# Relationships of Autism Spectrum Disorder subjective measures with objective measures of inflammation and immune function

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# Declaration

I, Siobhan de Lange, declare that the work contained in this thesis is my own, unaided work, except to the extent indicated in the acknowledgements section. This thesis is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg, South Africa. This work has not been submitted before for any degree or examination at any other university.

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(signature of candidate)

Date:

08th day of September 2022 in Kensington, Johannesburg

## Publications arising from this thesis

Four manuscripts arising from the work contained in this thesis have been submitted to journals for publication. The titles of the manuscripts are provided below, and the full manuscripts are provided in Appendix D.

1) *"Biomedical research on autism in South Africa: Re-thinking the informed consent process"* submitted to the Journal of Medical Ethics (manuscript number medethics-2022-108302).
2) *"Relationships between autistic symptom severity, motor skills and socioeconomic status"* submitted to the journal Autism (manuscript number AUT-22-0192).
3) *"Descriptive characteristics of children with autism at schools for autism in Johannesburg, South Africa"* submitted to the journal Autism Research (manuscript number AUR-22-0130).
4) *"Urinary cortisol and neopterin in autistic children: Relationships with balance"* submitted to the Journal of Autism and Developmental Disorders (manuscript number JADD-D-22-00389).

## Abstract

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterised by deficits in language use and social interaction, as well as restricted or repetitive interests and behaviours. In the past few decades, research on ASD has flourished in Europe and the Americas, but relatively little research on the disorder has been conducted in Africa. Individuals with ASD also commonly present with sensory dysregulation and motor impairment, and motor interventions are commonly employed for the alleviation of these symptoms as well as to improve language use, social interaction and repetitive behaviour in ASD. The pathophysiology underlying the disorder is not fully understood, but recent research has implicated dysregulation of the immune system as a central feature. Moreover, it has been theorised that motor interventions may be useful in ASD as a result of modulation of immune system function. The studies comprising this thesis therefore aimed to collect data describing the ASD population in South Africa, and to investigate the role of immune dysregulation in ASD symptomatology and how ASD symptoms may be improved by a motor intervention. Furthermore, as biomedical research on ASD is fraught with ethical concerns, especially in the African context, an in-depth discussion of such concerns and ways to consider them appropriately during the research conduct is included in this thesis. Hence, the thesis is constructed of four main aims: to discuss the ethical concerns inherent in biomedical research on ASD in South Africa, to collect data characterising the symptom profile of the population of ASD children in the Johannesburg area, to investigate relationships between the measures collected, and to assess the validity of an exercise intervention in improving the symptoms of ASD. As regards the first aim, future recruitment procedures may need to be re-evaluated in order to ensure recruitment of participants of a wider range of socioeconomic status and sociocultural background. I recommend the widespread implementation of support structures for community engagement processes as part of biomedical research in order to address this issue.

To achieve the second aim, children with ASD (total n=50) were recruited via the Fight with Insight clinic, which runs an exercise class for autistic children. Assessments of socioeconomic status (n=48), autistic symptomatology (n=41), and motor skills (n=25) were conducted. Additionally, urine samples (n=40) were collected in order to assess levels of activation of the stress system and cellular immunity as indicators of the brain-immune axis. Cortisol was analysed as a marker of activation of the stress system, while neopterin was

analysed as a marker of cellular immune activation. Both markers have previously shown evidence of dysregulation in ASD. The data obtained, along with demographic data, were used to characterise the cohort. The results obtained indicated that the cohort were of a relatively middle to high socioeconomic status with a male to female ratio of 10:1. The average motor skills for the cohort were lower than expected for typically-developing children of the same age, supporting the high incidence of motor impairment previously reported in ASD. Autistic symptomatology was shown to be less severe in this cohort than in a Saudi Arabian cohort assessed using the same instrument, which may be suggestive of potential differences in presentation in geographically distinct populations, or may be due to sampling differences. No evidence was found for increased activation of the stress system or of increased activation of cellular immunity.

The second aim relied on the same measures stated above, wherein correlations between each collected measure were analysed. Significant relationships were found between motor impairment and the domains of autistic symptomatology specifically relating to social/communicative behaviour and sensory awareness ($n=19$, $p<0.05$). This data, alongside previous research, suggests a neurological interlinking and interdependence of the structures responsible for the development of social/communicative behaviours, sensory awareness and motor skills in ASD. Additionally, the results of this thesis contribute to the body of research assessing the impact of the brain-immune axis on ASD. No relationships were found between autistic symptomatology and urinary cortisol or neopterin ($n=30$), and therefore no evidence for a central role of the activation of either cellular immunity or the stress system in ASD symptoms. However, a relationship was observed between increased urinary cortisol and decreased balance performance ($n=19$, $p<0.05$). This data is in line with previous research that suggests that activation of the stress system may be neurologically interlinked with postural control, and provides the first evidence of this relationship occurring specifically in children with ASD. No evidence was found for a relationship between socioeconomic status and ASD symptom severity ($n=39$). However, this may be due to a potential bias in recruitment in which only participants of a higher socioeconomic status were recruited as a result of the recruitment procedure. Future studies need to consider alteration of the recruitment procedure to involve community engagement practices in order to increase recruitment of people of lower socioeconomic status.

To achieve the third aim, the same measures mentioned above were collected over an 18-week period during which recruited children with ASD were involved in an exercise intervention (n=3). Changes in the measures over the course of the 18 weeks were assessed. No statistically significant evidence was found for improvements in either autistic symptomatology or motor skills, however, a non-significant decrease in autistic symptom severity was observed. The low sample size may be responsible for the lack of statistical significance and further study of the exercise intervention is therefore warranted. No trends in either cortisol or neopterin were observed over the course of the 18 weeks. The lack of trends suggests that the exercise intervention was not significantly modulating either activation of the stress system or cellular immunity and oxidative stress, when measured on a weekly basis.

In conclusion, the results of this thesis provide previously unknown insight into the characteristics of autistic children attending the Fight with Insight exercise classes in the Johannesburg region of South Africa. Furthermore, these results add to the body of knowledge on the pathophysiology of ASD, the role of motor skills in the disorder and the regulation of the stress system and the immune system in ASD.

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# List of Abbreviations

| | |
|---|---|
| ABA | Applied Behavioural Analysis |
| ABC | Autism Behaviour Checklist |
| ACTH | adrenocorticotropic hormone |
| ADHD | Attention-deficit/hyperactivity disorder |
| ADI-R | Autism Diagnostic Interview, Revised |
| ADOS-2 | Autism Diagnostic Observation Schedule (Second Edition) |
| APC | antigen-presenting cell |
| ASD | Autism Spectrum Disorder |
| ATEC | Autism Treatment Evaluation Checklist |
| BOT-2 | Bruininks-Oseretsky Motor Proficiency Test (Second Edition) |
| CARE | Centre for Autism Research and Education |
| CARS | Childhood Autism Rating Scale |
| CMI | The Children's Memorial Institute |
| CRH | corticotropin-releasing hormone |
| DSM-5 | Diagnostic and Statistical Manual (Fifth Edition) |
| ELISA | enzyme-linked immunosorbent assay |
| GC | glucocorticoid |
| HLA | human leukocyte associated |
| HPA | hypothalamic-pituitary-adrenal |
| IFN | interferon |
| Ig | immunoglobulin |
| IL | interleukin |
| IQR | inter-quartile range |
| IRS | inflammatory response system |
| JSA | Johannesburg School for Autism |
| LMICs | low/middle-income countries |
| MHC | major histocompatibility complex |
| MIA | maternal immune activation |
| NA | noradrenaline |
| NAFP | neuron-axon-filament protein |
| NK | natural killer |

| | |
|---|---|
| NO | nitric oxide |
| REC | Research Ethics Committee |
| ROS | reactive oxygen species |
| SD | standard deviation |
| SG | specific gravity |
| SNS | sympathetic nervous system |
| TCR | T-cell receptor |
| TGF | transforming growth factor |
| TNF | tumour necrosis factor |
| Tregs | regulatory T-cells |

# Preface

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that presents with restricted interests, stereotyped behaviours and social/communicative impairment. While biomedical research on ASD in Europe and the Americas has flourished in recent decades, the ASD population in South Africa has not been well-researched. Data on the South African ASD population is needed in order to provide adequate support and services to meet their needs. Importantly, children with ASD are a vulnerable study population and South Africa is a culturally diverse country with great socioeconomic disparity. This means that biomedical research on the ASD population in South Africa requires a careful ethical consideration in order to be conducted appropriately. The pathophysiology underlying ASD is not currently understood, but there is evidence that immunological dysregulation plays a significant role. ASD also commonly presents with motor deficits, and exercise interventions of various kinds have been investigated for improvement of both the motor deficits as well as the social and behavioural symptoms of the disorder. In the South African context, exercise interventions present the possibility of cost-effective and scalable interventions for children with ASD that may improve their quality of life by alleviating symptoms and promoting motor capability. However, the physiological mechanisms by which exercise interventions improve the symptoms of ASD are not understood. The impact that exercise has on regulating inflammation and immune function provides a potential route of enquiry into these mechanisms. The sections that follow describe how this body of work intends to contribute to our understandings of the aetiology of ASD, as well as how to continue biomedical research on the population in an ethical way.

**Chapter 1** provides a general background of ASD, including a neuropsychological description of the disorder, an overview of motor impairments in ASD, and a review of how the disorder is diagnosed and treated. **Chapter 1** ends with a review of the current state of research on the disorder in Africa in general.

**Chapter 2** follows with an in-depth discussion of the known aetiology of ASD, beginning with an overview of the immune system and the mechanisms by which the brain and immune system interact. The specific immune dysregulations observed in ASD are then discussed within this context. An overview of exercise interventions used to treat ASD is then provided, followed by a review of the literature on how exercise impacts immune system functioning.

**Chapter 2** ends with the rationale for the studies undertaken in this thesis, as well as the specific aims of the thesis.

**Chapter 3** comprises the results of the first aim of this thesis, with an in-depth discussion of the ethical challenges facing biomedical research on the ASD population in South Africa. Recommendations for widespread institutional support of community engagement practices going forward are given as a result of the discussion in **Chapter 3**.

**Chapter 4** provides the details of the methodology by which data was collected for this thesis.

**Chapter 5** describes the results obtained from the data collected via the methods described in the previous chapter.

**Chapter 6** comprises a discussion of the results obtained and places them in the context of the current literature on the aetiology of ASD. Possible theories for the results observed are discussed, as well as the limitations of the studies undertaken and suggestions for possible future studies. This chapter ends with the conclusions drawn from the data collected during this thesis.

**Chapter 7** is a complete list of all the references used in the thesis.

**Chapter 8** includes the following appendices: ethical clearance certificate, permission to use figure 2.1, information sheet used for recruitment, Turnitin report and manuscripts arising from this thesis that have been submitted for publication.

# Chapter 1: Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterised primarily by deficits in social interaction and restricted or repetitive behaviour patterns or interests, presenting from early childhood and impairing everyday functioning (American Psychiatric Association, 2013). ASD also very commonly presents with motor impairment and sensory disturbances (Dunn, Myles and Orr, 2002; Baranek *et al.*, 2013). The pathophysiology underlying the development of ASD is not fully understood and appears to involve a variety of interrelated mechanisms, not all of which will be present in each individual diagnosed with ASD. The social deficits inherent in the diagnosis of ASD make it a socially and culturally situated disorder. However, research implicates a biological cause for the development of ASD. Importantly, a common thread amongst medical research on ASD is the dysregulation of the immune system. The sociocultural understanding of ASD needs to be looked at it conjunction with a physiological understanding of the pathomechanisms underlying the disorder. Research on ASD has grown in recent decades, particularly in the United States of America and Europe. There are still many gaps in the data on the ASD population in Africa, and in South Africa more specifically. Biomedical research on ASD in Africa is ethically sensitive due to the vulnerability of the population to exploitation in a research setting, a factor which is compounded by the resource-poor nature of many communities in Africa. Given the sensitive nature of the topic, this thesis involves a theoretical discussion of the ethical concerns of conducting medical research on the ASD population in South Africa, incorporating insights gleaned from experience conducting such research. This thesis will furthermore add to the body of biomedical research on ASD by characterising a cohort of children with ASD in the Johannesburg area of South Africa. Additionally, this thesis will examine relationships between motor impairment in ASD, the social or behavioural symptoms of the disorder, socioeconomic status and urinary measures of inflammation and stress. Finally, this thesis also involves a pilot investigation of an exercise intervention aimed at treating the motor and behavioural symptoms of ASD. As an introduction to the thesis, the following sections will provide a description of ASD, a neuropsychological perspective on the disorder, an overview of motor impairment in ASD, background on the diagnosis and treatment options for ASD, and a discussion of the current state of biomedical research on ASD in Africa.

### 1.1 Description of Autism Spectrum Disorder

ASD was first described by Kanner (1943), with the term "autism" coined from the Greek root word "auto", meaning "self". This term was used in reference to the anti-social symptoms Kanner observed in his case studies. The latest edition of the Diagnostic and Statistical Manual of the American Psychiatric Association (DSM-5) recognises ASD as a spectrum disorder which now includes the previously disparate disorders of classic autism, Asperger's Syndrome and Pervasive Developmental Disorder (Not Otherwise Specified) (American Psychiatric Association, 2013). Disorders such as Asperger's were initially categorised as separate, but in practice are difficult to distinguish clinically using consistent criteria, which has led to the creation of ASD as a diagnosis (Happe and Frith, 1996). There remains some debate around the usefulness of diagnosing each incorporated disorder separately (de Giambattista *et al.*, 2019). The primary diagnostic criteria for ASD are persistent deficits in social communication and interaction (such as abnormal social reciprocity, verbal and non-verbal communication deficits and difficulties in the development or maintenance of relationships) and restricted or repetitive behaviour patterns and interests (involving stereotyped speech patterns, excessive adherence to routine, fixated focus of interest as well as sensory abnormalities). All three of the social communication and interaction criteria need to be met, along with two of the four restricted/repetitive behaviour criteria, in order for a diagnosis of ASD to be given. In addition, the symptoms described need to have been present since early childhood, and together the symptoms limit or impair the everyday functioning of the individual.

While ASD is primarily recognised by its social and behavioural symptomatology, individuals with ASD very commonly present with delayed motor development and sensory dysregulation (Miller *et al.*, 2014), higher incidences of psychiatric comorbidities such as social anxiety disorder and attention-deficit/hyperactivity disorder (ADHD) (Simonoff *et al.*, 2008) as well as other physiological complaints such as sleep disturbances and gastrointestinal issues (Geschwind, 2009). Epilepsy is a particularly common co-morbidity (Gillberg *et al.*, 1986; Gillberg and Steffenburg, 1987; Olsson, Steffenburg and Gillberg, 1988; Volkmar and Nelson, 1990; Ritvo and Ritvo, 1992), but the presence of seizures is not correlated with the severity of ASD (Elia *et al.*, 1995). Motor impairments such as toe-walking, nystagmus, motor posturing, hypotonia, spasticity and persistence of infantile reflexes have been observed in ASD (Minderaa *et al.*, 1985). A retrospective clinical record review has estimated hypotonia to occur in 51% of children with ASD, and motor apraxia to

occur in 34% of ASD children (Ming, Brimacombe and Wagner, 2007). Symptom severity can vary widely, and severity of one symptom does not necessarily correlate with severity in all other symptoms, which makes ASD a very heterogenous disorder. Initially, ASD was believed to develop in response to inadequate or inappropriate parenting strategies, but research has provided evidence that the condition has a strong biological basis (Geschwind, 2009). Importantly, no consistent difference of attachment style has been observed in autistics (Sigman and Ungerer, 1984). There is evidence for genetic causes, with increased incidence observed amongst siblings compared to the general population, however many genes appear to be involved (Happe and Frith, 1996). There is also evidence that environment may have an impact on the development of ASD, with some studies showing relationships between socioeconomic status and diagnosis (King and Bearman, 2011; Rai *et al.*, 2012; Thomas *et al.*, 2012). ASD has been estimated to occur at a global prevalence of between 0.6 and 1% (Elsabbagh *et al.*, 2012), although some estimates have shown increases in prevalence over the past few decades (Geschwind, 2009). There is a higher incidence of ASD amongst males than females, but sex ratio varies according to severity, with more males observed particularly with ASD without intellectual impairment (Lord and Schopler, 1987). Given the spectrum nature of ASD, the disorder presents cognitively in a wide variety of ways. However, some patterns have been observed, which are discussed in the next section.

### 1.2 The Cognitive Neuropsychology of ASD

ASD has been described as a developmental disorder with a behavioural definition and a biological basis (Happe and Frith, 1996). Therefore, a full analysis of the disorder needs to incorporate both the cognitive psychological symptoms and the underlying physiology. An understanding of the specific cognitive difficulties experienced by autistics is useful in the design and evaluation of appropriate treatment strategies.

While some cognitive functions are impaired in ASD, autistics also present with “islets of ability” such as superior rote memory, solving of jigsaw puzzles, calendar calculations, drawing, music, prime number calculations and mnemonism (O’Connor, 1989; Happe and Frith, 1996). “Savant skills”, in which an individual has abnormally high capacity in a particular cognitive task, have been estimated to occur in 1 out of 10 autistics (Rimland and Hill, 1984). Characterisation of the way the syndrome presents showed that the manifestation varies with age and ability (Wing and Gould, 1979). However, Wing and Gould (1979) described a consistent triad of cognitive deficits evident in ASD that present in the areas of

socialisation, communication and imagination. This triad has been theorised to be associated with the capacity for theory of mind, and that impairment in theory of mind may be responsible for some of the symptoms observed in ASD (Wing and Gould, 1979). For example, autistic children have been demonstrated to have difficulty attributing mental states independently of an actual state of affairs (an ability which is also known as "mentalising") (Baron-Cohen, Leslie and Frith, 1985). An illustration of this is how many autistics will assume that another person will look for an object where it actually is, rather than where that person last saw it. Impairment of imagination has been shown by an absence of pretend play in ASD, which is also considered a manifestation of mentalising (Wing and Gould, 1979). However, some autistics show evidence of the ability to understand subjective thoughts and feelings as well as a capacity for social insight (Frith and Happe, 1994), which indicates some level of theory of mind.

Significant among the social and behavioural deficits observed in ASD is impairment in language use and development. Happe and Frith (1996) theorised that language delay may be due to difficulties in the behaviours required for word acquisition, such as joint attention (in which the child is able to place their attention on the object of another's attention simultaneously). Deficits in joint attention have been documented in ASD, such as failure to share eye gaze, perform eye contact, and co-ordinate and respond to emotional expressions (Baron-Cohen, Allen and Gillberg, 1992; Kasari *et al.*, 1993). Autistics do not display a total lack of capacity for language, as they develop idiosyncratic vocalisations, and many use echolalia and develop syntax normally (Tager-Flusberg, 1981; Tager-Flusberg, 1993). However, ASD appears to present universally with impaired pragmatics (the way in which context contributes to language use and meaning) (Frith, 1989).

In addition to the language deficits present in ASD, memory deficits and alterations in attention have also been observed (Happe and Frith, 1996), as well as abnormalities in emotional recognition (Hobson, Ouston and Lee, 1988). A lack of interpersonal relatedness is observed in ASD, and interpersonal relatedness has been argued to lie at the core of social understanding (Hobson, 1986*a*; Hobson, 1986*b*; Hobson, 1993). Young autistics also display a lack of early imitation, which may lead to failure to share affect later on in their development (Rogers and Pennington, 1991; Meltzoff, 1993). Hermelin & O'Connor (1970) distinguished central processing from input/output processes, and argued that it is an alteration in central processing that causes the cognitive symptoms of ASD, rather than a

general retardation or problems with peripheral input. Evidence has been found for impairments in central coherence in ASD (Happe and Frith, 1996), with autistics not exhibiting better processing of meaningful than random stimuli in the way that typically-developing individuals do (for example, autistics tend to retain words without extracting meaning) (Hermelin & O'Connor, 1967; Tager-Flusberg, 1991). Autistics also show preferential attention to parts rather than wholes, which also explains some of the cognitive assets observed in ASD (Shah and Frith, 1993; Happe, 1996).

Impairments in executive function more generally have also been observed in ASD (Pennington and Ozonoff, 1996). Executive function deficits are theorised to be related to the repetitive and stereotyped behaviour patterns exhibited by autistics (Happe and Frith, 1996). Executive function describes a variety of abilities, including the ability to disengage from context, inhibition of inappropriate responses, planning of action sequences, monitoring of performance using feedback and shifting attention (Duncan, 1995). Evidence of executive function deficits in ASD include problems with planning and organisation (Prior and Hoffmann, 1990; Ozonoff, Pennington and Rogers, 1991; Hughes, Russell and Robbins, 1994), problems with attention-switching (i.e. perseveration) (Rumsey and Hamburger, 1988; Hughes, Russell and Robbins, 1994) and decreased generativity (Turner, 1996). However, a theory of executive dysfunction underlying ASD does not explain the intact and superior skills observed (Happe and Frith, 1996).

Neuropsychological research has attempted to connect the cognitive features of ASD (discussed above) with neuroanatomical abnormalities. However, no single neurological abnormality has been found to characterize all subjects with ASD (Happe and Frith, 1996). Regions associated with social perception and cognition (specifically the medial prefrontal cortex, superior temporal sulcus, temporo-parietal junction, amygdala and fusiform gyrus) have shown to be hypoactive regions in ASD (Varghese *et al.*, 2017). Increased cell packing, decreased cell size and decreased connectivity in the limbic system have been observed in autistics, as well as abnormalities in the cerebellum and inferior olive, which may be indicative of a curtailment of development of these parts of the brain (Bauman and Kemper, 1985). There is also evidence for cerebellar and cortical lesions that would occur at or before 30-32 weeks gestation (Bauman, 1991), and neural migration deficits during the first 6 months of gestation (Piven *et al.*, 1990). Decreased size of the cerebellum and brainstem has also been found in very young children with ASD (Hashimoto *et al.*, 1995). Executive

dysfunction is typically associated with frontal lobe damage, but executive dysfunction may also be indicative of damage to the basal ganglia (Robbins *et al.*, 1994) and hippocampus (Upton and Corcoran, 1995). A more recent study using functional magnetic resonance imaging to investigate brain function during mentalizing tasks in ASD individuals found that diagnosis affected neither task performance nor brain response (Moessnang *et al.*, 2020). This illustrates the lack of current understanding of how neurology may underlie ASD symptoms. Hence, while the cognitive features of ASD are fairly well described, the changes in physiology and anatomy that underlie those features have yet to be adequately characterised.

### 1.3 Motor Impairments in ASD

In addition to the cognitive symptoms of ASD, motor impairment in the disorder has been well documented, with movement disturbances observed in autistic children as early as 4 – 6 months of age (Teitelbaum *et al.*, 1998). Movement disturbances observed in ASD include difficulties with starting or stopping movement, switching from one action to another and combining different motor skills (Leary and Hill, 1996). Autistic children have been found to have impaired basic motor skills and postural knowledge compared with typically developing controls (Dowell, Mahone and Mostofsky, 2009). Decreased postural stability has also been found in autistic children, with age analysis showing that postural stability is developmentally delayed in ASD (Minshew *et al.*, 2004). Abnormalities in gait have been found in autistic children (Vilensky, Damasio and Maurer, 1981; Hallett *et al.*, 1993; Rinehart *et al.*, 2006), as well as impairments in motor control (Jansiewicz *et al.*, 2006), praxis (Mostofsky *et al.*, 2006; Bhat *et al.*, 2018), dynamic balance (Freitag *et al.*, 2007), locomotor and object-control skills (Berkeley *et al.*, 2001), handwriting function (Mayes and Calhoun, 2003; Fuentes, Mostofsky and Bastian, 2009; Kushki, Chau and Anagnostou, 2011) and catching ability (Whyatt and Craig, 2013). Fournier *et al.* (2010) performed a meta-analysis of studies on motor impairment in ASD and found substantial evidence of motor co-ordination deficits in ASD.

Table 1.1: Studies on motor impairment in ASD

| Paper | ASD sample size | Age | Main findings |
|---|---|---|---|
| Teitelbaum, *et al.*, 1998 | 17 | 4-6 months | Abnormalities in lying, walking, rolling over, crawling and standing |
| Dowell, Mahone and Mostofsky, 2009 | 37 | 8-13 years | Impaired basic motor skills and postural knowledge |
| Minshew *et al.*, 2004 | 79 | 5-52 years | Impaired postural stability |
| Vilensky, Damasio and Maurer, 1981 | 21 | Not available | Gait abnormalities |
| Hallett *et al.*, 1993 | 5 | Adult | Gait abnormalities |
| Rinehart *et al.*, 2006 | 11 | 4-7 years | Gait and postural abnormalities |
| Jansiewicz *et al.*, 2006 | 40 | 6-17 years | Impaired motor control |
| Mostofsky *et al.*, 2006 | 21 | Not available | Praxis deficits |
| Bhat *et al.*, 2018 | 11 | 5-14 years | Dyspraxia |
| Freitag *et al.*, 2007 | 16 | 14-22 years | Impairments in dynamic balance and diadochokinesis |
| Berkeley *et al.*, 2001 | 15 | 6-8 years | Delayed object control skills |
| Mayes and Calhoun, 2003 | 164 | 3-15 years | Graphomotor delay |
| Fuentes, Mostofsky and Bastian, 2009 | 14 | 8-13 years | Handwriting impairments |
| Whyatt and Craig, 2013 | 18 | 7-10 years | General motor impairment and specific difficulties in catching a ball and static balance. |

Motor disturbances are so common in ASD that they may represent a core feature of the disorder, and the dysregulation of movement may be integrally neurologically and cognitively linked with other aspects of the disorder. Previous research has investigated the relationship between motor impairment and autistic symptomatology. In deaf autistic children, poorer receptive language has been found to correlate with poorer praxis and severity of ASD (Bhat *et al.*, 2018), which is indicative of potential relationships between motor processing, communicative processing and autistic symptomatology. In very young children (14 – 33 months of age), fine and gross motor skills have been found to predict calibrated severity of ASD (MacDonald, Lord and Ulrich, 2014). Motor impairment in autistic children has also been found to associate with the degree of social withdrawal exhibited (Freitag *et al.*, 2007). Hilton *et al.* (2012) used the Bruininks-Oseretsky Motor Proficiency Test (Second Edition) (BOT-2) to assess motor impairment in sibling pairs in which one sibling had ASD and one did not. They found significant association of motor impairment with autistic severity by group. ASD symptoms have been found to relate to postural stability in adolescents and adults (Travers *et al.*, 2013). Motor skills have been found to relate to adaptive behaviours and daily living skills in autistic children (MacDonald, Lord and Ulrich, 2013). Risk of motor impairment in ASD has been found to increase with impairments in social communication, functioning, cognition, language and repetitive behaviour (Bhat, 2021). It has been suggested that movement disturbance may delay the initiation of social actions such that the appropriate moment passes (Leary and Hill, 1996). The results of this body of research indicate a high likelihood that severity of motor impairment and severity of autistic symptomatology are closely linked, although there is a lack of research investigating correlations between specific motor impairments and ASD symptoms. Movement disturbances in ASD are commonly attributed to differences in the cognitive processes necessary for movement regulation, such as perception, attention and assigning of emotional valence (Leary and Hill, 1996). The disruption of these cognitive processes is theorised to lead to a dysregulation of movement in which movement can no longer be used effectively in social and communicative behaviours (such as the initiation of speech or responses to other people) (Leary and Hill, 1996).

While motor impairment appears to be a core component of the pathophysiology and symptomatology of ASD, the neurological causes of the motor impairment are not well understood. In studies on gait, disturbances in the basal ganglia have been implicated (Vilensky, Damasio and Maurer, 1981; Rinehart *et al.*, 2006), as well as cerebellar

abnormalities (Rinehart *et al.*, 2006). Results from fMRI scanning during performance of visuomotor co-ordination tasks have shown atypical and diffuse patterns of connectivity in associative, orbitofrontal, oculomotor and motor circuits in autistic participants, with inefficient connectivity occurring between the caudate nuclei and the cerebral cortex (Turner *et al.*, 2006). Abnormalities in mirror-neuron behaviour has also been observed in ASD (Oberman *et al.*, 2005; Dapretto *et al.*, 2006; Williams *et al.*, 2006). Although the aetiology of movement impairment is not well understood, the widespread nature of motor impairment in ASD is evident in the development of myriad motor interventions aimed at improving the symptoms of the disorder. The high proportion of motor interventions for ASD suggests that treatment of the motor impairment aspects of the disorder may be useful in targeting other ASD symptoms.

### 1.4 Diagnosis and Treatment of ASD

Despite the glaring evidence for a motor impairment component of ASD, motor impairment is not considered to be a key diagnostic feature for ASD. Due to the ubiquity of abnormal social and behavioural features, the standard method for diagnosis of ASD is observation of the child by an experienced clinician (Geschwind, 2009). In addition to the definitions set out in the DSM-5, other standardised screening tools have been developed. The Childhood Autism Rating Scale (CARS) and the Autism Behaviour Checklist (ABC) are two such tools that are not considered to require a trained clinician for administration. However, the fact that ASD is diagnosed by observation means that there is a subjectivity issue inherent in the process of diagnosis. While research is beginning to elucidate some of the pathophysiology behind ASD, we do not as of yet have a physical or physiological marker that can be reliably used to diagnose ASD in a living individual. The most consistent physiological indicators of ASD currently are results of neurological histology in autopsy (Geschwind, 2009).

In addition to the limitations in diagnostic criteria, the understanding that we currently have of ASD pathophysiology limits the development of effective pharmacotherapies for the disorder. The pharmacotherapies that currently exist for ASD target symptoms of the disorder rather than treating underlying physiology (Ji and Findling, 2015). Such pharmacotherapies are also associated with high levels of unfavourable side-effects and as such are not always considered appropriate treatments by parents and caregivers. The more commonly employed treatments are physical and behavioural regimens (Hess *et al.*, 2008). Physical and behavioural therapies are generally both more cost-effective and have fewer side-effects than

pharmacological therapies, and are therefore preferred by many individuals with ASD as well as their parents and caregivers. Physical exercise therapies for children with ASD have shown to associate with improvements in scores of motor proficiency as well as social behaviour (Casey *et al.*, 2015; Srinivasan *et al.*, 2015; Najafabadi *et al.*, 2018). Furthermore, interventions aimed at improving language have been found to be successful, particularly for expressive and composite language outcomes (Sandbank *et al.*, 2020). A review of naturalistic developmental behavioural interventions for ASD found that there is evidence that such interventions improve communication, language, play, and cognition (Crank *et al.*, 2021). However, many therapies employed in treating individuals with ASD have not been rigorously scientifically validated. Hess *et al.*, (2008) found that the treatment options most commonly used for ASD in Georgia, USA (behavioural strategies including but not limited to sensory integration techniques, cognitive behavioural modification and assistive technologies) lack strong scientific evidence for their effectiveness. Some of the sensory integration-based strategies deemed successful for treating ASD have only undergone scientific study with vanishingly small sample sizes (Lang *et al.*, 2012). Applied Behavioural Analysis (ABA)-based therapies are a mixed bag in terms of scientific validation, with some therapies having been well validated using large sample sizes and others that have not been well validated but show promise in various outcomes (Vismara and Rogers, 2010). ABA is usually practiced in a more individual-oriented way, with the treatment programme altered according to the individual's personal responses to the environment in which the treatment is applied. Although ABA shows promising outcomes when applied sufficiently early and intensively, it appears to only be helpful in about 50% of cases, with the other 50% of children in ABA-programs showing little to no progress (Vismara and Rogers, 2010). This indicates a possibility that specific individuals may respond better to specific therapies, and differences in response to therapies may be linked to symptom severity. There is currently a need to develop economical and effective therapies for ASD that are validated for improving the debilitating symptoms of the disorder.

### 1.5 Previous research on ASD in Africa

Research on ASD for the current thesis is situated in South Africa and as such needs to be contextualised. Most of our understanding of ASD comes from high income countries, but it is expected that a high proportion of people with ASD live in low/middle income countries (LMICs) (World Health Organization, 2011). In Africa there are few prevalence studies to date, however there may be higher rates of ASD in Africa than there are in high-income

countries, since there are higher rates of intellectual disability (de Vries, 2016). In a study of over one million school children in the Western Cape province of South Africa, 0.08% are diagnosed with ASD (Pillay, Duncan and de Vries, 2021). It is important to note that this prevalence co-occurs with a bias in race and language, suggesting that some communities may not have access to a diagnosis for cultural or socioeconomic reasons.

Previous research on ASD in South Africa specifically has found that the demographics of families with an ASD child reflect the proportional demographics of the country as a whole, specifically with regards to population group, nationality, household size, household income and marital status (Erasmus, Kritzinger and van der Linde, 2022). Families receive limited governmental support and are under a high financial burden to pay for school fees, transport, medical insurance and extra-curricular therapies (Erasmus, Kritzinger and van der Linde, 2022). On average, caregivers in South Africa notice developmental delay in their ASD children at around 2 years of age, with nationality and qualification levels of the parents associating with later intervention (Erasmus, Kritzinger and Van der Linde, 2021). Importantly, low awareness of ASD is an underlying factor that leads to later diagnosis and therefore, later intervention (Erasmus, Kritzinger and Van der Linde, 2021). Autism-specific public schools contribute significantly to the education of ASD children in South Africa, but ASD children attending autism-specific private schools are likely to get diagnosed earlier and to begin school earlier (Van der Linde, Erasmus and Kritzinger, 2019). Caregivers of ASD children in South Africa experience high levels of stress (Simelane, 2020) as well as frustration with the limitations in treatment options and improvement attained thereby (Mthombeni and Nwoye, 2017). While these studies have provided some insights into the sociocultural aspects of the disorder in South Africa, there remains very little research connecting such findings to the biomedical side of ASD in this context.

Systematic research on the autistic population in Africa in general is lacking, which is a barrier to adequate provision of support to the African autistic community (Abubakar, Ssewanyana and Newton, 2016; Bakare *et al.*, 2022). Research shows that in sub-Saharan Africa there is a low level of professional and general knowledge about ASD as a disorder, and a need for improved education and training in this area (Franz *et al.*, 2017). Importantly, we lack standardised and culturally-validated phenotyping tools for ASD (de Vries, 2016). Tools that can provide a shared language to describe ASD in South Africa, but without the financial and proprietary barriers of the established tools (such as the Autism Diagnostic

Observation Schedule (ADOS-2) and the Autism Diagnostic Interview, Revised (ADI-R)) are required for the African setting but need to be systematised and validated. Springer *et al.* (2013) found racial differences in verbal ability amongst a cohort of autistic children in South Africa, but without tools validated for the African setting we cannot conclude that ASD in Africa is phenotypically different from ASD elsewhere. Indeed, it is likely that the differences observed thus far may be due to cultural, language or socioeconomic variables that affect the detection and presentation of ASD in Africa. For example, culturally-specific expectations of child development and behaviour (such as responsiveness to adults, use of eye contact and initiation of communication) affect the perception and thus diagnosis of developmental delays in children (Grinker *et al.*, 2012).

Another important variable to consider is that of communicable infectious disease as a precipitating or contributory agent to the development of ASD, the role of which has not been well-researched in Africa as of yet (Franz *et al.*, 2017). Previously, research in LMICs has focused on communicable diseases (such as malaria and HIV) and as such there is a lack of research on non-communicable conditions such as ASD (Bakare and Munir, 2011; de Vries, 2016; Franz *et al.*, 2017). Overall, research on ASD in Africa contains some methodological weaknesses, such as small sample sizes (Franz *et al.*, 2017). Almost all clinical data on ASD in Africa comes from clinical centres with a biased patient population (Bakare and Munir, 2011; de Vries, 2016; Franz *et al.*, 2017), and the small number of studies looking at a wide range of interventions means that none of the interventions has a sufficient evidence base for reliability (Franz *et al.*, 2017). There is thus a need for biomedical research on ASD in Africa in general, and South Africa more specifically. Data on the characteristics of the ASD population as well as on interventions that have the potential to assist the population is needed. Importantly, the sociocultural context of such research needs to be carefully considered and navigated in an ethical way.

# Chapter 2: The Role of the Brain-Immune Axis in ASD

Given the lack of biomedical research on ASD in Africa, further investigations into the disorder are required. As mentioned previously, the pathophysiology of ASD is not currently fully understood. ASD is an incredibly complex disorder, with both inherited and environmental factors contributing to its aetiology (Goyal and Miyan, 2014). While many genes have been found to associate with ASD (Chen *et al.*, 2015), the roles of only a few of these genes in the pathogenesis of ASD are understood. In some cases the ASD genetic impact seems to be cumulative or co-operative, and Chen *et al.* (2015) suggest that convergence in pathogenic mechanisms is at the transcriptomic level. It is thus likely that, despite the significant role that genetics may play in ASD, common pathways of pathogenesis are more likely to be found on a biochemical than a genetic level. Recent research has begun to elucidate connections between dysregulations in immune and inflammatory function, and the progression of various neuropsychiatric disorders, such as bipolar disorder, post-traumatic stress disorder, major depressive disorder and schizophrenia (Heim, Ehlert and Hellhammer, 2000; Rosenblat *et al.*, 2014). Some movement disorders have also been found to correspond with immune dysfunction, particularly the presence of autoantibodies (Singer, 2017). It is thus not surprising that emerging research in ASD has found strong connections between the disorder and various dysregulations in the immune system and inflammatory responses (Meltzer and Van de Water, 2017). Interestingly, specific measures of immune function have been found to correlate with specific characteristics of ASD (Meltzer and Van de Water, 2017). Increased inflammatory cytokines in mid-gestational mothers increases the chances of the child being diagnosed as ASD with intellectual disability (Jones *et al.*, 2017), and higher levels of autoantibodies in an ASD individual associate with increased brain volume (Nordahl *et al.*, 2013) and decreased cognitive functioning (Goines *et al.*, 2011*a*). Children with ASD have also been found to have higher levels of advanced glycation end-products in their urine, which may relate to imbalances in inflammatory processes (Anwar *et al.*, 2018). Moreover, the well-established link between the stress hormone cortisol and the immune system raises the question of what role cortisol may play in these pathways. Importantly, the African ASD population is sorely underrepresented in studies focusing on the biological determinants of the disorder.

This section focuses on the aetiology of ASD, and will begin with an overview of the immune system and the axes of interaction between the brain and the immune system, providing background for a review of the immunological dysregulation evident in ASD. The use of motor interventions for treating ASD is then discussed, followed by an investigation of the literature on the role of exercise interventions in regulating immune function. Chapter 2 ends with an explanation of the rationale behind the studies undertaken in this thesis and a list of the specific aims of those studies.

**2.1 Overview of the immune system**

As noted, extant ASD research has documented deficits in autistic immune systems (Ashwood and Van de Water, 2004; Meltzer and Van de Water, 2017) (detailed in section 2.3). The role of the mammalian immune system is to protect the organism from harmful pathogens while limiting damage both to self-tissues as well as to host-beneficial microbes (Chaplin, 2010). The immune system consists of physical barriers and constitutive elements of the body (such as the skin and the mucous membranes) as well as specialised cells of lymphoid and myeloid origin. There are two major immune response systems: the innate response and the adaptive response. The innate immune response consists of hard-wired responses encoded by germ-line genes to recognize molecular patterns in microbes that are not in the mammalian host (Chaplin, 2010). The hard-wired nature of the innate immune system means that it can act rapidly and therefore constitutes the first response to an infection. Additionally, physical barriers such as tight cell-cell contacts, the antimicrobial-rich mucous layer that protects epithelial cells in many organs, as well as epithelial cilia are integral to the innate immune system (Chaplin, 2010).

The adaptive immune system, on the other hand, comprises antigen-specific mechanisms and immunological memory (Dranoff, 2004). The adaptive immune system communicates with the innate system to activate T-cells and B-cells via their antigen-specific receptors. These receptors are assembled by somatic rearrangement of genetically-encoded elements to form the highly specific T-cell receptors (TCR) and immunoglobulins (Ig) (Chaplin, 2010). The small numbers of cells maintained that recognize specific pathogens means that the adaptive response requires time for these cells to proliferate before they can act to protect the host. However, due to maintaining cells that recognize specific pathogens, the cells of the adaptive response also retain memory of previous encounters with pathogens. Components of the innate system contribute to the activation of the adaptive system, while the adaptive system

recruits mechanisms of the innate system to assist in fighting off microbes identified by the adaptive system as foreign (Chaplin, 2010). Thus, the two major immune response systems complement and co-operate with one another.

The two arms of the immune system (innate and adaptive) require a system of communication to ensure smooth interactions. In the immune system communication is achieved by specific messenger molecules which are released from and act on immune cells in a variety of ways. Cytokines are a group of such intercellular messenger molecules which act on cells via transmembrane cell surface receptors (Chaplin, 2010). Cytokines are important regulators of immune function as they determine the activation and maturation of immune cells (Benoit, Desnues and Mege, 2008), and alterations in cytokine patterns have been observed in ASD (Croonenberghs *et al.*, 2002; Vargas *et al.*, 2005; Molloy *et al.*, 2006; Ponzio *et al.*, 2007; Li *et al.*, 2009; Ashwood *et al.*, 2011*a*; Krakowiak *et al.*, 2017) (detailed in section 2.3). Alternative macrophages are induced by IL-4, IL-10, IL-13 (Gordon, 2003). Interleukin (IL)-12, tumour necrosis factor (TNF)-α and interferon (IFN)-γ are the major pro-inflammatory cytokines and stimulate the functional activity of T-cells, NK cells and activated macrophages (cellular immunity) as well as nitric oxide (NO) synthesis and other inflammatory mediators that drive chronic delayed-type inflammatory responses (Elenkov *et al.*, 2000). IL-4 and IL-10 are the major anti-inflammatory cytokines and stimulate humoral immunity via activation of mast cells and eosinophils and the differentiation of B-cells to the antibody-secreting type. These anti-inflammatory cytokines also inhibit macrophage activity, T-cell proliferation and the production of pro-inflammatory cytokines (Abbas, Murphy and Sher, 1996; Fearon and Locksley, 1996; Mosmann and Sad, 1996).

A critical feature of immune system functioning and communication is discrimination between self and non-self cells and molecules, in order to limit damage to self-tissues (Chaplin, 2010). Therefore, mechanisms exist for the innate immune system to present invasive pathogens to the adaptive immune system for elimination. Major histocompatibility complex (MHC) molecules, also known as human leukocyte associated (HLA) antigens are cell surface glycoproteins determined by the genetic makeup of an individual. Alterations in specific MHC molecules have been observed in ASD (Warren *et al.*, 1986; Warren *et al.*, 1991; Warren, *et al.* 1996) (detailed in section 2.3). MHCs bind peptide fragments that have either been synthesized within the cell (class I) or that have been ingested by the cell and proteolytically digested (class II) (Chaplin, 2010). The MHC forms a complex with the

antigenic peptide, and the whole complex is then expressed on the surface of the antigen-presenting cell (APC), anchored into the extracellular surface of the cell membrane. This complex is the binding target of the T-cell receptor (TCR), and a specific TCR will bind to an MHC complex containing a unique peptide (Zinkernagel and Doherty, 1997).

While the immune system must recognize pathogenic cells, many invasive pathogens are intracellular in nature and the immune system must therefore be able to recognise infected cells as well. T-cells are particularly important in the recognition of infected cells. T-cells develop in the bone marrow and then migrate to the thymus where they form their MHCs and undergo maturation to different subpopulations, becoming either CD4+ helper cells, CD8+ cytotoxic cells or regulatory T-cells/Tregs (Parkin and Cohen, 2001). CD8+ T-cells act to kill cells infected with intracellular microbes and tumour cells, while CD4+ T-cells act to regulate the cellular and humoral immune responses. A portion of CD8+ cells are not cytotoxic but regulatory (Tregs), and act to suppress immune responses. Tregs are CD4+ cells that modulate immune responses. The natural Tregs (CD25+) secrete TGF-β and IL-10 (Sakaguchi *et al.*, 2006). Adaptive or induced Tregs (which develop their regulatory function in the periphery rather than in the thymus) develop in the presence of IL-10, and also secrete IL-10 and transforming growth factor (TGF)-β. Tregs suppress the activation and proliferation of effector T-cells (cytotoxic and helper T-cells) and help to maintain tolerance to self-antigens (Bettelli *et al.*, 2006; Campbell and Turner, 2018).

T-cells that become helper T-cells undergo a second functional differentiation following exposure to an antigen. There is the possibility of dysregulation of this differentiation process of helper T-cells in ASD, as evidenced by alterations in systemic cytokine levels (Croonenberghs *et al.*, 2002; Vargas *et al.*, 2005; Molloy *et al.*, 2006; Ponzio *et al.*, 2007; Li *et al.*, 2009; Ashwood *et al.*, 2011*b*; Krakowiak *et al.*, 2017) (detailed in section 2.3). Resting naïve CD4+ T-cells release low levels of cytokines. After stimulation by antigen and APC they secrete IL-2 and are designated as Th0. IL-2 is an important co-stimulatory molecule in T-cell proliferation (Elenkov *et al.*, 2000). T-cells then differentiate into sub-types, primarily Th1 or Th2, but also Th17, and cytokines produced by innate cells are the most important factor for determining this differentiation (Fearon and Locksley, 1996). The cytokines present in the environment of the maturing T-cell polarise the cell into one of the subtypes (Sallusto and Lanzavecchia, 2009) and each T-cell subtype secretes a signature pattern of cytokines (Table 2.1). The Th1 and Th2 systems often work together, but immune responses can easily

become polarised in such a way that either Th1 or Th2 dominates, as Th1 and Th2 are mutually inhibitory: IL-12 and IFN-γ inhibit Th2, while IL-4 and IL-10 inhibit Th1 (Elenkov *et al.*, 2000). This polarisation is sometimes functionally necessary but can also become pathogenic. Generally, Th1 cells support the cell-mediated immune responses while Th2 cells support humoral and allergic responses (Ernst *et al.*, 1999). Th-1 dominance is involved in organ-specific autoimmunity, kidney allograft rejection, Crohn's disease and unexplained recurrent abortions. In many autoimmune diseases there is a skew towards Th1 dominance, with increased levels of IL-12 and TNF-α (Wilder, 1995; Mosmann and Sad, 1996; Elenkov, Hoffman and Wilder, 1997). Th-2 dominance is involved in allergen-specific atopic disorders as well as other disorders such as systemic sclerosis and fibrosing alveolitis (Romagnani, 2000).

Table 2.1: Different T-cell types (Information from Mosmann & Sad (1996); Fearon & Locksley (1996); Abbas et al. (1996) and Chaplin (2010))

| *T-cell type* | *Induced by* | *Secretes* | *Functions* |
|---|---|---|---|
| *Th1* | IL-12 from NK cells | IL-2, IFN-γ, TNF | Cell-mediated immunity<br>Phagocyte-dependent inflammation |
| *Th2* | IL-4 from NK-T cells, basophils or mast cells | IL-4, IL-5, IL-9, IL-10, IL-13, GM-CSF | Antibody responses<br>Eosinophil accumulation<br>Inhibition of phagocytosis |
| *Th17* | TGF-β and IL-6 | IL-6 and IL-17 | Early adaptive response<br>Recruitment of neutrophils for extracellular bacteria |

Proper T-cell functioning is critical for preserving self-tolerance in the immune system. These mechanisms are relevant in ASD as it has been suggested that ASD is an autoimmune disorder (Ashwood and Van de Water, 2004). Autoimmune diseases appear to occur when there is a breakdown in self-tolerance, inducing activation of humoral and cellular immunity against self-tissues. These pathological responses usually show either a Th1 or a Th17 dominance, suggesting a potential mechanism for the pathology in T-cell differentiation or activation (Annunziato *et al.*, 2009). Atopic diseases (inappropriate IgE-mediated immune responses) on the other hand appear to associate with an overly aggressive Th2 response, leading to hypersensitivity to normally occurring environmental antigens. The central role of

Tregs in regulating the CD4+ T-cell response implies a crucial role for Tregs in the development of both autoimmune and atopic diseases. However, autoimmune diseases and atopic diseases do not represent over-polarisations of the T-cell response generally, as patients with autoimmune disease are more likely to also have atopic disorders, suggesting a common underlying aetiology (Simpson *et al.*, 2002). Activation of the mother's immune system during pregnancy may play a central role in over-polarisations of the child's immune system, as evidenced by various studies on the maternal immune activation (MIA) model (Shi *et al.*, 2003; Shi *et al.*, 2009; Smith *et al.*, 2007; Malkova *et al.*, 2012; Bauman *et al.*, 2014; Meltzer and Van de Water, 2017) (detailed in section 2.3). During pregnancy, the maternal immune response is normally suppressed against the semi-allogeneic foetus and placenta. Mid-gestation human foetuses have higher than normal levels of Treg cells and TGF-β (Burlingham, 2009), and show poor lymphocyte activation (Mold *et al.*, 2008). High levels of IL-10 appear to be important for maintaining tolerance of the foetus (Li and Guo, 2009).

**2.2 The brain-immune axis**

Given the complex nature of the immune system and its capacity to cause damage to self-tissues while performing its function, the immune system must be regulated by other systems in the body. The brain-immune axis describes the interactions between neuronal and endocrine messengers, and the immune system. There is significant interaction between the brain and the immune system in a way that affects neural processes and cognition, which is of relevance in the aetiology of ASD (Onore, Careaga and Ashwood, 2012; Goyal and Miyan, 2014). In animal models, depression behaviours have been shown to associate with proinflammatory cytokines (Grippo *et al.*, 2005; Hodes *et al.*, 2014), and cytokines have also been implicated in sleep regulation (Kapsimalis *et al.*, 2005; Krueger, Rector and Churchill, 2007). IL-1β, TNF and lipopolysaccharide have all been shown to promote proinflammatory genes in the brain (van Dam *et al.*, 1992; Layé *et al.*, 1994; Quan *et al.*, 1999) which induce a group of behaviours (social withdrawal, loss of appetite, decreased motor activity and cognitive deficits) known as "sickness behaviour" (Dantzer *et al.*, 2008). Although brain endothelial cells can produce and secrete cytokines (Verma *et al.*, 2006), these responses are also relevant in the cases of peripheral immune responses as it has been shown that cytokines cross the blood-brain barrier and act on astrocytes, neurons and microglia (Banks, Kastin and Gutierrez, 1994; Banks, Kastin and Broadwell, 1995; Hodes *et al.*, 2015). Immune responses that cause increases in cytokines in the brain may therefore be of relevance in the behavioural aspects of ASD.

The brain and the immune system therefore modulate one another through a system of "functionally relevant cross-talk" (Elenkov *et al.*, 2000). The cross-talk occurs via two pathways: direct neural influence from the autonomic nervous system, and humoral neuroendocrine messages from the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis and the sympathetic branch of the autonomic nervous system (SNS) constitute the peripheral limbs of the "stress system" first described by Selye (1956), and both are important for the maintenance of homeostasis. Activation of the stress system results in a redistribution and activation of immune cells in anticipation of tissue damage and infection. The stressful trigger need not be physical and may even be social, such as in the cases of conflict, rejection and exclusion (Zefferino, Di Gioia and Conese, 2021). Importantly, low socioeconomic status and concomitant stressors are likely to present with a higher incidence of both physiological and psychological stressors which impact the amygdala in the limbic system. The amygdala triggers the endocrine component of the stress system primarily via activation of the HPA axis. The amygdala provides stimulatory input to the HPA axis (Herman and Cullinan, 1997), activating the endocrine component of the stress system in response to perceived threat or fear. Initiation of the HPA axis occurs with the secretion of corticotropin releasing hormone (CRH) from the hypothalamus into portal blood vessels leading to the pituitary gland. The pituitary is then triggered to release adrenocorticotropic hormone (ACTH) into the systemic blood stream. ACTH stimulates the adrenal cortex to then secrete cortisol into the circulation. The HPA axis is regulated by a negative feedback loop, in which cortisol inhibits HPA activation at the hypothalamus, pituitary and hippocampus (Nicolson, 2008). Adrenal glucocorticoids (GCs) and other HPA end-products are generally immune-suppressive and anti-inflammatory in acute responses.

Cortisol is the human GC and is known as the "stress hormone", as it increases in response to alarm states. The enzyme 11β-hydroxysteroid dehydrogenase regulates cortisol levels in humans by converting cortisone to cortisol (Tomlinson *et al.*, 2004). Cortisol secretion follows a circadian rhythm, in which it is lowest around midnight, peaks in the morning and then decreases throughout the day in a pattern referred to as the diurnal slope. Cortisol regulates many organ functions via this rhythm (see Figure 2.1), and the rhythm itself is regulated by input from the suprachiasmatic nucleus (Debono *et al.*, 2009). Interference with the circadian rhythm of cortisol is associated with disease states including adrenal insufficiency (Chan and Debono, 2010) and cancer (Sulli, Lam and Panda, 2019). Flatter

diurnal cortisol slopes in particular are associated with poorer health, with a large effect size for immune and inflammatory outcomes (Adam *et al.*, 2017). Importantly, evidence for alterations in cortisol's circadian rhythm has been observed in ASD (Taylor and Corbett, 2014). Normally, GCs bind the GC receptor and thereby affect protein synthesis. Binding to GC response elements upregulates transcription of immune and metabolic proteins, while interactions with transcription factors result in down-regulation of pro-inflammatory and immunosuppressive proteins (Argentieri *et al.*, 2017). Cortisol is the major regulator of the immune system, controlling inflammation by inducing apoptosis in monocytes, macrophages and T-cells under homeostatic conditions (Amsterdam, Tajima and Sasson, 2002). In a review, strong evidence was found for relationships between increased cortisol and cognitive impairment, depression and hippocampal atrophy (Brown, Varghese and McEwen, 2004), which begs the question of the involvement of cortisol in autistic cognitive symptoms. Cortisol leads to decreased IL-1β, and IL-1β is significant in inflammatory processes in the brain (Zefferino, Di Gioia and Conese, 2021), which suggests a potential mechanistic link between cortisol abnormalities and changes in brain structure and function. In cases of prolonged stress, the body may enter into a state of "allostatic overload" (McEwen, 2008), in which the body no longer responds to the stress system in the normal homeostatic ways. The effects of acute and chronic stress differ, and while acute stress promotes adaptive immunity, chronic stress leads to sustained inflammation and leukocytosis (Zefferino, Di Gioia and Conese, 2021). In acute stress, cortisol increases then decreases. However, in chronic stress there is a loss of cortisol's circadian rhythm and the induction of GC resistance in which the GC receptor loses sensitivity to stimulation by cortisol. HPA hyperactivity and GC resistance may form the mechanistic link between chronic stress and the development of major depressive disorder (Brown, Varghese and McEwen, 2004; Ménard *et al.*, 2017). Chronic activation of the HPA axis can cause an increase in inflammation as a result of GC resistance (Schleimer, 1993; Avitsur, Stark and Sheridan, 2001; Miller, Cohen and Ritchey, 2002) and chronic stress therefore results in prolonged inflammation (Cohen *et al.*, 2012) which affects mental and physical health (McEwen, 1998; McEwen, 2008; McEwen and Seeman, 1999; Marques, Silverman and Sternberg, 2009). In a state of GC resistance, cortisol polarizes T-cells to the Th2 subtype, which increases susceptibility to infection, autoimmune diseases and cancer (Elenkov and Chrousos, 2002).

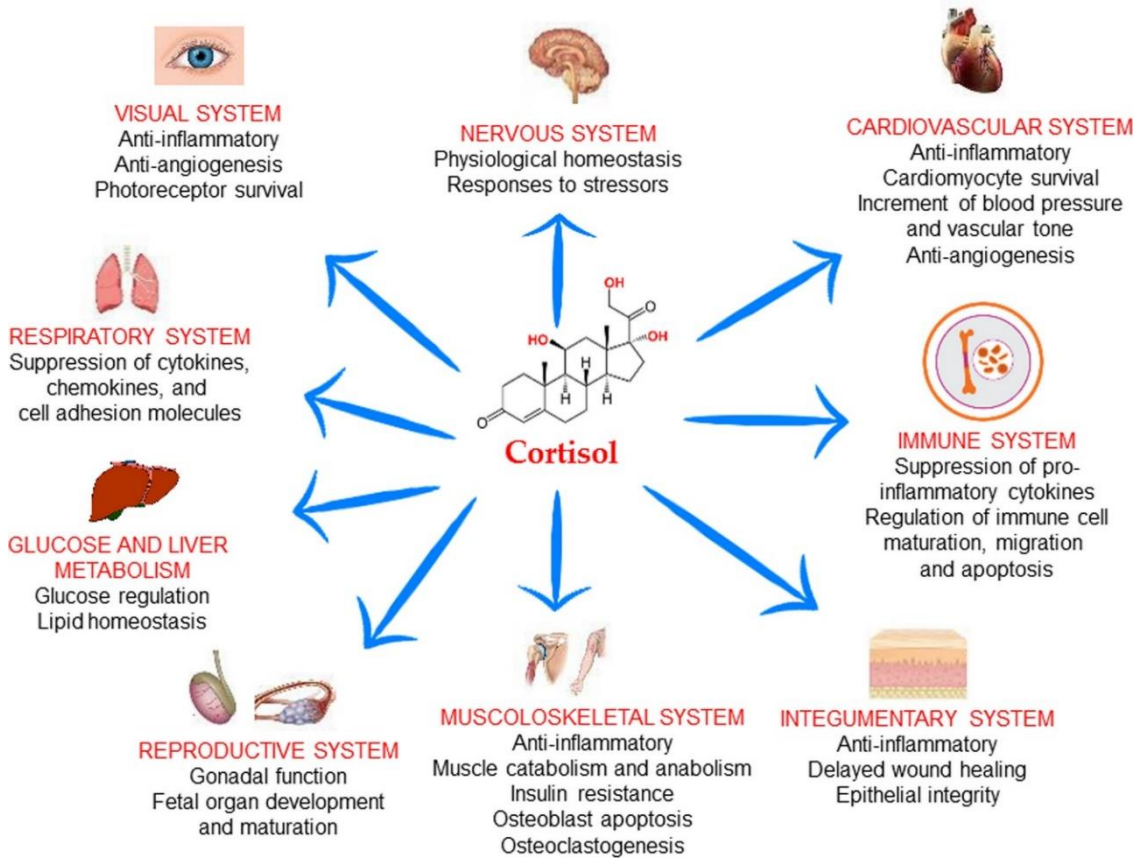


Figure 2.1: The physiological effects of cortisol (Zefferino, Di Gioia and Conese, 2021), reproduced with permission (see Appendix B)

The other branch of the stress system, the neural branch, is the SNS. The SNS is one of three branches of the autonomic nervous system and is the most important for regulating the immune system. Autonomic dysfunction has also been documented in ASD (Goyal and Miyan, 2014). Catecholamines are the end-point signalling molecules of the sympathetic nervous system, consisting of noradrenaline (NA) which is synthesized from dopamine and released primarily synaptically, and adrenaline which is converted from NA in the adrenal medulla and released into the blood stream. The SNS innervates all lymphoid organs (Tonkoff, 1899) and catecholamines modulate many aspects of the immune system. Sympathetic nerve terminals release NA as a neurotransmitter, which occurs when an electrical signal reaches the axon terminal and triggers depolarisation. Immune cells are influenced by locally released NA or circulating adrenaline. For example, injection of adrenaline causes a pronounced leukocytosis in humans (Loeper and Crouzon, 1904).

As a hormone, cortisol has longer-term effects on the physiological systems of the body, whereas the SNS is responsible for more immediate stress responses. Lymphoid organs (the

thymus, spleen, lymph nodes, tonsils, bone marrow and gut- and bronchus-associated lymphoid tissues) receive predominantly sympathetic innervation (Elenkov *et al.*, 2000). Noradrenergic innervation of the lymphoid organs precedes the development of the immune system and NA may therefore be involved in the maturation of the immune system (Elenkov *et al.*, 2000). Immunocytes have receptors to NA (Benesics *et al.*, 1997) and the most common NA receptor is the β-adrenoreceptor, with virtually all lymphoid cells (with the exception of Th2 cells) expressing them (Elenkov *et al.*, 2000). The differential expression of β-adrenoreceptors between the Th1 and Th2 cells facilitates the differential effects of catecholamines on immune function (Sanders *et al.*, 1997). Catecholamines selectively inhibit Th1 functions and favour Th2 functions rather than enacting a general immunosuppression. Activation of the β2-adrenoreceptors on immune cells by catecholamines leads to decreased production of IL-2, TNF-α and IL-12 but increased production of IL-10 (Elenkov *et al.*, 2000). Additionally, catecholamines inhibit APCs and the production of cytokines by Th1 cells, and thereby potentiate the production of type 2 cytokines by Th2 cells, while inhibiting the Th1 response (Elenkov *et al.*, 2000).

While a stress response causes activation of the SNS and HPA axis, which impact immune functionality, the system is non-linear and composed of multiple interacting factors (McEwen, 2008), evidenced by the effects of the immune response feeding back to the SNS and HPA. Immune responses lead to increased concentrations of corticosteroids in the plasma, alteration of the activity of hypothalamic noradrenergic neurons and decreased concentrations of NA in the spleen (Besedovsky *et al.*, 1975; Besedovsky, *et al.* 1979; Besedovsky, *et al.* 1983; Besedovsky, *et al.* 1986). Furthermore, during an immune response, cytokines such as IL-1, IL-6 and TNF-α can signal the brain, triggering SNS and HPA activation (Besedovsky *et al.*, 1986; Elenkov *et al.*, 2000). The SNS and HPA respond together by releasing NA and cortisol, which then results in decreased IFN-γ and increased IL-1, TNF and IL-6 (Cole *et al.*, 1998; Cole, *et al.* 2010; Lee *et al.*, 2000), and thereby an increase in inflammatory activity (Grebe *et al.*, 2010). The relationship between the stress system and the immune system is therefore complex and multi-factorial, and involves regulatory feedback mechanisms. Importantly, while catecholamines and GCs appear to be generally immunosuppressive, they influence the immune system in more complex ways, exerting both pro- and anti-inflammatory effects (Karalis *et al.*, 1991; Chrousos, 1995). In some cases, the immune response to stress may even be involved in causing inflammatory diseases (Slavich and Irwin, 2014). Evidently, the relationship between the brain and the

immune system, as mediated by the two arms of the stress system, is highly complex and bi-directional.

### 2.3 Immune dysregulation in ASD

Given the close relationship between the functioning of the brain and the immune system, it is not altogether surprising that there is significant evidence of an immune dysregulation component of ASD. There is evidence for altered functioning of immune cells, dysregulation of cytokine and chemokine cascades and the presence of autoantibodies specific to neuronal proteins in individuals with ASD (discussed in detail below). It has been suggested that in light of the substantial evidence for immune dysregulation in ASD, the condition should be considered to be an autoimmune disorder (Ashwood and Van de Water, 2004). Larger brain volume has been observed in ASD children (Courchesne *et al.*, 2007; Schumann *et al.*, 2010), and the disorder is also associated with neuroanatomical changes in both grey and white matter (including in the amygdala, hippocampus, precuneus, and the arcuate and uncinate fasciculi) (Bourgeron, 2015; Eissa *et al.*, 2018; Mei *et al.*, 2020). Post-mortem studies have shown signs of persistent neuroinflammation and alteration of dendritic spines (Hutsler and Zhang, 2010), which indicates a crucial role for the immune system in the observed neuroanatomical alterations. Croonenberghs *et al.* (2002) argue that products of the inflammatory response system (IRS) may be associated with some of the behavioural symptoms of ASD, such as social withdrawal, resistance to novelty and sleep disorders. This is comparable with the behavioural changes observed in "sickness behaviour", wherein elevated levels of IFN-γ, IL-1 and IL-6 are associated with sleep disturbance, social withdrawal, social/locomotor/exploratory suppression and anhedonia (Dantzer *et al.*, 1998; Linthorst and Reul, 1998). Indeed, inflammation and immune dysregulation have been found in other psychiatric and neurological conditions, including bipolar and PTSD (Jones and Thomsen, 2013) as well as schizophrenia (Brown *et al.*, 2004; Patterson, 2009; Michel, Schmidt and Mirnics, 2012). Further investigation of the role of the immune system in the pathogenesis of ASD may prove helpful in the diagnosis and treatment of the condition. Meltzer & Van de Water (2017) suggest that markers of immune function present the possibility of acting as biomarkers for ASD. Targeted immunotherapies, as well as holistic interventions that affect overall immune regulation (such as exercise) have the potential to be effective in treating the debilitating symptoms of ASD. All of the ASD-specific research discussed below was conducted in humans, unless specified otherwise.

Various immune-related gene variants have been found to associate with ASD, including variants of MHC genes (Warren *et al.*, 1990; Warren *et al.* 1995). Relationships have repeatedly been found between ASD and variants of the class II DRB1 gene specifically (i.e. the gene that affects the formation of the beta chain of the class II HLA) (Warren *et al.*, 1995; Chien *et al.*, 2012; Torres, Westover and Rosenspire, 2012; Mostafa, Shehab and Al-Ayadhi, 2013; Al-Hakbany, Awadallah and Al-Ayadhi, 2014). A particular 14 base-pair insertion on the HLA-G gene has also been found in mothers of ASD children (Guerini *et al.*, 2015). HLA-G is a non-canonical class I MHC which interacts with NK cells as part of the innate branch of the immune system. It is expressed in placental tissues and involved in immune tolerance during pregnancy (Carosella *et al.*, 2008). Additionally, a variant in the promoter of the MET oncogene has been found to associate with ASD incidence (Campbell *et al.*, 2006). The MET oncogene encodes a receptor tyrosine kinase that is critical for neuronal migration during cerebellar and cortical development and functions as a negative immune regulator. The MET promoter variant associated with ASD has also been found to have a relationship with the presence of maternal autoantibodies and a decrease in maternal IL-10 (Heuer *et al.*, 2011). In analysis of gene expression in the brains of people with ASD, evidence has been found for upregulation of immune and inflammatory response functions, Ig domains and other immune regulatory aspects including those that regulate astrocyte and microglia function (Voineagu *et al.*, 2011). Furthermore, a network designed to assess the interactome of genes associated with ASD (AutDB) suggests a central role for the immune system in ASD. This central role specifically implicates cytokine signalling, which may be the point of convergence of various genetic and environmental dysregulations that all cause ASD (Basu, Kollu and Banerjee-Basu, 2009). Epigenetic changes have also been found to associate with ASD, specifically methylation of genes involved in microglial cell specification and synaptic pruning during brain development (De Rubeis *et al.*, 2014; Nardone *et al.*, 2017). A review of genome-wide expression studies found a strong connection between ASD and the immune system, with immune dysregulation particularly affecting NK cells and cytokines (Lintas, Sacco and Persico, 2012). The authors proposed a model of dysregulation of the immune system that leads to increased metabolism and thus increased reactive oxygen species in the pre- and post-natal environment, which would cause changes to neural proliferation, migration, wiring and synapse formation.

In terms of alterations to immune cell function, both increased lymphocyte responsiveness (Ferrari *et al.*, 2013) and decreased lymphocyte responsiveness (Stubbs *et al.*, 1977) have

been observed in ASD. Decreased lymphoproliferative responses have been observed in ASD (Warren *et al.*, 1986) as well as decreased IL-2R on lymphocytes (Denney, Frei and Gaffney, 1996). Additionally, incomplete or partial T-cell activation (Plioplys *et al.*, 1994; Warren *et al.*, 1995), decreased numbers of T-cells and a selective decrease in CD4+ T cells (Warren *et al.*, 1990; Denney, Frei and Gaffney, 1996) have also been observed in ASD. Importantly, decreased NK cell activity in ASD has been observed (Warren, Foster and Margaretten, 1987), but no decrease in NK cell count which suggests the cells themselves are dysfunctional (DeLisi *et al.*, 1983). Increased expression of NK cytotoxicity genes has also been found to associate with ASD (Enstrom *et al.*, 2009). High plasma neopterin and monocyte counts in children with ASD have also been observed (Sweeten, Posey and McDougle, 2003). Neopterin has also been found to be elevated in the urine of children with ASD as compared with controls (Harrison and Pheasant, 1995; Messahel *et al.*, 1998). Neopterin is a pteridine that is produced by monocytes and macrophages when they are activated by IFN-γ. Increased levels of neopterin in the urine indicate Th1 dominance and heightened cellular immune activity, as well as increased levels of oxidative stress (Hamerlinck, 1999; Murr *et al.*, 2002). Evaluation of neopterin levels may provide further detail of immune system activation in ASD.

Cytokine disruption has been shown to associate with neurodevelopmental defects including ASD (Boulanger and Shatz, 2004; Brown *et al.*, 2004; Ponzio *et al.*, 2007; Deverman and Patterson, 2009; Hsiao *et al.*, 2013). Meltzer & Van de Water (2017) point out some disagreement in evidence for whether the cytokine and chemokine dysregulation present in ASD is skewed towards a pro-inflammatory Th1 response or an anti-inflammatory Th2 response. A couple of studies have found evidence of a Th2 dominance, with increased IL-4, IL-5 and IL-13 (Molloy *et al.*, 2006; Krakowiak *et al.*, 2017). However, a greater number of studies have found evidence of a Th1 dominance, with increased IL-1RA, IFN-γ, IL-8, IL-6, TNF-α and GM-CSF (Croonenberghs *et al.*, 2002; Vargas *et al.*, 2005; Ponzio *et al.*, 2007; Li *et al.*, 2009; Ashwood *et al.*, 2011*a*). One study found an association between Th1 response and the severity of impairment, while Th2 response associated with increased cognitive and adaptive functioning (Ashwood *et al.*, 2011*b*). Another study found elevated plasma levels of IL-1β, IL-6, IL-17, IL-12p40 and TNF-α in 3-9 year-olds with ASD, and that the levels of cytokines correlated with the level of impairment to communication and behaviour (DiSabato, Quan and Godbout, 2016). Increased TNF-α has been found to associate with disrupted melatonin release and sleep dysfunction in ASD (da Silveira Cruz-Machado *et al.*,

2021). Increased TGF-β1, MCP-1, IL-6 have been found in the middle frontal gyrus of ASD brains, while increased IL-10 was observed in the anterior cingulate gyrus and increased MCP in the cerebrospinal fluid (Vargas *et al.*, 2005). Increased IL-6 has been found in frontal cortices of individuals with ASD, with elevation of Th1-type cytokines in general (Li *et al.*, 2009), and increased IL-6 has also been observed in ASD cerebellums (Wei *et al.*, 2011). Additionally, increased IFN-γ (Stubbs, 1995; Singh, 1996), IL-12 (Singh, 1996), IL-2 and T8 antigen (Singh *et al.*, 1991) have been found in the plasma of autistic individuals, which once again implicate activation of the inflammatory response system and a role for neopterin as a potential biomarker.

Moreover, there is evidence that pre- or post-natal viral infections may also trigger the development of ASD (Singh *et al.*, 1993). Links have been observed between congenital rubella syndrome and incidence of ASD (Chess, 1971; Deykin & MacMahon, 1979). Other congenital infections have also shown relationships with ASD, including measles and mumps (Deykin & MacMahon, 1979), cytomegalovirus (Libbey *et al.*, 2005), polyomavirus (Lintas *et al.*, 2010) and influenza (Shi *et al.*, 2003; Zhang *et al.*, 2010; Atladóttir *et al.*, 2012). Additionally, maternal infection at hospital admission has been found to correlate with ASD diagnosis (Zerbo *et al.*, 2015), and risk of ASD has been found to increase with any hospitalisation of the mother in the year before pregnancy (Lee *et al.*, 2015). The Maternal Immune Activation (MIA) model suggests that activation of the immune system of the mother during pregnancy is responsible for immune system alterations in the child. Existing evidence on MIA models and infections relating to ASD suggest an underlying common aetiology involving the immune system (Meltzer and Van de Water, 2017). MIA models have been evaluated via infection of pregnant mice with either influenza or poly(I:C). These challenges to pregnant mice have been found to result in abnormal behaviour, exploration and social interaction in offspring, implicating the maternal immune response in the pathogenesis of these behavioural symptoms (Shi *et al.*, 2003; Shi *et al.*, 2009; Malkova *et al.*, 2012). The MIA model has also been evaluated in rhesus macaques, with poly(I:C) maternal activation leading to ASD-relevant phenotypes in the offspring (Bauman *et al.*, 2014), and ASD traits showing evidence of persisting epigenetically through the paternal line (Weber-Stadlbauer *et al.*, 2017). Interestingly, injection of IL-6 into pregnant mice has also been found to induce ASD-relevant behaviours in offspring (Smith *et al.*, 2007), suggesting that it may be the cytokine response to the immune challenge that mediates the pathogenesis of ASD. IL-6 leads to production of IL-17, and it is therefore possible that the Th17 pathway may be

particularly relevant in the pathogenesis of ASD. MIA models using injection of IL-6 cause increases in maternal TNF-α, IFN-β and IL-1β but not IL-10 (Choi *et al.*, 2016). Importantly, maternal IL-10 may play a role in attenuating the impact of immune activation on the child (Meyer *et al.*, 2008). Compellingly, cytokine dysregulation during human pregnancy has also been found to associate with risk of ASD diagnosis in the child. Increased IFN-γ, IL-4 and IL-5 in mid-gestational mothers (Goines *et al.*, 2011*b*), increased MCP-1, IL-4, TNF-α and TNF-β in amniotic fluid (Abdallah *et al.*, 2012; Abdallah *et al.*, 2013), and increased GM-CSF, TNF-α, IFN-γ, IL-1α, IL-1β, IL-4 and IL-6 in plasma during mid-gestation have all been found to associate with ASD diagnosis of the child (Jones *et al.*, 2017). Abdallah *et al.* (2013) suggest three possible pathways of a cytokine response during MIA leading to ASD: (1) a maternal pathway, in which cytokines cross the placenta; (2) a placental pathway, in which the immune response in the mother leads to a reaction in which cytokines are produced by the placenta; (3) a foetal pathway, in which immune and gene dysregulation is stimulated inside the foetus by the MIA during pregnancy.

Additionally, autoantibodies to neuronal proteins have been found both in individuals with ASD as well as their mothers (Dalton *et al.*, 2003; Zimmerman *et al.*, 2007; Singer *et al.*, 2008; Brimberg *et al.*, 2013). An association has also been observed between the presence of anti-foetal brain antibodies in the mother and ASD-related deficits in the child (specifically those relating to expressive language) (Braunschweig, Duncanson, *et al.*, 2012). Children with ASD present with autoreactivity to brain proteins (Singer *et al.*, 2006; Zimmerman *et al.*, 2007; Wills *et al.*, 2009; Goines *et al.*, 2011*a*; Mazur-Kolecka *et al.*, 2014). Antibodies specific to myelin basic protein and neuron-axon-filament protein (NAFP) have been found in individuals with ASD (McClelland *et al.*, 1992; Singh *et al.*, 1993; Singh *et al.*, 1997; Singh, Lin and Yang, 1998), as well as increased levels of autoantibodies to brain endothelial cells (Connolly *et al.*, 1999) and abnormal reactivity to cerebellar neurofilaments (Plioplys, Greaves and Yoshida, 1989). Importantly, the presence of autoantibodies has been found to correlate with the presence and severity of ASD-relevant behaviours (Goines *et al.*, 2011*a*; Piras *et al.*, 2014), and immunotherapy has shown to improve speech, learning, attention and sleep in individuals with ASD who present with NAFP antibodies (Singh *et al.*, 1988). Investigation of the role of autoantibodies in the pathogenesis of ASD using the MIA model has produced some compelling results. Anti-brain autoantibodies injected into pregnant mice led to ASD-relevant symptoms in the offspring (Dalton *et al.*, 2003; Singer *et al.*, 2009; Braunschweig, Golub, *et al.*, 2012; Camacho *et al.*, 2014), as well as a proliferation of radial

glial cells and an increase in brain volume and neuron size (Martínez-Cerdeño *et al.*, 2016). Additionally, auto-reactive IgG injected into pregnant macaques produced offspring with ASD-relevant behaviours (Martin *et al.*, 2008; Bauman *et al.*, 2013). The theory has been proposed that an environmental trigger (such as a virus) provokes faulty immune regulation and leads to the production of antibodies specific to brain tissues (Singh, 2009). There is, therefore, a wealth of evidence implicating immune dysregulation as a central component of the pathophysiology underlying the disorder. Despite this evidence, there is still a lack of understanding for the mechanisms behind specific symptoms of ASD. While the the aetiology of motor impairment has not been elucidated, many interventions for ASD involve a motor component.

### 2.4 Motor interventions for ASD

As mentioned previously, the complexity of the pathophysiology underlying ASD and our incomplete understanding of it means that few effective pharmocotherapies exist for the disorder. However, the ubiquity of motor impairment in ASD has led to a host of physical exercise interventions being researched for the disorder in children. Many of the physical exercise interventions that have been researched in children with ASD show promise in improving motor skills and alleviating some of the social and behavioural symptoms associated with the disorder. Physical exercise in the form of jogging has been found to have a positive impact on emotional regulation and behavioural function (Tse, 2020). A motor program of activities, games and sports was found to have significant positive effects on perceptual motor skills (Rafie *et al.*, 2017). A 14-week karate training program resulted in significant improvements in communication (Bahrami *et al.*, 2016), and similarly, kata techniques training has shown to be helpful in alleviating social dysfunction (Movahedi *et al.*, 2013). A 12-week physical exercise intervention involving table tennis resulted in significantly improved motor skill proficiency and executive function (Pan *et al.*, 2017). A skating intervention led to improved balance, motor behaviour and functional capacity in two boys with ASD (Casey *et al.*, 2015), while horse-riding has shown to improve adaptive behaviour (Gabriels *et al.*, 2012). A creative yoga intervention led to improvements in motor and imitation skills (Kaur and Bhat, 2019), and a yoga and dance intervention showed assistance in improving social dysfunction and behaviour (Rosenblatt *et al.*, 2011). Physical exercise has also been found to associate with decreased sleep disorders in ASD (Wachob and Lorenzi, 2015). One meta-analysis found that physical activity has a significant positive effect on social interaction, communication, motor skills and severity of ASD, but no effect

on stereotyped behaviour (Huang *et al.*, 2020). In contrast, a systematic review with meta-analysis found that physical exercise does reduce stereotypical behaviours (Ferreira *et al.*, 2019). One study pointed out that specific exercise may be more beneficial for specific stereotypies, and it may be important to match the exercise intervention to the child's specific needs and the desired outcome (Tse, Pang and Lee, 2018). Another systematic review found that exercise interventions (including jogging, horse-riding, martial arts, swimming and yoga/dance) improve stereotypical behaviours, social-emotional function, cognition and attention, with martial arts and horse-riding showing the largest and next-largest effect sizes respectively (Bremer, Crozier and Lloyd, 2016). A systematic review with meta-analysis found that chronic exercise has beneficial effects on executive function in children and adolescents with ASD (specifically cognitive flexibility and inhibitory control) (Liang *et al.*, 2021). Exercise of various forms has shown to be beneficial for children with ASD, including sessions from 15 to 90 minutes long and including training programs of 8 to 48 weeks (Ferreira *et al.*, 2019). However, short 10-min sessions of a light to moderate intensity have better outcomes on stereotypies than more prolonged and intense sessions (Olin *et al.*, 2017).

Table 2.2: Studies on exercise interventions for ASD

| Paper | ASD sample size | Age | Intervention | Main findings |
|---|---|---|---|---|
| Tse, 2020 | 27 | 12-18 years | Jogging (12 weeks) | Improved emotional regulation, reduced behavioural problems |
| Rafie *et al.*, 2017 | 20 | Adolescents | Mixed motor program (10 weeks) | Improved perceptual-motor skills |
| Bahrami *et al.*, 2016 | 30 | 5-16 years | Karate (14 weeks) | Reduction in communication deficit |
| Movahedi *et al.*, 2013 | 30 | 5-16 years | Kata techniques (14 weeks) | Improved social dysfunction |
| Pan *et al.*, 2017 | 22 | 9.08 ± 1.75 years | Physical activity (12 weeks) | Imprved motor skills and executive function |
| Casey *et al.*, 2015 | 2 | 7 and 10 years | Skating (12 weeks) | Improved balance, motor behaviour and functional capacity |
| Gabriels *et al.*, 2012 | 42 | 6-16 years | Horseback riding (10 weeks) | Improved self-regulation, expressive language, motor skills and verbal praxis |
| Kaur and Bhat, 2019 | 24 | 5-13 years | Creative yoga (8 weeks) | Improved motor and imitation skills |
| Rosenblatt *et al.*, 2011 | 24 | 3-16 years | Yoga, dance and music therapy (8 weeks) | Improved core and behavioural features of ASD |
| Tse, Pang and Lee, 2018 | 30 | 9-12 years | Ball-tapping exercise (12 weeks) | Hand-tapping sterotypy reduced but body-rocking not reduced |
| Olin *et al.*, 2017 | 7 | 13 ± 1.4 years | Five interventions of varying intensities | High-intensity exercise may exacerbate stereotypies while low- to moderate-intensity exercise reduces stereotypies. |

Despite the research into the effectiveness of exercise interventions in ASD, little research has focused on the mechanistic links between exercise interventions and the improvement of autistic symptomatology. It is known that physical exercise has beneficial effects for people with neuropsychiatric disorders more generally (Chen *et al.*, 2020), and it is possible that this effect is due to physical exercise promoting an anti-inflammatory state (Ignácio *et al.*, 2019). Exercise-stimulated cytokine release modulates neuronal metabolism, neuroinflammation and neuroplasticity (Bay and Pedersen, 2020; Murphy, Watt and Febbraio, 2020), which may be

crucial in the physiological pathways by which exercise impacts ASD symptoms. It has also been theorised that increased physical activity causes a decrease in obesity, which leads to a decrease in obesity-related inflammation (Toscano *et al.*, 2021). A 48-week exercise-based intervention led to beneficial effects on ASD traits and parent-perceived quality of life, as well as cholesterol, indicating potential benefit for the lipid-associated inflammatory metabolism (Toscano, Carvalho and Ferreira, 2018). A mini-basketball intervention resulted in improved social communication as well as increased white matter integrity, providing some evidence for neurological mechanisms underlying the role of exercise in improving the symptoms of ASD (Cai *et al.*, 2020). In research using a mouse-model, voluntary wheel running was used as an exercise intervention and it was found that the exercise led to a decrease in abnormalities in anxiety, sociability and repetitive behaviour (Andoh *et al.*, 2019). The authors observed that the exercise induced a microglial activation that results in synapse engulfment in the hippocampus, thereby normalising the hyper-density of synapses in that area that resulted from MIA-induce microglial deficits (Andoh *et al.*, 2019). Exercise may therefore have effects on neurophysiology that mediate positive outcomes for individuals with ASD.

### 2.5 The impacts of exercise on immune function

As described, exercise interventions have shown to be helpful in varying degrees for ASD. However, there is a lack of understanding of the physiological mechanisms underlying the improvement observed. While the physiological effects of exercise interventions in ASD have not been well-researched, there is some literature pertaining to the effects of exercise on immunology more generally. The effects of acute exercise and chronic exercise on the immune system differ (Alack, Pilat and Krüger, 2019). Acute exercise refers to a single bout of exercise within a short time frame, whereas chronic exercise refers to multiple consistent and regular bouts of exercise over a sustained period of time. Acute exercise results in a lymphocytosis during and immediately after the exercise, with lymphocyte numbers decreasing during the early recovery period (Krüger and Mooren, 2014). A first rapid neutrophilia has been observed in response to acute exercise, followed by a second delayed neutrophilia a few hours later (Walsh *et al.*, 2011). Unstimulated neutrophils respond to acute exercise stress with increased degranulation, oxidative burst and phagocytosis responses (Walsh *et al.*, 2011). Acute exercise has also been observed to induce a transient monocytosis for about two hours following exercise, with the monocytes migrating from the marginalised areas to the circulation (Walsh *et al.*, 2011). Acute exercise furthermore stimulates an

increase in the CD14+/CD16+ pro-inflammatory monocyte subtype relative to levels of the classic CD14+/CD16- subtype, as well as a rapid mobilisation of natural killer cells (Krüger, Mooren and Pilat, 2016). Innate immunity is enhanced by acute physical activity, which appears to provide a protection from infection (Dhabhar, 2000). Acute exercise has therefore shown to promote a transient activation of the immune system and a pro-inflammatory state.

Chronic exercise training, on the other hand, exhibits an anti-inflammatory effect on the human body (Nieman, 2012). Adults with higher activity levels consistently show decreased white blood cell counts, C-reactive protein, IL-6 and TNF-α levels (Nieman, 2012). Unlike acute exercise, chronic exercise results in a decrease in the pro-inflammatory monocyte subtype (Steppich *et al.*, 2000; Krüger, Mooren and Pilat, 2016). However, while T-cells and B-cells consistently show functional modulation by regular exercise (Alack, Pilat and Krüger, 2019), the effects of chronic exercise on natural killer cell counts and cytotoxicity have shown varying results (Zimmer *et al.*, 2017). Training increases the resistance of lymphocytes to apoptosis and promotes the stability of homeostasis (Alack, Pilat and Krüger, 2019), while resting lymphocyte levels appear to be unaffected by chronic exercise training (Nieman, 2000). Adjustments for BMI and fat mass attenuate, but do not negate, the relationship observed between activity level and inflammatory markers. Physical inactivity is associated with low grade systemic inflammation and increased total adipose tissue (Parsons *et al.*, 2017). Critically, an association between maternal obesity and risk of ASD in the child has been documented (Dodds *et al.*, 2011; Krakowiak *et al.*, 2012; Bilder *et al.*, 2013). Increased adipose tissue leads to an increase in immune cells in the adipose tissue and subsequently pro-inflammatory mediators spill over into the blood. Natural killer cells and T-cells are increased in the adipose tissues, while Tregs and regulatory macrophages are decreased (Huh *et al.*, 2014). Chronic exercise exerts an anti-inflammatory effect by decreasing fat mass and by inducing an anti-inflammatory environment in the body (Nieman, 2012). By decreasing adipose tissue, exercise furthermore decreases recruitment of leukocytes to the adipose tissue, and induces a switch to the anti-inflammatory phenotypes of macrophages, monocytes and T-cells (Gleeson *et al.*, 2011). Importantly for inflammatory conditions, exercise has also been observed to induce an increase in anti-oxidative enzymes (Tarnopolsky, 2015).

There are also differing effects on the immune system according to the intensity of the exercise. Regular physical activity improves immune function and decreases the risk of

contracting an upper respiratory tract infection, but sustained and intense exertion has the opposite effect (Malm, 2004). Acute prolonged exercise results in a decrease in leukocyte numbers in the blood (Mackinnon, 1999; Nieman, 2000). The cytotoxicity of NK cells can be reduced for hours after exhausting and prolonged exercise (Walsh *et al.*, 2011). Intensity of exercise also affects the behaviour of mobilised T-cells, with increased intensity of exercise leading to an increase in apoptosis of T-cells (Krüger *et al.*, 2016) as well as increased mobilisation of regulatory T-cells (Dorneles *et al.*, 2019). Prolonged and intense exercise stimulates the muscle fibres to produce IL-6, which in turn causes the increase of IL-1ra and IL-10 in the circulation. IL-6 also inhibits TNF-α production and stimulates lipolysis and fat oxidation (Petersen and Pedersen, 2005). Moderate exercise, however, acutely increases circulation of immunoglobulins, neutrophils and natural killer cells, with no elevation in stress hormones (which can suppress immunity) or pro/anti-inflammatory cytokines (Nieman, 2012). Moderate exercise leads to a Th1-type cytokine response (increased IL-2 and IL-12) while strenuous exercise leads to a Th2-type cytokine response (increased IL-6 and IL-10) (Malm, 2004).

Importantly, the change in leukocyte number following exercise has been attributed to the release of cortisol and catecholamines (Benschop, Rodriguez-Feuerhahn and Schedlowski, 1996). Exercise stimulates the release of cortisol and adrenaline (activation of the sympathetic nervous system and the HPA-axis) (Viru, 1992; Gleeson *et al.*, 2011). While cortisol inhibits immune cell function (McGregor *et al.*, 2016), T-cells, monocytes and NKs all have adrenergic receptors (Elenkov *et al.*, 2000) and are modulated by the activity of the sympathetic nervous system and HPA-axis. Catecholamine release causes an increase in circulating lymphocytes during exercise, but levels return to normal or even below normal within a few hours after exercise has ceased (Malm, 2004). In summary, exercise acts on the brain-immune axis by activating the stress system and may play a role in helping the body to regulate immune function. This provides a line of investigation into the potential mechanisms by which exercise may improve the symptoms of ASD.

### 2.6 Rationale for the investigations undertaken

Firstly, there is a dearth of data in general on the characteristics of the ASD population in Johannesburg, and further data is needed on this population in order to adequately support them. Previous research has indicated that motor impairments are ubiquitous in the symptomatology of ASD, and that exercise interventions are helpful in alleviating both motor

and behavioural symptoms of ASD. It has also been well-documented that dysregulation of the immune system is a core physiological feature of ASD. However, the mechanisms by which exercise interventions work for ASD have not been investigated, and given the immune-regulatory effects that exercise is known to have, the brain-immune axis presents a likely target for mechanistic elucidation. The physiological connection between motor impairment and the social/behavioural symptoms is also yet to be understood. Additionally, the role that socioeconomic status as a psychological stressor may play in modulating the brain-immune axis in ASD is not understood. While some epidemiological research has indicated that higher socioeconomic status is associated with higher chances of ASD diagnosis (Thomas *et al.*, 2012), conflicting findings in this arena have raised the question of education and awareness about the disorder being the mediating factor for such a relationship (King and Bearman, 2011). Indeed, in Sweden, where access to healthcare is universal, lower socioeconomic status has been found to correlate with a higher risk of ASD (Rai *et al.*, 2012). For these reasons, data was collected from autistic children in the Johannesburg area (South Africa) looking at autistic symptomatology, motor skills, socioeconomic status and biomarkers of inflammation and the brain-immune axis in order to characterise the population. Relationships were then assessed between the different measures collected to characterise the population. Additionally, a pilot study was conducted assessing the longitudinal effects of an exercise intervention on motor skills, autistic symptomatology and the chosen biomarkers. While biomedical research on ASD in South Africa is sorely needed, the population is vulnerable due to the communication impairments present in ASD and the likelihood for research to be conducted in children, and South Africa's sociocultural context presents with a number of ethical considerations. Therefore, the ethical considerations of conducting research on children with ASD in South Africa were also researched and discussed in detail in Chapter 3, providing the background for a theoretical paper (manuscript provided in the appendices).

In order to assess the brain-immune axis in ASD, physiological samples needed to be collected. Urine collection is a non-invasive way of collecting biomarkers, and urinary metabolomic profiles have previously been found to differ in children with ASD as compared with controls, indicating dysregulation in metabolic pathways and increased oxidative stress (Yap *et al.*, 2010; Gevi *et al.*, 2016; Anwar *et al.*, 2018). Urinary biomarkers found in ASD have provided information for the development of theories for the pathophysiology of ASD (Reichelt and Knivsberg, 2003). However, given the resource limitations of conducting

research in a middle-income country, as well as the ethical imperative to find diagnostic markers that are accessible in this setting, assessing a metabolomics profile in the urine was not feasible. Instead, it was decided to assess two biomarkers in the urine that can be less expensively measured via ELISA. The two biomarkers chosen for assessment were neopterin and cortisol as they both bear relationships with the brain immune axis and have shown dysregulation in ASD previously. Neopterin is a pteridine that is produced by activated monocytes and macrophages, and increased levels of neopterin in the urine indicate increased oxidative stress and Th1 dominance (Hamerlinck, 1999; Murr *et al.*, 2002). Neopterin has previously been found to be elevated in the urine of children with ASD as compared with controls (Harrison and Pheasant, 1995; Messahel *et al.*, 1998). Cortisol is the major stress hormone, and cortisol rhythm and responses to stressors have been also found to be altered in ASD. In a review of the literature Taylor & Corbett (2014) found that most studies looking at the circadian variation of cortisol in ASD found evidence of a dysregulated rhythm. Additionally, they found that there is evidence for a hypo-activation of the HPA axis in response to social threat and physiological manipulation, but a hyper-responsiveness to unpleasant stimuli. However, most studies did not show abnormalities in the cortisol awakening response in ASD. For these reasons, it was decided that urine samples would be collected first thing in the morning to assess the awakening cortisol levels.

Given the nature of the brain-immune axis and the role of the stress system as a mediator therein, life stressors may also play a role in the development of ASD. Importantly, there is evidence for a potential relationship between socioeconomic status and ASD, and low socioeconomic status is likely to present with increased stress. Studies in the United States have found evidence for a relationship between ASD diagnosis and socioeconomic status, where children of higher socioeconomic status are more likely to get a diagnosis of ASD, which may be due to increased knowledge about the disorder and increased access to healthcare services in higher socioeconomic levels (King and Bearman, 2011; Thomas *et al.*, 2012). In contrast, a study in Denmark found no association between socioeconomic status of the family and probability of receiving an ASD diagnosis (Larsson *et al.*, 2005), which may be due to the fact that healthcare services are available to all people in Denmark irrespective of socioeconomic status. However, a study in Sweden found that lower socioeconomic status associated with an increased chance of being diagnosed with ASD, and healthcare services are similarly widely available in Sweden as in Denmark (Rai *et al.*, 2012). The relationship between diagnosis and lower socioeconomic status in Sweden indicates the potential for

socioeconomic status to play a role in the development of ASD. Unfortunately, there is very little data on the relationship between socioeconomic status and severity of ASD symptomatology, with a single study in Malaysia finding employment status of the father to predict autistic symptom severity in the child (Eow *et al.*, 2020). There is the potential for higher socioeconomic status to act as a moderating influence on ASD symptom severity, with higher socioeconomic status providing access to earlier diagnosis and a wider variety of therapy and support options.

Given this background, as well as the sensitive nature of biomedical research on ASD in the South African sociocultural context, the first aim of this thesis involved a discussion of the ethical aspects of this type of research. Furthermore, the second aim of this thesis involved a characterisation of socioeconomic status, autistic symptom severity, motor skills, and levels of cortisol and neopterin in the urine of autistic children in the Johannesburg area. None of this data has previously been collected for the population of autistic children in Johannesburg and provides new insights into the needs and challenges of autistic children in the area, as well as providing a baseline from which to assess changes in the population. The third aim of the thesis assessed relationships between the measures collected during the characterisation process, in order to gain insight into which aspects of ASD may be interrelated. Finally, the same measures were collected over an 18-week period during which the participants were involved in an exercise-based intervention. It was theorised that the exercise would improve motor skills and may additionally influence the brain-immune axis, thereby improving autistic symptomatology.

### 2.7 Aims

The specific aims of the current thesis, based on the rationale above, are as follows:

1. To evaluate and discuss the ethical considerations for biomedical research on ASD in South Africa, and to provide suggestions for how to conduct such research in a more ethically-minded way in future.
2. To characterise socioeconomic status, ASD symptom severity, motor skills and urinary levels of cortisol and neopterin in a cohort of ASD children in the Johannesburg area.
3. To investigate relationships between the measures collected in (2).

4. To conduct a pilot assessment of changes in ASD symptom severity, motor skills and urinary levels of cortisol and neopterin in response to 18 once-weekly sessions of an exercise intervention.

# Chapter 3: Ethical Considerations for Biomedical Research on ASD in South Africa

While serious gaps exist in our knowledge of ASD, biomedical research on ASD presents with a host of ethical challenges which need to be carefully considered. Firstly, individuals with ASD and their families are considered a "vulnerable group" in research due to the effects of the condition socially and their need for greater levels of educational and therapeutic support. Since "vulnerable groups" (such as prisoners, the elderly or those with intellectual disabilities, and minors) are easier to exploit in research, stricter rules are applied to research within these groups. One of the critical requirements for research in established ethical guidelines is that of informed and voluntary consent (World Medical Association, 2013). For truly informed consent, participants need to understand and decide whether to accept the risks involved in participating. Thus, biomedical research on ASD has to consider the potential vulnerabilities of the population as research participants, and to ensure that participants are able to give genuine informed consent. The issue of informed consent is especially relevant to research on anyone who is not considered competent to consent, such as children or those with intellectual disabilities. The construction of this chapter arose out of my experience conducting the research comprising Chapters 4 and 5 of this thesis. While this chapter therefore did not inform the study protocols, engaging with this literature helped me to understand some of the potential reasons for poor participant retention, and I hope that some of these insights may prove beneficial for future research in this field.

In addition to these general ethical considerations for the research topic are considerations specific to the context in which the research is conducted. Up until the 1990s, South Africa was governed by a system of institutionalised racial segregation known as "apartheid". The history of the apartheid regime in the country, wherein society was sharply racially divided in terms of geography and resources, has produced a unique set of challenges. The legacy of the apartheid era is still present in the country today, with a huge disparity between the highest and lowest socioeconomic classes and black families largely occupying the lowest socioeconomic class. Black and other non-white families are therefore less likely to have access to medical, psychological and educational services. Families of a lower socioeconomic status are less likely to have the resources to support individuals with ASD (who may require special education, extra carers, therapy of various types and perhaps eventually housing in

specialised facilities) and thus will be more reliant on state resources. Families of a lower socioeconomic status are also at increased risk of exploitation, especially in a biomedical research setting, as they may see participation in research as an opportunity to access treatment options that they could not otherwise afford. Moreover, some studies have suggested that lower socioeconomic status is associated with delayed ASD diagnosis and treatment, as well as decreased access to support (King and Bearman, 2011; Rai *et al.*, 2012; Thomas *et al.*, 2012), which may translate to poorer overall outcomes for autistic individuals. While there are no studies assessing the relationship between socioeconomic status and ASD prognosis in South Africa as of yet, in reviewing studies looking at the phenotype of ASD in Africa, Franz *et al.* (2017) concluded that the samples for such studies are biased towards a higher socioeconomic status. This bias may be due to a lack of awareness of ASD as well as decreased access to diagnosis opportunities for families of a lower socioeconomic status, and indicates a gap in the research as regards autistics of lower socioeconomic status in Africa. Families of lower socioeconomic status may not have the time, energy or educational resources to engage with the information surrounding research projects in a meaningful way.

In addition to the impacts of sharp socioeconomic differentials are the considerations of cultural context on the conceptualisation of ASD itself. There has been a burgeoning of research on ASD in recent years, particularly in the Western contexts of Europe and North America. However, as a disorder that presents socially and behaviourally, the critical question arises as to whether Western descriptions and information are applicable to the South African context. South Africa is a melting-pot of cultures, with communities of Chinese, Taiwanese, Indian and Middle-Eastern origin, as well as the descendants of European settlers and the Bantu peoples, to name a few. Does ASD present differently within South African cultures and sub-cultures? Furthermore, do presentations of ASD found here associate with different difficulties in management and societal integration? Do individuals and communities with ASD in various South African contexts therefore have different support needs? Further research on the autistic population in South Africa is required to answer these questions and to ensure adequate provision of appropriate resources to individuals and communities affected by the condition. Additionally, the multicultural nature of South Africa includes multiple spoken languages, and language spoken by participants may present barriers to understanding the nature of research projects on ASD. The different cultural worldviews present in South Africa mean that there are also many different possible

ways of conceptualising ASD, and using only the Western framework in research projects is likely to cause exclusion of people from different backgrounds.

While research on ASD in South Africa is required in order to meet the needs of affected communities and individuals, such research presents a number of ethical challenges. As noted, autistic individuals and their families are already a vulnerable population group. The vulnerability of autistic individuals and their families is exacerbated in the case of people historically socioeconomically disadvantaged by the apartheid system. The multicultural nature of South Africa presents with language barriers and different worldview frameworks, which need to be taken into consideration when recruiting participants for research projects. South Africa is also a resource-scarce nation, and this impacts the resources available for the conduct of research. These challenges call for a revision of the standard procedures for recruitment and obtaining of informed consent in biomedical research, to allow for a more flexible approach that incorporates communities in a more inclusive way. The following sections will discuss the ethical issues mentioned above in more depth, beginning with a discussion of the ethics of conducting biomedical research with minors, since much of ASD research is by necessity conducted in children. That will be followed by discussions of aspects more specific to ASD, i.e. the communication difficulties inherent in the disorder and the effects of social stigmatisation. Ethical considerations more specific to the South African context will then be discussed, followed by an outline of some of the challenges inherent in the current informed consent process. An argument for the inclusion of community engagement processes will then be made, as well as an outline of how the community engagement process works. Finally, some recommendations are given as to how to implement community engagement as a part of biomedical research. This chapter provides the literature review for a submitted paper, which is provided in Appendix D.

### 3.1 Ethical challenges in research on children

Much of ASD research is conducted in children, as there is no other way to assess the true efficacy of early-life interventions. Children are considered a vulnerable group, as they are still in a process of cognitive development and are often not considered competent to assess potential risks and benefits and make the best choice for themselves. Children may choose to participate in order to please their parents or caregivers, or because they think that gaining clinical improvement will garner them approval from their parents or caregivers (Tan and Koelch, 2008). Most ethical guidelines call for the consent of a caregiver in the case of any

person who is not considered competent to consent for themselves, which applies both to minors and to individuals with reduced cognitive capacity. However, caregivers may not always be the best judges of what is in their charge's best interest (Worku, Davis and Morrow, 2016). In some cases, the risks of participating fall on the participant, while the benefits apply to the person consenting (Glass and Speyer-Ofenberg, 1996), such as where a caregiver stands to benefit from having their ward improve clinically. Parents' desperation for assistance or answers about their child's condition may also cause them to be more vulnerable to exploitation in a research environment (Daley, Singhal and Krishnamurthy, 2013). Parents in resource-scarce environments may consent to their children participating in very risky interventions out of desperation due to not having access to any other kind of intervention or support. In some cases, parents may carry the misconception that because an intervention is being researched it is an effective treatment method. When research is conducted in schools, parents may consent out of fear that their child may be expelled from the school if they do not consent. Importantly within the African context more generally, some cultures have higher expectations of children's subservience to their elders (Kruger, Ndebele and Horn, 2014). The consideration of cultural expectation is particularly pertinent to assessment of the sincerity of a child's assent.

### 3.2 Communication difficulties and the impact on informed consent

Not all ASD research is conducted in children, but some of the difficulties around informed consent remain applicable. There is a wide spectrum of impairment associated with ASD, and while some autistic individuals are fully cognitively capable, some are not. In individuals with intellectual disabilities more generally, there exists a wide range of competencies, with different faculties affected in different individuals (Fisher, 2003), and this is evidently the case for ASD as well. Some autistic individuals have cognitive impairment and may struggle to understand the purpose of the research or what procedures they are consenting to. Some individuals may have higher capacity than others to understand and weigh up risks. However, in autistic individuals the abilities to understand information and rationalise a decision do not necessarily co-occur with the ability to communicate their choice, and vice versa. These communication challenges may make it particularly difficult to assess an autistic individual's true level of understanding of the information provided. Many autistic individuals may be unable to communicate despite understanding the information they have received. It is furthermore important to bear in mind that those individuals with cognitive impairment may

still wish to be treated as autonomous members of the community, and to be meaningfully involved in the process of obtaining informed consent or assent (Fisher, 2003).

For individuals who are not considered competent to consent for themselves, an assent process is recommended, whereby a legal guardian consents but the participant is still informed about the process and given opportunities to ask questions and express dissent. A personalised assent process that responds to the needs of the individual and that is continuous throughout the duration of the study as opposed to a once-off activity has been suggested (Giesbertz, Bredenoord and van Delden, 2014). In addition, it has been suggested that assent processes should be designed and piloted before use in a study, and that quizzes should be used to assess understanding of what participants are assenting to (Crane and Broome, 2017). Cascella and Aliotta (2014) discuss examples of consent processes with individuals with communication disabilities, and provide suggestions for overcoming potential hurdles. While these may be valuable as ethical practices, they have the potential to place an even bigger hurdle in front of the researcher, especially in resource-scarce settings, thereby preventing this kind of research from occurring. These practices are time-consuming and delay the conduct of necessary research.

### 3.3 Stigma in ASD

Another ethical factor specific to ASD is that of stigma. Autistic individuals experience stigma in the form of social exclusion and lack of understanding for their challenges (Gillespie-Lynch *et al.*, 2015). Stigma occurs as a result of stereotyped assumptions regarding the competence of autistic individuals in social roles and the causes and characteristics of ASD. The flourishing of research on ASD in recent decades, with progress specifically in the fields of genetics and neuroscience, has led to excitement about the development of a possible “cure” for ASD. However, some advocates for the ASD community have expressed concerns that the idea that ASD requires a cure contributes to the stigma surrounding ASD (Pellicano and Stears, 2011). Autistic advocates challenge the ideas that ASD needs to be cured or prevented, as these ideas reinforce the stigma that ASD is something undesirable that requires correction (Pellicano and Stears, 2011). The lack of understanding of ASD has even led to the use of inhumane treatment programmes, in which autistics are dangerously restrained or physically and emotionally punished in order to “correct” undesirable behaviours (Neumeier and Brown, 2020). Importantly, the question has been raised of what is and is not “normal” and that a broader conception of neurodiversity could be more helpful.

While many people on the ASD spectrum are disabled, they may also have increased abilities in particular areas as compared with typically-developing individuals (Pellicano & Stears, 2011). For example, there is evidence to suggest that autistic individuals have enhanced visuospatial ability as compared with typically-developing individuals (Shah and Frith, 1983; Jolliffe and Baron-Cohen, 1997; Plaisted, O'Riordan and Baron-Cohen, 1998; Pellicano *et al.*, 2006). Many autistics consider their condition to be an integral part of their identity and not something that they would want to change about themselves. The recent progress that has been made in identifying genetic variants associated with ASD raises concerns about a "liberal eugenics" approach that deems ASD as unwanted. In many countries, selective abortion is seen as an acceptable health intervention when potential disability in the form of certain genetic markers is identified in a foetus. Thus it is evident that the ethical implications of genetic research in ASD are considerable, however an in-depth discussion of these implications is outside the scope of this thesis.

Researchers investigating ASD in humans need to be aware of the social implications of their work and how the questions they ask or the answers they produce will influence the way autistic individuals are viewed by society and therefore treated. In South Africa, evidence for social stigma against ASD has been documented (Fewster and Gurayah, 2015; Guler *et al.*, 2018). The effects of stigma on parents of autistic children in the South African context have been described as blame towards parents for the child's behaviour; caregiver isolation, secrecy and shame; and labelling of the autistic child as naughty (Guler *et al.*, 2018). Additionally, community stigma may lead to an aversion to accessing support services for an autistic child in the South African context (Guler *et al.*, 2018). When approaching biomedical research in ASD, stigma is a particularly pertinent consideration as often biomedical research frames ASD as something that requires prevention or a cure. In light of the difficulties experienced by autistic people, it is easy to see how biomedical scientists may come to view ASD as a problem that needs solving. However, the cause of the difficulties experienced by autistics may lie less in the condition itself and more in the ways in which society treats individuals. In the current global capitalist framework, people are treated as dispensable if they cannot easily form part of the productive workforce driving the economy. It is important to frame research questions around improving the lives of autistics by addressing the symptoms that are problematic in their daily lives, rather than trying to eliminate the disorder entirely.

### 3.4 Ethical concerns for biomedical research in the African context

Most African countries are LMICs, and as such are faced with a specific host of challenges to health research that are not experienced by their high-income counterparts. While South Africa is considered an upper-middle income country, large portions of the population fall into the LMIC category and the more general challenges to health research in Africa are therefore also applicable to the South African context. General challenges include impoverished and refugee populations with low access to healthcare, and barriers to communication and understanding such as low literacy levels, language barriers and cultural differences (Kruger, Ndebele and Horn, 2014). South Africa in particular has a high number of refugee occupants, who lack political status and are also subject to the xenophobia of the South African population. These factors make potential research subjects more susceptible to exploitation, for example via inducement or the "therapeutic misconception" wherein subjects confuse research participation for routine healthcare (Lema, 2009).

Additionally, the rate of health research in Africa has increased lately without concomitant improvements in the oversight of such research by Research Ethics Committees (RECs) (Benatar, 2002). Oversight is a crucial duty of RECs, whereby they monitor the progress and activities of approved research projects in order to ensure that they abide by ethical stipulations and are working in accordance with the approved protocol. RECs in Africa often lack the resources and capacity to conduct sufficient oversight (Silaigwana and Wassenaar, 2015). For example, while active and on-going monitoring processes (such as site visits) are deemed necessary as a part of ethics oversight, particularly for any studies involving vulnerable participants, there is not always the possibility for this to be carried out (Kruger, Ndebele and Horn, 2014).

Furthermore, researchers from developed countries have historically come to Africa to conduct research where they are less limited by the red-tape that they encounter in their own countries (Kruger, Ndebele and Horn, 2014). Higher income countries often have higher standards of care than LMICs, and RECs in LMICs have therefore historically demanded a relatively lower standard of care for research projects (Gisselquist, 2009). Consequently, the burden of the research is carried by LMIC populations while the benefits of the research are distributed to the high-income home countries of the researchers. Pfizer's testing of Trovan as a drug for meningitis on illiterate children in Nigeria provides one such example (Washington, 2006).

Importantly, many of the debates on medical and research ethics have historically been dominated by scholars from the Western world and it is only since the 1990s that African voices have been internationally heard and published in this arena (Kruger, Ndebele and Horn, 2014). This implies a potential gap in the suitability of ethical guidelines to the health research environment in Africa. In their 2015 review of the sparse literature, Silaigwana and Wassenaar found that the effective functioning of RECs in Africa was hampered by challenges such as low resource availability, inadequate training and diversity of members and lack of national guidelines. As of 2019, there is still very little research on the functioning of RECs in Africa (Silaigwana and Wassenaar, 2019). However, there is evidence that the issues of informed consent and respect for participants are frequently queried in REC meetings (Tsoka-Gwegweni and Wassenaar, 2014; Silaigwana and Wassenaar, 2019).

### 3.5 Challenges with the current informed consent process in South Africa

In order to develop appropriate strategies to support the African autistic population, we need to understand more about their specific needs. To achieve this goal, research on the population is required. In our experience, outdated frameworks around the informed consent process present a major barrier to recruitment and retention of research participants. REC requirements for how consent is to be obtained may have the impact of biasing the study population. Currently, consent forms are more likely to be written in English and in a parlance aimed at people of higher socioeconomic status. Critically, in South Africa cultural differences may also influence understanding and interpretation of information presented, and historic inequalities mean that there is huge variation in the level of education parents may have. A key point in the Declaration of Helsinki (World Medical Association, 2013) is the stipulation that groups that are underrepresented in medical research should be provided with appropriate access to participation. In order to understand and provide for the needs of poorly researched communities, their participation in research is a necessity. However, low education levels in impoverished and previously disadvantaged communities, along with language and cultural barriers, further complicate the acquisition of genuine informed consent or assent. How much detail does a participant need? At what point does it become too much information, such that it overwhelms a potential participant who is under-resourced in terms of time, education background or energy? These factors may create a participation bias that falls along socioeconomic lines and keeps these groups of people from being represented in

research. Information sheets can become overly lengthy as a result of ethics committees' requirements that specific pieces of information should be included in the typed information sheet. Parents of autistic children already have resource constraints due to the special care needs that autistic children often have. In the South African context, many families of colour in particular are of lower socioeconomic status, which further decreases parental resource availability. Consequently, there is a potential bias to inclusion in research, especially when information sheets for fairly complex research projects end up being over two pages long. Considering the factors at play, we can expect that such a bias would inadvertently skew the proportion of African families of colour included in a given research project. The fact that studies on ASD in Africa generally have shown a bias towards participants of a higher socioeconomic status (Franz *et al.*, 2017) lends credence to this theory.

In addition to the issues inherent in medical research on ASD are considerations of the cultural context in which research takes place. The cultures of the region in which a given research project takes place influences many aspects of the research. The sociocultural lens shapes how local communities view and treat members with a given mental health condition; as well as their understandings of and receptiveness to research investigating the condition. It is therefore important for the researcher to interrogate their own ideological biases when entering into a community in order to perform research projects, as their worldview may differ to that of potential participants. Culturally speaking, scientific research is situated within a reductionist, materialist framework with predominantly Western origins. Researchers often take this worldview for granted as fact, while the reality is that such a perspective may differ significantly from the worldviews of their research subjects. Participants' beliefs about disease causation may differ to those of the researcher (Tangwa, 2000), which affects their understanding and comprehension of information provided for informed consent. For example, while Western medicine focuses on the structures and functions of bodily organs, traditional African medicine views disease as caused by socio-religious factors (Onuoha, 2007). For a genuine informed consent process to occur, true levels of understanding of the information provided needs to be determined, and the vast differences in worldview in some instances make this very difficult (Kruger, Ndebele and Horn, 2014). It is important for researchers not to adopt a dismissive attitude towards the community's frameworks for understanding the world and lived experience. The information provided in informed consent materials and the questions used to determine comprehension of such materials need to be culturally sensitive and locally appropriate. It is important to

note that in such a culturally diverse context as South Africa, researchers need to be very careful about assuming that certain traditions or beliefs observed in one community will be relevant or applicable to another community.

Furthermore, the requirement for lengthy typed/written information sheets biases the recruitment process. Firstly, for many people in Africa, English is not the mother tongue. Secondly, as mentioned, the differences in worldview may render parts of the provided information completely unintelligible. It has been suggested that understanding of consent materials be assessed in order to ascertain whether consent provided is "genuinely informed". However, this also has the potential to exclude large sections of the population if we are assessing understanding of information provided in English and/or constructed within the Western biomedical framework. African RECs need to start incorporating some flexibility into their requirements around the information provided to participants for the informed consent process. Finally, and also very importantly, there is a low level of literacy in many parts of the African continent. This is partly due to historically having a predominant oral tradition as the primary means of transmitting information. In South Africa particularly, the Bantu system of education (implemented during the Apartheid era) has left a legacy of severe educational and socioeconomic inequality.

The way consent and assent are usually collected is also based on an individualist conception of selfhood. Western cultures utilise a more individualist construction of personhood than that in many African communities (Eaton and Louw, 2000). African culture has a more communitarian view of personhood and this influences decision-making processes and therefore, the process of obtaining informed consent. Communitarian systems in Africa require consensus with elders, who summarise the prevailing opinions of the group as a whole (Tindana, Kass and Akweongo, 2006). This can be compared with the Western form of consent in which an individual consents for him/herself only, or consent is given by a caretaker on behalf of a minor or adult of limited capacity. Therefore, the standard Western process of collecting consent, whereby a person (or caretaker, in the case of a non-competent subject) is approached in their individual capacity and makes an autonomous decision about participation, may not apply appropriately to all African cultural settings.

### 3.6 Argument for community engagement as an adjunct to the informed consent process

In light of the challenges presented above, the process of obtaining informed consent in research needs to be re-evaluated. However, ethics committees have requirements on what needs to be included in information sheets in order to protect participants and attempt to ensure that they aren't coerced into participation. Providing sufficient information about a research project is aimed at ensuring autonomy of participants and that they can make informed choices about what they may be agreeing to when they consent. Doing away with written information sheets is therefore not an ethical solution to the conundrum presented here. Alternative ways to ensure the protection and autonomy of participants engaged in research projects need to be explored and importantly, implemented. Given the communitarian nature of many African cultures, a contextually appropriate alternative to consider is that of community engagement. This process should involve the community members in the research process at every stage, but particularly in the earlier stages of problem identification and question construction. Such processes have the potential to provide choice and autonomy to community members in a more profound way than simply agreeing or disagreeing to participate. Kruger *et al.* (2014) suggest questions to consider when analysing the ethics of a research project, the most pertinent to this subject centring around addressing community needs, involving the community in identifying problems, priorities and protocols for research and finally establishing whether research findings have made a difference. In ASD research involvement of the community, as both a vulnerable group and a culturally-sensitive issue, these questions are critical. Ethically-minded scientists and research groups should consider engagement with their research population as an essential component of their vocation (Pellicano and Stears, 2011). Kruger *et al.* (2014) suggest that it is both ethical and feasible for the community to establish and own research monitoring structures that would perform similar functions to RECs.

Furthermore, for researchers to effectively help communities and produce relevant research, finding out what the community actually wants from research is important. The beliefs of parents of autistic children have been found to differ significantly to the beliefs of ASD researchers as regards the causes of ASD and priorities for future research (Fischbach *et al.*, 2016). Some parents may want ASD to be "fixed", while other parents may just want to know that their children will be taken care of if they are no longer able to fulfil that role. A fair analysis of the impact of research on the affected communities requires engagement with real-world situations and culturally-situated narratives. Daley et al. (2013) describe the

importance of confidentiality in ASD research in India since lay beliefs about the disorder may negatively impact the marriageability of female relatives. In South Africa, there is evidence for social stigma surrounding ASD (Fewster and Gurayah, 2015; Guler *et al.*, 2018). In light of this, researchers investigating ASD in South Africa need to interrogate what social harm may come to the individual or their family if they are publicly known as autistic. Community engagement processes may help researchers to assess levels of stigma within a community. Such an assessment would help to inform practices within the research project in order to minimize the risk of stigmatization to the participants involved. Moreover, within a community engagement setting, members of the community have the opportunity to learn more about ASD as a condition. Inappropriate or judgmental ideas about the condition can be "respectfully challenged" in this kind of environment. Community engagement practices therefore have the potential to reduce social stigmatization of ASD. Finally, community engagement as an adjunct to informed consent may allow for greater flexibility in the process of informing the community about research and helping research to adapt to the context in which it occurs.

Additionally, scientists have a tendency to focus on academic information, and another aspect of community engagement may be the incorporation of "experience-based expertise" into the design and conduct of research projects (Pellicano and Stears, 2011). Occupational therapists who treat children with ASD may have experiential insight into the disorder that can be of benefit to biomedical researchers. Another group of experts who may have a lot to contribute to developing an understanding of ASD (and other social and cognitive disorders) in South Africa are the historically sidelined traditional healers. In South Africa, traditional healers are sought out for mental health disorders fairly commonly, and traditional healing is reported to have a positive influence on individual and community mental health (Campbell-Hall *et al.*, 2010). Traditional healers operate within culturally appropriate explanatory models, which has a positive impact on treatment adherence. It has been suggested previously that Western medical institutions collaborate with traditional healers in order to increase quality of care, access to care and adherence to treatment protocols (Campbell-Hall *et al.*, 2010). These positive effects may translate to biomedical research as well. Traditional healers may also have historical knowledge of mental health disorders within African communities, which would be useful in evaluating prevalence and potential changes in prevalence over time. Traditional healers may also help to mediate communication between researchers and the participant population in a way that facilitates greater participant understanding of the

purposes and procedures involved in a given research project. Jecker & Atuire (2021) discuss the importance of partnerships between biomedically trained practitioners and traditional healers in the African setting, wherein the work that traditional healers have already been doing for their communities is affirmed and diverse parties work together towards the common goal of helping people in need.

### 3.7 The community engagement process

Community engagement in research, however, is a complicated process, especially where differing cultural expectations come into play. Community engagement entails establishing a trust relationship between researchers and the community and facilitating communication between researchers and the community (Kruger, Ndebele and Horn, 2014). Thus community engagement requires an awareness of hierarchical structures and power dynamics, which are relevant in how they manifest between the community, the researcher and recognised community leaders. In South Africa, the diversity of communities and cultures means that this process will have to be flexible and adaptable. Pellicano & Stears (2011) make the point that disagreement is inevitable in community engagement, and that consensus is not always the ultimate goal of engagement, rather raising awareness of concerns and uncertainties. Importantly, autistic individuals and their families have more at stake than researchers do, as it will have more of an impact on their lives, and their input should be weighted accordingly. It is well-worth researchers' making the effort to engage the community, as fostering understanding between the community and the researchers facilitates recruitment and retention of participants by limiting or managing negative views or misunderstandings of the research.

An existing system of community engagement is that of community-based participatory research, wherein local communities participate with researchers through dialogic development to select and design research topics (Minkler and Wallerstein, 2003). The Early Autism Project in the Kwa-Zulu Natal region of South Africa provides an excellent example of how community engagement via interviews and focus groups with appropriate stakeholders helped to develop a culturally sensitive modus operandi for conducting the research project in question (Grinker *et al.*, 2012). The Early Autism Project was aimed at improving early detection of ASD and other developmental disorders, and elicited community input in order to adapt screening tools to be more appropriate to the cultural context. While community engagement processes already occur in some humanities and

public health research spaces, the implementation of increased community engagement alongside biomedical research would be invaluable. Community engagement that allows the participant population to be involved in the framing of questions and the problems to be solved grants an increased level of autonomy to the population group. This involvement also ensures that resources spent on research efforts are directed at tackling problems relevant to the community. Community engagement has been suggested as an important adjunct to ASD research before (Pellicano and Stears, 2011) and some researchers have started to put this into practice (Kim *et al.*, 2011; Grinker *et al.*, 2012). However, there is still very little structural support for community engagement in biomedical research spaces in South Africa.

### 3.8 Practical recommendations for the implementation of community engagement in biomedical research spheres in South Africa

While community engagement as an ethical research imperative has been given some attention in the literature recently, particularly as a recommendation in ASD research, in our experience there is an implementation gap in the biomedical research sphere. Ethical consideration as it currently occurs appears to be a task delegated to a distant committee who provides a list of checkboxes to be completed. Once these checkboxes are ticked, approval is awarded. Support in the activity of community engagement is something that biomedical researchers have to seek out, rather than it being par for the course. As noted, biomedical research on ASD from both a causation and treatment perspective are increasing in importance. Thus, a gap exists between what is discussed in the literature and what occurs in the biomedical research spaces of LMICs. The real-world implementation of this recommendation requires pointers for practical application.

It is important to note that in the LMIC context, researchers already experience barriers to research conduct in the forms of low resources, decreased access to financial capital and logistical hurdles, to name a few. Such barriers decrease the amount of important and relevant research that can be conducted. In making community engagement a requirement for biomedical researchers we should not be adding to the administrative burden that already falls onto the shoulders of individual researchers. Rather, implementation of community engagement processes requires some structural and institutional support. While the question has been raised about alterations to legislation in order to support this initiative, we argue that implementation can already begin at an institutional and departmental level. Biomedical schools and faculties can begin to build community engagement support blocs drawing on the

experiences of current research staff. The humanities departments (perhaps those in the same institution) as well as public health research teams are important resources to be explored by biomedical research departments in this regard. Community engagement has been a part of humanities research for some time now (for example, focus groups and ethnographic analysis), and their experience may help to build community engagement capacity within the departments that focus more exclusively on biomedical research.

While obviously important for ASD research, community engagement as a process adjunct to biomedical research in general should be made a routine part of research activity. To apply community engagement as something necessary only in ASD research, or even only in mental health research spaces, would impede its occurring at all. Wider implementation of community engagement practices would decrease the energy investment required for an individual research project. Having the structures already set up in a systematic way decreases the burden on individual researchers who may feel it is an ethical imperative in their own biomedical research projects. It may be important within biomedical research departments to have one or two staff members who are dedicated to the project of increasing community engagement alongside research. With decreased workload in other areas of their academic responsibilities, such staff members can research the appropriate methods for approaching the community within the locale of the institution, focusing on the types of research conducted within their specific department. Having staff dedicated to community engagement support would help to reduce the administrative burden of enacting community engagement that would then fall onto specialist researchers. This would assist in ensuring that necessary research on the ASD population in South Africa is carried out in an ethical and contextually-appropriate way into the future.

# Chapter 4: Methods

## 4.1 Ethical Approval

The experimental protocol for the studies comprising aims two, three and four was approved by the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg, South Africa (clearance number M180767). Informed consent was obtained from the parents or legal guardians of all participants from whom data was collected. Once informed consent was obtained from a parent or guardian, assent was obtained from each child participating in the study. If a child expressed (at any point during the study) that they did not wish to complete a certain data collection activity, this was respected.

## 4.2 Participants

Children between the ages of 8 and 18 years, who had been previously diagnosed with ASD were recruited through the Fight with Insight Clinic, which conducts structured exercise classes for autistic children out of the Children's Memorial Institute (CMI) and the Centre for Autism Research and Education (CARE). Recruitment was conducted by sending information sheets (provided in Appendix C) home with the children for parents/guardians to read and sign if they decided that they wanted their child to participate. Children who attend the Johannesburg School for Autism (JSA) at CMI or the CARE require a confirmed diagnosis of ASD according to the DSM-5 criteria by a qualified medical professional in order to be accepted as a pupil. Participants were not excluded for any health reason, but most participants were of medium- to low-support needs (as diagnosed by the occupational therapy department of their respective school), as these are the support needs levels that attend the exercise classes run by Fight with Insight.

## 4.3 Experimental Protocol

Subjective ASD symptoms were assessed using the Autism Treatment Evaluation Checklist (ATEC) by a parent or caretaker who knows the child well. A short questionnaire assessing socioeconomic status was also completed by a parent or caregiver. Motor skills were assessed using the Bruininks-Oseretsky Test of Motor Proficiency (Second Edition) (BOT-2). Urine samples were also collected from each participant to assess levels of cortisol and neopterin via ELISA. The details of each assessment are provided in the sub-sections below. For most of our cohort, these measures were collected only once each and all assessments and samples for each participant were collected within a month of each other. However, for the pilot

study, longitudinal data was collected from some of the children in order to assess their response to the exercise class conducted by Fight with Insight. In these children, the socioeconomic status questionnaire was completed only once, but the BOT-2 and the ATEC assessments were completed twice: once before starting the exercise class with Fight with Insight and once after 18 once-weekly sessions of the exercise class. For these children, urine samples were collected once a week every week over the 18-session period to assess the changes in cortisol and neopterin over the time period of being involved in the exercise class. Figure 4.1 shows the timeline of data collection points for children involved in the study. Ideally, all participants would have undergone all data collection procedures, but this was not possible due to the COVID-19 lockdown. Initially, it was intended to have two control groups – a group of non-autistic children who also attend classes with Fight with Insight at the CMI building, as well as a group of autistic children who do not attend the Fight with Insight classes. However, due to recruitment difficulties and the impact of the COVID-19 lockdown, this was not achieved.

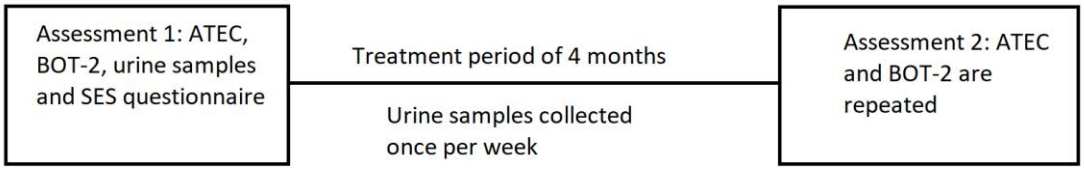


Figure 4.1: Timeline of data collection points.

### 4.3.1 Socioeconomic Status Questionnaire

The questionnaire used to assess socioeconomic status was based on Bagwath Persad *et al.*, (2017), which was chosen as it has been previously used in South African populations. The questionnaire assesses parent/caregiver level of education using four ordinal categories (none, primary school, high school and tertiary level). Employment status and assets owned in the home (refrigerator, television, car, washing machine, microwave) are assessed with yes/no items. A composite score was created for the questionnaire by numerically rating the possible answers on the items. A higher score indicates a higher socioeconomic status, with the maximum possible score on the questionnaire being 9 points.

4.3.2 The Autism Treatment Evaluation Checklist

We used the ATEC to assess autistic symptom severity, which was developed to be a tool for assessing changes in autistic symptomatology in response to intervention (Rimland and Edelson, 1999). The ATEC has been used previously to describe autistic characteristics in a sample (Deng *et al.*, 2007; Al Shirian and Al Dera, 2015). The ATEC scores autistic symptom severity in four different domains, namely: (1) Speech/Language/Communication, (2) Sociability, (3) Sensory/Cognitive Awareness and (4) Health/Physical/Behaviour. Each domain consists of between 14 and 25 items that are each scored on a Likert-type scale. Scores for each domain in addition to an overall score were obtained for each participant by numerically rating the possible answers. A higher score in each domain indicated a higher level of functioning, with the maximum scores for each domain being 28, 40, 36 and 75 respectively. The maximum possible score for the ATEC overall is therefore 179. The ATEC is freely available to download from https://www.aitinstitute.org/AIT_FORMS/ATEC.pdf and does not require training in order to be administered. The ATEC was completed by someone who knows the child well. The form was sent home to be completed by a parent/guardian or was otherwise completed by the respective child's teacher.

4.3.3 The Bruininks-Oseretsky Motor Proficiency Test (Second Edition)

The BOT-2 was developed in America based on standards from typically-developing children (Bruininks, 2005). The BOT-2 has been found to be a reliable and internally consistent motor skills measurement tool (Wuang and Su, 2009). Each child was individually assessed using the BOT-2 motor assessment kit. The BOT-2 assesses motor proficiency in four different domains, namely (1) Fine Manual Control, (2) Manual Co-ordination, (3) Body Co-ordination and (4) Strength & Agility. Each domain is scored on two sub-domains and each sub-domain consists of between 5 and 8 motor activities to be completed by the participant. The sub-domains are (1a) Fine Motor Precision, (1b) Fine Motor Integration, (2a) Manual Dexterity, (2b) Upper-limb Co-ordination, (3a) Bilateral Co-ordination, (3b) Balance, (4a) Running Speed & Agility and (4b) Strength. Performance in each domain and sub-domain was scored by the lead researcher according to rules laid out by the BOT-2 manual, where a higher score indicates a higher proficiency in that domain. A composite score for overall motor proficiency was obtained from all four domains. The raw scores for performance in each domain and subdomain were converted to an age-appropriate score using tables provided in the BOT-2 manual. The maximum score for each domain is 80 points, while the

maximum score for each subdomain is 35 points. The maximum composite score is also 80 points.

### 4.3.4 Urine Samples

Urine samples were collected by participants at home with the aid of their parent/caregiver. Parents/caregivers were asked to collect the samples from their child in the morning before 7 am, so as to obtain the morning void sample for each participant. Sterile collection bottles were sent home with the children for this purpose. Samples were aliquoted into Eppendorf tubes and placed into freezer storage by 11 am the same day of collection. Urine samples were frozen first at -4 °C and then moved to -80 °C freezers within a week of first freezing. Individual aliquots were then defrosted for each ELISA analysis. Specific gravity of each urine sample was assessed using a portable refractometer (ATC, reference number 312) previously calibrated with distilled water in order to correct ELISA results according to overall concentration of the urine, which may be affected by hydration status.

### 4.3.5 Cortisol and Neopterin ELISAs

Concentrations of cortisol and neopterin in each urine sample were assessed using enzyme-linked immunosorbent assay (ELISA) protocols. ELISAs work via the use of an antibody specific to the compound of interest, which is coupled to an enzyme that produces a colourful product (Gan and Patel, 2013). The amount of colour produced (assessed by spectrophotometry as absorbance at a particular wavelength) gives an indication of the concentration of antibody of interest in the sample, when compared with the absorbance values of samples of known concentrations of the antibody. Concentrations of cortisol in the urine were assessed using ELISA kits from Elabscience Biotechnology, Wuhan, China (Lot no. TKDP1LUSZU and EDBLSLY4H2). Concentrations of neopterin in the urine were assessed using an ELISA kit from Wuhan Fine Biotech Co., Ltd., Wuhan, China (Lot no. H3413G062 Y and H3413G106 Y).

Forty samples and eight standards were assessed in duplicate on each plate. Samples were moved from -80 °C storage to 4 °C storage 24 hours before assaying. ELISA results were obtained as absorbance values, from which concentration values were interpolated using the standard curve. Specific gravity of each sample was used to correct concentrations of neopterin and cortisol to a reference specific gravity of 1.030 (the mean specific gravity of the sample) according to the Levine-Fahy equation (where SG refers to specific gravity):

$$Concentration_{SG\ normalized} = Concentration_{specimen} \cdot (SG_{reference} - 1/(SG_{specimen} - 1)$$

### 4.4 Exercise Intervention

Once a week, children at the Johannesburg School for Autism and the Centre for Autism Research and Education attend a modified non-contact boxing class run by Fight with Insight. The boxing classes at the Fight with Insight Clinic in the Children's Memorial Institute were initiated as a diversion therapy for underage youths convicted of sexual assault. The classes have shown great success in that group, and other children from the surrounding area have also begun attending the boxing classes out of choice. The Johannesburg School for Autism is situated in the same building as the Fight with Insight Clinic in the Children's Memorial Institute. Occupational therapists at the school decided to take advantage of this placement and incorporated a session in the boxing class as part of the physical education component of their curriculum. In 2021, Fight with Insight began running a boxing class at the Centre for Autism Research and Education as well. Children were therefore recruited through Fight with Insight from both the Centre for Autism Research and Education and the Johannesburg School for Autism. The occupational therapists from the respective school decide which ASD children can participate in the boxing classes, according to their level of support needs and their likelihood to experience distress in the boxing class setting. ASD children attending the classes are classified as requiring low and medium support, according to the occupational therapists at the school. Support needs are relative, as children are grouped with peers of similar needs in their age group at the school. It is important to note that children with low support needs from these schools would struggle at a mainstream school. A qualified occupational therapist from the school also attends every boxing class with Fight with Insight, monitoring the children for any signs of distress and intervening where necessary. The classes, accompanied by music, consist of a specific and reliable routine, as described in Table 4.1 below. General exercises such as sit-ups, push-ups, squats and planks are performed, as well as boxing-specific exercises such as hitting a punching-bag and light sparring with the coach. The coach scales the sparring according to the level of proficiency of the individual.

Table 4.1: Structure of the boxing intervention class

| | **Round** | **Activities** |
|---|---|---|
| **Beginning: Warm-up** | **1** | Left leg forward, bouncing (skipping rope style – both legs off the ground) |
| | **2** | Legs open, knees bent, punch the air (to the rhythm of the music) |
| | **3** | Arms straight, parallel to the ground and hold (add different movements like: circles, up/down, twisting fists) |
| | **4** | Core stretch and warm-up: (1) bridge hold, (2) yoga snake pose, (3), yoga child pose, (4) yoga upward dog pose (30 secs each) |
| **Middle: Boxing** | **5** | Hit the bags: Any combination (usually start with 6 basic punches). NB: left leg forward & blocking. Take each child on hand pads, doing the combo once or twice. |
| | **6** | Hit the bags: Any combination (Keep it simple) NB: left leg forward & blocking. Take each child on hand pads, doing the combo once or twice. |
| | **7** | Individual hand pads taking it in turn, while all sit on bench watching each other. |
| | **8** | |
| **End: Conditioning & Breathing** | **9** | Leg exercise: (1) Pliometric jumps, (2) Touch heels in the middle, (3) heels to bum, (4) left/right leg in front and switch legs – 10 reps each. Can end with a hold in squat position. |
| | **10** | Arm exercise: 10 reps of each push-up, with a break in between each (can put knees on the ground if struggling) |
| | **11** | Stomach exercise: any sit up for about 20 reps and 6 inches (can include gentle stomach punches) |
| | **12** | Breathing exercise (No music). NB breathe in through the nose, and out through the mouth. Followed by sitting still, with hands on knees, back straight and head straight (assist with getting back straight) |

### 4.5 Sample Size

Based on a small effect size of 0.25 of a change in measures following the exercise intervention, performing an *a priori* sample size calculation for a repeated measures analysis of within and between factors (time and group) with an expected correlation of 0.5 between measurements and with an 85% power, a total sample size of 48 children (16 children per group) would be required in this study. An additional 12 children were intended to be recruited to account for possible drop outs and therefore we aimed to recruit a total of 60 children for the proposed study.

### 4.6 Data Analysis and Statistical Testing

The data collected as described above were analysed using the methods given below. Each sub-section refers to a specific aim.

#### 4.6.1 Sample Characteristics

Composite scores for the socioeconomic questionnaire, BOT-2 and ATEC, as well as the domain scores for the ATEC and BOT-2 and urinary concentrations of neopterin and cortisol were tested for normality using the Shapiro-Wilk test. Descriptive statistics (mean and standard deviation or median and interquartile range, as appropriate) were obtained for each variable. Data is presented as mean and standard deviation for normally-distributed variables, but as median and interquartile range for non-normally-distributed variables. The data obtained using the ATEC is furthermore presented in figures showing the percentages of respondents that selected a particular item. All statistical tests were performed using the core package and the plyr and tidyr packages in R statistical software (R Core Team, 2020) and significance was set at $p=0.05$. All figures were produced using the ggplot2 package in R statistical software.

#### 4.6.2 Correlations between socioeconomic status, ATEC scores, BOT-2 scores and urinary measures

The relationships between socioeconomic status, ATEC scores (both domain and composite), BOT-2 scores (subdomains, domains and composite) and corrected concentrations of neopterin and cortisol in the urine for each participant was assessed using different correlation approaches as appropriate. Spearman's correlation was used for the non-normally-distributed variables, while Pearson's correlation was used for the normally-distributed variables.

4.6.3 Longitudinal data assessing exercise intervention

Correlations between corrected urinary neopterin and corrected urinary cortisol scores for each child over the 18 sessions in the intervention were assessed using Pearson's or Spearman's correlation tests according to whether each data set was normally- or non-normally-distributed (as confirmed by Shapiro-Wilk test). Before and after scores on the ATEC and BOT-2 for the participants enrolled in the longitudinal assessment of the exercise intervention were analysed for difference using the Wilcoxon signed-rank test, since the sample size obtained in this pilot study was too small to use a parametric t-test. The corrected urinary concentrations of cortisol and neopterin were also averaged over the 18-week period for each participant. The averages thus obtained were also assessed for correlation with the initial ATEC and BOT-2 scores using Spearman's correlations. Visualisations of the urinary measures collected over the 18 sessions in the intervention were created using the ggplot package in R statistical software.

# Chapter 5: Results

Initially, participants were recruited only at CMI through information sheets and consent forms sent home with children for parents to read and sign. The bulk of the total sample (n=44) was recruited from JSA in this way, between late 2019 and early 2020. The first set of measures were collected from these children, and they were intended to start the intervention with Fight with Insight from March 2020. However, with the onset of the COVID-19 pandemic, the country went into a state of lockdown that included school closures from March 2020. Although schools reopened later that year, Fight with Insight was unable to restart classes for JSA due to the large number of children at the school, which necessitated a rotational attendance schedule and significant requirements for sanitizer, masks and disposable gloves in order to remain safe during the pandemic. Thus, the majority of the participants originally recruited did not have the opportunity to follow through with the intervention at all. In 2021, Fight with Insight began conducting their exercise classes at CARE, and I was therefore able to recruit some participants for the longitudinal study there. However, CARE is a much smaller facility, with only about 30 children in attendance (as compared with JSA, at over 300 students). I was only able to recruit 6 participants from CARE out of approximately 15 involved in the exercise class. One participant moved schools soon after the intervention began, and another underwent a surgery a few weeks into the intervention period that necessitated his exclusion from involvement in the class. Out of the remaining four, three completed the required 18 sessions, but the fourth started the intervention a few weeks later than his peers, and completion of the intervention period was cut short by another COVID-19-related school shutdown.

## 5.1 Sample Characteristics

Data were collected from 50 participants in total, and the median age of the sample was 13 years (IQR = 11 – 15). The sample was 92% male (n=46) and 8% female (n=4). In terms of support needs, 72% of the sample were of low-support needs (n=36), 24% of the sample were of medium-support needs (n=12) and 4% of the sample were of high-support needs (n=2). Most of the participants attended a government school for ASD (n=44, 88% of the sample), while a minority attended a private school for ASD (n=6, 12% of the sample). The race of the sample was 84% black (n=42), 8% white (n=4) and 8% Indian (n=4). Not all measures were collected for all 50 participants, as some refused to participate in certain procedures or

provide certain information, and some of the processes were impacted by COVID-19 shut-downs (particularly the conduct of BOT-2 assessments).

Table 5.1 summarises socioeconomic status as well as composite and subdomain scores for the ATEC and BOT-2. The distributions of each variable as confirmed by Shapiro-Wilk test, as well as the maximum possible scores for each variable are also given in Table 5.1. The highest score on the socioeconomic status questionnaire corresponds with a middle-class socioeconomic level and does not differentiate higher levels. The sample fell primarily on the higher end of the socioeconomic status score. In terms of the ATEC assessment, summary statistics for the sample are closer to the maximum score than the minimum score for each domain, which corresponds well with a sample comprised mostly of children regarded as having low- to medium-support needs. For the BOT-2, summary statistics for the subdomains of the sample are much closer to the minimum possible value than the maximum, which indicates very low motor competence in all domains. This is as expected since the BOT-2 was developed using standards from typically developing children. The summary statistics for the domains and the composite are a bit below the 50% score, which also indicates low motor competence. In addition, Table 5.1 includes summary statistics for urinary concentrations of cortisol and neopterin (original and corrected by specific gravity) and urinary specific gravity measures. The Cronbach's alpha for the subset of the sample that completed the ATEC was 0.734, and 0.917 for the subset of the sample that completed the BOT-2.

Figures 5.1 to 5.4 show the results of the ATEC questionnaire from the cohort. Figure 5.1 shows the results from the Speech/Language/Communication sub-domain of the ATEC, which comprises 14 items scored from "not true" to "very true". The results indicating an item was "very true" are given in Figure 5.1. While 100% of the children knew their own name, only 37% had a normal ability to communicate for their age. Figure 5.2 shows results from the Sociability sub-domain of the ATEC, which comprises 20 items scored from "not descriptive" to "very descriptive". The results indicating an item was "very descriptive" are given in Figure 5.2. Temper tantrums and lacking friends showed up the most commonly, at 29% and 37% respectively. Figure 5.3 shows results from the Sensory/Cognitive Awareness sub-domain of the ATEC, which comprises 18 items scored from "not descriptive" to "very descriptive". The results indicating an item was "very descriptive" are given in Figure 5.3.

While 100% of the children responded to their name, only 27% were “tuned in” to their surroundings. Figure 5.4 shows results from the Health/Physical/Behaviour sub-domain of the ATEC, which comprises 25 items scored from “not a problem” to “serious problem”. The results indicating an item was either a “serious problem” or a “moderate problem” are given in Figure 5.4. None of the cohort reported diarrhoea as a problem, but fixations were identified as problematic in 39% of the cohort.

Table 5.1: Summary statistics for socioeconomic status, ATEC and BOT-2 scores and measures of urinary cortisol, neopterin and specific gravity in a sample of autistic children (total n=50)

| **Variable** | **Distribution** | **Mean ± SD** | **Maximum score** |
|---|---|---|---|
| Socioeconomic status (n=48) | Non-normal | 7.96 ± 1.40 | 9 |
| ATEC scores (n=41) | | | |
| Speech/Language/Communication | Non-normal | 20.22 ± 6.10 | 28 |
| Sociability | Non-normal | 28.27 ± 8.07 | 40 |
| Sensory/Cognitive Awareness | Non-normal | 27.0 ± 6.63 | 36 |
| Health/Physical/Behaviour | Non-normal | 58.90 ± 14.93 | 75 |
| ATEC composite | Non-normal | 134.46 ± 28.73 | 179 |
| BOT-2 scores (n=25) | | | |
| Fine Motor Precision | Normal | 7.9 ± 3.3 | 35 |
| Fine Motor Integration | Normal | 9.0 ± 4.4 | 35 |
| Manual Dexterity | Normal | 5.1 ± 2.3 | 35 |
| Bilateral Co-ordination | Non-normal | 9.04 ± 4.72 | 35 |
| Balance | Non-normal | 10.56 ± 5.08 | 35 |
| Running Speed & Agility | Non-normal | 7.44 ± 3.12 | 35 |
| Upper-limb Co-ordination | Normal | 11.9 ± 7.1 | 35 |
| Strength | Normal | 6.8 ± 2.6 | 35 |
| Fine Manual Control | Normal | 35.2 ± 6.9 | 80 |
| Manual Co-ordination | Normal | 34.6 ± 8.7 | 80 |
| Body Co-ordination | Normal | 37.4 ± 10.7 | 80 |
| Strength & Agility | Normal | 33.4 ± 5.8 | 80 |
| BOT-2 Composite | Normal | 32.6 ± 6.6 | 80 |
| Urinary measures (n=40) | | | |
| Urine Specific Gravity | Normal | 1.03 ± 0.01 | N/A |
| Urinary cortisol concentration (ng/ml) | Non-normal | 49.81 ± 46.98 | N/A |
| Urinary neopterin concentration (ng/ml) | Non-normal | 3.54 ± 2.13 | N/A |
| Corrected urinary cortisol (ng/ml) | Non-normal | 53.69 ± 47.13 | N/A |
| Corrected urinary neopterin (ng/ml) | Normal | 4.2 ± 2.1 | N/A |

ATEC = Autism Treatment Evaluation Checklist, BOT-2 = Bruininks-Oseretsky Motor Proficiency Test Second Edition. Distribution of data was assessed using Shapiro-Wilk test..

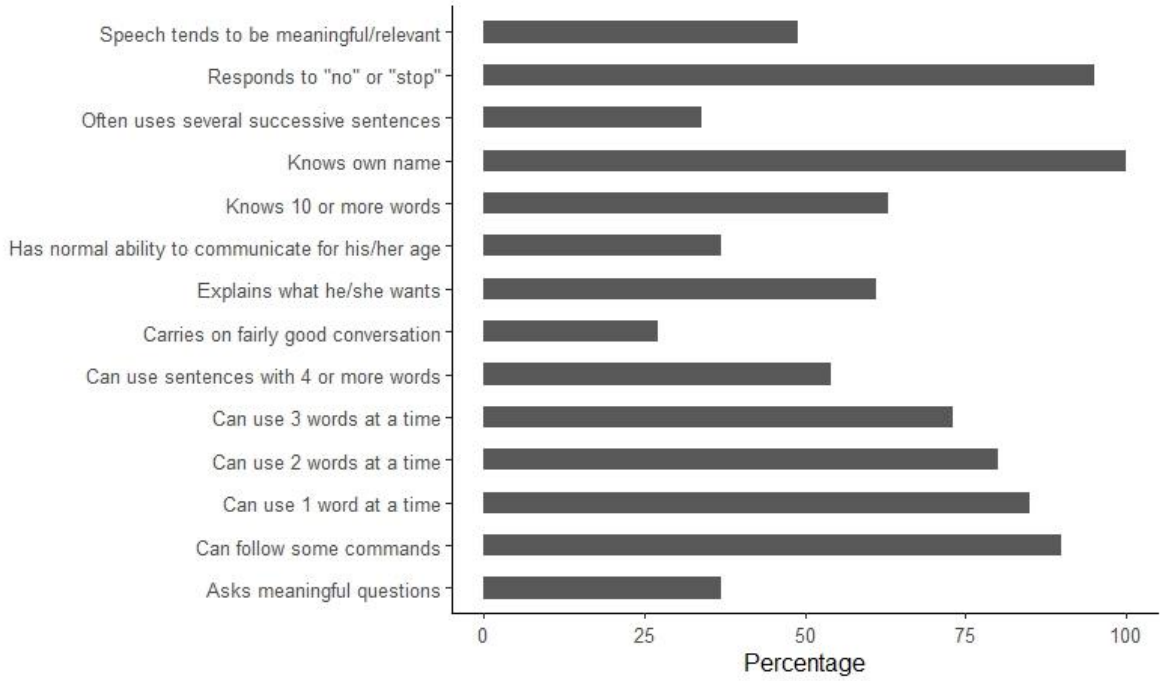


Figure 5.1: Graphical representation of "very true" response counts for the Speech/Language/Communication sub-domain of the ATEC (n=41)

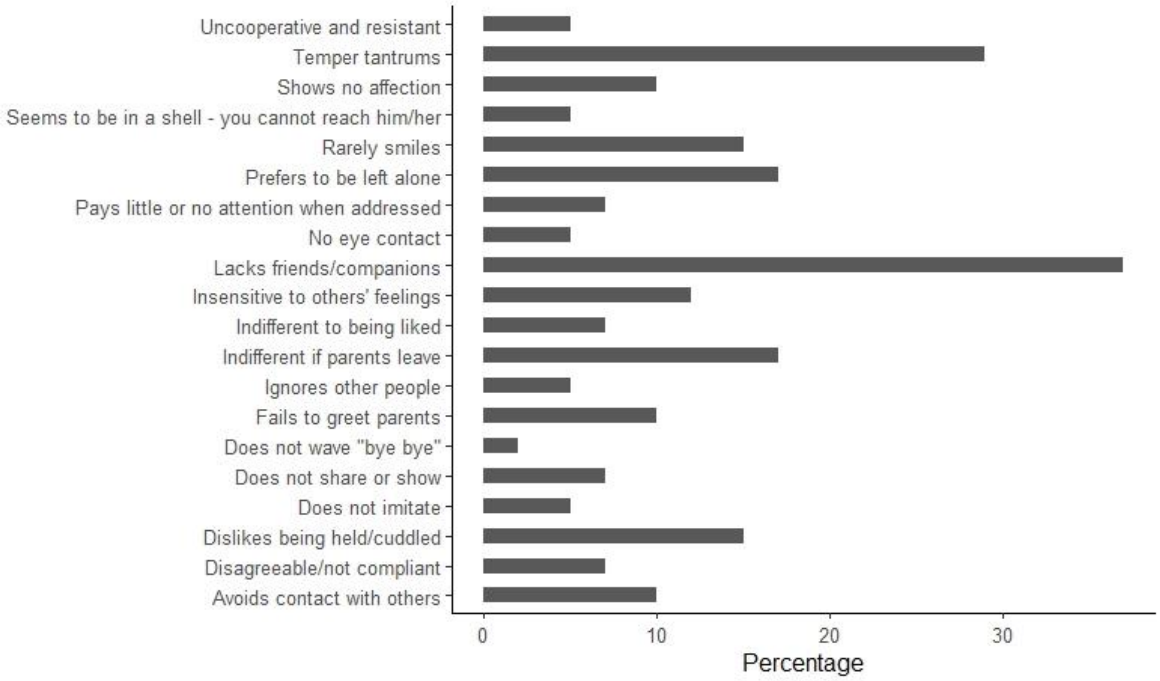


Figure 5.2: Graphical representation of "very true" response counts for the Sociability sub-domain of the ATEC (n=41)

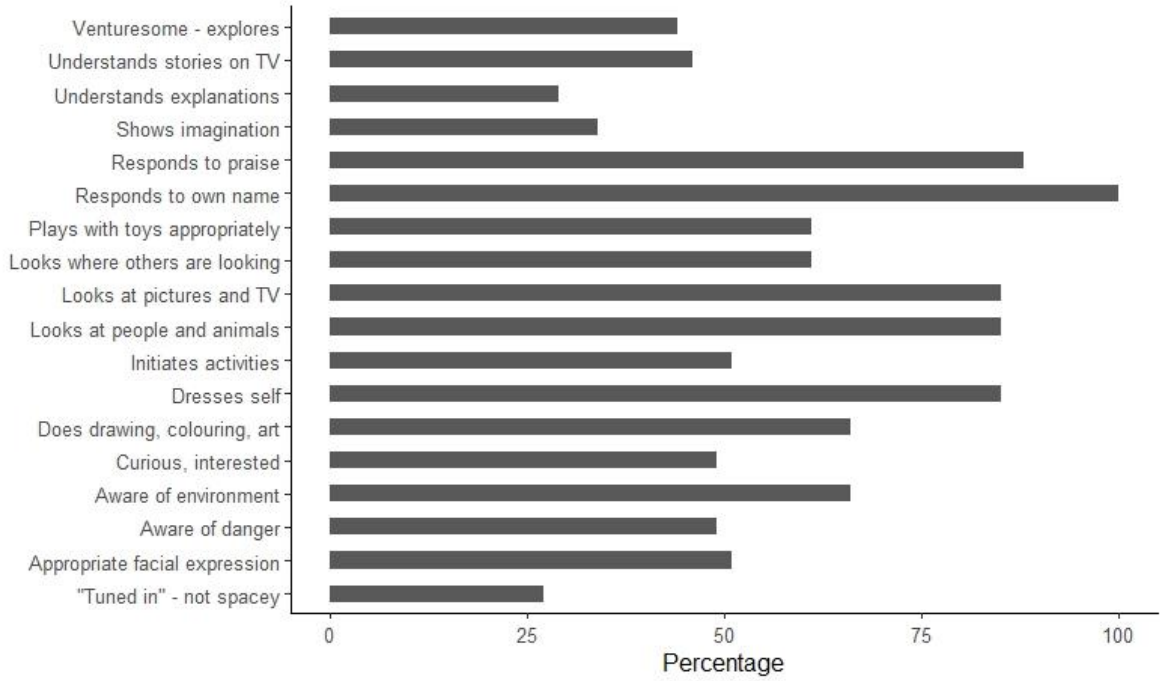


Figure 5.3: Graphical representation of "very true" response counts for the Sensory/Cognitive Awareness sub-domain of the ATEC (n=41)

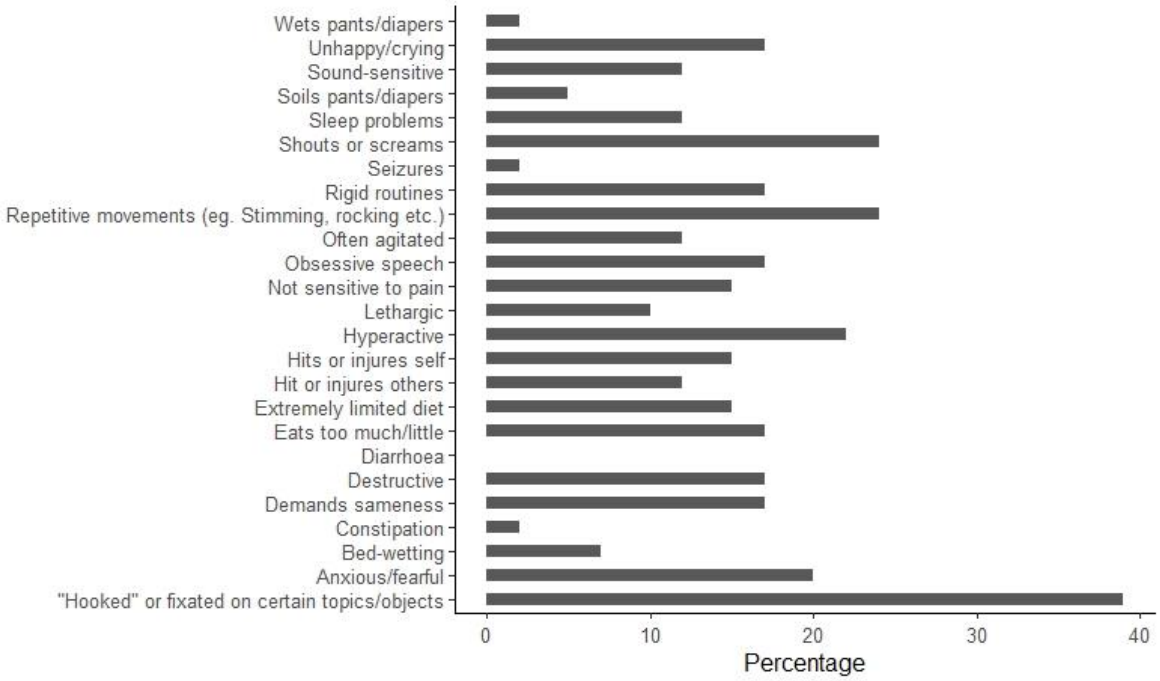


Figure 5.4: Graphical representation of "very true" response counts for the Health/Physical/Behaviour sub-domain of the ATEC (n=41)

### 5.2 Correlations between socioeconomic status, ATEC scores, BOT-2 scores and urinary measures of cortisol and neopterin

Table 5.2 shows correlation co-efficients and p-values for relationships between ATEC scores and BOT-2 scores, analysed from the sub-set of the sample that completed both the ATEC and the BOT-2 (n=19) using the Spearman's correlation test. Many significant relationships were found between ATEC and BOT-2 scores. No significant relationships were found between any of the ATEC scores and any of the urinary measures (Table 5.3, n=30), as assessed with the Spearman's correlation test. Scores for Balance and Running Speed & Agility were found to correlate with urinary cortisol concentrations (Table 5.4, n=19), as assessed by Spearman's correlation test. No significant relationships were found between socioeconomic status and any of the ATEC scores (Table 5.5, n=35) as assessed by Spearman's correlation, or between socioeconomic status and any of the BOT-2 scores (Table 5.6, n=25) as assessed by Spearman's correlation. The correlation coefficients (p-values) for relationships between socioeconomic status and corrected urinary cortisol, and socioeconomic status and corrected urinary neopterin were found to be 0.190 (0.253) and -0.082 (0.624) respectively, as assessed by Spearman's correlation. All correlation analyses were conducted using the data obtained from the initial measures for each participant.

Table 5.2: Summary statistics for relationships between ATEC scores (across) and BOT-2 scores (down) for an autistic sample (n=19) as assessed by Spearman's correlation

| | Speech/ Language/ Communication | Sociability | Sensory/ Cognitive Awareness | Health/ Physical/ Behaviour | ATEC Composite |
|---|---|---|---|---|---|
| Fine Motor Precision | **0.615 (0.005)*** | **0.633 (0.004)*** | **0.714 (<0.001)*** | **0.493 (0.032)*** | **0.637 (0.003)*** |
| Fine Motor Integration | **0.567 (0.011)*** | **0.490 (0.033)*** | **0.591 (0.008)*** | 0.405 (0.085) | **0.523 (0.022)*** |
| Fine Manual Control | **0.631 (0.004)*** | **0.558 (0.013)*** | **0.671 (0.002)*** | 0.401 (0.089) | **0.575 (0.010)*** |
| Manual Dexterity | **0.616 (0.005)*** | 0.362 (0.128) | **0.503 (0.028)*** | 0.102 (0.678) | **0.469 (0.043)*** |
| Upper-limb Co-ordination | **0.694 (0.001)*** | **0.505 (0.0528)*** | **0.622 (0.004)*** | 0.320 (0.181) | **0.598 (0.007)*** |
| Manual Co-ordination | **0.703 (0.001)*** | **0.522 (0.022)*** | **0.640 (0.003)*** | 0.290 (0.229) | **0.629 (0.004)*** |
| Bilateral Co-ordination | **0.616 (0.005)*** | **0.524 (0.021)*** | **0.510 (0.026)*** | 0.437 (0.062) | **0.625 (0.004)*** |
| Balance | **0.701 (0.001)*** | **0.770 (<0.001)*** | **0.762 (<0.001)*** | **0.485 (0.035)*** | **0.819 (<0.001)*** |
| Body Co-ordination | **0.669 (0.002)*** | **0.760 (<0.001)*** | **0.742 (<0.001)*** | **0.553 (0.014)*** | **0.830 (<0.001)*** |
| Running Speed & Agility | **0.605 (0.006)*** | **0.551 (0.015)*** | **0.602 (0.006)*** | 0.423 (0.071) | **0.607 (0.006)*** |
| Strength | **0.767 (<0.001)*** | **0.573 (0.010)*** | **0.729 (<0.001)*** | 0.281 (0.245) | **0.573 (0.010)*** |
| Strength & Agility | **0.702 (0.001)*** | **0.658 (0.002)*** | **0.775 (<0.001)*** | 0.415 (0.077) | **0.693 (0.001)*** |
| BOT-2 Composite | **0.780 (<0.001)*** | **0.670 (0.002)*** | **0.775 (<0.001)*** | **0.458 (0.049)*** | **0.760 (<0.001)*** |

ATEC = Autism Treatment Evaluation Checklist, BOT-2 = Bruininks-Oseretsky Motor Proficiency Test Second Edition. Correlation co-efficients (p-values) are presented, with significant relationships given in bold and marked with an asterisk (*). Significance was set at $p<0.05$.

Table 5.3: Summary statistics for relationships between ATEC scores and urinary measures of cortisol and neopterin (after correction to a specific gravity of 1.030) for an autistic sample (n=30) as assessed by Spearman's correlation

| | Urinary Cortisol | Urinary Neopterin |
|---|---|---|
| Speech/Language/Communication | 0.089 (0.634) | 0.261 (0.157) |
| Sociability | -0.179 (0.336) | 0.072 (0.699) |
| Sensory/Cognitive Awareness | -0.026 (0.890) | 0.192 (0.300) |
| Health/Physical/Behaviour | 0.100 (0.591) | 0.219 (0.237) |
| ATEC Composite | -0.063 (0.737) | 0.128 (0.492) |

ATEC = Autism Treatment Evaluation Checklist. Correlation co-efficients (p-values) are presented, with significant relationships given in bold and marked with an asterisk (*). Significance was set at $p<0.05$.

Table 5.4: Summary statistics for relationships between BOT-2 scores and urinary measures of cortisol and neopterin (after correction to a specific gravity of 1.030) for an autistic sample (n=19). All relationships were tested using Spearman's correlation, with the exception of Balance vs neopterin, Bilateral Co-ordination vs neopterin, and Running Speed &Agility vs neopterin.

| | Urinary Cortisol | Urinary Neopterin |
|---|---|---|
| Fine Motor Precision | -0.244 (0.314) | 0.208 (0.392) |
| Fine Motor Integration | -0.442 (0.058) | -0.152 (0.534) |
| Fine Manual Control | -0.388 (0.101) | -0.090 (0.714) |
| Manual Dexterity | -0.067 (0.786) | -0.291 (0.226) |
| Upper-limb Co-ordination | 0.008 (0.974) | 0.098 (0.691) |
| Manual Co-ordination | -0.067 (0.786) | -0.028 (0.909) |
| Bilateral Co-ordination | -0.295 (0.220) | -0.017 (0.946) |
| Balance | **-0.466 (0.044)*** | -0.106 (0.666) |
| Body Co-ordination | -0.453 (0.051) | -0.102 (0.678) |
| Running Speed & Agility | **-0.483 (0.036)*** | -0.098 (0.688) |
| Strength | -0.342 (0.152) | -0.177 (0.468) |
| Strength & Agility | -0.447 (0.055) | -0.104 (0.671) |
| BOT-2 Composite | -0.342 (0.152) | -0.108 (0.660) |

BOT-2 = Bruininks-Oseretsky Motor Proficiency Test Second Edition. Correlation co-efficients (p-values) are presented, with significant relationships given in bold and marked with an asterisk (*). Significance was set at $p<0.05$.

Table 5.5: Summary statistics for relationships between ATEC scores and socioeconomic status for an autistic sample (n=39) as assessed by Spearman's correlation.

| | Socioeconomic status |
|---|---|
| Speech/Language/Communication | 0.008 (0.963) |
| Sociability | 0.074 (0.655) |
| Sensory/Cognitive Awareness | -0.083 (0.614) |
| Health/Physical/Behaviour | 0.125 (0.447) |
| ATEC Composite | 0.082 (0.621) |

ATEC = Autism Treatment Evaluation Checklist. Correlation co-efficients (p-values) are presented, with significant relationships given in bold and marked with an asterisk (*). Significance was set at $p<0.05$.

Table 5.6: Summary statistics for relationships between BOT-2 scores and socioeconomic status for an autistic sample (n=25) as assessed by Spearman's correlation

| | Socioeconomic status |
|---|---|
| Fine Motor Precision | -0.206 (0.323) |
| Fine Motor Integration | -0.161 (0.442) |
| Fine Manual Control | -0.195 (0.349) |
| Manual Dexterity | -0.195 (0.351) |
| Upper-limb Co-ordination | 0.016 (0.938) |
| Manual Co-ordination | 0.028 (0.893) |
| Bilateral Co-ordination | -0.064 (0.763) |
| Balance | -0.165 (0.431) |
| Body Co-ordination | -0.104 (0.621) |
| Running Speed & Agility | -0.265 (0.200) |
| Strength | -0.123 (0.558) |
| Strength & Agility | -0.225 (0.279) |
| BOT-2 Composite | -0.162 (0.440) |

BOT-2 = Bruininks-Oseretsky Motor Proficiency Test Second Edition. Correlation co-efficients (p-values) are presented, with significant relationships given in bold and marked with an asterisk (*). Significance was set at $p<0.05$.

### 5.3 Longitudinal data assessing exercise intervention

A full data set (before and after measures) for the 18 sessions in the intervention was only collected for three participants. A nearly complete data set was collected from a fourth participant, but due to a COVID-19 shutdown, the last measures could not be collected for that participant. Data and a short history of each participant are provided below.

Figures 5.5 and 5.6 show the changes in morning void urinary cortisol concentrations over the course of the 18 weeks the children were enrolled in the study. Figures 5.7 and 5.8 show the changes in morning void urinary neopterin concentrations over the course of the 18 weeks the children were enrolled in the study. In Figures 5.5 and 5.7 the changes in concentrations for each child are depicted individually, with changes calculated by defining the first measurement as the baseline measure and the baseline measure minused from each subsequent measure. In Figures 5.6 and 5.8 the original concentrations are averaged for the group at each time point. There are a few missing data points where a child was sick for that week or away from school for another reason. Notably, Participant 44 did not complete the full 18 weeks of the intervention due to a COVID-19 shutdown.

Participant 39 was a 10-year-old girl of medium-support needs. She had limited verbal language skills and very specific interests akin to obsessions. Her parents communicated well with the school in which she was enrolled and also communicated well with the lead researcher during the research project. Although she was placed in a medium-support class, she had the lowest composite ATEC score out of the four children presented here. She had the lowest scores specifically in the Sociability and Sensory/Cognitive Awareness domains. While all of the children assessed with the BOT-2 in this study scored below average for their ages, participant 39 scored particularly low, especially in the subdomains of Fine Motor Precision, Manual Dexterity, Bilateral Co-ordination, Balance and Strength. Her lowest scoring domain was Body Co-ordination. Participant 39 showed fairly consistent urinary profiles, with the largest increases in both cortisol and neopterin occurring at Week 11.

Participant 40 was a 10-year-old girl of low-support needs. Although she did not often express herself verbally, she clearly understood instructions and used actions to express her desires. She did well academically, specifically in reading, writing and mathematics. Her parents also communicated well with the school and communicated well with the lead researcher during the research project. Participant 40 had the highest composite ATEC score

out of the four children presented here, with particularly high scores in Sociability, Sensory/Cognitive Awareness and Health/Physical/Behaviour. Participant 40 had the second highest composite score for the BOT-2 out of the four children, with her highest domain score in Fine Manual Control. Her lowest subdomain score was in Manual Dexterity. While her cortisol levels initially decreased, largely she underwent increases in cortisol levels from baseline. However, her neopterin levels initially increased and then mostly showed decreases from baseline.

Participant 42 was an 11-year-old boy of low-support needs. His verbal skills were much more developed than the other three participants enrolled in the longitudinal study. However, he was a highly anxious child and seemed very concerned with approval from authority figures. Teachers at the school found his family difficult to communicate with. His father in particular appeared to be uncooperative. Participant 42 had the second highest composite ATEC score, closely following participant 40. He scored the highest of all four children in the Speech/Language/Communication domain, and also scored quite high in the Sensory/Cognitive Awareness domain. Participant 42 had the highest composite score on the BOT-2 out of the four children, scoring the highest in the Manual Co-ordination domain. His ball skills were particularly good, and he scored well in the Upper-limb Co-ordination sub-domain. Participant 42's cortisol pattern shows the most extreme increase of all four children, occurring at week 5. The measurements at weeks 3 and 4 are missing as he was away from school those weeks with a short-term illness, which may account for the massive increase in cortisol.

Participant 44 was a 13-year-old boy of high-support needs. His verbal language skills were extremely limited. He was highly distractible and struggled to understand instructions. His family showed good communication although there was evidence that he moved around a lot between different family members. Participant 44 was the only child out of the four presented here to not score a full 9 out of 9 on the socioeconomic status questionnaire, with a score of 7. Although participant 44 was placed in a high-support class, he did not score the lowest on the ATEC composite out of the four children presented here. However, he had the lowest scores in the Speech/Language/Communication and Health/Physical/Behaviour domains out of the four children. Participant 44 had the lowest composite score on the BOT-2 out of the four children, scoring particularly low on the Strength & Agility and Body Co-ordination

subdomains. While his urinary cortisol profile exhibits much decrease, his neopterin profile only shows increases over the course of the 18 weeks.

Table 5.7 gives correlation coefficients and p-values for the relationships between urinary neopterin and urinary cortisol for each participant, collected weekly over the 18-week period enrolled in the intervention. No significant relationships between urinary neopterin and urinary cortisol were found. Table 5.8 provides p-values for the changes in ATEC and BOT-2 scores as well as urinary measures from before starting the intervention to after 18 weeks enrolled in the intervention. No significant changes were observed in any of the measures taken. Scores for all sub-sections of the ATEC increased non-significantly in each individual, with the exception of Participant 40's Sociability score, which decreased slightly. Scores for the sub-sections of the BOT-2 were variable in the changes exhibited by each individual, with some scores increasing, some decreasing and some staying the same. Fine Motor Precision decreased in all three participants, and Upper-limb Co-ordination increased in all three participants. No other sub-sections showed consistent changes across all three participants. Table 5.9 provides correlation co-efficients and p-values for the relationships between the initial BOT-2 scores, and the corrected urinary measures of cortisol and neopterin averaged for each participant over the 18 weeks of samples. Table 5.10 provides correlation co-efficients and p-values for the relationships between the initial ATEC scores, and the corrected urinary measures of cortisol and neopterin averaged for each participant over the 18 weeks of samples.

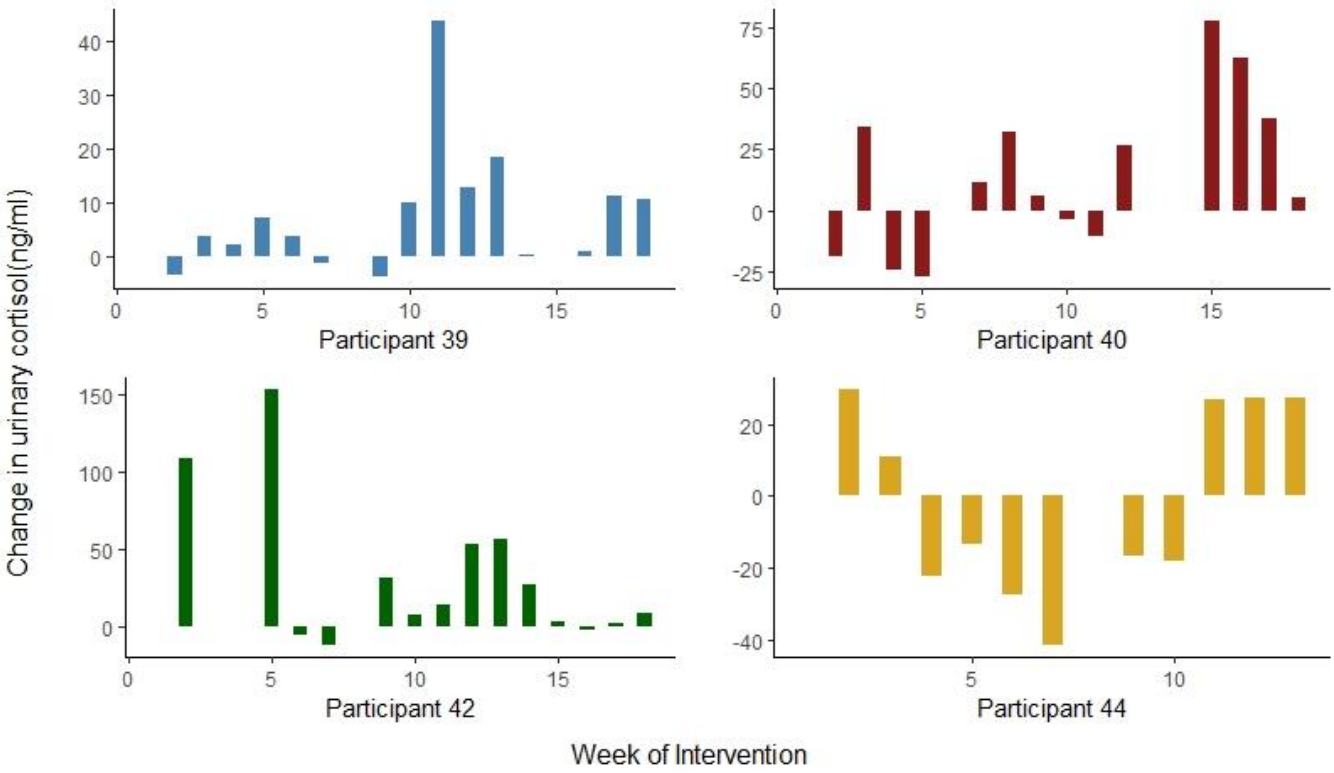


Figure 5.5: Changes in morning void urinary cortisol concentrations (corrected by specific gravity) for each autistic participant over the course of 18 weeks enrolled in an exercise intervention.

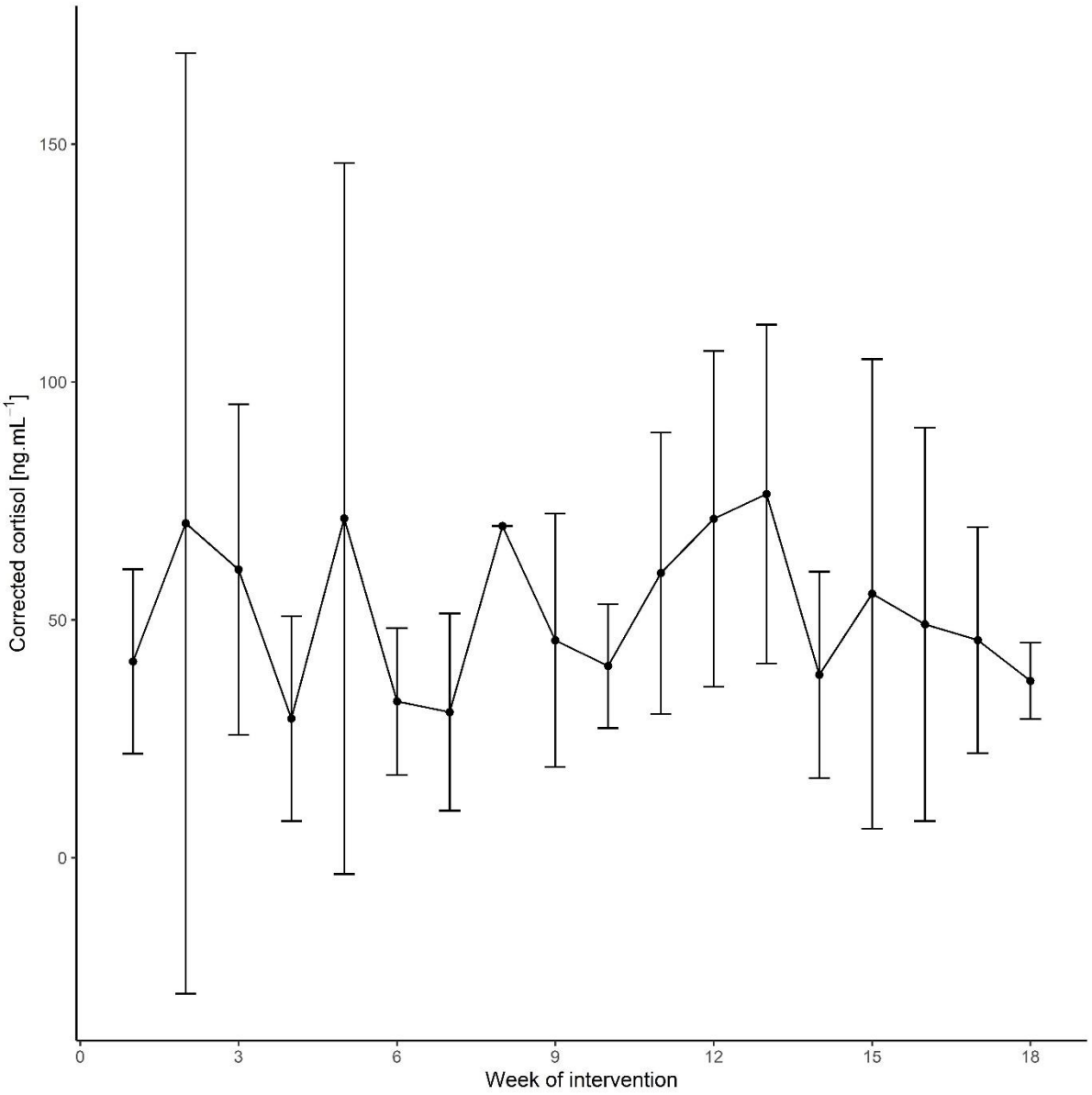


Figure 5.6: Average morning void urinary cortisol concentrations (corrected by specific gravity) for an autistic sample over the course of 18 weeks enrolled in an exercise intervention (n=4).

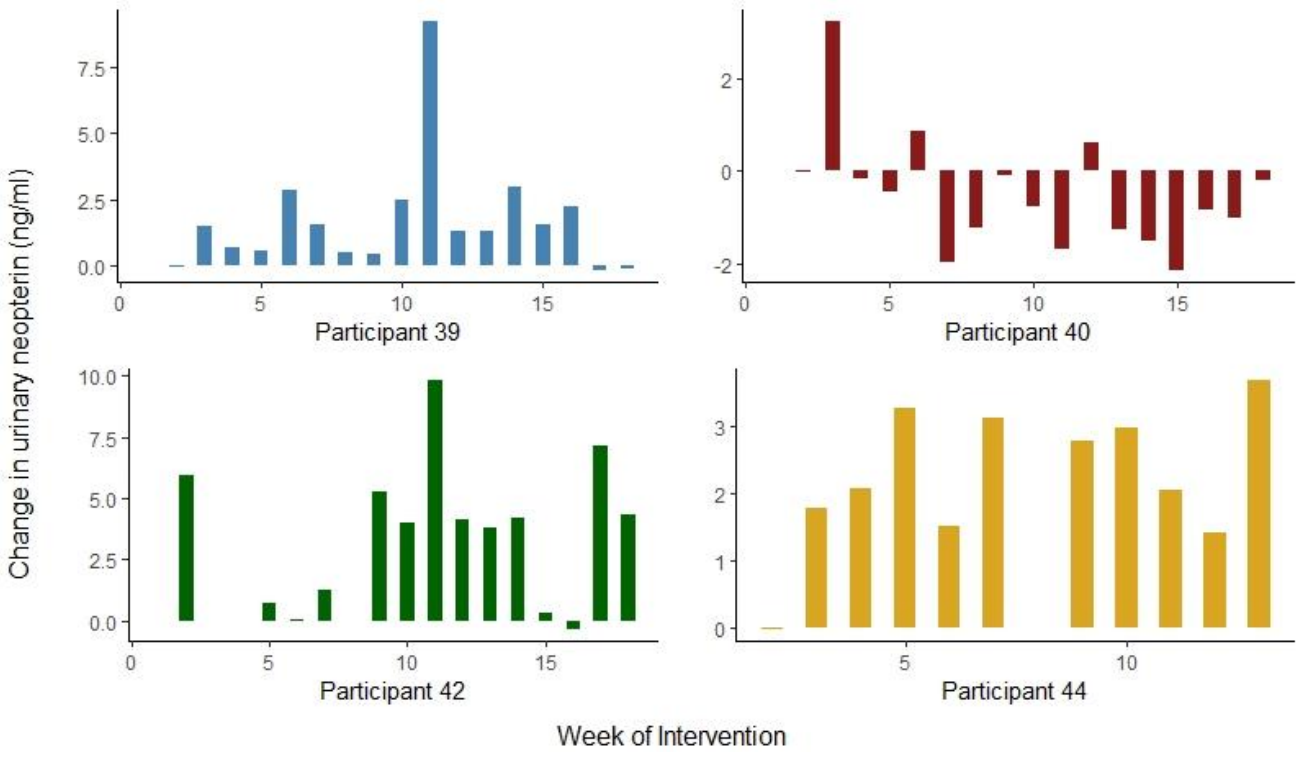


Figure 5.7: Changes in morning void urinary neopterin concentrations (corrected by specific gravity) for each autistic participant over the course of 18 weeks enrolled in an exercise intervention.

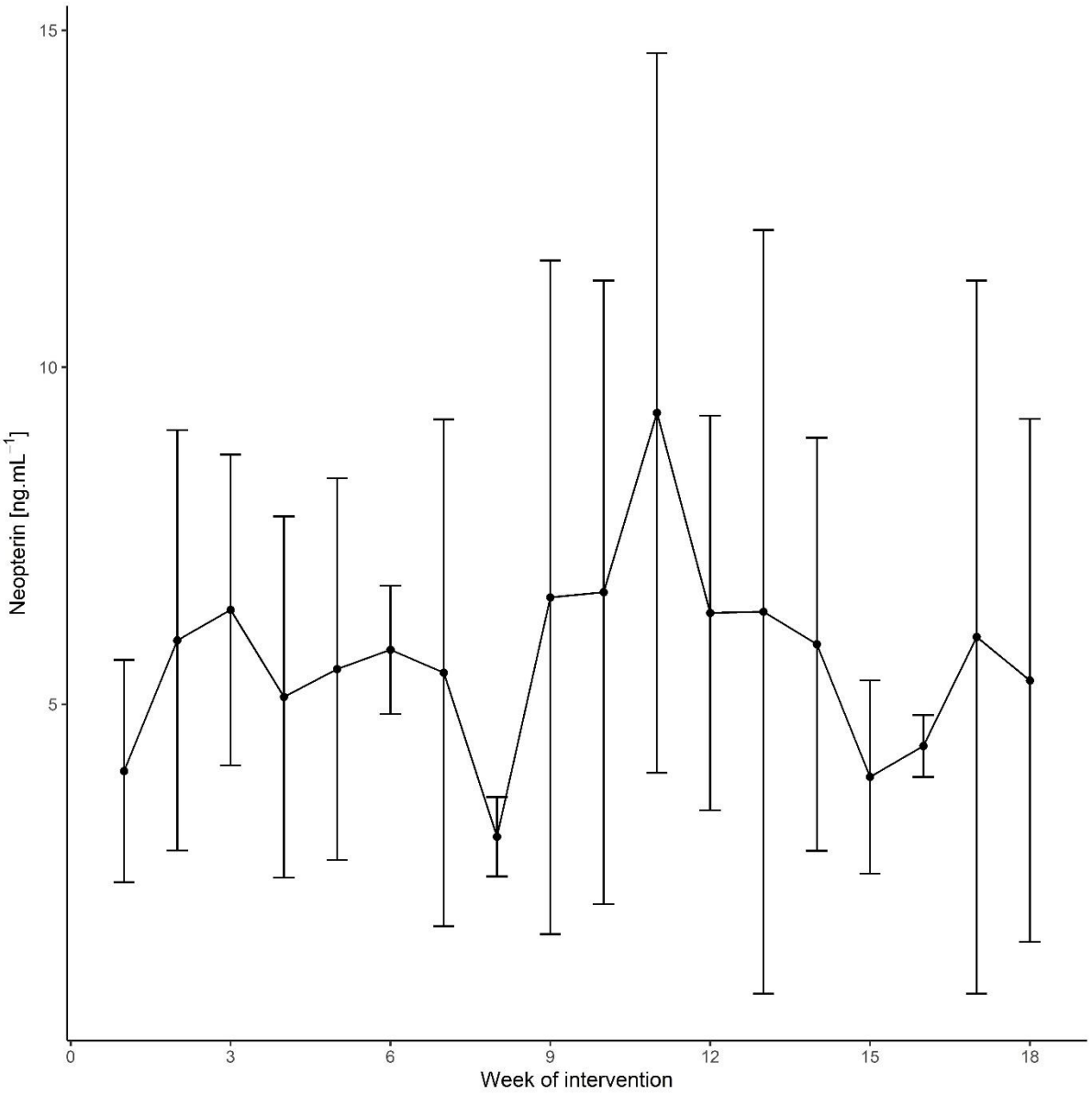


Figure 5.8: Average morning void urinary neopterin concentrations (corrected by specific gravity) for an autistic sample over the course of 18 weeks enrolled in an exercise intervention (n=4).

Table 5.7: Summary statistics for relationships between urinary cortisol and neopterin values (corrected to a specific gravity of 1.030) for four autistic participants, collected weekly over the 18 weeks of the intervention

| Subject | |
|---|---|
| 39 | 0.108 (0.680) |
| 40 | -0.264 (0.340) |
| 42 | 0.443 (0.100) |
| 44 | -0.345 (0.299) |

Correlation co-efficients (p-values) are presented, with significant relationships given in bold and marked with an asterisk (*). Significance was set at $p<0.05$.

Table 5.8: Summary statistics for Wilcoxon signed-rank tests assessing changes in ATEC, BOT-2, and urinary measures in an autistic sample from before starting the intervention to after 18 weeks in the intervention (n=3)

| **Variable** | **p-value** | **Mean score before** | **Mean score after** |
|---|---|---|---|
| ATEC scores | | | |
| Speech/Language/Communication | 0.250 | 17.0 | 22.0 |
| Sociability | 0.500 | 24.7 | 29.3 |
| Sensory/Cognitive Awareness | 0.250 | 22.7 | 26.7 |
| Health/Physical/Behaviour | 0.250 | 61.0 | 65.0 |
| ATEC composite | 0.250 | 126.0 | 143.3 |
| BOT-2 scores | | | |
| Fine Motor Precision | 0.174 | 7.0 | 5.3 |
| Fine Motor Integration | 0.500 | 13.3 | 11.0 |
| Manual Dexterity | 1.000 | 5.0 | 4.0 |
| Bilateral Co-ordination | 0.346 | 6.0 | 6.7 |
| Balance | 1.000 | 7.3 | 9.7 |
| Running Speed & Agility | 1.000 | 6.7 | 6.7 |
| Upper-limb Co-ordination | 0.250 | 12.7 | 10.7 |
| Strength | 0.371 | 6.7 | 5.7 |
| Fine Manual Control | 0.371 | 39.3 | 33.7 |
| Manual Co-ordination | 0.250 | 34.7 | 30.7 |
| Body Co-ordination | 1.000 | 30.7 | 33.7 |
| Strength & Agility | 0.773 | 31.3 | 30.7 |
| BOT-2 Composite | 0.500 | 31.7 | 30.0 |
| Urinary measures | | | |
| Corrected urinary cortisol (ng/ml) | 0.250 | 28.9 | 37.2 |
| Corrected urinary neopterin (ng/ml) | 1.000 | 4.0 | 5.4 |

ATEC = Autism Treatment Evaluation Checklist, BOT-2 = Bruininks-Oseretsky Motor Proficiency Test Second Edition. Significant relationships are given in bold and marked with an asterisk (*). Significance was set at $p<0.05$.

Table 5.9: Summary statistics for relationships between initial BOT-2 scores, and corrected urinary cortisol and neopterin concentrations averaged over 18 weeks of samples for an autistic sample (n=4)

| | Urinary Cortisol | Urinary Neopterin |
|---|---|---|
| Fine Motor Precision | -0.400 (0.750) | 0.000 (1.000) |
| Fine Motor Integration | -0.316 (0.684) | 0.211 (0.789) |
| Fine Manual Control | -0.400 (0.750) | 0.000 (1.000) |
| Manual Dexterity | 0.105 (0.895) | 0.632 (0.368) |
| Upper-limb Co-ordination | 0.200 (0.917) | 0.400 (0.750) |
| Manual Co-ordination | -0.200 (0.917) | 0.400 (0.750) |
| Bilateral Co-ordination | -0.400 (0.750) | 0.000 (1.000) |
| Balance | 0.105 (0.895) | 0.632 (0.368) |
| Body Co-ordination | -0.200 (0.917) | 0.400 (0.750) |
| Running Speed & Agility | -0.316 (0.684) | 0.211 (0.789) |
| Strength | -0.400 (0.750) | 0.000 (1.000) |
| Strength & Agility | -0.400 (0.750) | 0.000 (1.000) |
| BOT-2 Composite | -0.200 (0.917) | 0.400 (0.750) |

BOT-2 = Bruininks-Oseretsky Motor Proficiency Test Second Edition. Correlation co-efficients (p-values) are presented, with significant relationships given in bold and marked with an asterisk (*). Significance was set at $p<0.05$.

Table 5.10: Summary statistics for relationships between initial ATEC scores, and corrected urinary cortisol and neopterin concentrations averaged over 18 weeks of samples for an autistic sample (n=4)

| | Urinary Cortisol | Urinary Neopterin |
|---|---|---|
| Speech/Language/Communication | -0.400 (0.750) | 0.200 (0.917) |
| Sociability | 0.200 (0.917) | 0.400 (0.750) |
| Sensory/Cognitive Awareness | 0.316 (0.684) | 0.632 (0.368) |
| Health/Physical/Behaviour | 0.400 (0.750) | 0.000 (1.000) |
| ATEC Composite | 0.200 (0.917) | 0.400 (0.750) |

ATEC = Autism Treatment Evaluation Checklist. Correlation co-efficients (p-values) are presented, with significant relationships given in bold and marked with an asterisk (*). Significance was set at $p<0.05$.

# Chapter 6: Discussion and Conclusion

This thesis focused on a sample of children with ASD in Johannesburg and began with an investigation of the literature pertaining to conducting biomedical research on ASD in an ethical manner. The investigation of literature resulted in some suggestions regarding implementation of structural support for community engagement processes in biomedical research spaces. Such support would prove invaluable in assisting researchers in the creation and execution of research projects that have a greater positive impact on the community.

Furthermore, the results of the data-driven studies conducted provide insight into the characteristics of a sample of children with ASD in the Johannesburg region of South Africa. Specifically, data was collected describing autistic symptomatology, motor skills and socioeconomic status. This cohort showed a bias towards higher socioeconomic status and a great disparity between males and females diagnosed with ASD. Additionally, motor skills in this cohort are lower than what would be expected in typically-developing children of the same age, but better than the motor skills observed in autistic populations in previous research. The autistic symptoms in the cohort were less severe than those observed in an autistic cohort in Saudi Arabia assessed with the same instrument, and our cohort also displayed different patterns in terms of which characteristics were the most and least severe. Additionally, in order to investigate the role of the stress-immune axis in ASD, the urinary levels of cortisol and neopterin present in the sample were also assessed. No evidence was found for significantly altered urinary cortisol or neopterin in the cohort.

The data collected on the cohort also allowed for correlation tests. Of greatest note are the many correlations observed between the sub-domains of the ATEC and the sub-domains of the BOT-2, indicating relationships between the social/behavioural symptoms of ASD, and the motor impairments that co-occur. These relationships are suggestive of a common underlying physiological aetiology between motor impairment and behavioural symptoms in ASD. Additionally, correlations were found between urinary cortisol and the motor skill domains related to balance, which may be a result of shared circuitry between the neural pathways controlling balance and the neural pathways involved in the stress response. Finally, a small pilot study of an exercise intervention for ASD was conducted in order to assess the effects of exercise on the brain-immune axis. No statistically significant evidence was found that the exercise intervention improved motor skills or ASD symptomatology,

however non-significant improvements were observed in ASD symptomatology. Additionally, while cortisol showed a relationship with balance in the correlation assessments, no evidence was found that the exercise intervention was impacting the brain immune axis, either by influencing the HPA axis or by decreasing activation of cellular immunity. Possible reasons for this could be the very small sample size obtained for the pilot study, and that urinary cortisol was assessed on a weekly basis.

### 6.1 Sample Characteristics

Following an investigation of ethical issues inherent in biomedical research on ASD, the second aim was to characterize a sample of children with ASD in the Johannesburg area. Specifically, socioeconomic status, autistic symptomatology, motor skills and urinary levels of cortisol and neopterin in a cohort of children with ASD were assessed. This sample consisted primarily of children classified as having low-support needs since the children involved in the exercise classes at Fight with Insight were primarily low-support children. The relative percentages of support needs in the sample are not reflective of the relative percentages of support needs in the schools from which the sample was drawn. The sample is therefore unlikely to be fully representative of the support needs of the autistic population in the region. The terms “low-support”, “medium-support” and “high support” can be interpreted differently in different contexts. Importantly, although some of the children in the cohort were considered to be of low-support needs, all the children included in the cohort had a level of symptom severity that would translate to great difficulty incorporating into a mainstream school. While the cohort was not fully reflective of relative support needs, the male/female division of the sample is a better representation of what was observed in the schools from which the sample was drawn, at a ratio of about 10:1. In contrast, previous findings in the Western Cape of South Africa have estimated the male to female ratio in ASD at 5.5:1 (Pillay, Duncan and de Vries, 2021). Moreover, the race representation in the sample is close to the racial proportions of the Johannesburg area, which are 75.4% Black, 12.3% White and 4.9% Indian/Asian according to a 2016 census (*Johannesburg Population (2020/2021)*, no date), although the cohort assessed in this thesis had a slightly lower proportion of White participants and a slightly higher proportion of Black participants. The sample scored high on the socioeconomic status questionnaire, with a median score of nine, which is the maximum score on the questionnaire, despite the fact that most of our participants were drawn from a government school with very low school fees and learners known to be of low socioeconomic status. This may be due to a lack of sensitivity in the

socioeconomic status questionnaire, since it does not discriminate socioeconomic levels above middle-class. It may also be due to barriers to participation experienced by people of very low socioeconomic status (as discussed in detail in 6.4.3).

### 6.1.1 Motor Skills

The cohort studied showed very low scores in all domains and sub-domains of the BOT-2, which is consistent with the wealth of previous research that has demonstrated impairment of motor skills in autistic children (Vilensky, Damasio and Maurer, 1981; Hallett *et al.*, 1993; Teitelbaum *et al.*, 1998; Berkeley *et al.*, 2001; Mayes and Calhoun, 2003; Minshew *et al.*, 2004; Jansiewicz *et al.*, 2006; Mostofsky *et al.*, 2006; Rinehart *et al.*, 2006; Freitag *et al.*, 2007; Dowell, Mahone and Mostofsky, 2009; Fuentes, Mostofsky and Bastian, 2009; Fournier *et al.*, 2010; Kushki, Chau and Anagnostou, 2011; Whyatt and Craig, 2013; Bhat *et al.*, 2018). The BOT-2 was developed in the United States and uses a standardised scoring system based on typically-developing children (Bruininks, 2005). The BOT-2 has been found to be a reliable and internally consistent motor skills measurement tool (Wuang and Su, 2009), and a study on children with Down's Syndrome found the BOT-2 to be excellent at identifying motor impairment in that sample (Nocera *et al.*, 2021). However, in a study in Greece, the BOT-2 was found to not be a reliable way to assess motor impairment in five-year-old children (Venetsanou *et al.*, 2007), which may be due to the very young age of the children. Many studies looking at the validity of the BOT-2 have focused on the short form (Cairney *et al.*, 2009; Venetsanou *et al.*, 2009; Lucas *et al.*, 2013; Brown, 2019). The studies in this thesis, however, used the long form version of the test. In autistic populations, the BOT-2 has been used to assess the effects on motor skills of physical exercise interventions (Srinivasan *et al.*, 2015; Pan *et al.*, 2017; Rafie *et al.*, 2017; Najafabadi *et al.*, 2018; Kaur and Bhat, 2019), and to characterise motor deficits of the population (Pan, 2014; Jeoung, 2018; Alsaedi, 2020; Wuang, Huang and Tsai, 2020). The results of this thesis add to the extant body of literature confirming the ubiquity of motor impairments in ASD. Although slightly higher, the scores obtained in this cohort are comparable to those obtained in the studies by Wuang *et al.* (2020) and Alsaedi (2020). Wuang *et al.* (2020) reported domain scores that are all lower than the domain scores obtained for this cohort, with the exception of the Strength & Agility domain, where their cohort scored higher than this cohort. Alsaedi (2020) reported sub-domain scores which are all lower than the subdomain scores obtained for this cohort, without exception. These comparisons indicate that overall, the cohort studied here has a greater motor capability than those studied by Wuang *et al.* (2020) and Alsaedi (2020). This

is possibly due to both studies having larger sample sizes with lower mean ages than this cohort, and may also relate to our cohort being of a relatively low support needs level.

### 6.1.2 Autistic Symptomatology

I used the ATEC to assess autistic symptom severity, which was developed to be a tool for assessing changes in autistic symptomatology in response to intervention, specifically looking at language and communicative behaviours, social function, sensory and cognitive awareness as well as general health symptoms (Rimland and Edelson, 1999). Other ASD assessment instruments, such as the Autism Diagnostic Interview-Revised (Lord, Rutter and Le Couteur, 1994), the Autism Diagnostic Observation Schedule (ADOS) (Lord *et al.*, 1999) and the Childhood Autism Rating Scale (CARS) (Schopler, Reichler and Renner, 1986) were primarily developed as diagnostic tools and are less sensitive to changes in autistic symptomatology over time. The ATEC has been used previously to assess changes in autistic symptoms in response to neurological and biological interventions (Jarusiewicz, 2002; Coben and Padolsky, 2007; Meiri, Bichovsky and Belmaker, 2009; Hadoush *et al.*, 2020). It has also been used to assess changes in response to music therapy (LaGasse, 2014), dolphin therapy (Salgueiro *et al.*, 2012) and physical therapy (Najafabadi *et al.*, 2018). Additionally, the ATEC has been used to assess changes in symptomatology over time without reference to any intervention, but rather assessing changes that occur with age and development (Mahapatra *et al.*, 2020). While the ATEC was not developed to describe ASD characteristics in a population, it is a free to use assessment tool that requires no training to administer and is therefore well-suited to use for research and assessment in low-resource settings such as South Africa. The ATEC has been found to be consistent and reliable in measuring ASD symptoms, although it may be less sensitive at the extreme ends of the spectrum (Magiati *et al.*, 2011). The ATEC compares favourably with the CARS as an assessment instrument and shows evidence of validity in assessing autistic symptomatology (Geier, Kern and Geier, 2013).

Previously, the ATEC has been used to characterise an autistic population in Saudi Arabia (Al Shirian and Al Dera, 2015). The results suggest that my cohort has a lesser degree of symptom severity than the cohort in Al Shirian and Al Dera's 2015 study. In particular, in the Speech/Language/Communication sub-domain, 100% of my cohort knew their own name, while only 77% of Al Shirian and Al Dera's cohort knew their own name. Their cohort scored the lowest on asking meaningful questions (reported by 4% of the sample), while the

lowest score in my cohort was for carrying on fairly good conversation (reported by 27% of the sample). On all items in the Speech/Language/Communication category, my cohort showed a higher percentage of scores for "very true", indicating overall a higher degree of speech and communication capability. In the second sub-domain, Sociability, my cohort scored the highest for lacking friends or companions (37% of the sample), while their cohort scored highest for disliking being held or cuddled (69% of the sample). The least common social impairment in their cohort was being insensitive to others' feelings (reported by only 12% of their cohort), while the least common social impairment in my cohort was not waving goodbye (reported by only 2% of this cohort). The two cohorts showed very similar scores on rarely smiling (15% in both studies), being insensitive to others' feelings (12% in both studies) and having temper tantrums (27% in the Saudi Arabian cohort and 29% in my cohort). However, on all other items, my cohort scored lower than the Saudi Arabian cohort, indicating once again a greater level of social capability in my cohort than in theirs. In the Sensory/Cognitive Awareness sub-domain, 100% of my cohort could respond to their name, while that was true of only 27% of the Saudi Arabian cohort. The highest scoring item in the Saudi Arabian cohort was looking at pictures at 58%, which is significantly less than the score of 85% on that item in my cohort. The lowest score in my cohort was for being "tuned in" (27%) while the lowest score in their cohort was for looking where others look (8%). My cohort exhibited a greater level of sensory and cognitive awareness, with higher scores on all items. In the last sub-domain, Health/Physical/Behaviour, my cohort exhibited the highest scores for fixation at 39%, which was also one of the highest scoring items in the Saudi Arabian cohort at 65%. My cohort showed the lowest scores on constipation and diarrhoea (at 2% and 0% respectively), while their cohort showed the lowest scores on injuring others, demanding sameness and having an extremely limited diet (at approximately 40% each). Overall, my cohort scored lower on all items than their cohort did, indicating that their cohort has more severe health and physical difficulties than ours does. The differences highlighted here may be due to the fact that my cohort was of a higher age than theirs was, and symptom severity assessed by the ATEC has been shown to decrease with increasing age, especially for younger children (Mahapatra *et al.*, 2020). It is significant, however, that my cohort showed differences to their cohort in terms of what were the most severe and least severe symptoms, which supports the idea that geographically distinct populations may present with differences in symptomatology.

The ATEC has also been used in Nigeria to characterise a clinical population of children presenting with ASD (Izuwah, Okoh and Alikor, 2016). The results of the ATEC in their study are not reported in full as they are in the study by Al Shirian and Al Dera, however some comparisons could be made. While only 5% of my cohort presented with poor eye contact, poor eye contact was present in 93% of the Nigerian cohort. Poor imitation was present in 15.9% of the Nigerian cohort, but in only 5% of my cohort. Repetitive behaviour was reported as a problem in 94.1% of the Nigerian cohort, while only 24% of my cohort reported repetitive behaviours as a significant problem. Hyperactivity was a problem for 87.1% of the Nigerian cohort, but only 22% of my cohort. From the comparisons here it is evident that my cohort had a lesser degree of ASD symptom severity than the cohort studied in Nigeria by Izuwah, Okoh and Alikor (2016). However, it must be noted that their cohort was a bit younger on average than mine, and was also recruited through hospital and clinic visits. Recruitment through clinics and hospitals may bias the sample towards the children that have more severe symptoms. While this data may also suggest that geographically distinct populations present with different symptomatology, further study is required using the same instruments and controlled sampling procedures across different regions in order to validate this notion.

### 6.1.3 Urinary Measures of Cortisol and Neopterin

The average level of urinary neopterin in the sample is comparable to that found previously in healthy subjects (Lhee *et al.*, 2006), which suggests that the neopterin levels are not abnormally high in this cohort of ASD children. Previously, neopterin has been found to be increased in autistic subjects (Harrison and Pheasant, 1995; Messahel *et al.*, 1998; Sweeten, Posey and McDougle, 2003), but our findings do not appear to agree with this observation. Neopterin is closely associated with activation of cellular immunity, as macrophages release neopterin in response to stimulation by T-cell-derived IFN-γ (Hamerlinck, 1999). Neopterin is also a marker of oxidative stress (Murr *et al.*, 2002). The data obtained here suggest that oxidative stress and activation of cellular immunity are not significantly increased in this cohort, and therefore are unlikely to be integrally related to the physiological aetiology underlying the symptoms of ASD. However, it must be noted that further study with a matched non-ASD control group from the same region would strengthen this finding.

There is very little existing data on levels of urinary cortisol in ASD as cortisol is more commonly assessed either by blood draw or in saliva. Attempts to collect salivary cortisol

were hindered by obstacles in that many of the children had motor difficulties spitting into a small tube, were not able to produce sufficient saliva for analysis or would spit food and other substances into the tubes. For these reasons, urine was decided on as the analytical fluid of choice in these studies. The chosen analytes, neopterin and cortisol, are both easily measured in the urine using ELISAs, only requiring correction by specific gravity to account for how hydration status may affect concentrations (Lindsay and Costello, 2017).

Previous research on urinary cortisol in ASD has found that the circadian rhythm of urinary cortisol excretion is normal, although there may be some hypersecretion of cortisol during the day (Richdale and Prior, 1992). Additionally, research that indicates that children with ASD have a heightened stress response with an increased secretion of cortisol when encountering a stressor (Tordjman *et al.*, 1997), which may account for the hypersecretion observed during the day by Richdale and Prior (1992). One study by Spratt et al. (2012) reported mean morning void urinary cortisol levels of children with ASD at 315.32 μg/dl, which is comparable to the mean obtained here which translates to 380 μg/dl. In their study they found that the morning void urinary cortisol concentration in individuals with ASD is not significantly different to a group of non-ASD controls (Spratt *et al.*, 2012). It is likely then that our results, although a little increased compared to theirs, do not represent an abnormally high level of cortisol in the urine. However, as with neopterin, further study comparing these results with a matched non-ASD cohort from the same region would bolster the strength of this finding. Further study would also benefit from collecting more history on the children as well as information on events occurring at home that may also be influencing cortisol and neopterin levels.

### 6.2 Correlations between Autistic Symptomatology, Motor Skills, Socioeconomic Status and Urinary Measures of Cortisol and Neopterin

The third aim was to assess relationships between autistic symptomatology, motor skills, socioeconomic status and urinary measures of cortisol and neopterin in the cohort of autistic children. Many significant relationships were observed to occur between autistic symptomatology and motor impairment. Additionally, significant relationships were observed to occur between urinary cortisol concentration and measures of motor skill involving balance. However, no significant relationships were observed between socioeconomic status

and any of the other variables, and no significant relationships were observed between urinary neopterin concentration and any of the other variables in this sample.

### 6.2.1 Motor Impairment vs Autistic Symptomatology

Strong relationships between all motor skill domains and subdomains and the Speech/Language/Communication and Sensory/Cognitive Awareness domains of the ATEC were found in the cohort. Sociability correlated with every motor skill domain and subdomain with the exception of Manual Dexterity. The Health/Physical/Behaviour section of the ATEC showed weak correlations with two subdomains, namely Fine Motor Precision and Balance, as well as the Body Co-ordination domain of the BOT-2. The presence of stronger and more consistent relationships between motor skills and the first three sections of the ATEC indicate that motor skills may have either a role in, or a shared underlying mechanism with Speech/Language/Communication, Sociability and Sensory/Cognitive Awareness which is more separate to the mechanisms underlying the Health/Physical/Behaviour aspects of ASD.

Significant and consistent relationships were found between all motor domains and three of the ATEC domains, which is suggestive of a shared aetiology between environmental awareness, social and communicative skills and motor capability. These relationships are consistent with previous findings on associations between various aspects of ASD symptom severity and motor skills (Freitag *et al.*, 2007; Hilton *et al.*, 2012; MacDonald, Lord and Ulrich, 2013; MacDonald, Lord and Ulrich, 2014; Travers *et al.*, 2013; Bhat *et al.*, 2018). Moreover, development of social skills in young autistic boys has been found to correlate with gross motor skills and core stability, with specific impairments of stability, motor accuracy and object manipulation predicting social function (Holloway, Long and Biasini, 2018). Relationships between motor ability and IQ (Green *et al.*, 2009; Kaur, Srinivasan and Bhat, 2018; Surgent *et al.*, 2021) and between motor ability and sensory symptom severity (Surgent *et al.*, 2021) have been found in children with and without ASD. Magnitude of risk for motor impairment has also been found to relate to severity of impairment in social communication, functioning, cognition, language and repetitive behaviours (Bhat, 2021). Hilton *et al.* (2012) used the BOT-2 to assess motor impairment in sibling pairs in which one sibling had ASD and one did not. They found significant association of motor impairment with autistic severity by group. In line with these data, it has been theorized that motor difficulties in ASD may decrease play and thereby impair social interaction and subsequent social development (Stins and Emck, 2018). Notably, Kaur *et al.* (2018) also used the BOT-2

in assessment of typically-developing children as compared with low IQ autistic children and high IQ autistic children. They found that motor impairment correlated with IQ but not with scores on the ADOS, which is the only finding directly in opposition to the correlations found in this thesis. To our knowledge, the ATEC has not previously been used to assess relationships between autistic symptom severity and motor skills. Our findings add to the literature investigating the relationships between motor impairment in ASD and autistic symptoms, and provide support to the idea that motor impairment is a core component of autistic symptomatology.

Motor skills may either be a requirement for social and communicative behaviours, or may share underlying neurological infrastructure with social/communicative behaviours. The relationships between motor impairment and language difficulties observed in several studies support theories of a neurological link between motor functionality and language development (Noterdaeme *et al.*, 2002; Gernsbacher *et al.*, 2008; McPhillips *et al.*, 2014; Bedford, Pickles and Lord, 2016; Bhat *et al.*, 2018; Bhat, 2021). The requirement for motor capacity in the oral formation of words (and the manual formation of words in the case of deaf children and those who use assisted communication methods such as Makaton) presents an obvious link between the two functions, but many other underlying relationships could be occurring.

A study comparing autistic children with children with ADHD or a disorder of developmental co-ordination found similar motor impairment across groups, but gestural impairment only in the autistic group, which suggests that gestural impairment may not be solely due to the motor deficits present (Dewey, Cantell and Crawford, 2007). In their study of dyspraxia in ASD, Mostofsky *et al.* (2006) also suggest that the body-part-for-tool errors observed in ASD imply that dyspraxia is not solely due to motor deficits. Neurologically, the authors suggest an impairment of the circuitry responsible for acquisition and representation of motor sequences, specifically the subcortical circuitry between the frontal and parietal regions. In alignment with this theory, abnormalities in frontal and parietal morphology have also been observed in ASD, specifically increases in grey matter and surface area in the pre-central and post-central gyri as well as the inferior parietal cortex (Mahajan *et al.*, 2016). The observation by Carmo *et al.* (2013) that autistic children perform known gestures better than novel gestures supports a theory of impairment in the learning and acquisition of motor sequences.

Additionally, cognitive and sensory awareness is likely to be necessary for accurate motor actions and responses. In a study on postural stability, Minshew *et al.* (2004) found that somatosensory disruption increased postural instability in autistic children. Vanvuchelen *et al.* (2007) have suggested that the impairment of imitation observed in ASD implicates an impairment of perceptual-motor integration. Impaired perceptual-motor integration may be responsible for difficulties in adapting movements to changing task demands (Whyatt and Craig, 2013). This is supported by fMRI evidence that there is asynchrony between the visual and motor systems in ASD, and that the severity of this asynchrony correlates with the severity of the ASD (Nebel *et al.*, 2016). Severity of social/communicative ASD symptoms has also been found to associate with decreased connectivity between the posterior cerebellum and the left inferior parietal lobule, which is an important circuit for visuomotor processing (Lidstone *et al.*, 2021). Interestingly, relationships have been observed between social responsiveness and sensory processing in ASD (Hilton, Graver and LaVesser, 2007), which suggests a role for sensory processing in social behaviour as well. Social motor synchronisation has been found to associate with severity of ASD symptoms in a way that is not fully accounted for by motor deficits (Fitzpatrick *et al.*, 2017). The results obtained in this thesis together with the previous research discussed above suggest that the triad of deficits discussed here (social/communication, motor and sensory processing) are neurologically inter-related and functionally interdependent.

### 6.2.2 Urinary Measures of Cortisol and Neopterin

No relationships between neopterin and any of the ATEC or BOT-2 scores was found, which suggests that increased oxidative stress and activation of the cellular immune system (Th1-type response) are not closely associated with ASD severity or motor impairment. However, the evidence for an immune dysregulation component of ASD is substantial and possibly other indicators of immune function could provide more insight into the relationship between immune dysregulation and ASD symptom severity.

Furthermore, no relationships were found between any of the ATEC measures and urinary cortisol. This indicates that morning cortisol levels measured once a week are unlikely to be related to the severity of autistic symptoms. These results suggest that daily levels of activation of the HPA axis are not significantly linked with ASD symptom severity. The lack of relationships observed between urinary cortisol and neopterin, and the ATEC scores could

be due to the fact that cortisol and neopterin are both fluctuating significantly according to circadian changes as well as over days and weeks. However, in light of this fact, the longitudinal cortisol and neopterin measures collected over 18 weeks for the four participants enrolled in the intervention study were averaged and assessed for relationships with ATEC and BOT-2 scores as well. No relationships were found in that analysis, indicating that long term activation of the stress system and cellular immunity are not significantly related to motor skills or ASD symptom severity in this sample. The pathophysiology of ASD may be more significantly affected by alterations in the stress system and cellular immunity that occur during gestation and in the very early developmental stages. Given that our cohort are all over the age of eight years, they may be at a stage where the stress system and cellular immunity levels are no longer significantly influencing the development of their physiology to the extent that cognitive and motor symptoms are affected.

Additionally, no relationships were observed between socioeconomic status and neopterin, which suggests that socioeconomic status does not significantly influence activation of cellular immunity in children with ASD. No relationships were observed between cortisol and socioeconomic status either, which suggests that the level of stress encountered as a result of socioeconomic status is not a significant influencing factor over morning void cortisol levels. The lack of observed relationship may be due to habituation of the HPA response which occurs in response to daily or frequently experienced psychological stressors (Grissom and Bhatnagar, 2009).

However, urinary cortisol levels measured once-off in the full cohort were found to associate with two of the BOT-2 scores, namely Balance and Running Speed & Agility. These were both negative relationships with scores on the Balance and Running Speed & Agility sub-domains decreasing with increasing levels of cortisol in the urine. Pertinently, many of the tasks comprising the Running Speed & Agility sub-domain of the BOT-2 involved balance (for example, hopping tasks). These results suggest that regulation of cortisol levels may be neurologically intertwined with the balance aspect of motor capability.

Balance can be described as the ability to maintain the body's centre of mass over its base of support (Shumway-Cook and Woollacott, 2001). Balance is particularly complex as it requires a combination of exteroceptive (cues from the environment), proprioceptive (cues

from the muscles and sensory organs) and interoceptive (cues from the viscera about the internal state of the body) input in order to function (Balaban and Thayer, 2001). Balance allows humans to integrate visual information, identify the orientation of the body in space, identify the direction and speed of movement and make adjustments to maintain stability. The system that maintains balance in humans incorporates sensory input from the visual, proprioceptive and vestibular systems, which is integrated and used to direct motor output (Vestibular Disorders Association, 2016). Visual information is collected through the eyes, proprioceptive information is collected via touch receptors and specialized sense organs in the muscles, and vestibular information is collected via the vestibular apparatus in the ear. The vestibular apparatus consists of the utricle and the saccule (which detect gravity and linear movement), as well as the semi-circular canals which identify the directionality of rotational movement. All of this somatosensory information is integrated through centres in the brain stem, the cerebellum and higher cortical structures that provide learned input to movement. The repeated practice of an action leads to facilitation of that action, which is a strengthening of the nerve-to-muscle pathway involved in producing the action (Vestibular Disorders Association, 2016). Balance is not controlled by a single central system but rather through the complex interaction of various physiological mechanisms, including biomechanical variables, movement strategies, sensory input, dynamic control and cognitive processing (Horak, 2006). Different tests are required to identify the specific impairment in an individual that is giving rise to balance disorder, but analysis of impairments in the sub-components of the system allow the prediction of context-specific instability. However, such specific tests of the balance system have not been conducted in this cohort. Further study could benefit from assessing different aspects of the balance control system in ASD. Critically for autistics, the amount of cortical input required in the balance system is dependent on both the complexity of the task as well as the capability of the individual's balance-control system (Horak, 2006).

As discussed previously, there is a wide range of motor impairment observed in ASD. Balance is no different, with ASD children scoring lower on tests assessing balance than controls (Berkeley *et al.*, 2001; Green *et al.*, 2009; Staples and Reid, 2010; Breslin and Rudisill, 2011; Whyatt and Craig, 2012; Liu and Breslin, 2013). Vestibular processing has been found to be impaired in autistics as compared with controls (Kern *et al.*, 2007), and vestibular dysfunction has been suggested to play a role in the development of ASD (Ornitz,

1970). Postural instability has been found to be increased in children with ASD when standing on one leg, as compared with controls (Travers *et al.*, 2013). Postural instability in response to changing the angle of the support surface (Minshew *et al.*, 2004) and closing eyes (Stins *et al.*, 2015) has been found to worsen more in ASD individuals as compared with controls. Additionally, providing unreliable sensory information causes increased instability in individuals with ASD as compared with controls (Doumas, McKenna and Murphy, 2016), and individuals with ASD exhibit greater postural benefit from touching a wall lightly than controls do (Chen and Tsai, 2016). These data all indicate that in ASD there may be a heightened reactivity to sensory disturbance in the control of balance. Additionally, postural asymmetry (Travers *et al.*, 2013) and instability (Radonovich, Fournier and Hass, 2013) in individuals with ASD has shown to be predictive of the presence and severity of repetitive behaviours, indicating a link between postural dysregulation and autistic symptoms. In a meta-analysis, dysregulation of sensory processing has been suggested to lie at the core of the postural deficits observed in ASD (Lim *et al.*, 2017).

Balance is known to be affected by neuro-endocrine and psychological factors. Anxiety in particular affects clinical outcomes of balance, however our understanding of the mechanisms behind this is limited (Saman *et al.*, 2020). Previous research has indicated that the ability to balance is linked to anxiety levels (Balaban and Thayer, 2001; Erez *et al.*, 2004; Stins *et al.*, 2009). It has been theorised that anxiety may alter basic sensory processing and thereby affect balance (Horslen and Carpenter, 2011). Balaban & Thayer (2001) propose that disconcerting input about our relationship with the environment induces panic cues in order to motivate a quick adjustment to the environmental change, which provides an evolutionary explanation for the neurological link between balance and anxiety. The parabrachial nucleus is a site of convergence for vestibular and visceral information and has been suggested as a circuit underpinning the relationship between balance and anxiety (Balaban and Thayer, 2001). This theory is based on the neuroanatomical connections between the vestibular nuclei and the parabrachial nucleus (Balaban, 1996; Porter and Balaban, 1997), which provide a link between the vestibular system and the networks associated with fear and anxiety (Pratt, 1992; Fanselow, 1994). Pathways carrying visceral and vestibular information converge at the parabrachial nucleus, which then modulates the information sent onwards to the amygdala, hypothalamus, basal forebrain and cortical regions, also affecting autonomic output (Balaban and Thayer, 2001). It is therefore possible that activation of the stress systems (i.e. activation

of the SNS and HPA axis) may involve the parabrachial nucleus and therefore increased cortisol release would co-occur with modulation of the balance system. The relationship between cortisol as a marker of stress and performance on balance tasks has been assessed in only a handful of studies, and never in ASD to my knowledge. In a study on subjects with multiple sclerosis, no correlations were observed between hair cortisol levels and balance (Pereira *et al.*, 2019). In opposition to our findings, increased cortisol has been found to associate with decreased postural sway (Smyth *et al.*, 2019). However, an increased cortisol awakening response and increased stress levels have been observed to have a relationship with decreased balance performance (Coco *et al.*, 2020). Additionally, increased cortisol levels have been found to relate to decreased balance scores in healthy individuals (Cay *et al.*, 2018), and increased cortisol levels have been found to associate with poorer postural control during menstruation (Şenol *et al.*, 2021). Our findings of a relationship between increased urinary cortisol and decreased balance performance therefore add to the evidence for a relationship between activation of the stress system and modulation of balance control. Our findings also specifically highlight the occurrence of this relationship in individuals with ASD.

### 6.2.3 Socioeconomic Status

Although we hypothesized a link between ASD and socioeconomic status, we found no correlation between socioeconomic status and either autistic symptom severity or motor skills. Our results on the lack of relationships between autistic symptom severity and socioeconomic status may indicate that socioeconomic status is not a primary environmental factor involved in the pathogenesis of ASD. However, socioeconomic status may still be a moderating factor in the prognosis of ASD, since families of higher socioeconomic status are more likely to have access to support services and informative resources. A study in Australia found socioeconomic status to act as a moderating factor on the relationship between parental competency and autistic symptom severity (Mathew *et al.*, 2019), which supports this hypothesis. The fact that we found no evidence of a relationship could be due to the complex nature of the relationship, or it could also be due to the fact that our sample was not fully representative of the socioeconomic levels present in the population.

## 6.3 Exercise Intervention Pilot Study

The fourth aim was to assess the validity of an exercise intervention in improving motor skills and ASD symptoms in ASD children. No statistically significant change was observed

in either the BOT-2 scores or the ATEC scores. These results suggest that the intervention was not particularly helpful in improving the social and behavioural symptoms of ASD, or in improving the motor deficits present in this sample. However, non-significant improvements in almost all domains of the ATEC were observed between the scores before and the scores after the 18-week intervention. Scores in the Speech/Language/Communication, Sensory/Cognitive Awareness and Health/Physical/Behaviour domains went up for all three children, while only one child showed a decrease in score in the Sociability domain. Furthermore, all three children showed increases in their ATEC composite scores. The non-significant improvements in the ATEC scores suggest that further study of the intervention is warranted, as the lack of a statistically significant result may be due to the very small sample size of only three participants who completed the longitudinal study.

No trends were evident in the changes in cortisol and neopterin concentrations over the course of the 18-weeks the children were involved in the study. This suggests that the exercise intervention did not have a significant impact on either cellular immunity and oxidative stress levels (in the case of neopterin), or the level of activation of the HPA axis (in the case of cortisol). Additionally, no relationships were found between cortisol and neopterin levels in the urine of each child. This is in line with previous research that has found no evidence of a relationship between neopterin excretion and cortisol levels (Garbutt *et al.*, 1985), as well as a study that found no apparent change in neopterin following an ACTH challenge (O'Toole, Chiappelli and Rubin, 1998). However, further research assessing the relationship between exercise and the brain-immune axis would benefit from collection of samples directly preceding and directly following an exercise session, in order to evaluate acute changes.

### 6.4 Future Directions

While some interesting and useful data was obtained in the course of the studies conducted, there are some limitations to the studies that could potentially be addressed by future research. One of the biggest hurdles was the conduct of physiological research during the COVID-19 pandemic, which had a significant impact on recruitment and data collection. This was especially significant during the hard lockdowns in South Africa, which interrupted longitudinal data collection and limited the possible sample size. Increased sample size would help to determine the significance of the results. Furthermore, our sample had a bias to lower support levels and higher socioeconomic status, and a more comprehensive characterisation

of the Johannesburg ASD population would require inclusion of all support levels and socioeconomic statuses. Other limitations which could be addressed in future research include the lack of control groups, limitations of the instruments used, aspects of the recruitment procedure, and the use of regression analysis, which was not conducted in this thesis.

### 6.4.1 Control Groups

As mentioned previously, some of the findings discussed here would be strengthened by the use of control groups. The preliminary findings of the pilot study suggest that ASD symptoms may be improved, although the improvement was not statistically significant. Further study on the intervention with a larger experimental group as well as an ASD control group not involved in the intervention would provide more conclusive evidence on the effects of the intervention. Furthermore, in assessing the levels of urinary neopterin and cortisol and determining whether there are increased levels of these markers in ASD, further study should compare levels obtained from an ASD group with levels obtained in a matched non-ASD group. A non-ASD group would also be useful in determining whether the relationship observed between cortisol levels and balance is particular to ASD or is something common amongst all children of school-going age in the central Johannesburg region.

Additionally, it may be useful to perform studies that compare ASD with other developmental disorders, as some studies have shown that similar motor impairment exists in other developmental disorders as that observed in ASD. One study found that autistic children have similar fine and gross motor profiles to children with developmental delay (Provost, Heimerl and Lopez, 2007). Similarly, Noterdaeme *et al.* (2002) found that both autistic children and children with either an expressive or receptive language disorder have more motor impairments than typically-developing controls. Asperger's Syndrome is a previously distinct diagnosis that has more recently been incorporated into the diagnosis of ASD (American Psychiatric Association, 2013). Children with Asperger's Syndrome have been noted to overlap with children with a specific disorder of motor function in their motor impairment profiles (Green *et al.*, 2002). In a study comparing children with Asperger's Syndrome to children with learning disabilities, motor delay was found in both groups, with similar impairment profiles once again. However, a significant difference in manual dexterity was observed between the two groups, with the Asperger's group showing poorer performance in that domain (Miyahara *et al.,* 1997). Autistic children and children with a

specific language impairment have been found to show similarly impaired motor profiles, with the exception once again where autistic children showed poorer performance on a manual dexterity task that involved both hands (McPhillips *et al.*, 2014). Vanvuchelen *et al.* (2007) found that boys with low-functioning ASD performed worse than boys with mental retardation at both a motor task as well as an imitation task. For future studies it may be important to look at comparing autistic motor deficits with those present in other populations with developmental disorders to identify syndrome-specific deficits and therefore more targeted therapies.

### 6.4.2 Instrument Limitations

For these studies, instruments were chosen on the basis of their previous usage in published research and their availability and ease of use for the context in which the research is conducted. However, it is important to note that data collection instruments do have their limitations and that these may affect the quality of data obtained. Specific limitations were observed in the ATEC, the BOT-2 and in the urine collection procedure.

The ATEC is free to use and does not require training to administer, which makes it appropriate to use in our setting. However, the ATEC form is presented in English and the diversity of first languages present in the Johannesburg population mean that it is likely that parents filling out the ATEC may not always have full comprehension of each item. A notable example was the item “Lethargy” in the Health/Physical/Behaviour sub-domain, which was often left incomplete. The word “lethargy” is not often used in South African parlance outside of a medical context and individuals whose first language is not English are unlikely to know what the word means. Further study evaluating the applicability of the ATEC to the South African socio-cultural context would be useful as this instrument does not have the financial and proprietary barriers present in the case of other ASD assessment instruments. An example of successful cross-cultural adaptation of the ATEC for use in low-resource environments is given by Borissov *et al.* (2021), who adapted the ATEC for use in Ethiopia and found the adapted version to be reliable and consistent for use in the Ethiopian linguistic and cultural context. The ATEC therefore has the potential to be successfully adapted for use in the South African context too.

In terms of the BOT-2, some aspects of the scoring system cause a decrease in score as age increases, which does not always appear to make sense. For example, participant 42 scored

100% on the tasks involving ball skills (the Upper-limb Co-ordination sub-domain) at both time points in the pilot study. However, at the second time point the standard score he obtained was lower as he had passed into the next age category. Therefore, while his ball skills had not actually decreased over the 18-weeks involved in the intervention, the score he obtained appeared to show that his ball skills were poorer. This means that sub-domain scores of the BOT-2 need to be interpreted with care, especially when assessing changes in motor competence in response to a longitudinal intervention. The use of raw scores for interpretation may be useful in overcoming this issue.

Finally, although the procedure of collecting urine once a week over the course of the exercise intervention was more achievable under the conditions of the COVID-19 pandemic, more information regarding activation of the HPA axis and its relationship with immune activation could be obtained from 24-hour urine collection procedures, or daily collection of urine. Additionally, future research should involve the collection of more background information on the children's lives and families, as knowledge of family circumstances and the occurrence of daily stressors would help to rule out extraneous influences on the HPA axis.

### 6.4.3 Recruitment

While the population attending the government school was known to include a fair proportion of individuals with very low socioeconomic status, it is notable that the majority of our sample scored high on the socioeconomic status questionnaire. The questionnaire is more sensitive to differentiating levels from middle-class to very low-class, and does not discriminate levels above upper-middle class. The maximum score on the questionnaire indicates that the family has an (unspecified) income, at least one parent has a tertiary education and the family owns a car, a television, a washing machine, a microwave and a refrigerator. Moreover, I suspect that children of a very low socioeconomic status often did not participate in the study due to the low resources of their families. Recruitment occurred primarily by sending an information sheet home with the child with an invitation to consent to the child's participation in the study. The information sheet was constructed according to the requirements of the Human Research Ethics Committee of the University of the Witwatersrand, and is provided in Appendix C. Due to the nature of the research project and these requirements, the information sheet was lengthy and information-dense. Parents and caregivers of a lower socioeconomic status are under-resourced in terms of time and energy

and are therefore less likely to read such an information sheet. Furthermore, lower socioeconomic status is often associated with lower levels of formal education, and a lengthy and information-dense sheet is therefore likely to be daunting to approach and difficult to understand. In addition, many families of a lower socioeconomic status in the Johannesburg area do not speak English as their first language, and this serves as an additional barrier to comprehension of the information sheet. This may explain the bias in our sample, in which no participant was recruited who has a socioeconomic status score of less than 5 out of a possible 9. Future studies on ASD in the Johannesburg area need to try to include a greater range of socioeconomic statuses in their studies, which may require alteration of recruitment and informed consent procedures. In Chapter 3, the suggestion of implementing community engagement practices alongside biomedical research projects was made, which may prove fruitful in overcoming the barriers to recruiting participants of lower socioeconomic status. Community engagement practices have also been suggested to be useful for increasing awareness of ASD in African contexts (Ruparelia *et al.*, 2016).

### 6.5 Conclusion

The first aim of this thesis was to research the ethical considerations inherent in conducting biomedical research on ASD in South Africa. It was found that community engagement practices would significantly enhance the applicability of research to the needs of the community. Furthermore, biomedical studies were conducted on a sample of children with ASD in the Johannesburg area. Regarding the collected data, three aims were addressed: firstly, to characterize the population of children attending the Fight with Insight classes in the Johannesburg area, secondly to investigate relationships between the measures collected and thirdly to assess the validity of an exercise intervention in improving the symptoms of ASD.

With respect to the first aim, the data collected here provides information on the demographics, socioeconomic status, motor skills and ASD symptom severity of children with ASD attending the Fight with Insight exercise classes in the central Johannesburg region of South Africa. The results indicate a 10:1 disparity between male and female children, with the racial proportions of the cohort being comparable to the general racial proportions in Johannesburg. The results indicate that the sample is of a higher socioeconomic status and has significant motor impairment. No evidence was found for differences in levels of cortisol

or neopterin in the cohort as compared with values obtained previously for the general population. Finally, our results also describe the relative severity of different ASD symptoms in the cohort.

With respect to the second aim, relationships were identified between motor skills and autistic symptom severity in the domains of social behaviour, language/communication and sensory/cognitive awareness. These results support previous research highlighting the centrality of motor impairment in autistic symptomatology. The data here, together with previous research, suggest an interlinking and interdependence of neurological structures underlying social/communicative function, sensory processing and motor development in ASD. Interventions strategies for ASD therefore need to be aimed at integrating these three domains. Additionally, these results contribute to the body of research assessing the impact of the brain-immune axis on ASD. No relationships were found between autistic symptomatology and cortisol as a marker of HPA activation. Additionally, no relationships were found between autistic symptomatology and neopterin as a marker of cellular immunity and oxidative stress. However, a relationship was observed between increased levels of morning void urinary cortisol and decreased performance on balance-related tasks. This data supports previous research indicating that activation of the stress system may be neurologically interlinked with modulation of postural control, and provides the first evidence of this relationship occurring specifically in children with ASD. Importantly, we did not find any evidence for a relationship between socioeconomic status and ASD symptom severity. However, future studies assessing the relationship between socioeconomic status and autistic symptoms need to make extra effort to include participants of lower socioeconomic status in their cohorts. Specifically, community engagement practices need to be employed in order to assist recruitment and retention of participants in an ethical way.

Finally, with respect to the third aim, no statistically significant evidence was found showing that the exercise intervention improves the symptoms of ASD or motor skills. However, ATEC scores did show a non-statistical improvement which warrants further investigation into the exercise intervention. Future study of this intervention should recruit an increased sample size and use control groups. Additionally, future studies assessing urinary markers of the HPA axis and immune function in ASD would benefit from more frequent and even continuous sampling of urine of possible, as well as assessment of the acute effects of exercise on the brain-immune axis.

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# Chapter 8: Appendices

## Appendix A: Ethical Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Ms Siobhan de Lange et al

**HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)**

**CLEARANCE CERTIFICATE NO. M180767**

| | |
|---|---|
| **NAME: (Principal Investigator)** | Ms Siobhan de Lange et al |
| **DEPARTMENT:** | School of Physiology<br>Fight with Insight Clinic |
| **PROJECT TITLE:** | Relationships of Autism Spectrum Disorder subjective measures with objective measures of inflammation and immune function |
| **DATE CONSIDERED:** | 27/07/2018 |
| **DECISION:** | Approved unconditionally |
| **CONDITIONS:** | |
| **SUPERVISOR:** | Dr Dee Muller and Dr Chloe Dafkin |
| **APPROVED BY:** | CB Penny<br>Dr CB Penny, Chairperson, HREC (Medical) |
| **DATE OF APPROVAL:** | 05/12/2018 |

**This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.**

**DECLARATION OF INVESTIGATORS**

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **July** and will therefore be due in the month of **July** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date 08/01/2019

**PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES**

## Appendix B: Permission from Zefferino to use cortisol diagram (Figure 2.1)

**Siobhan de Lange** <712538@students.wits.ac.za> Wed, 19 Jan, 10:12

to roberto.zefferino

Good day,

I am a PhD candidate with Wits University in South Africa. I would like to use your cortisol image (figure 1) from your paper "Molecular links between endocrine, nervous and immune system during chronic stress" in the literature review for my thesis. Please let me know if that would be okay.

Kind regards,
Siobhan de Lange

**Roberto Zefferino** <roberto.zefferino@unifg.it> Wed, 19 Jan, 13:42

to me

Ok, don't worry.
I authorize you.
Best regards

•••

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*Prof. Roberto Zefferino*
*Medicina del Lavoro Universitaria*
*71122 FOGGIA ITALY*
*E-MAIL: roberto.zefferino@unifg.it*

## Appendix C: Information sheet for parents, used for recruitment

School of Physiology

Faculty of Health Sciences, University of the Witwatersrand, Johannesburg
7 York Road, Parktown, 2193 • Tel: +27-11-717-2363 • Fax: +27-11-643-2765 • www.wits.ac.za/health/Physiology
E-mail: physiology.health@wits.ac.za

**Parent/Guardian Information Sheet (ASD with boxing)**
**Inflammation and immune function in Autism Spectrum Disorder**
**Protocol no. M180767**

Hello,

I, Siobhan de Lange, am a PhD student in the School of Physiology and I am running a project looking at Autism Spectrum Disorder (ASD), and would like to invite your child to participate. ASD includes a range of developmental challenges, characterized by difficulties in social interaction, expressive communication and forming of relationships.

Before consenting to your child's participation, it is important to understand what you are consenting to. Please read carefully the information about the study presented below. Your child will also be given both written and verbal explanations of the study before participation. Please go through the Child Information Sheet with your child to help him/her understand what is happening in this study. Please do not hesitate to ask any questions.

Purpose of the study

Many people suffer from ASD and experience the associated consequences. However, little is known about the causes of ASD, making it difficult to understand and treat. Through my project, I aim to connect behavioural characteristics with physical and chemical measures in order to better understand ASD. I also aim to assess whether the exercise class attended by children at the Johannesburg School for Autism improves the symptoms of ASD.

Details of the study

Children with ASD at the Johannesburg School for Autism attend a weekly exercise class that has elements of boxing with the Fight with Insight Clinic as part of their school program. The class has been running successfully for some time and the classes for ASD children are accompanied by a qualified occupational therapist from the school. In order to ascertain the benefits of this class for children with ASD, we would like to assess these children before they start the class and again after four months of participation in the class. We will also do an assessment of each participant at a random time point during the four month period. In order to know if the changes that we may see following this exercise class are definitely due to the class, we require control groups. One control group must consist of non-ASD children who also participate in the exercise class, in order to compare the effects of the class in ASD children against the effects seen in non-ASD children, which will tell us what benefits of the class are specific to children with ASD. The other control group must consist of ASD children who do not participate in the class, so that we can be sure that changes seen in the ASD group who do the exercise class are as a result of the exercise class and not due to other factors (such as school activities or conditions in the home). Your child has been selected as he/she has previously been diagnosed with ASD and will be attending the Fight with Insight class. Your child will be assessed three times; once before they start the Fight with Insight class, once four months following the first assessment, and a third time, at a random time point during the four month period.

We hope to recruit twenty children for each group (that is, twenty ASD children attending the exercise class, twenty ASD children not attending the exercise class and twenty non-ASD children attending the exercise class). Both boys and girls, between the ages of 8 and 18 years old, will be recruited. In addition to verifying the benefits of the exercise class for children with ASD, we hope to use our results to understand relationships between what goes on inside the body (using saliva and urine) with what we see on the outside (behaviour, cognitive, motor and activity measures). This may in turn improve therapies for ASD.

Please bear in mind that participation is completely voluntary, and you may withdraw your child from participation at any point should you wish to do so. Your child may also choose to withdraw him/herself from the study at any time point too. There are no negative effects of not participating, or from withdrawing from the study, for either you or your child. Allowing your child's participation in the study will not directly benefit you or your child, but we hope to gain a better understanding of how to treat ASD from the results.

What will be required from your child

Your child has been selected for this study because he/she has previously been diagnosed with ASD and because he/she is participating in the boxing class at Fight with Insight. Once a week, your child will perform directed boxing related exercises under the supervision of a boxing coach and an occupational therapist. The occupational therapists at the school decide which ASD children can participate in the boxing classes, according to their level of support needs and their likelihood to experience distress in the boxing class setting. ASD children attending the classes are classified as requiring low and medium support. A qualified occupational therapist from the school also attends every ASD class at Fight with Insight, monitoring the children for any signs of distress and intervening where necessary. Thus far, only once has a child required distress intervention, during which the child left the class and sat in the hallway outside for a few minutes, before choosing to rejoin the class. Your child will undergo an evaluation of ASD symptoms (including behavioural, cognitive and motor function) at each data collection point. This evaluation will take about an hour each time it is completed and involves asking your child to perform simple motor activities such as walking across a balancing beam or catching a ball. This evaluation will occur at your child's place of schooling. This evaluation will allow us to see whether the Fight with Insight programme improves the symptoms of ASD on both a long-term and short-term basis. Additionally, at the same time we will take voluntary saliva and urine samples to assess levels of cortisol (stress hormone) and immune system markers, which will allow us to connect any improvements we see with what is going on inside the body. We may require your assistance in obtaining urine samples from your child. Your child will also be given a small activity monitor to wear, which is about the size of a bottle cap and will not hinder movement. It is called an ActiGraph and is attached to the hip by a waistband and must be removed when showering or bathing. We would like your child to wear the ActiGraph all day for seven days. These devices will not hurt and once they are put on they can be ignored for the seven days of wear. After your child has completed the week of wearing the ActiGraph, we will arrange to collect the devices from you at a time that best suits you. All these measurements will be performed three times; before your child starts the Fight with Insight class, four months later and once during the four month period. You will also be required to fill in a short 5 minute questionnaire to help us assess your socioeconomic status.

The results will be analyzed and published in scientific papers and presented at a scientific conference, however your child's identity will remain anonymous. Your child's personal results will be made available to you if you wish. The results of the study may also be made available to the schools and to the government departments that have approved the study, but once again, the identities of participants will be kept confidential.

My supervisors and I have obtained approval for this study from the Human Research Ethics Committee (HREC) of the University of the Witwatersrand whose contact details are listed below. There are no risks involved in taking part in the study. However, your child may withdraw from participating at any time should they choose to, for whatever reason and without consequences. If after reading this information sheet you decide against participating in the study please be assured that this will not impact on you negatively in any way. If there is anything you don't understand, I will be happy to explain verbally so please do not hesitate to ask questions.

If after reading this, you would like your child to participate in the study, please confirm that you allow so by signing the consent form overleaf. If your child is able to understand the research and is happy to participate and can write their name, they will be asked to complete an "assent" form with you. You may keep this information sheet and copies of the signed consent and assent forms for your own reference.

If you decide to discontinue your child's participation at any point during the study, kindly inform me: Siobhan de Lange: 073 907 7631
There will be no negative effects of withdrawing from the study for either you or your child.

Sincerely,

Siobhan de Lange
0739077631
Siobhan_delange@yahoo.com

HREC contact details:

Dr Clement Penny, HREC (Medical) Chairperson:
Tel: 011 488 3820, Email: clement.penny@wits.ac.za.
Ms Z Ndlovu Secretariat:
Tel: 717 1252, Email: Zanele.ndlovu@wits.ac.za,
Mr L Moeng:
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# Appendix D: Turnitin report (first page)

Turnitin Originality Report

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**Appendix E: Articles submitted for publication**

1) *"Biomedical research on autism in South Africa: Re-thinking the informed consent process"* submitted to the Journal of Medical Ethics (manuscript number medethics-2022-108302).
2) *"Relationships between autistic symptom severity, motor skills and socioeconomic status"* submitted to the journal Autism (manuscript number AUT-22-0192).
3) *"Descriptive characteristics of children with autism at schools for autism in Johannesburg, South Africa"* submitted to the journal Autism Research (manuscript number AUR-22-0130).
4) *"Urinary cortisol and neopterin in autistic children: Relationships with balance"* submitted to the Journal of Autism and Developmental Disorders (manuscript number JADD-D-22-00389).