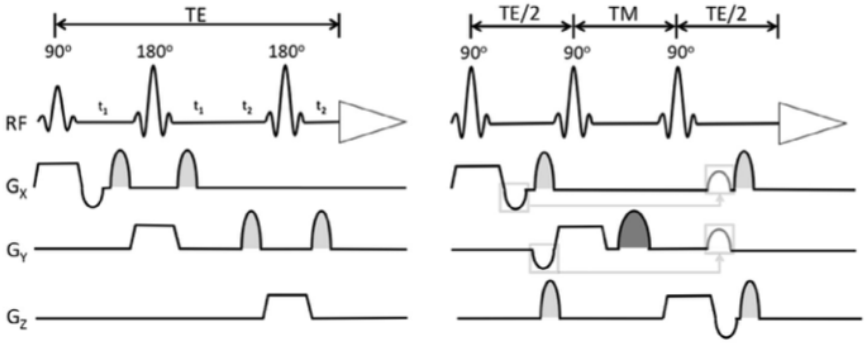

FIGURE 2 Point-resolved spectroscopy (right) and stimulated echo acquisition mode (left) sequences, taken from "In Vivo NMR Spectroscopy," (De Graaf, 2013)

echo acquisition mode (STEAM), and variants or extensions to these sequences, almost exclusively.[2] PRESS and STEAM both use three slice selective pulses (Figure 2):

1. PRESS: $1 \times 90^{\circ} + 2 \times 180^{\circ}$
2. STEAM: $3 \times 90^{\circ}$

STEAM decreases the specific absorption and because there is no T2 decay in the TM period is preferred for short T2 metabolites, but this approach does sacrifice the SNR of each acquired spectra.

3.5.3 | Water suppression and spectral editing

Because of the local environment, water suppression is required in order to resolve the much smaller spectral peaks induced by the target metabolites. Chemical shift selective and variable pulse powers and optimized relaxation delays (VAPOUR) are commonly used for water suppression, while it is also possible to include water into the macromolecular baseline utilized during molecular decomposition.

With an accurate high-order shim, it is possible to resolve GSH peaks directly. However, should the spectral linewidth be too large (or simply to enhance quantitative accuracy) spectral editing may also be used to extract specific metabolic signals. The MEscher-GArwood (MEGA) technique uses editing pulses aimed at spins which are J-coupled with the spin of interest, for instance, an editing pulse is applied at 4.56 ppm which targets a α-CH resonance J-coupled to the desired GSH spins at 2.95 ppm (Saleh et al., 2016). Sequences with and without editing are subtracted to produce a final spectrum that contains greatly decreases cross-talk between the spins of interest and other peaks within the spectra.

3.5.4 | Modifications and extensions

The predominant approach to spectral measurement in the studies considered here used highly optimized PRESS and STEAM sequences directly with, in some cases, editing sequences such as MEGA to home in on specific metabolites. However, a range of those studies considered also investigated novel spectroscopy acquisition protocols. Such approaches used relationships involving GSH, tested in vivo, to not only validate and test the novel protocol, but also to validate and explore the metabolic relationships.

- **Hadamard encoding and reconstruction of mega-edited spectroscopy:** The use of a Hadamard encoding in editing allows the simultaneous acquisition of two different voxels and two different metabolites simultaneously, quartering acquisition time (or doubling SNR).
- **Phase rotation**: Phase cycling is introduced into the radio frequency signal to remove undesired spin echo signals, and has been used with STEAM to isolate the stimulated echo.
- **2D J-differences editing**: Two dimensionally resolved MRS provides spectra over a range of editing frequencies and as such can be used to perform accurate MRS with no expectation as to the target metabolite.
- **MRS imaging:** While direct repetition of PRESS or STEAM for multiple voxels is possible, imaging (or spatial encoding of the spectroscopic signal) is generally conducted using phase encoded gradient fields.
- **Proton echo planar spectroscopic imaging (PEPSI):** PEPSI uses an approach to echo planar imaging, yet one dimension of echos are encoded with chemical shift information, rather than spatial information as in standard EPI. In studies considered that employ PEPSI large outer volume suppression slabs and advanced approaches to shimming (FAST(EST)MAP) were required.

3.6 | Data acquisition post-processing

Once the spectra have been acquired, extraction of the GSH signal is required. Signal extraction can take a number of directions and may be dependent on the approach taken to measurement. Should sufficient and accurate editing have been conducted, the GSH peak may then be measured directly (often using simple summation over the GSH peak). However, in the case that there is residual overlap between GSH and spectra from other metabolites present in the sample more sophisticated processing is required.

3.6.1 | Spectral deconvolution

A standard approach, in the case of overlapping peaks, is linear decomposition of the acquired spectra into metabolic basis functions.

Standard software packages (such as LCModel) are available, or in-house software may also be used for this approach. In all cases, a database of metabolic basis functions must be available that are either carefully measured (with the same system and sequence used for measurement), simulated using advanced modelling tools such as GAMMA, VERSI, FID-A (or in-house developed tools) or even a combination of the two where measurements of the macromolecular spectra may be measured and used in concert with simulated spectra of specific metabolites.

Once the basis metabolites are identified, they are used to estimate a linear decomposition of the measured spectra (based on some cost function such as least-squares). The decomposition provides an estimate of the contribution from each metabolite, while the residual allows for the presence of missing features to be assessed (ie, it should represent noise). Generally, such fits also provide a measure of uncertainty, as a percentage of the Cramer Rao lower bound (CRLB) in the case of LCModel, which can be used to accept or reject the measurement. Where acceptance levels were quoted as CRLB, most commonly >20% uncertainty measurements were rejected, but in some cases rejection was reserved only for >50% uncertainty. While it is not clear as to the impact on final values of the estimated value or its uncertainty, nor rejection levels utilized, using the %CRLB as a rejection threshold has been shown to be unreliable, particularly for low concentration metabolites (Kreis, 2016). Kreis (2016) argued that despite the unreliability of these widely used threshold levels of 20% to 50%, CRLB is a valuable tool to give an idea of minimal uncertainties in MRS, when the obtained error is understood to be an estimate of the lower bound of the fitting error.

Finally, once the metabolite concentration has been identified, the voxel heterogeneity was accounted for (in a similar way for most studies). The MRS voxel (or voxels) used in measurement is segmented based on the original magnetic resonance imaging image used for positioning. The contributing volume fractions from GM, WM and CSF are calculated (often using the FAST4 algorithm from FSL) and used to correct the measured concentration (or understood as covariates with this concentration).

3.6.2 | Measurement normalization and post-processing

Some note should be made regarding the comparison of spectroscopy techniques across the studies considered here. However, this is made difficult not only by the broad range of sequences used but also the range of parameters explored within each sequence. In each study different spectral bandwidths, sampling rates, and number of averages were considered. Each of these parameters will affect the FWHM and SNR of the recovered spectra, and hence the reliability of the spectral decomposition (as will the methods used to generate the basis metabolites). Even the units used in measurement of the metabolic concentration differed. While in many studies the absolute GSH concentration was used as the final measurement, in other studies GSH as a fraction of total Creatine (tCr) or Water peaks were used. Finally, comparison across studies that use different scanners is a potential confound, while the pooling of MRS data is becoming more important in terms of providing a larger sample size to increase confidence in effect size, or to facilitate collaborative work, the risk of systematic error is increased, since there is always variability in participant positioning, partial volume effects and image intensity inhomogeneity (Stonnington et al., 2008).

Such diversity may not impact the overall neuroscientific findings, yet each choice will impact the accuracy of each finding, hindering comparison. No study regarding the impact on quantification of the relevant tradeoffs has been conducted, so that generally in-house optimisations and conveniences have been used. While useful for immediate application, this approach may hinder a standardized approach for quantitative exploration.

4 | SUMMARY

There is evidence of perturbations in the oxidative stress/antioxidant response system across youth metal health, with emerging evidence implicating GSH in the eitiology of mental illness. However, the field is hampered by highly variable methodology, from basic sequence and voxel selection to analysis approach and choice of measurement units. This is before problems surrounding diagnostic heterogeneity are considered and the simple issue of statistical power. If this area of study is to progress, studies with larger participant numbers are required, most probably involving multiple collaborating sites where very close attention is paid to minimizing between-site variability in both clinical and spectroscopic data collection.

ENDNOTES

[1] Adapted from http://www.djmannion.net/psych_programming/data/power/power.html

[2] Exceptions include spin-echo full-intensity acquired localized spectroscopy (SPECIAL) and imaging studies.

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Exercise as a protective mechanism against the negative effects of oxidative stress in first-episode psychosis: a biomarker-led study

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Abstract

First-episode psychosis (FEP) is a psychiatric disorder, characterised by positive and negative symptoms, usually emerging during adolescence and early adulthood. FEP represents an early intervention opportunity for intervention in psychosis. Redox disturbance and subsequent oxidative stress have been linked to the pathophysiology of FEP. Exercise training can perturb oxidative stress and rebalance the antioxidant system and thus represents an intervention with the potential to interact with a mechanism of disease. The aim of this study was to assess the effect of exercise on markers of redox status in FEP. Twenty-two young men were recruited from Birmingham Early Intervention services and randomised to either a 12-week exercise programme or treatment as usual (control). Measures of blood and brain glutathione (GSH), markers of oxidative damage, inflammation, neuronal health, symptomology and habitual physical activity were assessed. Exercise training was protective against changes related to continued psychosis. Symptomatically, those in the exercise group showed reductions in positive and general psychopathology, and stable negative symptoms (compared to increased negative symptoms in the control group). Peripheral GSH was increased by 5.6% in the exercise group, compared to a significant decrease (24.4%) ($p = 0.04$) in the control group. Exercise attenuated negative changes in markers of neuronal function (brain-derived neurotrophic factor), lipid damage (thiobarbituric acid-reactive substances) and total antioxidant capacity. C-reactive protein and tumour necrosis factor-α also decreased in the exercise group, although protein and DNA oxidation were unchanged. Moderate-intensity exercise training has the ability to elicit changes in markers of oxidative stress and antioxidant concentration, with subsequent improvements in symptoms of psychosis.

Introduction

First-episode psychosis (FEP) is a psychiatric disorder, characterised by positive and negative symptoms, usually emerging during adolescence and early adulthood[1]. It is well described that a delay in FEP treatment can lead to cognitive alterations and worsened functional outcome[2]. FEP patients are highly heterogeneous in symptom profile, treatment response and cannabis consumption, and there is a need for improved characterisation of biomarkers related to disease pathophysiology in order to better understand pathology of disease. Many of the biochemical perturbations observed in this population are associated with the psychosis phenotype and thus represent targets for treatment.

Several aspects of (neuro) physiology are altered in FEP and schizophrenia patients, including dysfunctional neurotransmitter systems[3,4], decreased synaptic plasticity[5] and reduced hippocampal volume[6]. In addition, inflammation and cellular redox status is also altered. This is often termed oxidative stress, and represents an imbalance between reactive radicals or oxidants, which are naturally produced in aerobic metabolism, and

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antioxidants, which balance and quench oxidants to allow essential signalling processes, but prevent damage to biomolecules leading to cell injury or death[7]. Brain-derived neurotrophic factor (BDNF), which has an essential role in neural signal transmission and synaptic plasticity, is depleted in FEP[8]. It has also been suggested that BDNF is responsible for protecting against oxidative stress by upregulating the expression of antioxidant enzymes[9], highlighting the value of BDNF as a candidate biomarker of psychosis.

Glutathione (GSH) is the most abundant antioxidant that acts as a scavenger of reactive oxygen species, with a primary role of maintaining the intracellular redox balance[10]. GSH has been implicated in the pathogenesis of a range of neurodegenerative and psychotic diseases[11]. FEP patients have shown as much as a 52% reduction in GSH compared with controls[12,13]. Indeed, evidence of perturbed redox homeostasis in FEP and schizophrenia is plentiful. There have been reports of reduced total GSH[13–15], increased oxidised GSH (GSSG)[15], increased lipid[16,17], protein[18,19] and DNA damage[20,21]. However, conflicting data by Reddy et al.[22] also showed no impairment of antioxidant defences in comparison with a healthy control group, hypothesising that disease stage is related to progression of oxidative stress accumulation. A study by Fraguas et al.[14] assessed the relationship between grey matter volume and GSH in the brains of schizophrenia patients. The study highlighted a progressive blood GSH decline over the 2-year follow-up period, as well as a relationship between grey matter loss and blood GSH.

The brain is a particularly vulnerable target for free-radical-mediated damage due to a high level of oxygen consumption[23,24], and high lipid content[19]. When this high oxygen concentration is coupled with a modest endogenous antioxidant concentration[25], and inability of GSH to cross the blood–brain barrier (BBB), particularly from exogenous supplementation or circulating pools, the antioxidant defence system is stretched. Assessment of brain redox state in vivo is not easy, and peripheral markers are usually employed as an estimate of whole-body oxidative environment and antioxidant concentration. However, recent methodological advances in magnetic resonance spectroscopy (MRS) have allowed the quantification of GSH in the cerebral tissue[26].

Exercise has been identified as an adjunctive treatment for psychosis as it has the ability to improve clinical symptoms[27] and has the potential to restore redox homeostasis, as demonstrated in healthy populations[28]. However, no study has proven the efficacy of exercise in altering the underlying pathology of disease. Studies employing moderate-intensity aerobic exercise[29] and combined aerobic and resistance training[30] across 16 and 12 weeks, respectively, have resulted in reduced psychotic symptoms assessed by the Positive and Negative Symptom Scale (PANSS). Acil et al.[31] described a 14% increase in quality of life, as well as a decrease of positive and negative symptoms that define the schizophrenic phenotype.

Mechanisms underpinning exercise adaptation are well established in studies of healthy participants and are characterised by an antioxidant response to radical signals[32]. Elokda and Nielsen[33] demonstrated adaptations to either aerobic training or combined aerobic and circuit weight training (standardised workload). Both training programmes led to a significant increase in GSH, and reduced oxidised GSH (GSSG). Additionally, exercise training has the potential to modify other indices of redox status. Studies in health volunteers have shown that exercise training may elevate BDNF[34] and reduce oxidative damage markers, including malondialdehyde (lipid peroxidation) and protein carbonyl concentration[35].

Furthermore, exercise training is beneficial in reducing circulating proinflammatory cytokines[36]. There is robust evidence to implicate a systemic proinflammatory state in FEP. Inflammation and oxidative stress are strongly linked in a number of pathologies. Systematic review[37] confirms elevated proinflammatory cytokines in drug-naive FEP, including interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), IL-1β and sIL-2R, and there is clear evidence of elevated IL-6 in childhood, predicting risk for both psychosis and metabolic dysfunction. Regular physical activity can lead to reductions in circulating IL-6[38,39], TNF-α[40] and C-reactive protein (CRP)[41,42].

The aim of this exploratory pilot study was to assess the effect of 12 weeks of exercise training on the GSH system in FEP, via in vivo brain and peripheral blood measures. To add context around changes in GSH, measures of oxidative damage, inflammation and neuronal health were assessed, as well as symptoms of psychosis.

Methods

Participants

Male patients, aged 16–35 years, with a diagnosis of FEP (as identified by a psychiatrist in keeping with ICD-10 F 20-29, F31.2, 32.3 diagnostic criteria), were recruited from the community-based Birmingham Early Intervention service. Patients were within 3 years of first presentation of illness. Male patients only were recruited, since the oestrogen cycle has a significant effect on antioxidant concentration, in particular GSH[43]. Eligibility criteria were assessed initially by the primary care coordinators for each patient, followed by assessment of habitual activity to ensure a sedentary lifestyle, as well as the use of a general health questionnaire to assess cannabis use and confirm the patient was free from medical conditions that prevent participation in moderate-intensity aerobic exercise. A sample size of 28 was calculated, using the

G*Power software[44], based on intervention-associated change in GSH. Exclusion criteria included failure to adhere to pretesting requirements, for example, provide a blood sample, or significant risk to self of others as identified by the clinical team. Study assessments took place at baseline, mid-point (for the exercise group only) and post intervention. This study was commenced following approval from the NIHR HRA ethics committee (West Midlands- Edgbaston REC 17/WM/0412). Intervention design, quality and patient-oriented outcomes were assessed and are summarised by Fisher et al.[45].

Randomisation

Following consent, participants were randomised to either the exercise intervention group or the control arm (treatment as usual) of the study. A block randomisation method (http://www.randomization.com)[46,47] was used to allow for equal group distribution in the event of poor recruitment.

Exercise intervention

Exercise sessions were designed and supervised by a trained researcher at the School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham. The intervention was 12 weeks long, with each participant required to exercise at least 2 times per week, for 40–60 min per session. In an effort to maximise attendance and compliance, which has historically been difficult in this group[48], participants were given a choice of different activities to undertake at each session (available exercise: running; cycling; swimming; tennis; squash; badminton; circuit training and football). Each training session was standardised by heart rate target zone, based on 70–80% HRmax (maximum heart rate). The minimum training intensity for improvement in aerobic fitness is 55–65% HRmax[49]; therefore, in order to observe a meaningful and significant effect of exercise, intensity was set above this. The ACSM (American College of Sports Medicine) recommends 50% VO_2max (maximum rate of oxygen consumption) as a minimum intensity for exercise training[50], which corresponds to 65% HRmax. The relationship between HR and VO_2 reflect energy expenditure in a linear fashion, up to 85% HRmax[51]. Energy consumption, resting heart rate, active calories and intensity minutes (equivalent to moderate-intensity exercise) was tracked at three time-points in the intervention period (baseline, mid-intervention and post intervention), and for the duration of each exercise bout by Garmin VivoSmart™ HR activity monitor (Garmin, USA).

Blood sampling

Cephalic/cubital venous blood samples were taken at pre-intervention (0 weeks), mid-intervention (6 weeks) and post-intervention (13 weeks) time-points, into BD Vacutainer® MAP K_2EDTA 1.0 mg tubes (BD, USA). Following removal of whole blood (2 × 1 mL) for comet assay and GSH analysis, samples were centrifuged (2000 r.p.m., 15 min, 10 °C) and the resultant plasma was aliquoted for subsequent determination of FRAP (ferric-reducing ability of plasma), TBARS (thiobarbituric acid-reactive substances), 8-isoprostane, BDNF, IL-6, CRP, TNF-α and protein carbonyl. All samples were stored at −80 °C for a maximum of 9 months until analysis.

MRS GSH measurement

Scans were conducted at the Birmingham University Imaging Centre, using a 3 Tesla Phillips Achieva MRI (magnetic resonance imaging) scanner, with a 32-channel head coil. The ^{1}H single-voxel Mescher–Garwood point-resolved spectroscopy method of acquisition was employed, with repetition time = 2 s; echo time = 131 ms; 55 Hz bandwidth editing pulse at 4.56 p.p.m.; and 1024 complex data points acquired at a sampling frequency of 2000 Hz, followed by water suppression. The MRS protocol included a T1-weighted structural MRI for MRS planning (5 min). The volume of interest was located in the anterior cingulate cortex (30 × 30 × 20 mm^3) (Fig. 1). Total MRS scan time for GSH measurement was 18 min. Spectral alignment was completed using the RATS (Robust Alignment to a Target Spectrum) method[52], implemented in R (v3.5.0) (Vienna, Austria), and integrated into the SPANT (SPectroscopy ANalysis Tools) package (v0.12.0) for MRS analysis. GSH was then fitted using TARQUIN. An example spectrum is detailed in Fig. 1, following post-processing, with the GSH peak highlighted.

Blood GSH

Whole-blood GSH concentration was determined using a commercially available luminescence-based assay (GSH-Glo™ Glutathione Assay, Promega, WI, USA). The assay was undertaken according to the manufacturer's instructions.

Comet assay

Cells were prepared for assessment of single-strand DNA strand breaks, characterised by Singh et al.[53]. Thawed whole-blood samples were used for analysis, breaking away from the conventional use of isolated mononuclear cells, replicating the protocol described by Akor-Dewu et al.[54]. Active SYBR-GOLD nucleic acid gel staining solution (1:1000 dilution of stock solution in neutralisation buffer) was distributed across the slides and incubated at room temperature before scoring (tail length representing single-strand break density), using a fluorescent microscope (Zeiss Axiovert 10, Germany).

Lipid peroxidation

8-Isoprostane and TBARS concentrations were used to assess lipid peroxidation in the plasma. For TBARS, plasma

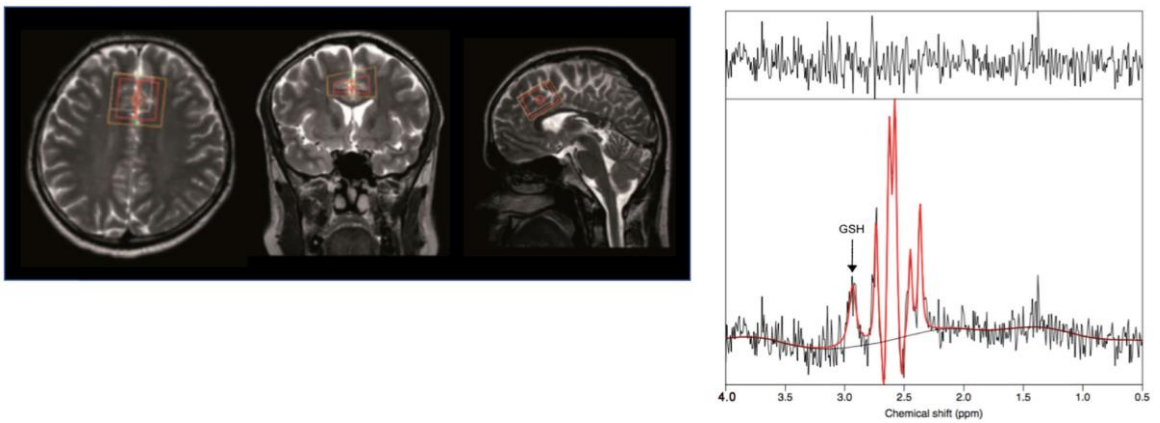


Fig. 1 T1 weighted MRI image demonstrating the volume of interest (VOI) pictured. 30 × 30 × 20 mm. Placed in the anterior cingulate cortex. Image above represents axial, frontal and sagittal views from left to right. The metabolite peak on the right shows an example, edited spectra with labelled glutathione peak at 2.95 mm.

samples and standards (1,1,3,3-tetramethoxypropane) (100 µl) were mixed with trichloroacetic acid (410 mM, 100 µl) and colour reagent (4.6 mM thiobarbituric acid, 1.74 M glacial acetic acid and 0.67 M butylated hydroxytoluene, 800 µl) in Eppendorf tubes. After boiling vigorously in water (100 °C) for 1 h, the reaction was stopped by placing the tubes in an ice bath for 10 min. Supernatants were transferred to a multiwell plate and the absorbance was measured at 540 nm. The concentration of 8-isoprostane in plasma samples was determined by competitive enzyme-linked immunosorbent assay using a commercially available kit (8-isoprostane EIA Kit 516351, Cayman Chemical, Ann Arbor, MI), according to the manufacturer's instructions. After pretreatment of the plasma sample to concentrate the total 8-isoprostane content, affinity sorbent (8-isoprostane affinity sorbent 401113-1, Cayman Chemical, Ann Arbor, MI) was used.

Protein carbonyls

Protein carbonyl concentration was determined in the plasma. Anti-DNP (2,4-dinitrophenol) antiserum primary antibody (1:1000) and peroxidase-labelled secondary antibody (rat anti-mouse IgE in blocking buffer 1:5000) were used, following the protocols described by Buss et al.[55] and Alamdari et al.[54].

Inflammatory markers

Plasma IL-6, CRP and TNF-α concentrations were determined using commercially available immunoassay kits (Human IL-6 Quantikine ELISA Kit (D6050), Human TNF-alpha Quantikine ELISA Kit (DTA00C) and Human C-Reactive protein/CRP Quantikine ELISA Kit (DCRP00)), from R&D Systems (Minnesota, USA). Concentrations were determined according to the manufacturer's instructions.

Total antioxidant capacity using the FRAP method

The FRAP method was developed by Benzie and Strain[56]. Standards were prepared using a 0–1000 µM concentration range of ascorbic acid. FRAP reagent—consisting of acetate buffer (300 mM sodium acetate at pH 3.6 (3.1 g) into neat 16 mL glacial acid per litre of buffer solution), TPTZ (2, 4, 6-Tris (2-pyridyl)-*S*-triazine) solution (160 mM: 0.05 g/mL–0.1 g TPTZ in 2 mL methanol, then 2 mL into 30 mL 40 mM HCl) and $FeCl_3{\cdot}6H_2O$ solution (0.332 g ferric chloride in 100 mL ddH_2O)—was added to samples/standards on the plate, incubated for 8 min at room temperature and read at 650 nm. FRAP concentrations were determined by linear regression relative to ascorbic acid.

Brain-derived neurotrophic factor

Plasma BDNF concentration was determined using a commercially available immunoassay kit (Human BDNF ELISA Kit (ab99978), Abcam, Cambridge, UK). Results using this sandwich ELISA Kit were determined according to the manufacturer's instructions.

Psychiatric outcomes

At each assessment time-point, participants completed the PANSS[57], via a structured clinical interview designed to monitor symptoms of psychosis. The PANSS interview assesses positive and negative symptoms, and is widely considered the 'gold-standard' method of quantifying psychotic behaviour. Interviewers were trained in the completion of PANSS.

Data analysis

Data analysis was performed using GraphPad Prism 8 software (version 8.0.1, 2018). At baseline, relationships between markers were determined using linear regression, and to assess any difference at baseline between the two groups, two-sample t tests were used. To assess changes between different time-points in the study, paired t tests were used. To compare relationships between marker pre-intervention/control period vs. mid or post, Pearson's correlation coefficient was employed. Biomarkers were assayed in triplicate, with average values calculated. Outlying values were identified using the ROUT (Robust regression and Outlier removal) method ($Q = 1\%$). Shapiro–Wilk test for normality was used for Gaussian distribution, with α significance level set at 0.05. Standardised mean difference (SMD) test was also used to assess effect size between time points and intervention groups, using the standard deviation of paired differences.

Results

Baseline

Twenty-two early intervention service users were recruited and randomised into the study, aged between 17 and 34 (average length of service use, at recruitment, was 19 months). Baseline characteristics are presented below (Table 1). From a potential caseload of 134, 67 patients did not meet eligibility criteria. Of the 67 remaining, with potential for inclusion into the study, 22 were randomised. Reasons for eligible patients not being randomised included discharge from the early intervention services, work/university time commitments, no interest in taking part in the study and an inability to make initial contact with the patient. Fifteen participants completed the trial, across the exercise ($n = 7$) and control ($n = 8$) groups. Peripheral biomarker analysis and symptom assessment was completed for all participants, whereas only four members of each intervention group were able to complete the MRS brain scan.

Table 1 Summary of baseline characteristics, for both exercise and control groups.

	Exercise		Control		
	Average	SD	Average	SD	*P* values
Weight (kg)	82.51	14.69	84.10	16.76	0.82
BMI (kg/m^2)	25.45	3.71	25.49	4.65	0.98
Age (years)	23.45	3.75	26.10	5.74	0.22
Duration of illness (months)	20.56	9.99	18.00	11.70	0.61
Tobacco consumption	73%		64%		NA
Cannabis consumption	50%		91%		NA
Brain GSH (mM/kg) (tNAA)	0.254	0.061	0.275	0.044	0.75
Blood GSH (μM)	0.72	0.23	0.77	0.22	0.62
Comet (%)	3.20	1.52	2.54	1.41	0.31
TBARS (μM)	12.82	2.94	12.31	3.34	0.72
Protein carbonyl (nmol/mg protein)	138.44	42.69	150.52	136.05	0.79
IL-6 (pg/mL)	1.88	0.49	1.6	1.2	0.34
TNF-α (pg/mL)	83.69	13.99	87.25	12.14	0.54
CRP (ng/mL)	2.51	1.37	2.79	1.43	0.65
FRAP (μM)	294.64	57.01	270	36.68	0.26
BDNF (ng/mL)	52.53	20.91	40.56	26.87	0.64

BDNF brain-derived neurotrophic factor, *BMI* body mass index, *CRP* C-reactive protein, *FRAP* ferric-reducing ability of plasma, *GSH* glutathione, *IL-6* interleukin-6, *TBARS* thiobarbituric acid-reactive substances, *TNF-α* tumour necrosis factor-alpha.

Brain and blood GSH

Peripheral whole-blood GSH increased by 6.13% in the exercise group, but significantly decreased by 24.37% in the control group ($p = 0.04$), in a group–time interaction (SMD = 0.47).

Ineligibility for scanning included foreign metal in the body ($n = 2$), anxiety/fear of enclosure in the scanner ($n = 6$) and injury preventing prolonged periods of time in the supine position ($n = 1$). Brain GSH concentration increased by 8.50%, and 8.80% in the exercise and control groups, respectively (SMD = −0.06). There was no significant change in brain GSH data as a result of 12 weeks of exercise ($p = 0.55$), but these data are limited by the small sample of the cohort that were able to be scanned (exercise group $n = 4$ and control group $n = 4$ for pre–post scans).

The Pearson's correlation coefficient for the relationship between blood and brain GSH showed that in the control group, pre and post r values were 0.37 and 0.23, respectively, indicating weak positive correlation at both time points. The r value for the exercise group pre-intervention was 0.91, and post intervention 0.06. These changes indicate that, in this exercising group of participants, exercise affected peripheral and brain GSH regulation differently. Both blood and brain GSH changes are presented in Fig. 2. Additionally, in the exercise group, the relationship between GSH change and intensity minutes increase, as a result of being in the intervention, was strongly positively correlated, for both blood ($r = 0.61$) and brain ($r = 0.68$) measures.

Markers of oxidative damage

Lipid peroxidation (assessed by TBARS (SMD = −0.67) and isoprostane concentration (SMD = 0.41)), protein oxidation (assessed by protein carbonylation (SMD = 0.31)) and DNA oxidation (assessed by strand breaks

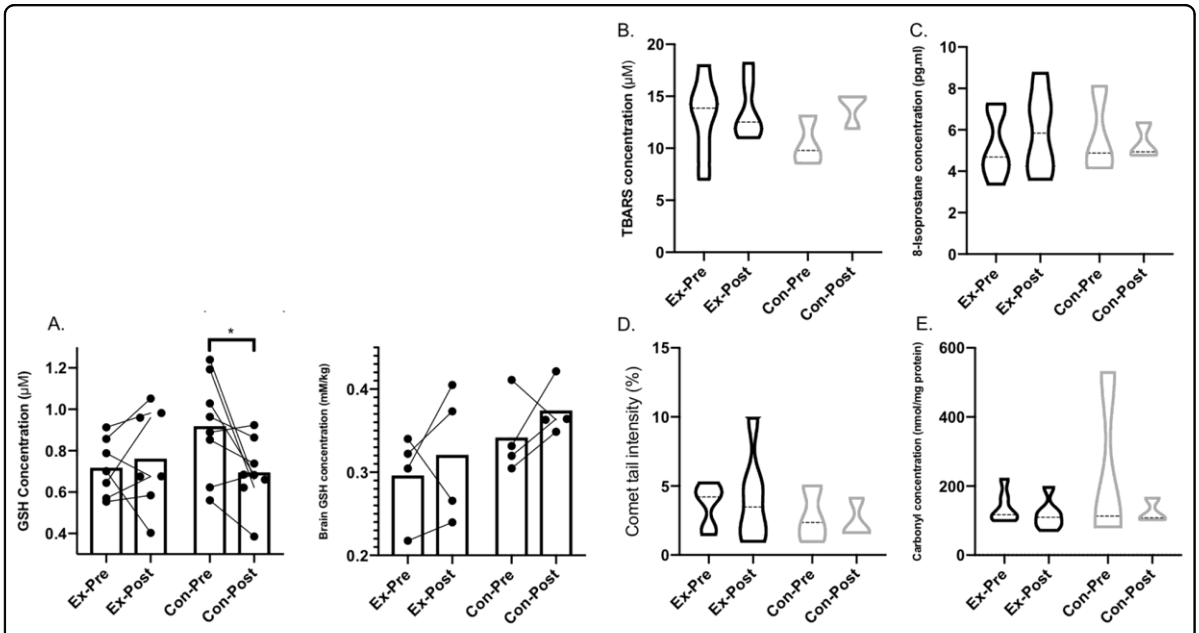


Fig. 2 Changes in indices of antioxidant capacity and oxidative stress. a A comparison of whole-blood GSH concentration (μM) (left) vs. cerebral GSH concentration (mM/kg) (right), pre-intervention vs. post intervention. **b** Plasma thiobarbituric acid-reactive substances concentration (μM), a measure of lipid peroxidation. **c** Plasma 8-isoprostane concentration (pg/mL), a measure of lipid peroxidation. **d** Single-strand DNA damage from whole blood (%). **e** Plasma protein carbonyl concentration (nmol/mg protein). Both GSH measures are expressed as mean and standard deviation. All oxidative damage markers are expressed as median and interquartile range. * indicates a significant difference between pre- and post measures.

(SMD = 0.18)) are presented in Fig. 2. Exercise did not cause any significant changes in markers of oxidative stress. Repeated-measures analysis of variance (ANOVA) showed no difference between time points for each marker of oxidative damage. Due to the small sample size represented in this study, median values were used to better describe the central tendency of the data, since each measure had a wide range[58].

Inflammatory markers

The markers of inflammation CRP, IL-6 and TNF-α each changed differently in response to the exercise intervention. IL-6 concentration decreased between pre and post time-points in both groups: 25.98% in the exercise group and 43.30% in the control group (SMD = 0.25). CRP decreased by 10.29% in the exercise group and increased by 12.05% in the control group (SMD = −0.37). TNF-α decreased in both groups: 4.28% and 6.80% for the exercise and control groups, respectively (SMD = 0.13). Repeated-measured ANOVA showed no difference between time points for each inflammatory marker.

BDNF concentration

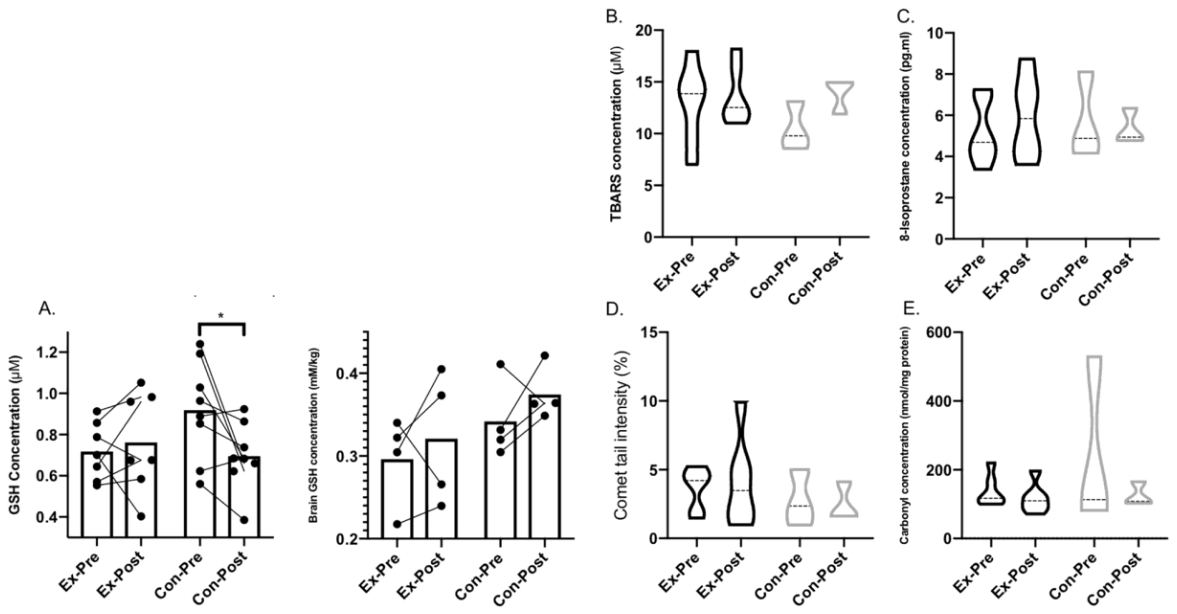
Plasma BDNF content increased by 10.38% in the exercise group ($p = 0.70$), and declined by 17.52% in the non-exercising controls ($p = 0.64$). Despite the negligible increase in BDNF content in the exercise group over the full 12 weeks of the intervention period, between mid and post time-points in the exercise group, plasma BDNF concentration increased by a much greater margin of 41.58% ($p = 0.12$, SMD = 0.23).

Cannabis and tobacco consumption

Subgroup analysis was undertaken in the exercise intervention group in participants who self-identified as regular cannabis smokers ($n = 4$), compared with non-smokers ($n = 3$). TBARS concentration decreased 1.30% in non-smokers, but increased 37.76% in smokers, and isoprostane concentration increased 5.22% in non-smokers compared to 17.72% in smokers. Protein carbonylation decreased 31.9% in non-smokers and decreased 5.84% in smokers. These effects may have been fortified by tobacco consumption, since all cannabis smokers were also tobacco smokers, and only one non-smoker consumed tobacco only. In an analysis of baseline values for tobacco smokers vs. non-smokers, there was no difference in any of the biomarkers assessed.

Intensity minutes and markers of inflammation

The potential effect of quantity of exercise (intensity minutes) on markers of inflammation was assessed (Fig. 3). 'Intensity minutes', measured by the Garmin



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Plasma BDNF content increased by 10.38% in the exercise group ($p = 0.70$), and declined by 17.52% in the non-exercising controls ($p = 0.64$). Despite the negligible increase in BDNF content in the exercise group over the full 12 weeks of the intervention period, between mid and post time-points in the exercise group, plasma BDNF concentration increased by a much greater margin of 41.58% ($p = 0.12$, SMD = 0.23).

Cannabis and tobacco consumption

Subgroup analysis was undertaken in the exercise intervention group in participants who self-identified as regular cannabis smokers ($n = 4$), compared with non-smokers ($n = 3$). TBARS concentration decreased 1.30% in non-smokers, but increased 37.76% in smokers, and isoprostane concentration increased 5.22% in non-smokers compared to 17.72% in smokers. Protein carbonylation decreased 31.9% in non-smokers and decreased 5.84% in smokers. These effects may have been fortified by tobacco consumption, since all cannabis smokers were also tobacco smokers, and only one non-smoker consumed tobacco only. In an analysis of baseline values for tobacco smokers vs. non-smokers, there was no difference in any of the biomarkers assessed.

Intensity minutes and markers of inflammation

The potential effect of quantity of exercise (intensity minutes) on markers of inflammation was assessed (Fig. 3). 'Intensity minutes', measured by the Garmin

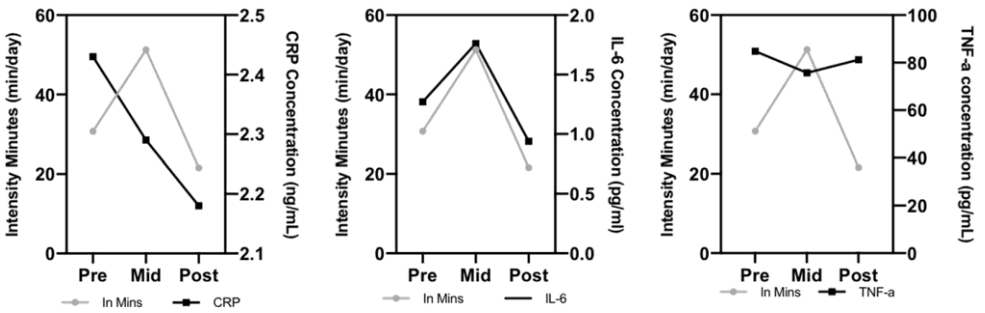

Fig. 3 Garmin device measured 'intensity minutes', alongside markers of chronic inflammation CRP, IL-6 and TNF-α. The grey points represent 'intensity minutes', as derived from the Garmin devices, and the black points respective inflammatory cytokine concnetrations at pre-, mid- and post-intervention time-points.

Table 2 Summary of bout distribution and HR data for each separate activity type.

	Exercise bout modality							
	Swim	**Run**	**Cycle**	**Tennis**	**Football**	**Squash**	**Badminton**	**Circuits**
% Total sessions	18.3	15.3	3.5	14.9	16.8	4.5	5.9	20.8
Average HR (BPM)	117.5	136.3	137	125.5	139.4	124.5	124.1	121.3
95% CI	111.2–123.8)	(130.2–142.4)	117.5–156.5)	118.2–132.8)	(132–146.7)	(119.2–129.8)	(119.8–128.4)	(118.7–123.8)

HR heart rate, *BPM* beats per minute, *CI* confidence interval.

devices, are representative of the number of minutes of moderate-intensity physical activity that contribute to continuous bouts of activity lasting 10 min or more. CRP, IL-6 and TNF-α concentrations at pre-intervention, mid-point and post intervention were compared with intensity minutes per day. It can be seen in Fig. 3 that as intensity minutes increased from baseline to mid-intervention, resting IL-6 concentration also increased. Levels then fell as activity levels decreased post intervention. TNF-α plasma concentration decreased in response to increased activity, and increased once again post intervention. CRP decreased throughout the intervention period and was still decreased post intervention.

Retention, exercise quality and psychopathology

Overall retention to the intervention was 68%, with attrition rates of 18% and 45% for the exercise and control groups, respectively. Four members of the exercise group dropped out, one immediately after baseline assessment, two during the first week of exercise and one after 10 weeks of intervention duration. Three members of the control group dropped out just before the 13-week post assessment was due. Attendance to the exercise sessions was 83% for the target two training sessions per week, and 41% for three sessions per week. The average duration of each exercise session was 37.74 min, and the mean number of sessions attended per week was 2.31, above the minimum target for the intervention. Exercise session data are summarised in Table 2. In the assessment of habitual activity at baseline, mid-intervention and post intervention, the exercise group demonstrated an increase from 30.7 min of activity per day to 51.3 during the intervention. This fell significantly to 21.6 min/day in the week following the intervention ($p = 0.05$). The control groups were similarly active at baseline, with an average of 25.6 min/day, which fell to 7.2 min/day.

There were strong negative correlations between exercise intervention adherence and TBARS ($r = -0.75$, $p = 0.08$) and protein carbonyl concentration ($r = -0.73$, $p = 0.09$). The PANSS interview assessed symptomology across positive, negative and general psychopathology domains. In each of the three domains, exercise either conferred protection against declining scores observed in the control group or resulted in a much greater change between baseline and post-intervention measurement. There was a 1.44% increase in negative score in participants in the exercise group, and a 13.89% increase in participants in the control group (SMD = −0.33). There was a 17.31% decrease in positive symptoms of PANSS in participants in the exercise group, and a 7.83%

decrease in the non-exercising controls (SMD = −0.18). Participants in the exercise group reported a 10.98% decrease in PANSS general score, compared to a 2.82% decrease observed in participants in the control group (SMD = −0.24). These data have been assessed alongside other functional measures that better shape the psychotic state, and the effects of the exercise intervention in Fisher et al.[45].

Discussion

This exploratory study demonstrated that 12 weeks of regular exercise training may be sufficient to elicit positive changes in biomarkers of redox status and inflammation in a cohort of young men with FEP. The study highlighted potential differences in the response of GSH to training in blood and brain. Although some results were not significant when analysing individual outcomes (due to small participant numbers), when taken together, the battery of outcomes suggest a shift in redox and inflammatory status. In many cases, exercise appeared to be protective of the negative changes that were observed in the control group, which are likely a result of disease status: either redox-linked disease progression, treatment or the impact the disease has on lifestyle factors. These are all representative indicators of poorer long-term outcomes for the patient.

Brain GSH vs. peripheral GSH

The data presented suggest that blood and brain GSH responded differently to exercise. Exercise caused increased blood GSH, which is a common response[32]; however, no detectable change was seen in brain GSH in this study. There was no correlation between changes in blood and brain GSH, for either study group. It should be noted that the number of participants able to be scanned was less than those that were able to give a blood sample, and as such assessment of brain GSH was only possible in a subset of an already small sample of FEP patients. However, Rai et al.[59] also reported differences in blood and brain GSH response to exercise. The BBB is impermeable to peripheral GSH and as such it is probable that the two pools of GSH are regulated separately, protecting the brain from peripheral chemical changes that could produce toxic effects to brain function and integrity[60]. The present study is the first to assess brain and blood GSH in response to exercise in a psychosis population.

In addition to the discordant regulation of GSH in brain and blood, there are a number of methodological considerations that are worthy of note. GSH determination by MRS does not discriminate between glial, neuronal or extracellular sources of GSH[61], and since GSH concentration in astrocytes is 10 times that of neurons, differential neuronal regulation may be masked. The complexity of FEP, as with most mental illnesses, means that brain GSH has not been identified as having a causal role in disease pathogenesis, but rather perturbed GSH metabolism[26] alongside other stress-related markers and antioxidants are believed to create the FEP phenotype. In the present study, there was most variation in brain GSH in the exercise group, and given that exercise training can cause changes in GSH, it may be appropriate to suggest that exercise may have indeed perturbed brain GSH, but participant number and confounding factors such as diet, smoking status and medication made it impossible to draw any conclusions from these data. From an exploratory standpoint, the data presented around GSH regulation warrants further investigation, given its critical role in health.

Inflammation and redox status

No exercise intervention studies have yet assessed the effects of training on markers of inflammation in an FEP population. Exercise resulted in a positive change in many of the markers of redox status and inflammation. For example, CRP concentration reduced throughout the study in response to exercise (−10.29%) compared to an increase (12.05%) in the control group. This response is highlighted in Fig. 3, where exercise intensity minutes increased across the study, and CRP concentration fell. In a schizophrenia population, a 10-week intervention of high-intensity exercise resulted in a 66% decrease in plasma CRP[62], matching the results shown in the present study. Other studies that have assessed CRP in psychotic disorders reported no change after exercise training[63,64]. IL-6 has been described as a myokine, which can act as a regulator of exercise-induced metabolic changes. IL-6 release is elevated during dynamic exercise[65] and thus explains the observed increased plasma IL-6 concentration observed at mid-point during this intervention. A review paper by Gómez-Rubio et al.[66] summarised that in schizophrenia, regular exercise leads to a reduction in disease-associated IL-6 elevation, as was observed at the post-intervention measure in the present study.

Exercise training conferred a protective effect for a number of blood biomarkers, including GSH, BDNF and TBARS. All of these measures demonstrated greater detrimental change in the control group, which may be associated with disease progression[67–69]. Increased TBARS, as a measure of lipid peroxidation, is thought to predate presentation of psychotic symptoms, in a comparison of unmedicated FEP patients, chronic schizophrenia patients and healthy controls[17]. In that study, the concentration of TBARS was greater in both patient groups, and greatest in the chronic schizophrenic population. This is in line with the results of the present study, which suggest that there is a disease course-dependent increase in lipid markers of free-radical-mediated damage, over time. BDNF, a regulator of neuronal health and synaptic plasticity, can be

depleted in FEP, via an inflammatory-mediated pathway[70]. Increased circulating proinflammatory cytokine concentration, demonstrated in this clinical group, down-regulates BDNF expression[71]. The current study observed a variety of responses to exercise in different inflammatory markers, which partially act to influence BDNF expression downstream. Most importantly, the protective effect of exercise was demonstrated as preventing the BDNF decline observed in the control group. BDNF concentration increased between the mid-intervention and post-intervention time-points, with a 22.03% decrease between baseline and mid-intervention, suggesting a lag-period between exercise initiation and the beginning of an adaptive response to training in this marker. Peripheral BDNF measures are used as a proxy for brain BDNF, since it is able to cross the highly restrictive BBB. Measurement in the plasma is representative of free BDNF that crosses the BBB, and a strong reflection of the influence of an intervention in the brain, rather than a serum measure that takes into account platelet secreted BDNF upon activation[72]. This study brings together many of the linked biochemical perturbations observed in FEP, and highlights the potential of exercise training to normalise indices of antioxidant depletion, oxidative damage, inflammation and neuronal function.

Cannabis and tobacco interaction with redox markers

Regular use of cannabis in mental illness is far greater than the general population (6.6%)[73], particularly in psychosis populations (23%)[74]. When data were assessed by subgroup in the current study, the positive effects of exercise that were seen in non-smokers were not observed in the subgroup of smokers. In a study assessing the redox effects of regular cannabis consumption in healthy young people, there was no difference in markers of lipid peroxidation or protein carbonylation between smokers and non-smokers[75]. However, there are many studies that show the negative effects of smoking on markers of redox status[76]. It is possible that changes in redox status as a result of cannabis smoking, followed by increases in markers of oxidative damage do contribute to the pathology of psychotic disease; however, more investigation of the specific effects of cannabis use on redox status in FEP is needed to draw any conclusions. Exercise did not confer complete protection for markers of free-radical medicated damage in the current FEP group. In addition, regular tobacco consumption was prevalent among the participants of this study (70% smokers, 30% non-smokers). Although the groups were too small to allow further analysis of the effects of tobacco smoking on adaptation to exercise, the protective effect of regular training on indices of oxidative stress in smokers has been highlighted. One 12-week intervention demonstrated significant improvements in total antioxidant capacity, GSH peroxidase and GSH reductase enzymes, alongside a reduction in lipid peroxidation products in smokers only, with no adaptive change in a non-smoking control group[77]. Although baseline oxidative stress is normally higher in smokers[78,79], the benefits of an exercise intervention
are particularly salient in this FEP population, given the high percentage of the population who habitually smoke tobacco.

The impact of exercise on symptoms of psychosis

The changes in biomarker described herein were accompanied by a positive change in symptom profile as a result of exercise. The observed changes in symptoms of FEP indicate that the current intervention design was sufficient to elicit beneficial changes in functional status (PANSS symptomology), in addition to changes in biomarkers of redox status and inflammation as a result of exercise training. This is in concordance with the current literature; exercise has been shown to be superior to control conditions in improving indices of symptomology, quality of life and depressive symptoms[27]. Additionally, another meta-analysis showed the value of 90 min of moderate-intensity exercise per week (below the World Health Organisation recommendation of 150 min moderate–vigorous-intensity exercise per week) in improving psychiatric symptoms[80].

Limitations

The principle limitation in this study was a small sample size, rendering many of our time-point and inter-marker comparisons statistically insignificant. However, the current study did observe a measurable change in redox status and clinical outcome measures. Not all participants were able to be assessed for brain GSH do to ineligibility or inability to enter the brain scanner. The main barrier to scanning was anxiety of participants to be scanned, but there were some incidences of metal compounds in the body, or injury preventing prolonged time in the supine position. Lastly, despite the opportunity to undertake a variety of indoor-based activities, poor and unpredictable weather in the United Kingdom was a factor in participants' willingness to engaging in the intervention. This effect was particularly salient during the winter months. Confounding factors such as adherence to the intervention, body mass index and cannabis consumption may have affected the outcomes of this exercise intervention, together with potential contributions from antipsychotic medication and duration of illness.

Conclusion

This study, in male patients with FEP, highlighted the beneficial effects of exercise training on markers of redox homeostasis. The study showed that 12 weeks of moderate-intensity exercise training was sufficient to

reduce markers of lipid oxidation and increase antioxidant status. In many of the markers assessed in this study, exercise was able to arrest the changes that were seen in the control group, which would usually be associated with FEP. Future studies should use these data to explore more definitive outcomes, given the feasibility of such an intervention, and the observed change in markers that describe several aspects of disease pathology.

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University of Birmingham - School of Sport & Exercise Sciences

General Health Questionnaire

All the information provided in this form is voluntary

Date:		**Occupation:**	
First Name:		**Email:**	
Middle Name:		**Home Phone:**	
Last Name:		**Mobile:**	
Address:			
		DOB:	
		Weight:	
		Height:	
Age:		**Gender:**	M / F

Medication (include prescribed and over the counter drugs/oral contraceptives/dietary supplements):

Names/Dosage/How often taken?

Allergies:

Smoking history:

Do you smoke?	Y / N	Cigarettes?	Y / N	Pipe/Cigar?	Y / N	Other?	Y / N

If you quit, what year did you quit?

packs smoked per day? For how many years?

Alcohol consumption history:

Do you currently drink alcohol? Y / N

If you drank alcohol previously, when did you stop?

If you ever did drink alcohol, what is (was) the volume consumed?

units /day for # of years

General Health Questionnaire - page 2		
Exercise history:		
Do you currently exercise?	Y / N	
How many years?		Duration:
Types of exercise:		Frequency:
Do you compete in endurance events?		
How many years?		
What events?		Frequency:
Are you curently sedentary, when did you last exercise?		
How many years?		Duration:
Types of exercise:		Frequency:

Medical history:		
NO	**YES**	**Please explain any yes answers.**
		high blood pressure
		chest pain / history of heart attack
		extra heart beats or racing
		abnormal electrocardiogram (ECG)
		other heart trouble (e.g. murmur, valve problems)
		high cholesterol
		diabetes
		seizures
		stroke
		fainting spells
		anxiety (diagnosed)
		depression (diagnosed)
		recurrent fatigue
		insomnia
		thyroid problems
		difficulty breathing
		emphysema/ asthma/ chronic bronchitis
		tuberculosis
		chronic infection
		stomach/ GI problems
		hepatitis
		blood or bleeding disorder (e.g. anaemia)
		kidney/ urinary disorder
		joint injuries/ joint pain
		arthritis (rheumatiod or osteoarthritis)
		autoimmune condition (e.g. lupus)
		migraine headache
		malignant disease (e.g. cancer)
		infectious blood disease (e.g. hepatitis)
		vision problems (exclude corrected near/far sightedness)
		surgical procedures

I have completed the questionnaire to the best of my knowledge and any questions I had have been answered to my full satisfaction.

Signature _____ **Date** _____

#	Item	1	2	3	4	5	6	7	Score
1	I enjoy it	○	○	○	○	○	○	○	I hate it
2	I feel bored	○	○	○	○	○	○	○	I feel interested
3	I dislike it	○	○	○	○	○	○	○	I like it
4	I find it pleasurable	○	○	○	○	○	○	○	I find it unpleasurable
5	I am very absorbed in this activity	○	○	○	○	○	○	○	I am not at all absorbed in this activity
6	It is not fun at all	○	○	○	○	○	○	○	It's a lot of fun
7	I find it energising	○	○	○	○	○	○	○	I find it tiring
8	It makes me depressed	○	○	○	○	○	○	○	It makes me happy
9	It is very pleasant	○	○	○	○	○	○	○	It is very unpleasant
10	I feel good physically while doing it	○	○	○	○	○	○	○	I feel bad physically while doing it
11	It is very invigorating	○	○	○	○	○	○	○	It is not at all invigorating
12	I am very frustrated by it	○	○	○	○	○	○	○	I am not at all frustrated by it
13	It is very gratifying	○	○	○	○	○	○	○	It is not at all gratifying
14	It is very exhilarating	○	○	○	○	○	○	○	It is not at all exhilarating
15	It is not at all stimulating	○	○	○	○	○	○	○	It is very stimulating
16	It gives me a strong sense of accomplishment	○	○	○	○	○	○	○	It does not give me any sense of accomplishment
17	It is very refreshing	○	○	○	○	○	○	○	It is not at all refreshing
18	I would rather be doing something else	○	○	○	○	○	○	○	There is nothing else I would rather be doing

FEPEX RG_17-195 IRAS: 234178

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

   ☐ Yes

   ☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

   _____ **days per week**

   ☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

   _____ **hours per day**
   _____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

   _____ **days per week**

   ☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**
_____ **minutes per day**

This is the end of the questionnaire, thank you for participating.

WHODAS 2.0

WORLD HEALTH ORGANIZATION
DISABILITY ASSESSMENT SCHEDULE 2.0

36-item version, self-administered

This questionnaire asks about difficulties due to health conditions. Health conditions include diseases or illnesses, other health problems that may be short or long lasting, injuries, mental or emotional problems, and problems with alcohol or drugs.

Think back over the past 30 days and answer these questions, thinking about how much difficulty you had doing the following activities. For each question, please circle only one response.

In the past 30 days, how much difficulty did you have in:						
Understanding and communicating						
D1.1	Concentrating on doing something for ten minutes?	None	Mild	Moderate	Severe	Extreme or cannot do
D1.2	Remembering to do important things?	None	Mild	Moderate	Severe	Extreme or cannot do
D1.3	Analysing and finding solutions to problems in day-to-day life?	None	Mild	Moderate	Severe	Extreme or cannot do
D1.4	Learning a new task, for example, learning how to get to a new place?	None	Mild	Moderate	Severe	Extreme or cannot do
D1.5	Generally understanding what people say?	None	Mild	Moderate	Severe	Extreme or cannot do
D1.6	Starting and maintaining a conversation?	None	Mild	Moderate	Severe	Extreme or cannot do
Getting around						
D2.1	Standing for long periods such as 30 minutes?	None	Mild	Moderate	Severe	Extreme or cannot do
D2.2	Standing up from sitting down?	None	Mild	Moderate	Severe	Extreme or cannot do
D2.3	Moving around inside your home?	None	Mild	Moderate	Severe	Extreme or cannot do
D2.4	Getting out of your home?	None	Mild	Moderate	Severe	Extreme or cannot do
D2.5	Walking a long distance such as a kilometre [or equivalent]?	None	Mild	Moderate	Severe	Extreme or cannot do

Please continue to next page ...

WHODAS 2.0

WORLD HEALTH ORGANIZATION
DISABILITY ASSESSMENT SCHEDULE 2.0

36
Self

In the past 30 days, how much difficulty did you have in:						
Self-care						
D3.1	Washing your whole body?	None	Mild	Moderate	Severe	Extreme or cannot do
D3.2	Getting dressed?	None	Mild	Moderate	Severe	Extreme or cannot do
D3.3	Eating?	None	Mild	Moderate	Severe	Extreme or cannot do
D3.4	Staying by yourself for a few days?	None	Mild	Moderate	Severe	Extreme or cannot do
Getting along with people						
D4.1	Dealing with people you do not know?	None	Mild	Moderate	Severe	Extreme or cannot do
D4.2	Maintaining a friendship?	None	Mild	Moderate	Severe	Extreme or cannot do
D4.3	Getting along with people who are close to you?	None	Mild	Moderate	Severe	Extreme or cannot do
D4.4	Making new friends?	None	Mild	Moderate	Severe	Extreme or cannot do
D4.5	Sexual activities?	None	Mild	Moderate	Severe	Extreme or cannot do
Life activities						
D5.1	Taking care of your household responsibilities?	None	Mild	Moderate	Severe	Extreme or cannot do
D5.2	Doing most important household tasks well?	None	Mild	Moderate	Severe	Extreme or cannot do
D5.3	Getting all the household work done that you needed to do?	None	Mild	Moderate	Severe	Extreme or cannot do
D5.4	Getting your household work done as quickly as needed?	None	Mild	Moderate	Severe	Extreme or cannot do

Please continue to next page ...

WHODAS 2.0

WORLD HEALTH ORGANIZATION
DISABILITY ASSESSMENT SCHEDULE 2.0

36
Self

If you work (paid, non-paid, self-employed) or go to school, complete questions D5.5–D5.8, below. Otherwise, skip to D6.1.

Because of your health condition, in the past 30 days, how much difficulty did you have in:						
D5.5	Your day-to-day work/school?	None	Mild	Moderate	Severe	Extreme or cannot do
D5.6	Doing your most important work/school tasks well?	None	Mild	Moderate	Severe	Extreme or cannot do
D5.7	Getting all the work done that you need to do?	None	Mild	Moderate	Severe	Extreme or cannot do
D5.8	Getting your work done as quickly as needed?	None	Mild	Moderate	Severe	Extreme or cannot do

Participation in society						
In the past 30 days:						
D6.1	How much of a problem did you have in joining in community activities (for example, festivities, religious or other activities) in the same way as anyone else can?	None	Mild	Moderate	Severe	Extreme or cannot do
D6.2	How much of a problem did you have because of barriers or hindrances in the world around you?	None	Mild	Moderate	Severe	Extreme or cannot do
D6.3	How much of a problem did you have living with dignity because of the attitudes and actions of others?	None	Mild	Moderate	Severe	Extreme or cannot do
D6.4	How much time did you spend on your health condition, or its consequences?	None	Mild	Moderate	Severe	Extreme or cannot do
D6.5	How much have you been emotionally affected by your health condition?	None	Mild	Moderate	Severe	Extreme or cannot do
D6.6	How much has your health been a drain on the financial resources of you or your family?	None	Mild	Moderate	Severe	Extreme or cannot do
D6.7	How much of a problem did your family have because of your health problems?	None	Mild	Moderate	Severe	Extreme or cannot do
D6.8	How much of a problem did you have in doing things by yourself for relaxation or pleasure?	None	Mild	Moderate	Severe	Extreme or cannot do

Please continue to next page ...

WHODAS 2.0

WORLD HEALTH ORGANIZATION
DISABILITY ASSESSMENT SCHEDULE 2.0

36
Self

H1	Overall, in the past 30 days, how many days were these difficulties present?	***Record number of days ____***
H2	In the past 30 days, for how many days were you totally unable to carry out your usual activities or work because of any health condition?	***Record number of days ____***
H3	In the past 30 days, not counting the days that you were totally unable, for how many days did you cut back or reduce your usual activities or work because of any health condition?	***Record number of days ____***

This completes the questionnaire. Thank you.

WHO/MSA/MNH/PSF/97.4
English only
Distr.: Limited

WHOQOL-BREF

PROGRAMME ON MENTAL HEALTH
WORLD HEALTH ORGANIZATION
GENEVA

For office use only

	Equations for computing domain scores	Raw score	Transformed scores*	
Domain 1	(6-Q3) + (6-Q4) + Q10 + Q15 + Q16 + Q17 + Q18 ☐ + ☐ + ☐ + ☐ + ☐ + ☐ + ☐	=	**4-20**	**0-100**
Domain 2	Q5 + Q6 + Q7 + Q11 + Q19 + (6-Q26) ☐ + ☐ + ☐ + ☐ + ☐ + ☐	=		
Domain 3	Q20 + Q21 + Q22 ☐ + ☐ + ☐	=		
Domain 4	Q8 + Q9 + Q12 + Q13 + Q14 + Q23 + Q24 + Q25 ☐ + ☐ + ☐ + ☐ + ☐ + ☐ + ☐ + ☐	=		

* Please see Table 4 on page 10 of the manual, for converting raw scores to transformed scores.

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I.D. number | | | | |

ABOUT YOU

Before you begin we would like to ask you to answer a few general questions about yourself: by circling the correct answer or by filling in the space provided.

What is your **gender**? Male Female

What is you **date of birth**? _____ / _____ / _____
Day / Month / Year

What is the highest **education** you received?
None at all
Primary school
Secondary school
Tertiary

What is your **marital status**?
Single Separated
Married Divorced
Living as married Widowed

Are you currently **ill**? Yes No

If something is wrong with your health what do you think it is?_____illness/ problem

Instructions

This assessment asks how you feel about your quality of life, health, or other areas of your life. **Please answer all the questions.** If you are unsure about which response to give to a question, **please choose the one** that appears most appropriate. This can often be your first response.

Please keep in mind your standards, hopes, pleasures and concerns. We ask that you think about your life **in the last two weeks.** For example, thinking about the last two weeks, a question might ask:

		Not at all	Not much	Moderately	A great deal	Completely
	Do you get the kind of support from others that you need?	1	2	3	4	5

You should circle the number that best fits how much support you got from others over the last two weeks. So you would circle the number 4 if you got a great deal of support from others as follows.

		Not at all	Not much	Moderately	A great deal	Completely
	Do you get the kind of support from others that you need?	1	2	3	4	5

You would circle number 1 if you did not get any of the support that you needed from others in the last two weeks.

Please read each question, assess your feelings, and circle the number on the scale for each question that gives the best answer for you.

		Very poor	Poor	Neither poor nor good	Good	Very good
1(G1)	How would you rate your quality of life?	1	2	3	4	5

		Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
2 (G4)	How satisfied are you with your health?	1	2	3	4	5

The following questions ask about **how much** you have experienced certain things in the last two weeks.

		Not at all	A little	A moderate amount	Very much	An extreme amount
3 (F1.4)	To what extent do you feel that physical pain prevents you from doing what you need to do?	1	2	3	4	5
4(F11.3)	How much do you need any medical treatment to function in your daily life?	1	2	3	4	5
5(F4.1)	How much do you enjoy life?	1	2	3	4	5
6(F24.2)	To what extent do you feel your life to be meaningful?	1	2	3	4	5

		Not at all	A little	A moderate amount	Very much	Extremely
7(F5.3)	How well are you able to concentrate?	1	2	3	4	5
8 (F16.1)	How safe do you feel in your daily life?	1	2	3	4	5
9 (F22.1)	How healthy is your physical environment?	1	2	3	4	5

The following questions ask about **how completely** you experience or were able to do certain things in the last two weeks.

		Not at all	A little	Moderately	Mostly	Completely
10 (F2.1)	Do you have enough energy for everyday life?	1	2	3	4	5
11 (F7.1)	Are you able to accept your bodily appearance?	1	2	3	4	5
12 (F18.1)	Have you enough money to meet your needs?	1	2	3	4	5
13 (F20.1)	How available to you is the information that you need in your day-to-day life?	1	2	3	4	5
14 (F21.1)	To what extent do you have the opportunity for leisure activities?	1	2	3	4	5

		Very poor	Poor	Neither	Good	Very good

				poor nor good		
15 (F9.1)	How well are you able to get around?	1	2	3	4	5

The following questions ask you to say how **good or satisfied** you have felt about various aspects of your life over the last two weeks.

		Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
16 (F3.3)	How satisfied are you with your sleep?	1	2	3	4	5
17 (F10.3)	How satisfied are you with your ability to perform your daily living activities?	1	2	3	4	5
18(F12.4)	How satisfied are you with your capacity for work?	1	2	3	4	5
19 (F6.3)	How satisfied are you with yourself?	1	2	3	4	5
20(F13.3)	How satisfied are you with your personal relationships?	1	2	3	4	5
21(F15.3)	How satisfied are you with your sex life?	1	2	3	4	5
22(F14.4)	How satisfied are you with the support you get from your friends?	1	2	3	4	5
23(F17.3)	How satisfied are you with the conditions of your living place?	1	2	3	4	5
24(F19.3)	How satisfied are you with your access to health services?	1	2	3	4	5
25(F23.3)	How satisfied are you with your transport?	1	2	3	4	5

The following question refers to **how often** you have felt or experienced certain things in the last two weeks.

		Never	Seldom	Quite often	Very often	Always
26 (F8.1)	How often do you have negative feelings such as blue mood, despair, anxiety, depression?	1	2	3	4	5

Did someone help you to fill out this form?.....

How long did it take to fill this form out?.....

Do you have any comments about the assessment?

.....

.....

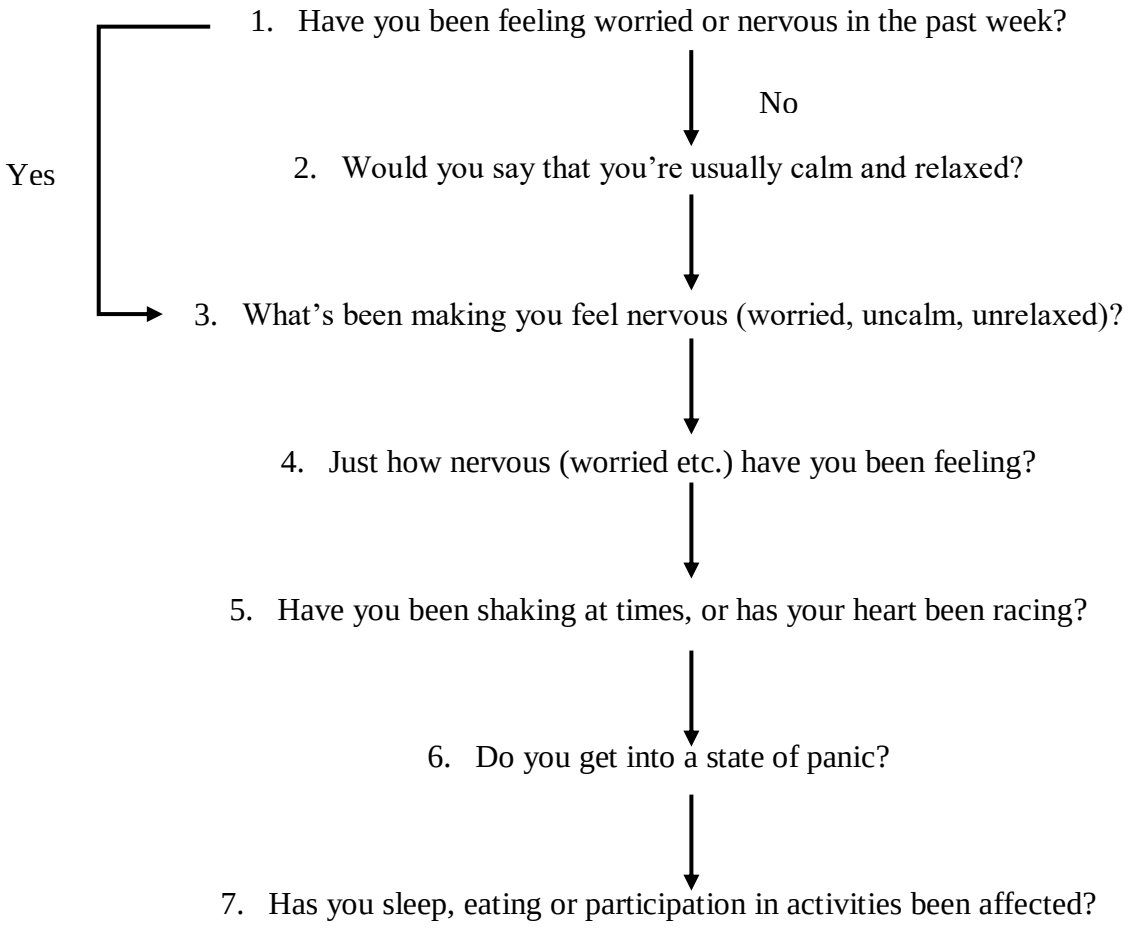
THANK YOU FOR YOUR HELP

Structured clinical interview for the positive and negative syndrome scale (SCI-PANSS)

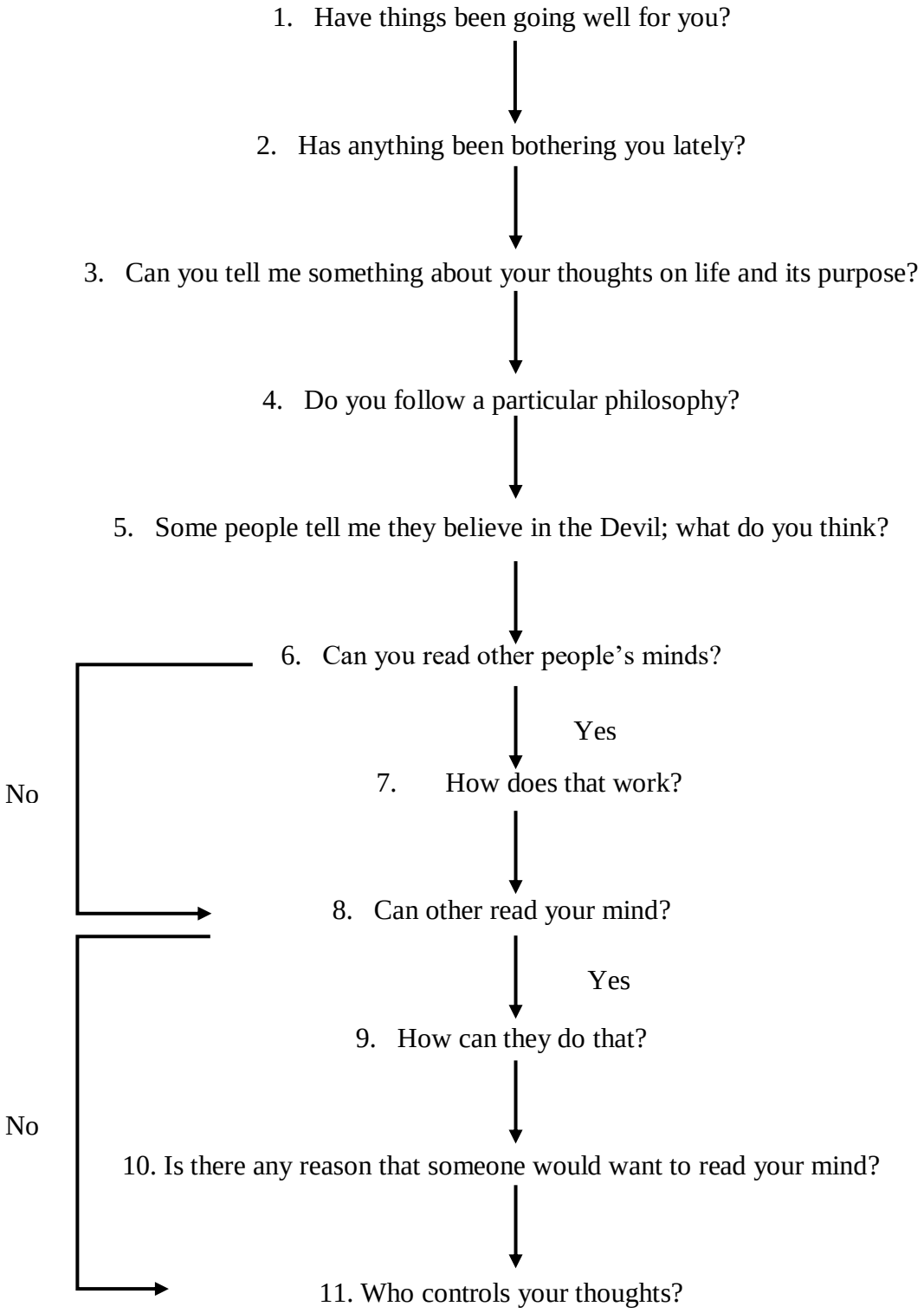
Data on lack of spontaneity, flow of conversation, poor rapport and conceptual disorganisation.

Hi, I'm…..We're going to be spending the next 20 or 30 minutes talking about yourself and the reasons for being here. Maybe you can start by telling be something about yourself and your background? (Allow at least 5 minutes for a non-directive phase serving to establish rapport in the context of an overview before proceeding to the specific questions listed below).

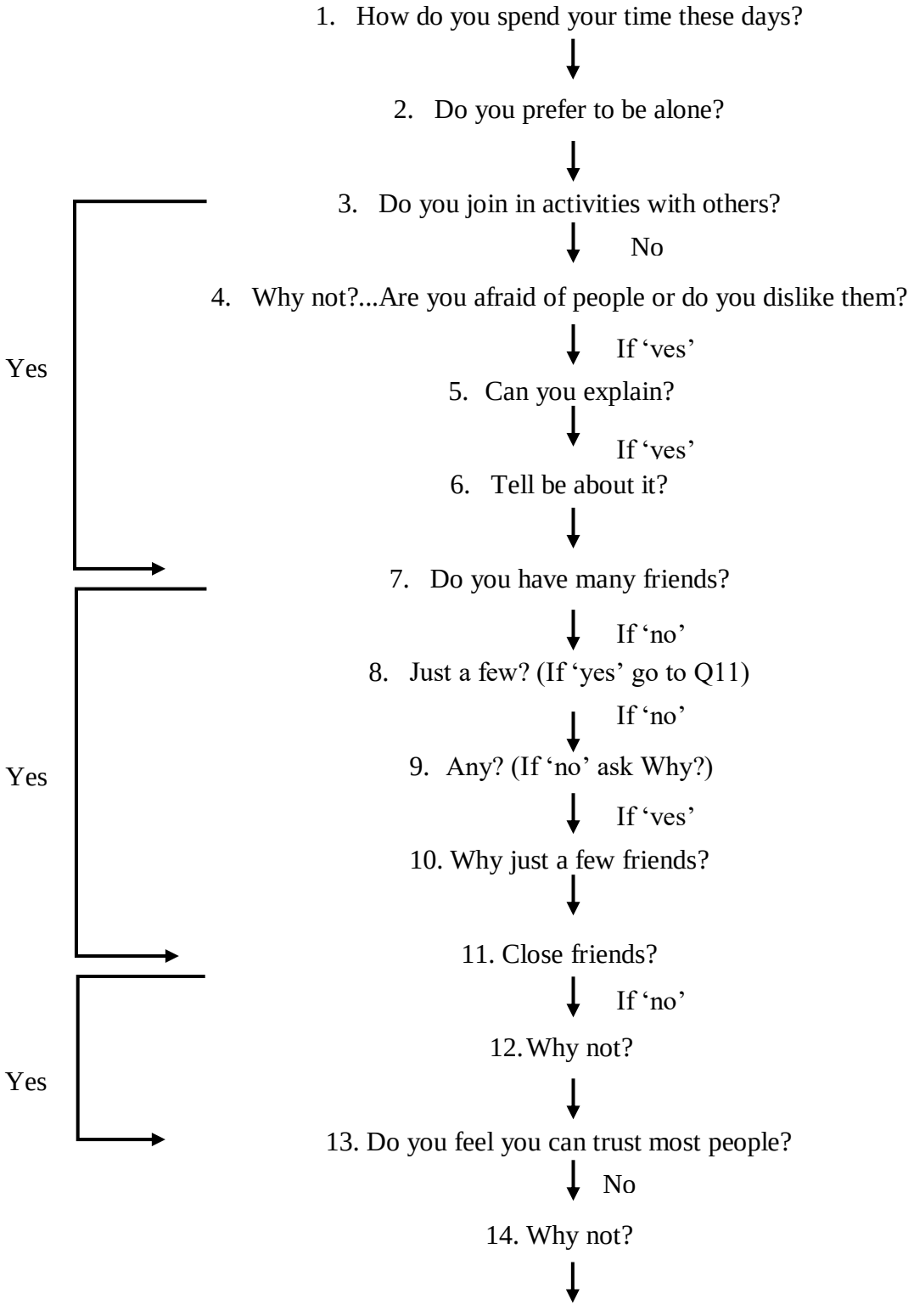
Data on ANXIETY

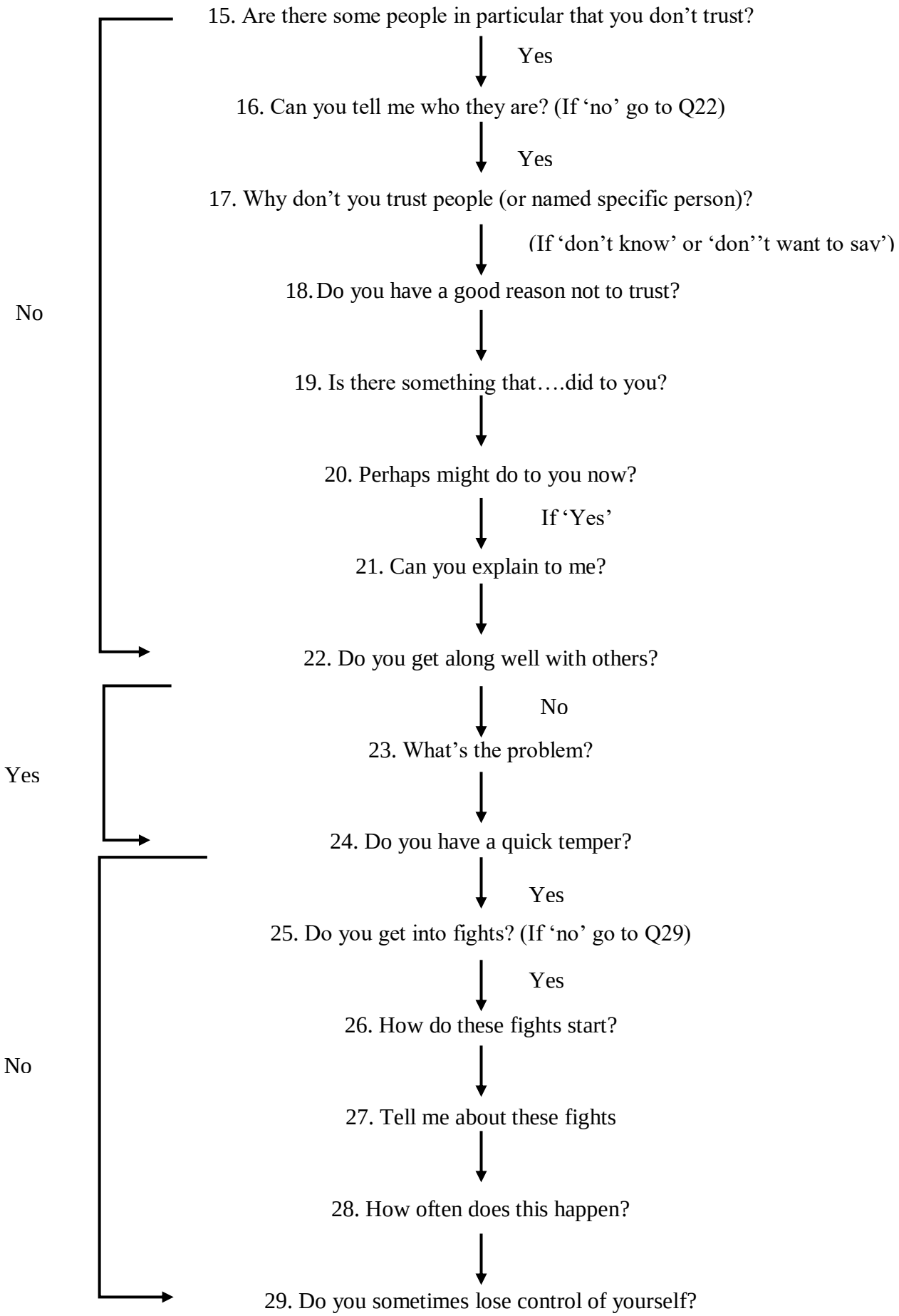


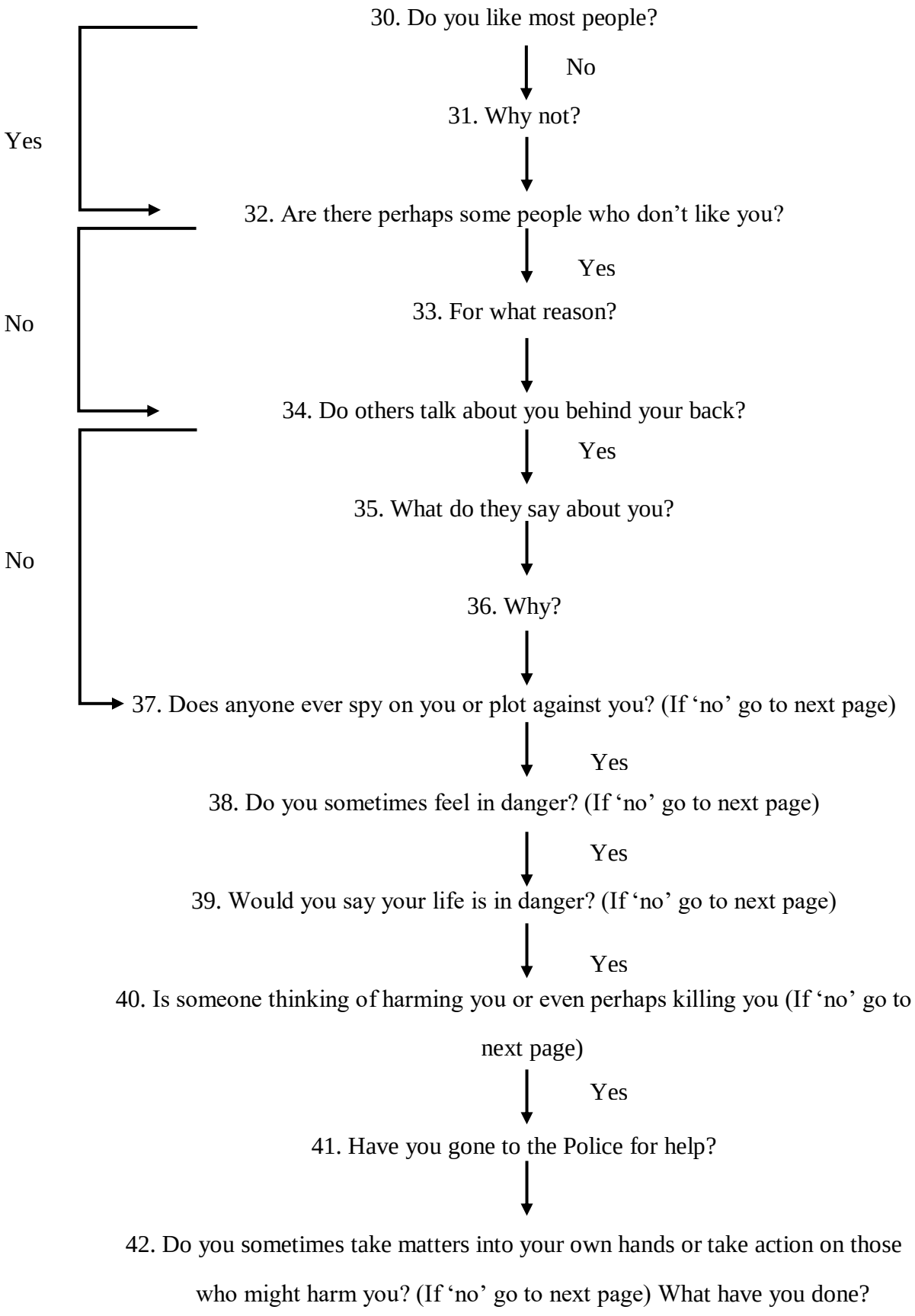
Date on DELUSIONS (GENERAL) and UNUSUAL THOUGH CONTENT.



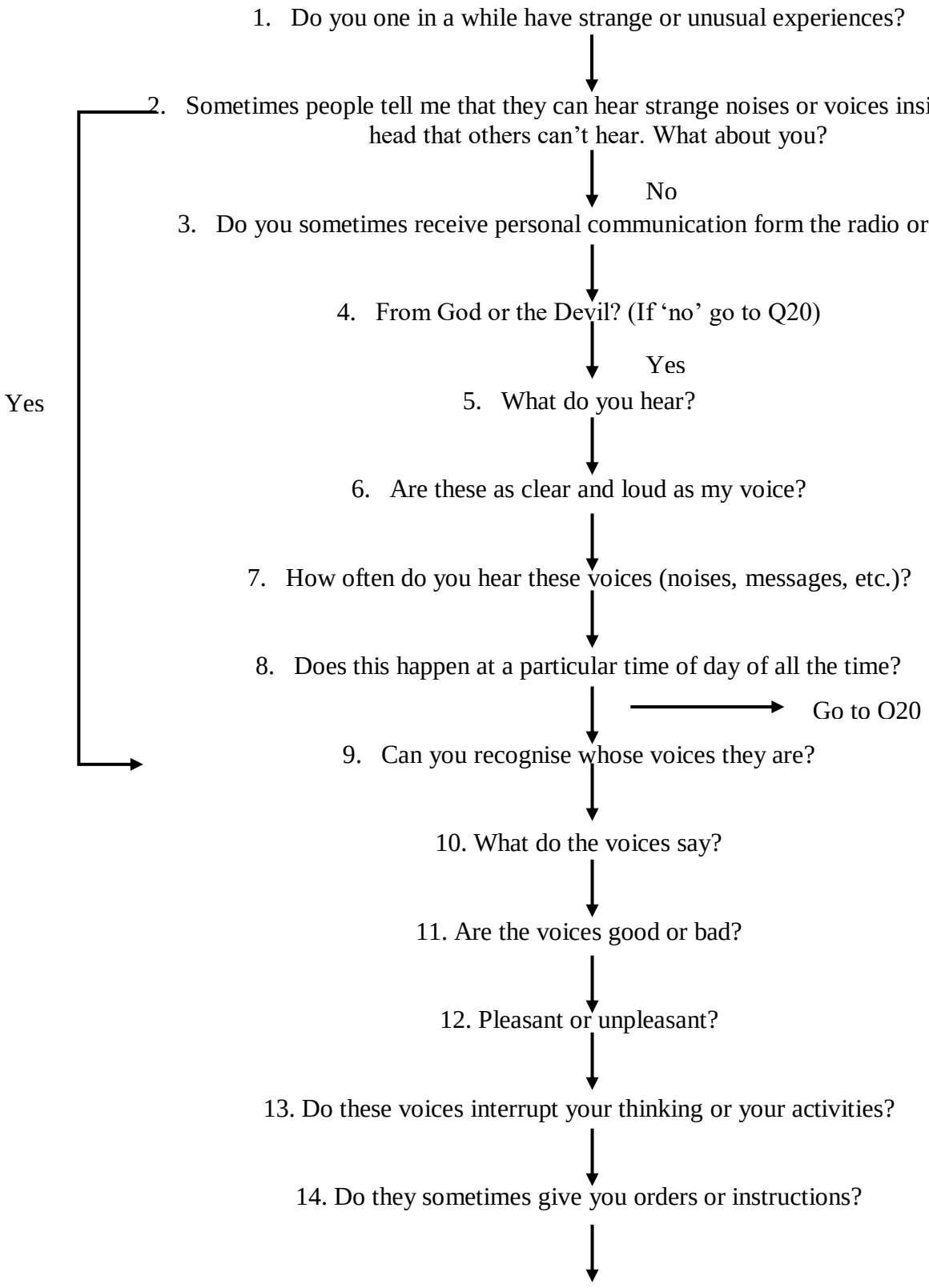
Data on SUSPICIOUSNESS/ PERSECUTION, PASSIVE/APATHETIC SOCIAL WITHDRAWAL, ACTIVE SOCIAL AVOIDANCE and POOR IMPULSE CONTROL.

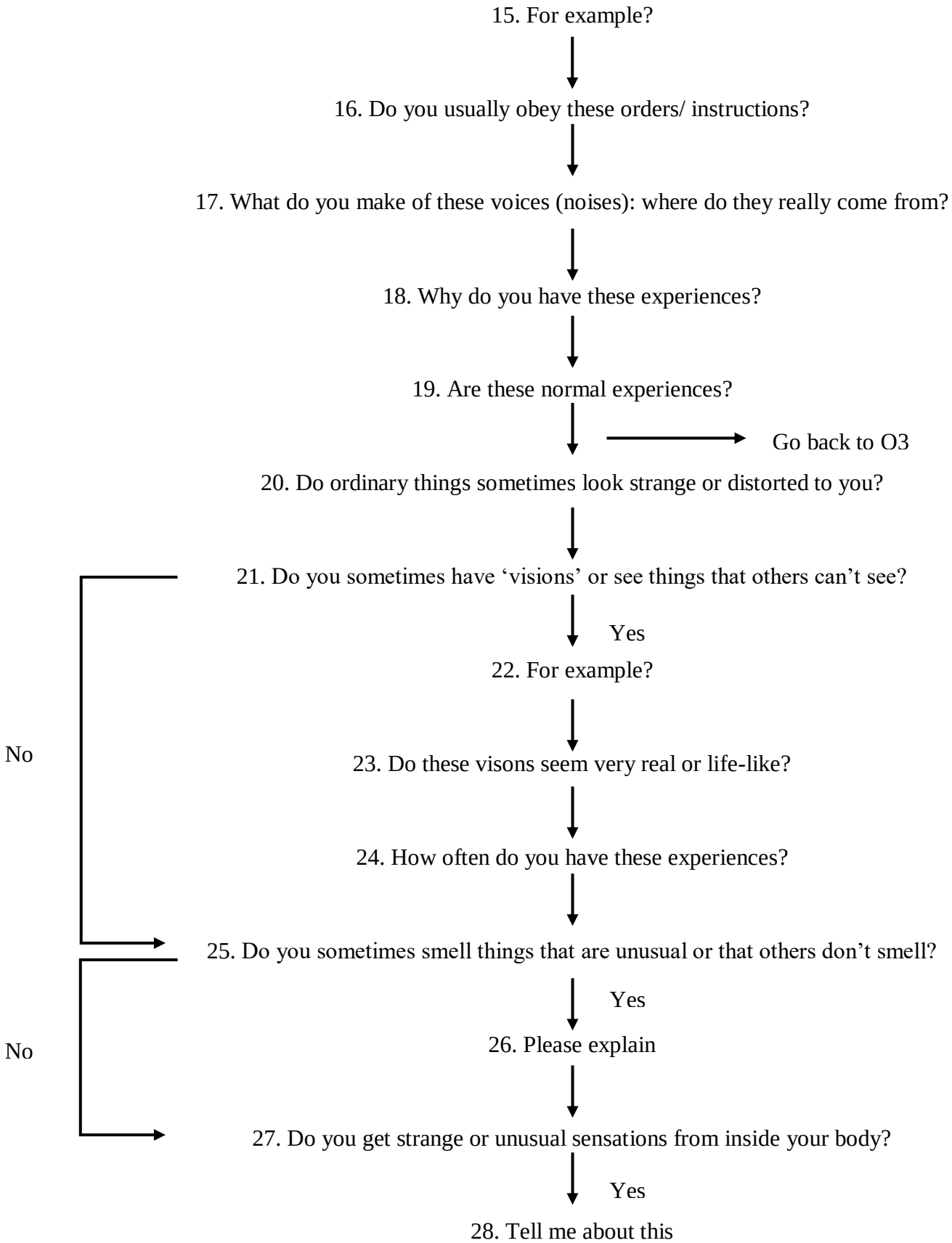



15. Are there some people in particular that you don't trust?
Yes
16. Can you tell me who they are? (If 'no' go to Q22)
Yes
17. Why don't you trust people (or named specific person)?
(If 'don't know' or 'don''t want to say')
18. Do you have a good reason not to trust?
No
19. Is there something that….did to you?
20. Perhaps might do to you now?
If 'Yes'
21. Can you explain to me?
22. Do you get along well with others?
No
23. What's the problem?
Yes
24. Do you have a quick temper?
Yes
25. Do you get into fights? (If 'no' go to Q29)
Yes
26. How do these fights start?
No
27. Tell me about these fights
28. How often does this happen?
29. Do you sometimes lose control of yourself?

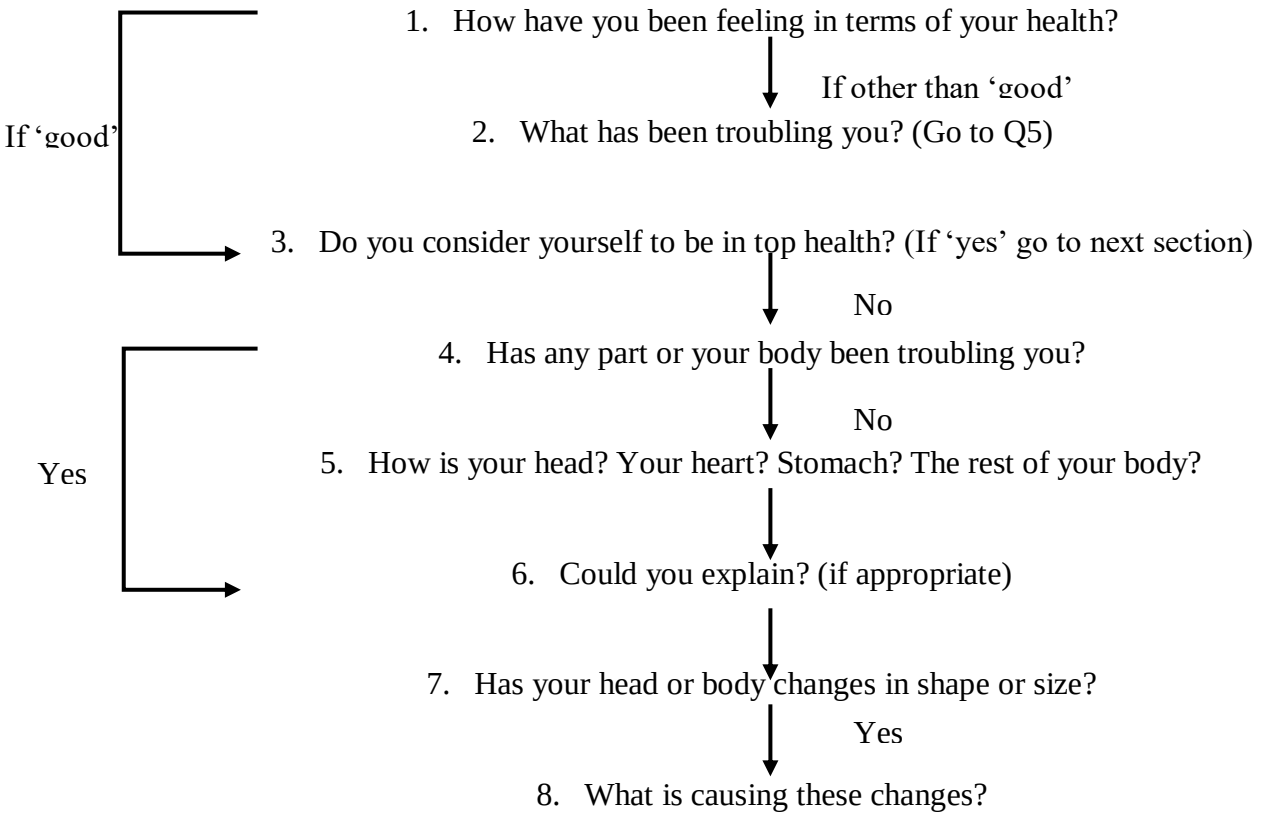

30. Do you like most people?
No
31. Why not?
Yes
32. Are there perhaps some people who don't like you?
Yes
33. For what reason?
No
34. Do others talk about you behind your back?
Yes
35. What do they say about you?
No
36. Why?
37. Does anyone ever spy on you or plot against you? (If 'no' go to next page)
Yes
38. Do you sometimes feel in danger? (If 'no' go to next page)
Yes
39. Would you say your life is in danger? (If 'no' go to next page)
Yes
40. Is someone thinking of harming you or even perhaps killing you (If 'no' go to next page)
Yes
41. Have you gone to the Police for help?
42. Do you sometimes take matters into your own hands or take action on those who might harm you? (If 'no' go to next page) What have you done?

Data on HALLUCINATORY BEHAVIOUR and ASSOCIATED DELUSIONS.

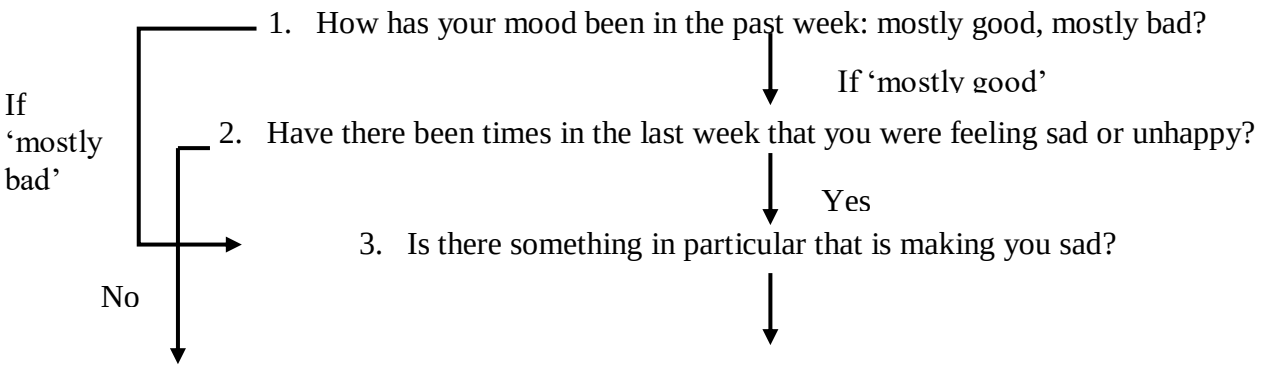



15. For example?
16. Do you usually obey these orders/ instructions?
17. What do you make of these voices (noises): where do they really come from?
18. Why do you have these experiences?
19. Are these normal experiences?
Go back to O3
20. Do ordinary things sometimes look strange or distorted to you?
21. Do you sometimes have 'visions' or see things that others can't see?
Yes
22. For example?
No
23. Do these visons seem very real or life-like?
24. How often do you have these experiences?
25. Do you sometimes smell things that are unusual or that others don't smell?
Yes
No
26. Please explain
27. Do you get strange or unusual sensations from inside your body?
Yes
28. Tell me about this

Data on SOMATIC CONCERN



Data on DEPRESSION.



4. How often do you feel sad?

5. Just how sad have you been feeling?

6. Have you been crying lately?

7. Has your mood in any way affected your sleep?

8. Has it affected your appetite?

9. Do you participate in less activities on account of your mood?

10. Have you had any thoughts of harming yourself?

11. Any thoughts about ending your life?

No

Yes

12. Have you attempted suicide?

Go to next section

Data on GUILT FEELINGS and GRANDIOSITY.

1. If you were to compare yourself to the average person, how would you come out: a little better, maybe a little worse, or about the same?

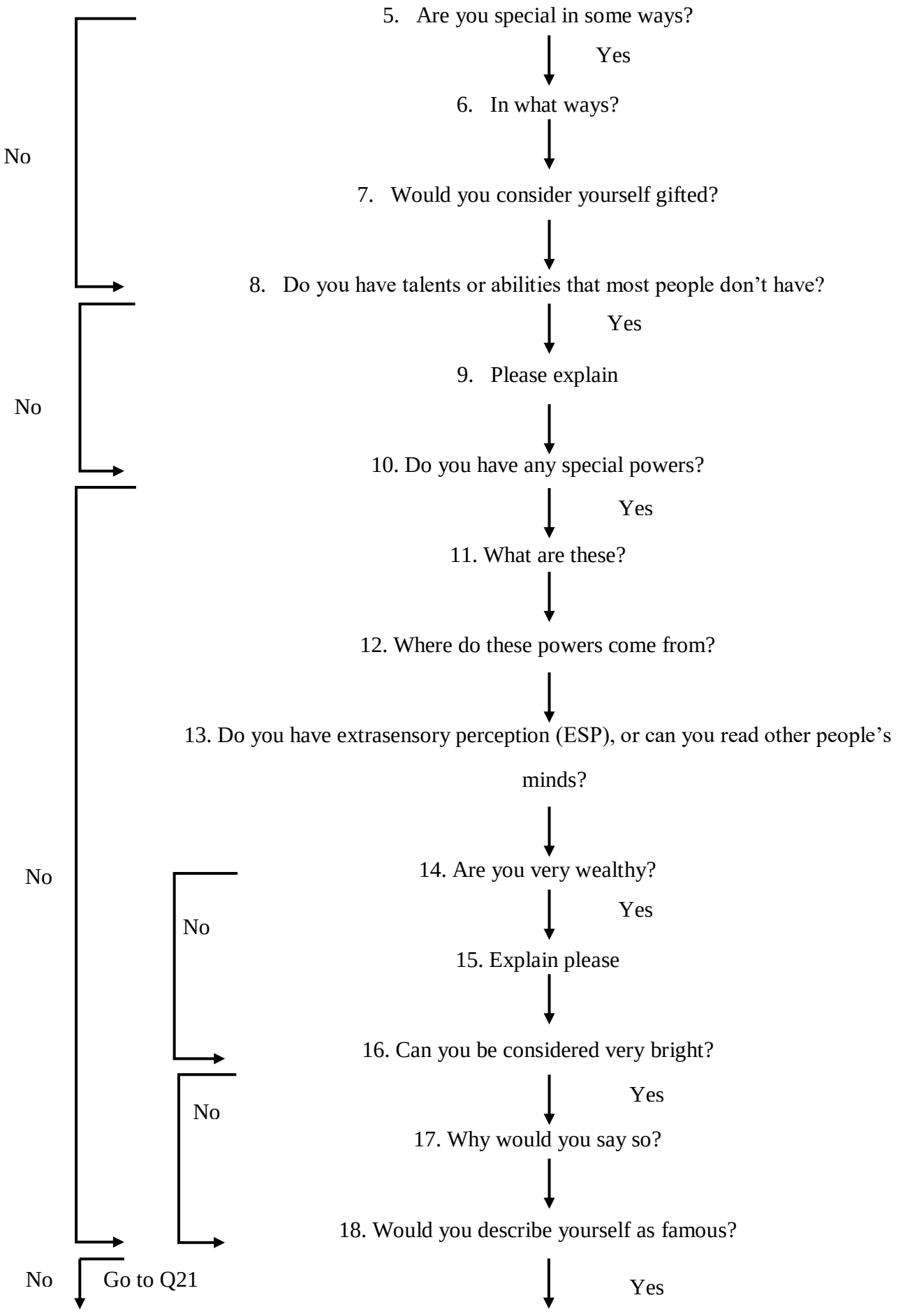
Worse

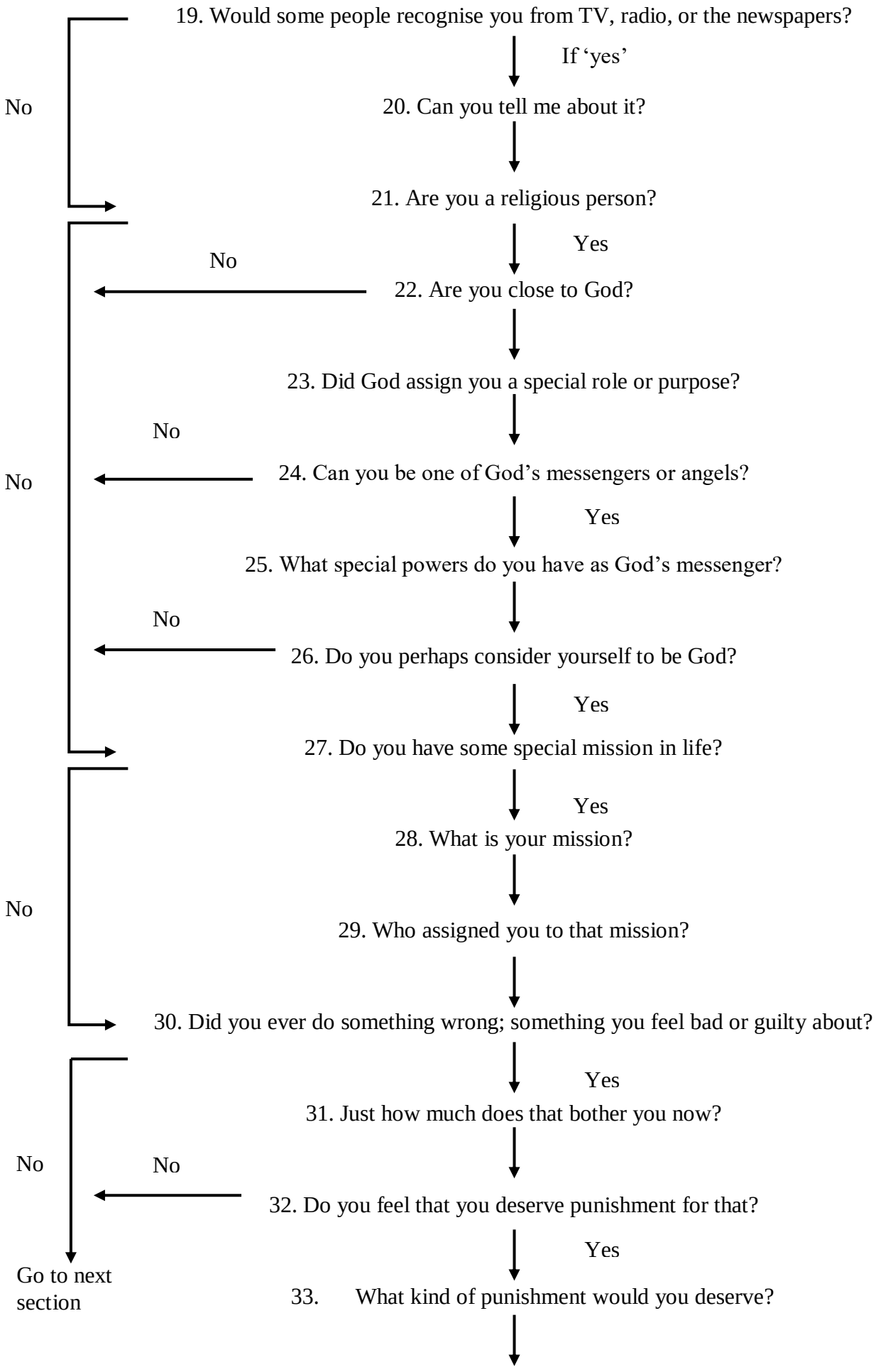
2. Worse in what ways?

Better

3. Just how do you feel about yourself? (Go to Q5)

4. Better in what ways? (Go to Q5)


5. Are you special in some ways?
Yes
6. In what ways?
No
7. Would you consider yourself gifted?
8. Do you have talents or abilities that most people don't have?
Yes
9. Please explain
No
10. Do you have any special powers?
Yes
11. What are these?
12. Where do these powers come from?
13. Do you have extrasensory perception (ESP), or can you read other people's minds?
14. Are you very wealthy?
No
Yes
15. Explain please
No
16. Can you be considered very bright?
Yes
17. Why would you say so?
No
18. Would you describe yourself as famous?
Yes
No
Go to Q21


19. Would some people recognise you from TV, radio, or the newspapers?
If 'yes'
No
20. Can you tell me about it?
21. Are you a religious person?
Yes
No
22. Are you close to God?
23. Did God assign you a special role or purpose?
No
No
24. Can you be one of God's messengers or angels?
Yes
25. What special powers do you have as God's messenger?
No
26. Do you perhaps consider yourself to be God?
Yes
27. Do you have some special mission in life?
Yes
28. What is your mission?
No
29. Who assigned you to that mission?
30. Did you ever do something wrong; something you feel bad or guilty about?
Yes
31. Just how much does that bother you now?
No
No
32. Do you feel that you deserve punishment for that?
Yes
Go to next section
33. What kind of punishment would you deserve?

34. Have you at times thought of punishing yourself?

Yes

35. Have you ever acted upon these thoughts?

Go to next section

Data on DISORIENTATION.

1. Can you tell me today's date (i.e. the day, the month, the year)?
2. What is the name of the place you are in now?
3. (If hospitalised) What ward are you on?
4. (If hospitalised) What is the address of where you now stay?
5. If someone had to reach you by phone, what number would they call?
6. What is the name of the doctor who is treating you now?
7. (If hospitalised) Can you tell me who else is one staff and what they do?
8. Do you know who is now the Prime Minister?
9. Who was the Prime Minister before?
10. Who is the leader of the opposition party?

Data on DIFFICULTY IN ABSTRACT THINKING.

1. I'm going to now say a pair of words, and I'd like you to tell me in what important way they are alike- Let's start for example, with the words….see Appendix A. How are they alike- what do they have in common?

If any other answer — If correct answer

2. Good. Now what about…? (Select the other items from the similarities list at varying levels of difficulty and based upon the interview stage and record the responses. Then go to Q4). (If we use 'apple and banana' as the items) The incorrect answer will be either CONCRETE ('they both have skins' or 'you can eat them', TANGENTIAL ('they're small'), or IDIOSYNCRATIC ('monkeys like them')- record this in the notes section

3. Okay, but they're both…give answer. Now how about…: how are these alike? (Select three other items from the similarities list at varying levels of difficulty and based upon the interview stage and record the responses.

4. You've probably heard the expression…select proverb from Appendix B. What does this really mean?

5. What about when people say….select second proverb from Appendix B. What do they mean?

6. There's a very old saying…see Appendix B for third proverb. What is the deeper meaning of this proverb? (Select 1 other proverb from the final set in Appendix B)

Data on LACK OF JUDGEMENT and INSIGHT.

1. How long have you been in the hospital (clinic etc.)?

2. When did you come to the hospital (clinic etc.)?

Yes, Q5

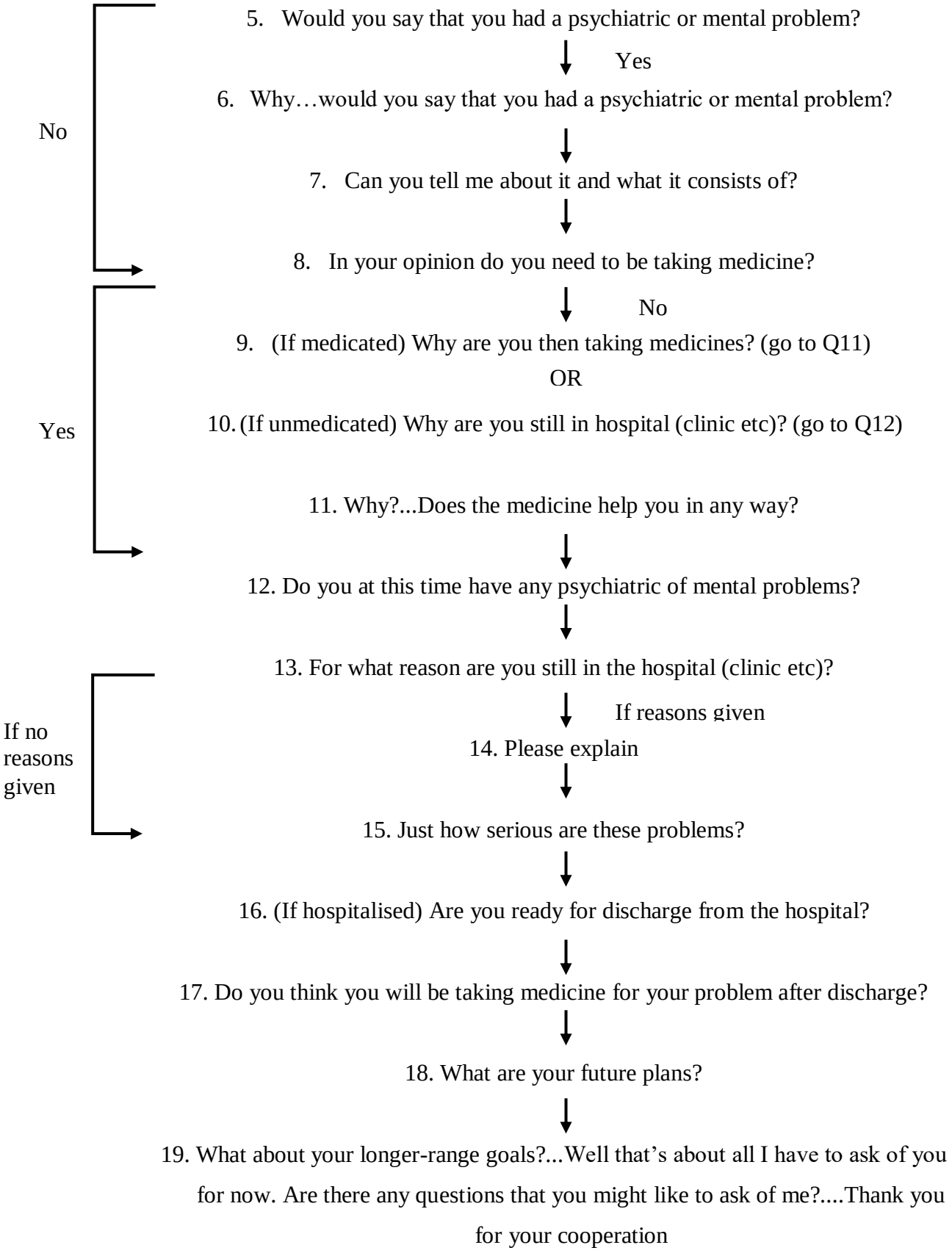
3. Do you need to be in the hospital (clinic etc.)?

No

No, Q8

4. Did you have a problem that needed treatment?

Yes


5. Would you say that you had a psychiatric or mental problem?
Yes
6. Why…would you say that you had a psychiatric or mental problem?
No
7. Can you tell me about it and what it consists of?
8. In your opinion do you need to be taking medicine?
No
9. (If medicated) Why are you then taking medicines? (go to Q11)
OR
Yes
10. (If unmedicated) Why are you still in hospital (clinic etc)? (go to Q12)
11. Why?...Does the medicine help you in any way?
12. Do you at this time have any psychiatric of mental problems?
13. For what reason are you still in the hospital (clinic etc)?
If reasons given
If no reasons given
14. Please explain
15. Just how serious are these problems?
16. (If hospitalised) Are you ready for discharge from the hospital?
17. Do you think you will be taking medicine for your problem after discharge?
18. What are your future plans?
19. What about your longer-range goals?...Well that's about all I have to ask of you for now. Are there any questions that you might like to ask of me?....Thank you for your cooperation

Appendix A: Items for assessing SIMILARITIES in the evaluation of DIFFICULTY IN ABSTRACT THINKING.

2-year assessment Baseline/ 6mnth assessment 1-year/discharge/18mnth assessment Acute ward assessment	1. How are a ball and orange alike? 2. Apple and banana? 3. Pencil and pen? 4. Nickel and dime?
2-year assessment Baseline/ 6mnth assessment 1-year/discharge/18mnth assessment Acute ward assessment	5. Table and chair? 6. Tiger and elephant? 7. Hat and shirt? 8. Bus and train?
2-year assessment Baseline/ 6mnth assessment 1-year/discharge/18mnth assessment Acute ward assessment	9. Arm and leg? 10. Rose and tulip? 11. Uncle and cousin? 12. The sun and the moon?
2-year assessment Baseline/ 6mnth assessment 1-year/discharge/18mnth assessment Acute ward assessment	13. Painting and poem? 14. Hilltop and valley? 15. Air and water? 16. Peace and prosperity?

Appendix B: Items for assessing PROVERB INTERPRETATION in the evaluation of DIFFICULTY IN ABSTRACT THINKING.

2-year assessment Baseline/ 6mnth assessment 1-year/discharge/18mnth assessment Acute ward assessment	1. "Plain as the nose on your face"? 2. "Carrying a chip on your shoulder" 3. "Two heads are better than one" 4. "Too many cooks spoil the broth"
2-year assessment Baseline/ 6mnth assessment 1-year/discharge/18mnth assessment Acute ward assessment	5. "Don't judge a book by its cover" 6. "One man's food is another man's poison" 7. "All that glitters isn't gold" 8. "Don't cross the bridge until you come to it"
2-year assessment Baseline/ 6mnth assessment 1-year/discharge/18mnth assessment Acute ward assessment	9. "What's good for the goose is good for the gander" 10. "The grass always looks greener on the other side" 11. "Don't keep all your eggs in one basket" 12. "One swallow does not make a Summer"
2-year assessment Baseline/ 6mnth assessment 1-year/discharge/18mnth assessment Acute ward assessment	13. "A stitch in time saves nine" 14. "A rolling stone gathers no moss" 15. "The acorn never falls far from the tree" 16. "People who live in glass houses should not throw stones at others"

Patient Information									
Patient Date			Day		Mth.		Year	Time	Hour	Min
Personal notes										

Scoring Procedure

Tick appropriate box for each item

P1. Delusions Beliefs which are unfounded, unrealistic, and idiosyncratic. Basis for rating thought content expressed in the interview and its influence on social relations and behavior.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Presence of one or two delusions which are vaque, uncrystallized, and not tenaciously held. Delusions do not interfere with thinking, social relations, or behavior.	☐
4 Moderate - Presence of either a kaleidoscopic array of poorly formed, unstable delusions or of a few wellformed delusions that occasionally interfere with thinking, social relations, or behavior.	☐
5 Moderate severe - Presence of numerous well-formed delusions that are tenaciously held and occasionally interfere with thinking, social relations, or behavior.	☐
6 Severe - Presence of a stable set of delusions which are crystallized, possibly systematized, tenaciously held, and clearly interfere with thinking, social relations, and behavior.	☐
7 Extreme - Presence of a stable set of delusions which are either highly systematized or very numerous, and which dominate major facets of the patient's life. This frequently results in inappropriate and irresponsible action, which may even jeopardize the safety of the patient or others.	☐

P2. Conceptual disorganization Disorganized process of thinking characterized by disruption of goal-directed sequencing, e.g., circumstantiality, tangentiality, loose associations non sequiturs, gross illogicality, or thought block. Basis for rating: cognitive-verbal processes observed during the course of interview.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Thinking is circumstantial, tangential, or paralogical. There is some difficulty in directina thoughts toward a goal and some loosening o7 associations may be evidenced under pressure.	☐
4 Moderate - Able to focus thoughts when communications are brief and structured, but becomes loose or irrelevant when dealing with more complex communications or when under minimal pressure.	☐
5 Moderate severe - Generally has difficulty in organizing thoughts, as evidenced by frequent irrelevances, disconnectedness. or loosening of associations even when not under pressure.	☐
6 Severe - Thinking is seriously derailed and internally inconsistent, resulting in gross irrelevancies and disruption of thought processes, which occur almost constantly.	☐
7 Extreme - Thoughts are disrupted to the point where the patient is incoherent. There is marked loosening of associations, which results in total failure of communication, e.g., "word salad. or mutism.	☐

P3. Hallucinatory behavior Verbal report or behavior indicating perceptions which are not generated by external stimuli. These may occur in the auditory visual, olfactory, or somatic realms. Basis for rating: Verbal report and physical manifestations during the course of interview as well as reports of behavior by primary care workers or family.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - One or two clearly formed but infrequent hallucinations, or else a number of vaque abnormal perceptions which do not result in distortions of thinking or behavior.	☐
4 Moderate - Hallucinations occur frequently but not continuously, and the patient's thinking and behavior are affected only to a minor extent.	☐
5 Moderate severe - Hallucinations are frequent, may involve more than one sensory modality, and tend to distort thinking and/or disrupt behavior. Patient may have a delusional interpretation of these experiences and respond to them emotionally and, on occasion, verbally as well.	☐
6 Severe - Hallucinations are present almost continuously, causing major disruption of thinking and behavior. Patient treats these as real perceptions, and functioning is impeded by frequent emotional and verbal responses to them.	☐
7 Extreme - Patient is almost totally preoccupied with hallucinations, which virtually dominate thinking and behavior. Hallucinations are provided a rigid delusional interpretation and provoke verbal and behavioral responses, including obedience to command hallucinations.	☐

P4. Excitement Hyperactivity as reflected in accelerated motor behavior, heightened responsivity to stimuli hypervigilance, or excessive mood lability. Basis for rating: Behavioral manifestations during the course of interview as well as reports of behavior by primary care workers or family.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Tends to be slightly agitated, hypervigilant, or mildly overaroused throughout the interview, but without distinct episodes of excitement or marked moolabitity. Speech may be slightly pressured.	☐
4 Moderate - Agitation or overarousal is clearly evident throughout the interview, affecting speech and general mobility, or episodic outbursts occur sporadically	☐
5 Moderate severe - Significant hyperactivity or frequent outbursts of motor activity are observed, making it difficult for the patient to sit still for longer than several minutes at any given time.	☐
6 Severe - Marked excitement dominates the interview delimits attention, and to some extent affects personal functions such as eating and sleeping.	☐
7 Extreme - Marked excitement seriously interferes in eating and sleeping and makes interpersonal interactions virtually impossible. Acceleration of speech and motor activity may result in incoherence and exhaustion.	☐

P5. Grandiosity Exaggerated self-opinion and unrealistic convictions of superiority, including delusions of extraordinary abilities, wealth, knowledge, fame, power, and moral righteousness. Basis for rating: thought content expressed in the interview and its influence on behavior.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Some expansiveness or boastfulness is evident, but without clear-cut grandiose delusions.	☐
4 Moderate - Feels distinctly and unrealistically superior to others. Some poorly formed delusions about special status or abilities may be present but are not acted upon.	☐
5 Moderate severe - Clear-cut delusions concerning remarkable abilities, status, or power are expressed and influence attitude but not behavior.	☐
6 Severe - Clear-cut delusions of remarkable superiority involving more than one parameter (wealth, knowledge, fame, etc.) are expressed, notably influence interactions, and may be acted upon.	☐
7 Extreme - Thinking, interactions, and behavior are dominated by multiple delusions of amazing ability, wealth knowledge, fame, power, and/or moral stature; which may take on a bizarre quality.	☐

P6. Suspiciousness/persecution Unrealistic or exaggerated ideas of persecution, as reflected in guardedness, a distrustful attitude, suspicious hypervigilance, or frank delusions that others mean one harm. Basis for rating: thought content expressed in the interview and its influence on behavior.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Presents a guarded or even openly distrustful attitude, but thoughts, interactions, and behavior are minimally affected.	☐
4 Moderate - Distrustfulness is clearly evident and intrudes on the interview andior behavoir, but there is no evidence of persecutorv delusions. Alternatively, there may be indication of loosely formed persecutory delusions, but these do not seem to affect the patient's attitude or interpersonal relations	☐
5 Moderate severe - Patient shows marked distrust fulness, leading to major disruption of interpersonal relations, or else there are clear-cut persecutory delusions that have limited impact on interpersonal relations and behavoir.	☐
6 Severe - Clear-cut pervasive delusions of persecution which may be svstematized and significantly interfere in interpersonal relations.	☐
7 Extreme - A network of systematized persecutory delusions dominates the patient's thinking, social relations, and behavior.	☐

P7. Hostility Verbal and nonverbal expressions of anger and resentment, including sarcasm, passive-aggressive behavior, verbal abuse, and assaultiveness. Basis for rating: interpersonal behavoir observed during the interview and reports by primary care workers or family.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Indirect or restrained communication of anger such as sarcasm, disrespect, hostile expressions, and occasional irritability.	☐
4 Moderate - Presents an overtly hostile attitude, showing frequent irritability and direct expression of anger or resentment.	☐
5 Moderate severe - Patient is highly irritable and occasionally verbally abusive or threatening.	☐
6 Severe - Uncooperativeness and verbal abuse or threats notably influence the interview and seriously impact upon social relations. Patient may be violent and destructive but is not physically assaultive toward others.	☐
7 Extreme - Marked anger results in extreme uncooperativeness, precluding other interactions, or in eoisede(s) of physical assault toward others.	☐

NEGATIVE SCALE (N)

N1. Blunted affect Diminished emotional responsiveness as characterized by a reduction in facial expression, modulation of feelings, and communicative gestures. Basis for rating: observation of physical manifestations of affective tone and emotional responsiveness during the course of interview.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Changes in facial expression and communicative gestures seem to be stilted, forced, artificial, or lacking in modulation.	☐
4 Moderate - Reduced range of facial expression and few expressive gestures result in a dull appearance.	☐
5 Moderate severe - Affect is generally ~flat-, with only occasional changes in facial expression and a paucity of communicative gestures.	☐
6 Severe - Marked flatness and deficiency of emotions exhibited most of the time. There may be unmodulated extreme affective discharges, such as excitement, rage, or inappropriate uncontrolled laughter.	☐
7 Extreme - Changes in facial expression and evidence of communicative gestures are virtually absent. Patient seems constantly to show a barren or "wooden" expression.	☐

N2. Emotional withdrawal Lack of interest in, involvement with, and affective commitment to life's events. Basis for rating: reports of functioning from primary care workers or family and observation of interpersonal behavoir during the course of interview.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Usually lacks initiative and occasionally may show deficient interest in surrounding events.	☐
4 Moderate - Patient is generally distanced emotionally from the milieu and its challenges but, with encouragement, can be engaged.	☐
5 Moderate severe - Patient is clearly detached emotionally from persons and events in the milieu, resisting all efforts at engagement. Patient appears distant, docile, and purposeless but can be involved m communication at least briefly and tends to personal needs, sometimes with assistance.	☐
6 Severe - Marked deficiency of interest and emotional commitment results in limited conversation with others and frequent neglect of personal functions, for which the patient requires supervision	☐
7 Extreme - Patient is almost totally withdrawn, uncommunicative, a nd neglectful of personal needs as a result of profound lack of interest and emotional commitment.	☐

N3. Poor rapport Lack of interpersonal empathy, openess in conversation, and sense of closeness, interest, or involvement with the interviewer. This is evidenced by interpersonal distancing and reduced verbal and nonverbal communication. Basis for rating: interpersonal behavior during the course of interview.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Conversation is characterized by a stilted strained or artificial tone. It may lack emotional depth or tend to remain on an impersonal, intellectual plane.	☐
4 Moderate - Patient typically is aloof, with interpersonal distance quite evident. Patient may answer questions mechanically, act bored, or express disinterest.	☐
5 Moderate severe - Disinvolvement IS obvious and clearly impedes the productivity of the interview. Patient may tend to avoid eye or face contact.	☐
6 Severe - Patient is highly indifferent, with marked interpersonal distance. Answers are perfunctory, and there is little nonverbal evidence of involvement. Eye and face contact are frequently avoided.	☐
7 Extreme - Patient is totally uninvolved with the interviewer. Patient appears to be completely indifferent and consistently avoids verbal and nonverbal interactions during the interview.	☐

N4. Passive/apathetic social withdrawal Diminished interest and initiative in social interactions due to passivity, apathy, anergy, or avolition. This leads to reduced interpersonal involvement and neglect of activities of daily living. Basis for rating: reports on social behavior from primary care workers or family.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Shows occasional interest in social activities but poor initiative. Usally engages with others only when approached first by them.	☐
4 Moderate - Passively goes along with most social activities but in a disinterested or mechanical way. Tends to recede into the background.	☐
5 Moderate severe - Passively participates in only a minority of activities and shows virtually no interest or initiative Generally spends little time with others	☐
6 Severe - Tends to be apathetic and isolated, participating very rarely in social activities and occasionally neglecting personal needs. Has very few spontaneous social contacts.	☐
7 Extreme - Profoundly apathetic, socially isolated, and personally neglectful.	☐

N5. Difficulty in abstract thinking Impairment in the use of the abstract-symbolic mode of thinking, as evidenced by difficulty in classification, forming generalizations, and proceeding beyond concrete or egocentric thinking in problemsolving tasks. Basis for rating: responses to questions on similarities and proverb interpretation, and use of concrete vs. abstract mode during the course of the interview.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Tends to give literal or personalized interpretations to the more difficult proverbs and may have some problems with concepts that are fairly abstract or remotely related.	☐
4 Moderate - Often utilizes a concrete mode Has difficulty with most proverbs and some categories. Tends to be distracted by functional aspects and salient features	☐
5 Moderate severe - Deals primarily in a concrete mode, exhibiting difficulty with most proverbs and many categories.	☐
6 Severe - Unable to grasp the abstract meaning of any proverbs or figurative expressions and can formulate classifications for only the most simple of similarities. Thinking is either vacuous or locked into functional aspects, salient features, and idiosyncratic interpretations.	☐
7 Extreme - Can use only concrete modes of thinking. Shows no comprehension of proverbs, common metaphors or similes, and simple categories. Even salient and functional attributes do not serve as a basis for classification. This rating may apply to those who cannot interact even minimally with the examiner due to marked cognitive impairment.	☐

N6. Lack of spontaneity and flow of conversation Reduction in the normal flow of communication associated with apathy, avolition, defensiveness, or cognitive deficit. This is manifested by diminished fluidity and productivity of the verbal-interactional process. Basis for rating: cognitive-verbal processes observed during the course of interview.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Conversation shows little initiative. Patient's answers tend to be brief and unembellished, requiring direct and leading questions by the interviewer.	☐
4 Moderate - Conversation lacks free flow and appears uneven or halting. Leading questions are frequently needed to elicit adequate responses and proceed with conversation.	☐
5 Moderate severe - Patient shows a marked lack of spontaneity and openness, replying to the mterviewer's questions with only one or two brief sentences.	☐
6 Severe - Patient's responses are limited mainly to a few words or short phrases intended to avord or curtail communication. (E g., "I don't know," "I'm not at libertv to say.") Conversation is seriously impaired as a result, and the interview is highly unproductive	☐
7 Extreme - Verbal output is restricted to, at most, an occasional utterance, making conversation not possible.	☐

N7. Stereotyped thinking Decreased fluidity, spontaneity, and flexibility of thinking, as evidenced in rigid, repetitious, or barren thought content. Basis for rating: cognitiveverbal processes observed during the interview.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Some rigidity shown in attitudes or beliefs. Patient may refuse to consider alternative positions or have difficulty in shifting from one idea to another.	☐
4 Moderate - Conversation revolves around a recurrent theme, resulting in difficulty in shifting to a new topic.	☐
5 Moderate severe - Thinkinq is rigid and repetitious to the point that despite the interviewer's efforts conversation is limited to only two or three dominating topics.	☐
6 Severe - Uncontrolled repetition of demands, statements, ideas, or questions which severely impairs conversation.	☐
7 Extreme - Thinking, behavior, and conversation are dominated by constant repetition of fixed ideas or limited phrases, leading to gross rigidity, inappropriateness, and restrictiveness of patient's communication.	☐

GENERAL PSYCHOPATHOLOGY SCALE (G)

G1. Somatic concern Physical complaints or beliefs about bodily illness or malfunctions. This may range from a vague sense of ill being to clear-cut delusions of catastrophic physical disease. Basis for rating: thought content expressed in the interview.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Distinctly concerned about health or somatic issues, as evidenced by occasional questions and desire for reassurance.	☐
4 Moderate - Complains about poor health or bodily malfunction, but there is no delusional conviction, and overconcern can be allayed by reassurance.	☐
5 Moderate severe - Patient expresses numerous or frequent complaints about physical illness or bodily malfunction, or else patient reveals one or two clearcut delusions involving these themes but is not preoccupied by them.	☐
6 Severe - Patient is preoccupied by one or a few clearcut delusions about physical disease or organic malfunction, but affect is not fully immersed in these themes, and thoughts can be diverted by the interviewer with some effort.	☐
7 Extreme - Numerous and frequently reported somatic delusions, or only a few somatic delusions of a catastrophic nature, which totally dominate the patient's affect and thinking.	☐

G2. Anxiety Subjective experience of nervousness, worry, apprehension, or restlessness, ranging from e xcessive concern about the present or future to feelings of panic. Basis for rating: verbal report during the course of interview and corresponding physical manifestations.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Expresses some worry, overconcern, or subjective restiessness, but no somatic and behavioral consequences are reported or evidence.	☐
4 Moderate - Patient reports distinct symptoms of nervousness, which are reflected in mild physical manifestations such as fine hand tremor and excessive perspiration.	☐
5 Moderate severe - Patient reports serious problems of anxiety which have significant physical and behavioral consequences, such as marked tension, poor concentration, palpitations, or impaired sleep.	☐
6 Severe - Subjective state of almost constant fear associated with phobias, marked restlessness, or numerous somatic manifestations.	☐
7 Extreme - Patient's life is seriously disrupted by anxiety, which is present almost constantly and at times reaches panic proportion or is manifested in actual panic attacks.	☐

G3. Guilt feelings Sense of remorse or self-blame for real or imagined misdeeds in the past. Basis for rating: verbal report of guilt feelings during the course of interview and the influence on attitudes and thoughts.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Questioning elicits a vague sense of guilt or selfblame for a minor incident, but the patient clearly is not overly concerned	☐
4 Moderate - Patient expresses distinct concern over his responsibility for a real incident in his life but is not preoccupied with it, and attitude and behaviour are essentially unaffected.	☐
5 Moderate severe - Patient expresses a strong sense of quilt associated with self-deprication or the belief that he deserves punishment. The guilt feelings may have a delusional basis, may be volunteered spontaneously, may be a source of preoccupation and/or depressed mood, and cannot be allayed readily by the interviewer.	☐
6 Severe - Strong ideas of guilt take on a delusional quality and lead to an attitude of hopelessness or worthlessness The patient believes he should receive harsh sanctions for the misdeeds and may even regard his current life situation as such punishment.	☐
7 Extreme - Patient's life is dominated by unshakable delusions of guilt, for which he feels deserving of drastic punishment, such as life imprisonment, torture, or death. There may be associated suicidal thoughts or attribution of others' problems to one's own past misdeeds.	☐

G4. Tension Overt physical manifestations of fear, anxiety, and agitation, such as stiffness, tremor, profuse sweating, and restlessness. Basis for rating: verbal report attesting to anxiety and, thereupon, the severity of physical manifestations of tension observed during the interview.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Posture and movements indicate slight apprehensiveness, such as minor rigidity, occasional restlessness, shifting of position, or fine rapid hand tremor.	☐
4 Moderate - A clearly nervous appearance emerges from various manifestations, such as fidgety behaviour, obvious hand tremor, excessive perspiration, or nervous mannerisms.	☐
5 Moderate severe - Pronounced tension is evidenced by numerous manifestations, such as nervous shaking, profuse sweating, and restlessness, but conduct in the interview is not significantly affected.	☐
6 Severe - Pronounced tension to the point that interpersonal interactions are disrupted. The patient for example, may be constantly fidgeting, unable to sit still for long, or show hyperventilation.	☐
7 Extreme - Marked tension is manifested by signs of panic or gross motor acceleration, such as rapid restless pacing and inability to remain seated for longer than a minute, which makes sustained conversation not possible	☐

G5. Mannerisms and posturing Unnatural movements or posture as characterized by an awkward, stilted, disorganized, or bizarre appearance. Basis for rating: observation of physical manifestations during the course of interview as well as reports from primary care workers or family.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Slight awkardness in movements or minor rigidity of posture.	☐
4 Moderate - Movements are notably awkward or disjointed, or an unnatural posture is maintained for brief periods.	☐
5 Moderate severe - Occasional bizarre rituals or contorted posture are observed, or an abnormal position is sustained for extended periods.	☐
6 Severe - Frequent repetition of bizarre rituals, mannerisms, or stereotyped movements, or a contorted posture is sustained for extended periods..	☐
7 Extreme - Functioning is seriously impaired by virtually constant involvement in ritualistic, manneristic, or stereotyped movements or by an unnatural fixed posture which is sustained most of the time.	☐

G6. Depression Feelings of sadness, discouragement, helplessness, and pessimism. Basis for rating: verbal report of depressed mood during the course of interview and its observed influence on attitude and behavior.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Expresses some sadness or discouragement only on questioning. but there is no evidence of depression in general attitude or demeanor.	☐
4 Moderate - Distinct feelings of sadness or hopelessness, which may be spontaneously divulqed, but depressed mood has no major impact on behavior or social functioning, and the patient usally can be cheered up.	☐
5 Moderate severe - Distinctly depressed mood is associated with obvious sadness, pessimism, loss of social interest psychomotor retardation, and some interference in appetite and sleep. The patient cannot be easily cheered up.	☐
6 Severe - Markedly depressed mood is associated with sustained feelings of misery, occasional crying, hopelessness, and worthlessness. In addition, there is major interference in appetite and/or sleep as well as in normal motor and social functions, with possible signs of self-neglect.	☐
7 Extreme - Depressive feelings seriously interfere m most major functions. The manifestations include frequent crying, pronounced somatic symptoms, impaired concentration, psychomotor retardation, social disinterest, self-neglect, possible depressive or nihilistic delusions, and/or possible suicidal thoughts or action.	☐

G7. Motor retardation Reduction in motor activity as reflected in slowing or lessening of movements and speech, diminished responsiveness to stimuli, and reduced body tone. Basis for rating: manifestations during the course of interview as well as reports by primary care workers or family.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Slight but noticeable diminution in rate of movements and speech Patient may be somewhat underproductive in conversation and gestures.	☐
4 Moderate - Patient is clearly slow in movements, and speech may be characterized by poor productivity, including long response latency, extended pauses, or slow pace.	☐
5 Moderate severe - A marked reduction in motor activity renders communication highly unproductive or delimits functioning in social and occupational situations. Patient can usually be found sitting or Iying down.	☐
6 Severe - Movements are extremely slow, resulting in a minimum of activity and speech. Essentially the day is spent sitting idly or lying down.	☐
7 Extreme - Patient is almost completely immobile and virtually unresponsive to external stimuli.	☐

G8. Uncooperativeness Active refusal to comply with the will of significant others, including the interviewer, hospital staff, or family, which may be associated with distrust, defensiveness, stubbornness, negativism, rejection of authority, hostility, or belligerence. Basis for rating interpersonal behavior observed during the course of interview as well as reports by primary care workers or family.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Complies with an attitude of resentment, impatience, or sarcasm. May inoffensively object to sensitive probing during the interview.	☐
4 Moderate - Occasional outright refusal to comply with normal social demands, such as making own bed, attending scheduled programs, etc. The patient may project a hostile, defensive, or negative attitude but usually can be worked with.	☐
5 Moderate severe - Patient frequently ~s incompliant with the demands of his milieu and may be characterized by others as an "outcast" or having "a serious attitude problem." Uncooperativeness is reflected in obvious defensiveness or irritability with the interviewer and possible unwillingness to address many questions.	☐
6 Severe - Patient is highly uncooperative, negativistic, and possibly also belligerent. Refuses to comply with most social demands and may be unwilling to initiate or conclude the full interview.	☐
7 Extreme - Active resistance seriously impact on virtually all major areas of functioning. Patient may refuse to join in any social activities, tend to personal hygiene, converse with family or staff, and participate even briefly in an interview.	☐

G9. Unusual thought content Thinking characterized by strange, fantastic, or bizarre ideas, ranging from those which are remote or atypical to those which are distorted, illogical, and patently absurd. Basis for rating: thought content expressed during the course of interview.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Thought content is somewhat peculiar or idiosyncratic, or familiar ideas are framed in an odd context.	☐
4 Moderate - Ideas are frequently distorted and occasionally seem quite bizarre.	☐
5 Moderate severe - Patient expresses many strange and fantastic thoughts (e.g., being the adopted son of a king, being an escapee from death row) or some which are patently absurd (e.g., having hundreds of children, receiving radio messages from outer space through a tooth filling).	☐
6 Severe - Patient expresses many illogical or absurd ideas or some which have a distinctly bizarre quality (e.g., having three heads, being a visitor from another planet).	☐
7 Extreme - Thinking is replete with absurd, bizarre, and grotesque ideas.	☐

G10. Disorientation Lack of awareness of one's relationship to the milieu, including persons, place, and time, which may be due to confusion or withdrawal. Basis for rating: responses to interview questions on orientation.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - General orientation is adequate but there is some difficulty with specifics. For example, patient knows his location but not the street address, knows hospital staff names but not their functions, knows the month but confuses the day of week with an adjacent day, or errs in the date by more than two days. There may be narrowing of interest evidenced by familiarity with the immediate but not extended milieu such as ability to identify staff but not the Mayo;, Governor, or President.	☐
4 Moderate - Only partial success in recognizing persons, places, and time. For example, patient knows he is in a hospital but not its name, knows the name of his city but not the burrough or district, knows the name of his primary therapist but not many other direct care workers, knows the year and season but not sure of the month.	☐
5 Moderate severe - Considerable failure in recognizing persons, place, and time. Patient has only a vague notion of where he is and seemsunfamiliar with most people in his milieu. He may identify the year correctly or nearly so but not know the current month, day of week, or even the season.	☐
6 Severe - Marked failure in recognizing persons, place, and time. For example, patient h as no knowledge of his whereabouts, confuses the date by more than one year, can name only one or two individuals in his current life.	☐
7 Extreme - Patient appears completely disoriented with regard to persons, place, and time. There is gross confusion or total ignorance about one's location, the current year, and even the most familiar people, such as parents, spouse, friends, and primary therapist.	☐

G11. Poor attention Failure in focused alertness manifested by poor concentration, distractibility from internal and external stimuli, and difficulty in harnessing, sustaining, or shifting focus to new stimuli. Basis for rating: manifestations during the course of interview.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Limited concentration evidenced by occasional vulnerability, to distraction or faltering attention toward the end of the interview.	☐
4 Moderate - Conversation is affected by the tendency to be easily distracted, difficulty in long sustaining concentration on a given topic, or problems in shifting attention to new topics.	☐
5 Moderate severe - Conversation is seriously hampered by poor concentration, distractibility, and difficulty in shifting focus appropriately.	☐
6 Severe - Patient's attention can be harnessed for only brief moments or with great effort. due to marked distraction by internal or external stimuli.	☐
7 Extreme - Attention is so disrupted that even brief conversation is not possible.	☐

G12. Lack of judgment and insight Impaired awareness or understanding of one's own psychiatric condition and life situation. This is evidenced by failure to recognize past or present psychiatric illness or symptoms, denial of need for psychiatric hospitalization or treatment, decisions characterized by poor anticipation of consequences, and unrealistic short-term and long-range planning. Basis for rating: thought content expressed during the interview.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Recognizes having a psychiatric disorder but clearly underestimates its seriousness, the implications for treatment, or the importance of taking measures to avoid relapse. Future planning may be poorly conceived.	☐
4 Moderate - Patient shows only a vague or shallow recognition of illness. There may be fluctuations in acknowledgement of being ill or little awareness of major symptoms which are present, such as delusions, disorganized thinking, suspiciousness, and social withdrawal. The patient may rationalize the need for treatment in terms of its relieving lesser symptoms, such as anxiety, tension, and sleep difficulty.	☐
5 Moderate severe - Acknowledges past but not present psychiatric disorder. If challenged, the patient may concede the presence of some unrelated or insignificant symptoms, which tend to be explained away by gross misinterpretation or delusional thinking. The need for psychiatric treatment similarly goes unrecognized.	☐
6 Severe - Patient denies ever having had a psychiatric disorder. He disavows the p resence of any psychiatric symptoms in the past or present and, though compliant, denies the need for treatment and hospitalization.	☐
7 Extreme - Emphatic denial of past and present psychiatric illness. Current hospitalization and treatment are given a delusional interpretation (e.g.. as punishment for misdeeds, as persecution by tormentors, etc.), and the patient may thus refuse to cooperate with therapists, medication, or other aspects of treatment.	☐

G13. Disturbance of volition Disturbance in the wilful initiation, sustenance, and control of one's thoughts, behavior, movements, and speech. Basis for rating thought content and behavior manifested in the course of interview.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - There is evidence of some indecisiveness in conversation and thinking, which may impede verbal and cognitive processes to a minor extent.	☐
4 Moderate - Patient is often ambivalent and shows clear difficulty in reaching decisions. Conversation may be marred by alternation in thinking, and in consequence verbal and cognitive functioning are clearly impaired.	☐
5 Moderate severe - Disturbance of volition interferes in thinking as well as behavior. Patient shows pronounced indecision that impedes the initiation and continuation of social and motor activities, and which also may be evidenced in halting speech.	☐
6 Severe - Disturbance of volition interferes in the execution of simple, automatic motor functions, such as dressing and grooming, and markedly affects speech.	☐
7 Extreme - almost complete failure of volition is manifested by gross inhibition of movement and speech, resulting in immobility and/or mutism.	☐

G14. Poor impulse control Disordered regulation and control of action on inner urges resulting in sudden, unmodulated, arbitrary, o; misdirected discharge of tension and emotions without concern about consequences. Basis for rating: behavior during the course of interview and reported by primary care workers or family.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Patient tends to be easily angered and frustrated when facing stress or denied gratification but rarely acts on impulse.	☐
4 Moderate - Patient gets angered and verbally abusive with minimal provocation. May be occasionally threatening, destructive, or have one or two episodes involving physical confrontation or a minor brawl.	☐
5 Moderate severe - Patient exhibits repeated impulsive episodes involving verbal abuse destruction of property, or physical threats. There may be one or two episodes involving serious assault, for which the patient requires isolation, physical restraint, or p.r n. sedation.	☐
6 Severe - Patient frequently is impulsively aggressive, threatening, demanding, and destructive, without any apparent consideration of consequences. Shows assaultive behavior and may also be sexually offensive and possibly respond behaviorally to hallucinatory commands.	☐
7 Extreme - Patient exhibits homicidal attacks, sexual assaults, repeated brutality, or self-destructive behavior. Requires constant direct supervision or external constraints because of inability to control dangerous impulses.	☐

G15. Preoccupation Absorption with internally generated thoughts and feelings and with autistic experiences to the detriment of reality orientation and adaptive behavior. Basis for rating: interpersonai behavior observed during the course of interview.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Excessive involvement with personal needs or problems, such that conversation veers back to egocentric themes and there is diminished concern exhibited toward others.	☐
4 Moderate - Patient occasionally appears selfabsorbed, as if daydreaming or involved with internal experiences, which interferes with communication to minor extent.	☐
5 Moderate severe - Patient often appears to be engaged in autistic experiences, as evidenced by behaviors that significantly intrude on social and communicational functions, such as the presence of a vacant stare, muttering or talking to oneself, or involvement with stereotyped motor patterns.	☐
6 Severe - Marked preoccupation with autistic experiences, which seriously delimits concentration, ability to converse, and orientation to the milieu. The patient frequently may be observed smiling, laughing, muttering, talking, or shouting to himself.	☐
7 Extreme - Gross absorption with autistic experiences, which profoundly affects all major realms of behavior. The patient constantly may be responding verbally and behaviorally to hallucinations and show little awareness of other people or the external milieu.	☐

G16. Active social avoidance Diminished social involvement associated with unwarranted fear, hostility, or distrust. Basis for rating: reports of social functioning by primary care workers or family.	
1 Absent - Definition does not apply	☐
2 Minimal - Questionable pathology; may be at the upper extreme of normal limits.	☐
3 Mild - Patient seems ill at ease in the presence of others and prefers to spend time alone, although he participates in social functions when required.	☐
4 Moderate - Patient begrudgingly attends all or most social activities but may need to be persuaded or may terminate prematurely on account of anxiety, suspiciousness, or hostility.	☐
5 Moderate severe - Patient fearfully or angrily keeps away from many social interactions despite others' efforts to engage him. Tends to spend unstructured time alone.	☐
6 Severe - Patient participates in very few social activities because of fear, hostility, or distrust. W hen approached, the patient shows a strong tendency to break off interactions, and generally he tends to isolate himself from others.	☐
7 Extreme - Patient cannot be engaged in social activities because of pronounced fears, hostility, or persecutory delusions. To the extent possible, he avoids all interactions and remains isolated from others.	☐

PANSS rating form

		Absent	Minimal	Mild	Moderate	Moderate-severe	Severe	Extreme
P1	Delusions	1	2	3	4	5	6	7
P2	Conceptual disorganization	1	2	3	4	5	6	7
P3	Hallucinatory behaviour	1	2	3	4	5	6	7
P4	Excitement	1	2	3	4	5	6	7
P5	Grandiosity	1	2	3	4	5	6	7
P6	Suspiciousness/persecution	1	2	3	4	5	6	7
P7	Hostility	1	2	3	4	5	6	7
N1	Blunted affect	1	2	3	4	5	6	7
N2	Emotional withdrawal	1	2	3	4	5	6	7
N3	Poor rapport	1	2	3	4	5	6	7
N4	Passive/ apathetic social withdrawal	1	2	3	4	5	6	7
N5	Difficulty in abstract thinking	1	2	3	4	5	6	7
N6	Lack of spontaneity and flow of conversation	1	2	3	4	5	6	7
N7	Stereotyped thinking	1	2	3	4	5	6	7
G1	Somatic concern	1	2	3	4	5	6	7
G2	Anxiety	1	2	3	4	5	6	7
G3	Guilt feelings	1	2	3	4	5	6	7
G4	Tension	1	2	3	4	5	6	7
G5	Mannerisms and posturing	1	2	3	4	5	6	7
G6	Depression	1	2	3	4	5	6	7
G7	Motor retardation	1	2	3	4	5	6	7
G8	Uncooperativeness	1	2	3	4	5	6	7
G9	Unusual though content	1	2	3	4	5	6	7
G10	Disorientation	1	2	3	4	5	6	7
G11	Poor attention	1	2	3	4	5	6	7
G12	Lack of judgement and insight	1	2	3	4	5	6	7
G13	Disturbance of volition	1	2	3	4	5	6	7
G14	Poor impulse control	1	2	3	4	5	6	7
G15	Preoccupation	1	2	3	4	5	6	7
G16	Active social avoidance	2	2	3	4	5	6	7

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