Biological Endophenotypes of Prodromal Psychosis and Depression

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DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given. The extent of collaboration with others has been stated clearly and fully in the thesis and the co-authors of all publications included in this thesis have provided written statements of the nature of their contribution. As the copyright owner of this thesis, I request the thesis to be embargoed until 30 June 2019 and grant James Cook University a permanent nonexclusive license to store, display or copy any or all of the thesis, in all forms of media, for use within the University after this date, and to make the thesis freely available online to other persons or organisations. Every reasonable effort has been made to gain permission and acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.

The research presented and reported in this thesis was conducted in accordance with the National Statement on Ethics Conduct in Research Involving Human Research (2007), the Joint NHMRC/AVCC Statement and Guidelines on Research Practice (1997) and the NHMRC Guidelines for Ethical Conduct in Aboriginal and Torres Strait Islander Health Research (2003). The relevant human ethics approval numbers are provided in each chapter.

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ABSTRACT

The identification of biomarkers to predict risk and clinical outcomes beyond clinical observation in psychiatry is a key priority in contemporary mental health research. Individuals at elevated risk for mental disorders have been the focus of such efforts as they represent a potentially viable target group for early intervention and indicated prevention. Early intervention and indicated prevention efforts for individuals at ultra-high risk (UHR) for psychosis might benefit from such biomarkers to enable better risk prediction and to discern who will benefit from treatment. Alterations in neuroendocrine, immune and oxidative stress markers as well as abnormalities in lipid biology are robust findings in biological psychiatry. On the other hand, mounting evidence suggests that First Nations people including the Aboriginal and/or Torres Strait Islander people of Australia have a higher risk for common and severe mental illness, most likely attributable to social and environmental health determinants. However the role of stress-related biomarkers in this context remains poorly understood. Consequently, the aims of this thesis were to examine (1) a multisystem biomarker index known as allostatic load in two high-risk populations and to (2) examine the role of lipid biology in these groups.

The first part of this thesis explores the role of allostatic load (AL) and omega-3 (ω-3) polyunsaturated fatty acids (PUFA) in two clinical studies of patients with schizophrenia, first-episode psychosis and individuals at UHR for psychosis. The second part explores the role of AL and dietary ω-3 PUFA in a community-based study of Aboriginal and/or Torres Strait Islander people, who are at increased risk for mental ill-health according to recent national reports. The above aims are achieved through meta-analysis of existing studies of cortisol in psychotic disorders, secondary analysis of two clinical studies, and in a cross-sectional multi-site community-based study of Aboriginal and/or Torres Straight Islander adolescents and young adults attending a health check.

First, flattened cortisol-awakening response is reported in patients with schizophrenia and first-episode psychosis relative to controls, but not in UHR individuals, suggesting neuroendocrine dysregulation during some stages of the psychosis spectrum. Secondly, informed by these findings, a study of AL demonstrated elevated AL in patients with schizophrenia and first-episode psychosis that was positively correlated with positive psychotic symptoms and negatively correlated with functioning at trend level. No prospective association of AL and treatment response and symptomatic

remission was identified. Next, secondary analysis of a multi-centre randomised controlled trial investigated AL in youth at UHR for psychosis. This study revealed prospective associations of high AL with impairments in social and occupational functioning. Next, the prospective association of PUFA status and clinical outcomes in a 12-month follow-up is reported, which demonstrated a selective relationship of low ω-3 PUFA levels with mood disorders and no association with psychotic or anxiety disorders. The second part of the thesis reports depression rates in three remote communities, explores the relationship of depression with hair cortisol and AL, and reports an association of low plasma levels of o ω-3 PUFA with depressive symptoms.

Collectively, the data presented in this thesis demonstrate complex associations of a multisystem biomarker index and ω-3 PUFA with mental ill-health. Ultimately, multisystem indices like AL may be used in future research to establish their utility as a clinical prognostic risk index. By studying membrane lipids in an ethnic minority group, this thesis was able to show an association of high dietary intake of ω-3 PUFA and low levels of depression, which may serve as a protective factor.

CONTENTS

1 Introduction

Common and severe mental disorders such as depression and psychotic disorders are among the largest contributors to disease burden globally. A substantial proportion of these disorders typically have their onset during adolescence and early adulthood and lead to considerable morbidity and disability throughout the lifespan. Understanding the processes characterising the period prior to the emergence of psychiatric disorders offers opportunities for potential prevention strategies. Consequently, a key research priority in current psychiatric research is the identification of individuals at risk for psychiatric disorders with the promise to improve long-term outcomes through early intervention. The last two decades have seen much progress in the development of clinical criteria and attempts to evaluate suitable interventions, yet risk factors for progression and adverse clinical outcomes remain poorly understood. If successful, such risk biomarkers may assist in identifying individuals with need for care, who will benefit from specific interventions, or point toward potential intervention targets.

In this thesis, two biological processes thought to contribute to the early phases of mental disorders are examined in regard to clinical symptoms and functional outcomes in a series of individual papers focusing on patients with psychotic disorders, help-seeking individuals with at-risk mental states (ARMS)[i] and non-clinical populations. More specifically, the allostatic load (AL) framework is applied to a psychiatric context and evaluated in regard to its cross-sectional association with symptoms and prospective relationship with clinical and functional outcomes. Similarly, polyunsaturated fatty acids (PUFA), known to be relevant for several pathophysiological processes related to inflammation, are examined in terms of their relevance for psychiatric disorders.

In accordance with the HDR Thesis Preparation Guidelines of James Cook University, this *thesis by publication* is presented as a series of published papers and manuscripts

[i] The terms “ultra-high risk (UHR) for psychosis” “clinical high risk (CHR) for psychosis” and “at-risk mental state (ARMS)” are used throughout the literature to refer to people who present with varying degrees of sub-clinical symptoms and are at increased risk of developing a psychiatric disorder. While the term UHR typically indicates the presence of a risk state identified through the application of operationally defined criteria such as those discussed in this chapter, ARMS is sometimes used to refer to at-risk groups more generally, although there is substantial overlap in the use of these two terms. Some authors have argued that ARMS is in fact the preferred terminology by service users, carries less stigma and reflects the pluripotent outcomes of these phenotypes that extend beyond psychotic disorders. In this thesis, the terms UHR and ARMS are used to reflect the different study populations as well as the preferences of the journals in which the thesis chapters have been published.

submitted or in preparation for publication, preceded by an introduction and followed by a discussion of the main findings.

The General Introduction (Chapter 1) outlines the general background to the topic, includes a focused review of literature relevant to the topic and concludes with the aims of the thesis and an overview of the following chapters. As the scope of this thesis is not confined to a single diagnostic entity but rather broadened to a range of possible outcomes in mood and psychotic disorders, the General Introduction is not focused on a specific disorder but rather on risk mechanisms more generally. First, the mental health burden and disorders relevant to this thesis are briefly introduced. Next, literature pertinent to the concepts discussed in the thesis is reviewed. Focused reviews relevant to the specific objectives of the thesis are presented in the introductions of the subsequent chapters.

1.1 Disease burden - depression and psychotic disorders

Mental disorders are major contributors to the non-fatal disease burden globally. In Australia, it is estimated that one in two people will experience a mental health condition at some point in their life, with mental disorders accounting for 24% of the non-fatal disease burden [1]. Importantly, 75% of all mental disorders have their onset before the age of 24 [2], highlighting the importance of mental health for young people. In fact, mental disorders are the largest contributor to disease burden in young people in developed countries [3], lead to significant disability in many cases and have consequently been termed "chronic diseases of the young" by some authors [4]. This is mirrored by the 2017 Australian Youth Survey, which highlighted mental health as the most critical concern raised by young people [5].

The largest contributors to the disease burden in mental disorders include high-prevalence conditions such as anxiety disorders, mood disorders, attention deficit and hyperactivity disorder (ADHD) but also disorders associated with substantial disability such as psychotic disorders [6]. As much of this thesis refers to patients with or at risk for psychosis as well as to depressive symptoms in the community, a brief overview is included here.

Mood disorders include unipolar depression (e.g. Major Depressive Disorder (MDD)) and disorders that cycle between elevated and depressed mood (e.g. Bipolar Disorder). These disorders are characterised by alterations in mood (depressed or

elevated), inability to experience pleasure, excessive rumination, hopelessness, inappropriate guilt, but also somatic symptoms including sleep disturbances or changes in appetite. In youth, irritability is more commonly seen and depressed mood less frequent [7]. MDD is one of the most common psychiatric disorders, with prevalence estimates of approximately 3% globally [8] and lifetime prevalence estimates of 3 to 20% between different countries [2, 9]. Higher rates are commonly observed in developed countries compared to developing countries [9] and in females compared to males (rate ratio: approximately 2:1). MDD is estimated to be among the largest contributors to non-fatal disease burden globally with an upward trajectory [10, 11], making it a significant public health concern. In young people, depression is similarly or even more common, affecting approximately 2% of children and 4 to 8% of adolescents [12]. Importantly, high rates of comorbidity and increased risk for suicide contribute to the morbidity and mortality of MDD and other depressive disorders.

The vulnerability for depression is determined by both genetic and environmental factors. In addition to family history, established risk factors include stressful life events such as childhood trauma and adversity [13, 14] or low socioeconomic status [15]. Alterations in monoaminergic neurotransmission are a core feature of the biology of depression and are addressed by antidepressant medication [16]. Mounting evidence suggests that systemic factors including immune activation [17], activation of the hypothalamic-pituitary-adrenal (HPA) axis [18] and alterations in the commensal bacterial flora contribute to the pathophysiology of depression [19, 20].

Psychotic disorders are illnesses characterised by psychotic symptoms, i.e. primarily delusions and hallucinations [7]. More generally, psychotic disorders can entail positive symptoms (characterised by an excess or alteration of normal functions), negative symptoms (reflecting a loss of function) and cognitive symptoms. In addition to delusions and hallucinations, positive symptoms include disordered thinking, speech and movement and are often evident in abnormal behaviour. Negative symptoms on the other hand consist of symptoms such as loss of emotional expression/affective flattening, lack of motivation, reduced verbal expression or social withdrawal. Psychosis is a characteristic feature of Schizophrenia Spectrum Disorders, a group of disorders consisting of Schizophrenia, Schizoaffective Disorder, Delusional Disorder, Substance-Induced Psychosis and others, but may also be present in other disorders. For example, Bipolar Disorder I, Depressive Disorder with Psychotic Features, and Obsessive Compulsive Disorder are also included in the umbrella category of psychotic disorders in the DSM-V [7]. Psychosis is sometimes conceptualised as a

psychosis continuum or “extended psychosis phenotype” [21], which recognises that psychosis is not limited to severe and chronic clinical presentations but may be present in attenuated form in otherwise healthy individuals in the general population. Similarly and in addition to the “horizontal” extended psychosis phenotype, a “vertical” psychosis continuum describes the progression from subtle clinical signs and functional impairment to psychosis below the diagnostic threshold and more severe stages [22, 23] (see 1.2).

The epidemiology of psychotic disorder has long been thought to be homogenous across countries and ethnicities, with the prevalence believed to be approximately 1% based on the WHO 10-country study [24]. More recent studies, however, have refined this assumption and called for a more nuanced view. These studies show substantial variation in the treated incidence of psychotic disorders between countries ranging from 8 to 60 per 100000 person years with higher rates in men compared to women (rate ratio 1.4:1) [25] and a lifetime prevalence of 0.55% for schizophrenia [26] and 3% to 3.5% for any psychotic disorder [27]. Additional to detection rates and access to care, several factors including sex, migration and urbanicity might explain some of this variation [28, 29].

Psychotic disorders, in particular schizophrenia, are serious disorders that are associated with significant morbidity and mortality. While long-term remission from a first psychotic episode and functional recovery are seen in some cases, a substantial proportion of cases does not recover and are affected by persisting symptoms and disability [30]. Importantly, suicide as well as cardiovascular and metabolic diseases are common causes of death in patients with schizophrenia and contribute to the excess mortality [31]. In addition to absence of clinically relevant symptoms, functional recovery (that is, meaningful relationships, education and employment, etc.) as well as absence of medication-related side effects are important outcomes.

The exact aetiology and pathophysiology of psychosis remain elusive. However, it is clear that psychotic disorders including schizophrenia entail a complex and heterogeneous constellation of disease processes, many of which are present before the onset of clinically relevant symptoms [32]. These include aberrations in neurotransmitter function, in particular dopamine and glutamate [33, 34], sensitisation of immune cells in the brain [35], alterations in energy metabolism [36, 37], synapse formation and dendritic arborisation and brain structural and functional abnormalities [38]. Aetiological factors include genetic predisposition [39], pre-natal and environmental

insults [40, 41] or cannabis use in adolescence [42] as well as pathophysiological processes including dopaminergic and glutamatergic dysfunction [23, 34], brain structural and functional abnormalities [43] and alterations in immune function and oxidative defence at least in a subset of patients [44]. Importantly, these processes likely interact with each other and do not occur in isolation, and it should be noted that many factors overlap with other condition and are likely transdiagnostic. For example, many genetic risk variants for schizophrenia are shared with bipolar disorder and vice versa [45] and childhood adversity predisposes to a variety of disorders [14].

1.2 Studying high-risk groups

The last two decades have seen growing interest in early intervention strategies for populations at risk for mental disorders, most notably for psychotic disorders. Such strategies are grounded in the paradigm of early intervention with the aim to improve clinical outcomes for patients by identifying individuals at elevated risk for psychosis early and offering targeted interventions to reduce the risk for progression. Considerable effort has been made to define and characterise high-risk groups, to estimate the risk for transition to frank psychosis, to identify predictors of transition and to evaluate novel interventions appropriate to prodromal stages. The translation of these research efforts has resulted in the development and implementation of clinical services for people – particularly adolescents and young adults – who seek help for symptoms below the threshold for conventional mental health services (e.g. *headspace* in Australia). In addition to clinical criteria aimed at identifying people at risk for mental disorders early on in the disease course, epidemiological studies also revealed population groups at increased risk for mental ill-health

While a multitude of social, environmental, biological and genetic risk factors for psychotic disorders have been identified (reviewed elsewhere [29]), their effect sizes are often small. A recent comprehensive umbrella review of 55 meta-analyses and systematic reviews of risk factors for psychosis concluded that the most convincing and robust evidence exists for the ultra-high risk (UHR) state for psychosis (OR=9.32, 95%CI 4.81 – 17.32) and ethnic minorities in the UK (OR=4.87, 95%CI 3.96 – 6.00), closely followed by ethnic minority status more generally and first and second-generation immigrants [29].

1.2.1 Ultra-high risk for psychosis and clinical staging

The observation that psychotic disorders are commonly preceded by a prodromal phase characterised by sub-clinical yet detectable and distressing symptoms (psychotic and non-psychotic) has led to the development of criteria to identify people at imminent risk for developing first-episode psychosis. First operationalised in the 1990s by Yung and colleagues [46], these criteria propose the prospective identification of people at UHR for psychosis to advance and overcome the retrospective concept of a prodromal phase. The construct of the UHR state is now widely used to study the phase preceding first-episode psychosis and inclusion requires one or more of the following criteria to be present: Attenuated psychotic symptoms (APS), characterised by sub-clinical psychotic features; brief limited intermittent psychotic episode (BLIPS), a psychotic episode of shorter duration than required for a DSM-V diagnosis; and genetic high risk accompanied by a marked decline in psychosocial functioning (Table 1.1). To date, several validated diagnostic interviews have been developed, for example the Comprehensive Assessment of At-Risk Mental States (CAARMS) [47] in Australia, the Structured Interview for Prodromal Syndromes (SIPS) [48] in the United States and the Basel Screening Instrument for Psychosis (BSIP) [49] in Switzerland. These interviews are now commonly used in help-seeking populations in research and in clinical services and have excellent sensitivity but only moderate specificity [50]. The prevention of psychosis in the UHR group ("indicated prevention") thus represents a promising strategy to reduce the disease burden and to improve long-term outcomes in individuals who might otherwise develop psychosis and has been recognised in guidelines in several countries including Australia [51], the United Kingdom [52] or by the European Psychiatric Association [53].

Table 1.1 Ultra-high risk (UHR) for psychosis criteria according to the Comprehensive Assessment of At-Risk Mental States (CAARMS)

APS	BLIPS	Genetic High Risk
• Sub-threshold attenuated positive symptoms (ideas of reference, "magical" thinking, perceptual disturbance, paranoid ideation, odd thinking and speech) • either sub-threshold frequency or sub-threshold intensity	• Transient psychotic symptoms (thought content, perceptual abnormalities, disorganized speech) • duration of the episode of 1 week in the past 12 months • spontaneous remission • decline in functioning *or*	• Family history of psychosis *or* an individual with schizotypal personality disorder • decline in functioning *or* sustained low functioning[1]

• present for 1 week in the past 12 months • decline in functioning *or* sustained low functioning[1]	sustained low functioning[1]

APS= attenuated psychotic symptoms, BLIPS= brief limited intermittent psychotic episode; [1]Defined as a decline in Social and Occupational Functioning Assessment Scale (SOFAS) score of 30% since the last assessment, or a score of 50 or less sustained for the past 12 months or longer

The notion that early phases of illness are detectable in clinical settings and indicative of risk for more severe conditions has led to the development of clinical staging frameworks. Such staging frameworks build on evidence for a continuum of psychopathological phenomena and propose distinct stages that can be differentiated by clinical presentation, risk for progression and need for care. Common to staging systems proposed in psychiatry in the last decade that purposefully include early risk stages is that they extend beyond traditional diagnostic boundaries to include less differentiated or unspecific symptoms, following the idea of the UHR phenotype. McGorry and colleagues [22] proposed a staging system consisting of five stages ranging from asymptomatic risk for mental disorders through family history (Stage 0); mild, non-specific symptoms and decline in functioning, overlapping substantially with the UHR criteria discussed above (Stage 1a/b), first episode of a severe mental disorder (Stage 2); incomplete remission, recurrence or multiple relapse (Stage 3a/b/c); and severe, persistent illness associated with significant disability (Stage 4). An overview of different staging systems and their criteria is provided in Table 1.2. In order to provide empirical support for clinical staging, the authors have argued for large, trans-diagnostic longitudinal studies including high-risk individuals to examine a multitude of outcomes, stage-adequate treatments and biomarkers for progression and treatment response [54, 55]. Such biomarkers may include markers for HPA-axis activity, oxidative stress, immune activation, lipid metabolism or AL [55-58] but also neuroimaging biomarkers [59]. Accordingly, staged-care approaches are currently being tested (e.g. ACTRN12616000098437). Ultimately, stratifying patients across molecular features instead of a diagnosis might assist in identifying subsets of patients that benefit for particular interventions. However, identifying biological signatures that are associated with discrete outcomes remains challenging for complex syndromes such as psychotic disorders. Unlike in oncology for example, where clear pathological boundaries exist between stages that are associated with distinct changes in mortality risk and treatment efficacy, translation from clinical to pathophysiological staging in psychiatry

is challenging and will likely involve a broad range of biological information rather than single biomarkers.

Table 1.2 Clinical Staging Criteria in Psychiatry

	McGorry et al. 2006	Fusar-Poli et al. 2017
Stage	Description	Description
0	Asymptomatic genetic risk	Asymptomatic genetic risk
1a	Mild or non-specific symptoms, including neurocognitive deficits, of psychosis or severe mood disorder. Mild functional change or decline	Negative and cognitive symptoms
1b	Ultra high risk: moderate but subthreshold symptoms, with moderate neurocognitive changes and functional decline (GAF < 70)	Attenduated psychotic symptoms
1c	N/A	Brief limited intermittent psychotic symptoms (BLIPS)
2	First episode of psychotic or severe mood disorder Full threshold disorder with moderate-severe symptoms, neurocognitive deficits and functional decline (GAF 30–50)	First-episode psychosis (FEP)
3a	Incomplete remission from first episode of care	N/A
3b	Recurrence or relapse of psychotic or mood disorder which stabilises with treatment at a level of GAF, residual symptoms, or neurocognition below the best level achieved following remission from first episode	N/A
3c	Multiple relapses, provided worsening in clinical extent and impact of illness is objectively present	N/A
4	Severe, persistent or unremitting illness as judged on symptoms, neurocogntion and disability criteria	N/A

The utility of the UHR criteria and their benefits for early intervention strategies rely heavily on their ability to predict who will develop psychosis and other mental

disorders. The most recent meta-analytical estimates suggest that 20% (95%CI 17-25%) of individuals at UHR for psychosis convert to first-episode psychosis (FEP) within 2 years [60]. Recent research has revealed critical issues regarding the risk for psychotic and non-psychotic outcomes. For example, the risk for transition is highly heterogeneous, with individuals who fulfil BLIPS criteria having the highest transition rates (38%), followed by people with APS (18%), while those at genetic high risk on the other hand have substantially lower risks for transition at approximately 4% [60], suggesting that these categories may represent distinct subgroups characterised by different prognoses [61]. In contrast, long-term non-psychotic outcomes do not differ between these sub-groups, which led some authors to argue that the current criteria should be maintained [62]. Another important observation is that transition rates have declined since the first UHR studies were published [63] with a recent large randomised-controlled trial (RCT) reporting only 11.2% in the placebo group within one year [64]. The reasons for the declining transition rates may include low pre-test risk enrichment due to the fact that with increasing awareness of UHR stages study participants are not only recruited from people seeking help at specialist services but also from a variety of settings ("false positives") [65, 66]. While psychotic-like experiences are common in the general population [67-69], particularly in youth [70, 71], with point prevalence estimates ranging from 5% to 17%, and are associated with future mental health service use [72, 73], applying CAARMS criteria to non-help-seeking populations may lead to excessive risk dilution and a higher rate of false-positive cases. Consequently, UHR criteria should only be applied in help-seeking populations. However, the hypothesis that risk enrichment contributes to declining transition rates has been also questioned based on the observation that symptom severity and level of functioning do not seem to differ between studies with high and low transition rates [74] and re-analyses of older studies using current criteria did not result in higher transition rates [75]. A complementary explanation for declining transition rates may be the fact that recent clinical trails and observational studies provided background interventions such as high quality psychosocial care to study participants, which may have led to lower transition rates even in the control groups [76].

Psychosis is not the only outcome of interest in the UHR group. In addition to the paradigmatic outcome of transition to psychosis, which arguably remains as an important outcome of interest in people at UHR for psychosis, it has become clear that other psychiatric disorders are common in this group. Lin and colleagues [77] conducted one of the largest long-term follow-up studies in non-transitioned UHR individuals to date (median duration of follow-up=5.7 years) and found that 49% experienced mood

disorders, 35% had an anxiety disorder, 29% a substance abuse disorder, and 28% had persistent attenuated psychotic symptoms. Of these, 90% were already present at baseline and 36% of participants received a new diagnosis during the follow-up period. Only 7.3% did not have an Axis I diagnosis at any point during the follow-up period. Other studies with longer follow-up duration showed similar rates of non-psychotic disorders in youth at UHR for psychosis [78]. An analysis of two large North American UHR cohorts on the other hand found that non-psychotic outcomes were in fact not more common in people at UHR for psychosis compared to those who were screened but did not satisfy UHR criteria when only incident (newly occurring) diagnoses are considered [79]. Non-psychotic outcomes in people at UHR for psychosis are further discussed in Chapter 5.

Currently, no biological tests are available to increase the accuracy of outcome prediction beyond clinical characteristics. This is in stark contrast to most other branches of medicine, where biomarkers are used to estimate the risk for a disease outcome of interest (e.g. pre-diabetes [80]) and consequently the need for care. This question is particularly relevant in the context of UHR for psychosis, as psychosis prediction criteria identify a substantial proportion of individuals who will not develop psychosis but still require care for other reasons. A recent meta-analysis examined the presence of known environmental risk factors for psychosis in the UHR group [81]. This study confirmed that obstetric complications, childhood trauma including emotional abuse and neglect, low functioning during childhood and adolescence, high perceived stress, social deficits and affective comorbidities, male gender and low educational attainment were significantly more prevalent in the UHR group. However, much less is known about risk factors that directly modify psychosis risk *within* the UHR group. Long-term follow-up studies of UHR cohorts provided insights into clinical risk factors for transition by showing that longer duration of symptoms before accessing specialist services [82, 83], low baseline functional capacity [82-89], negative symptoms and thought disorder [82, 83] and total Positive and Negative Symptoms Scale (PANSS) scores [84, 85] were associated with higher risk for transition. Biological predictors of transition have been explored less with only a few smaller clinical trials and case-control studies providing evidence. These include increases in the omega-3 (ω-3) PUFA EPA during supplementation trials [84, 90], low total ω-3 PUFA [85], low levels of the ω-9 FA nervonic acid [85, 91], low levels of glutathione (GSH) [92] and cortisol [93-95] (see Chapter 2). Collectively, these studies provide preliminary evidence that antioxidant and immune mechanisms might be involved in modulating risk for psychosis transition. Even less is

known about the antecedents of non-psychotic outcomes with the limited available evidence pointing to negative symptoms at baseline [77].

1.2.2 Indigenous mental health

Indigenous people and other ethnic minority groups are often confronted with social challenges that can affect their mental health. Indigenous people suffer from a broad range of communicable and chronic conditions at higher rates compared to the mainstream society [96]. Similarly, there is evidence that Indigenous people experience poor mental health more commonly, including Aboriginal Canadians [97, 98], Maori in New Zealand [99] and the Aboriginal and Torres Strait Islander people in Australia, who are more than twice as likely to be hospitalised due to a mental disorder [100]. In the Australian context, mental health disparities are also documented through higher rates of non-specific psychological distress, with 30-44% reporting high or very high levels of distress [100-103]; significantly higher rates of suicide, particularly in youth [104, 105]; higher rates of most mental and substance abuse disorders in Aboriginal and Torres Strait Islander youth [103]; high prevalence of risk factors for mental illness in adolescent populations [106]; and evidence of higher rates of psychotic disorders of approximately twice the Australian national estimate in the geographical area relevant to this thesis [107]. Higher depression rates have been described in several studies: in one of the largest community surveys in Aboriginal youth in Melbourne, Luke et al. [108] report prevalence rates for moderate to severe depressive symptoms measured with the Patient Health Questionnaire-9 (PHQ-9) of 18-23%. The Darwin Region Urban Indigenous Diabetes (DRUID) study, a cross-sectional study of 185 adults, reported rates of 13%[109]. More recently, Taylor et al. reported comparatively low rates of moderate/severe depressive symptoms (PHQ-9) in the Torres Strait of 12%. Finally, in the adolescent population from which participants for Chapter 7 were recruited, moderate/severe symptoms measured with the PHQ-9 were found in 18% of study participants. Together, these studies provide evidence for high rates of depression at population level, with higher rates possibly found in youth.

Evidence for health inequalities between ethnic minority groups and the general population is not limited to First Nations groups. In fact, migration remains as one of the best established risk factors for poor mental health [110, 111]. For example, the psychosis risk in migrant groups in several European countries ranges from 1 to 2-fold increases (intra-European migrants) to 5 to 10-fold increases (Caribbean and African migrants) compared to the majority white population [111-114]. There is also evidence for

higher treated incidence of psychotic disorders in ethnic minority groups across Europe and South America [25] and that ethnic minorities are overrepresented in first-episode psychosis services [115]. While psychotic disorders are arguable the most studied outcome, the existing evidence suggests that this trend extends to other disorders including affective and anxiety disorders [110]. Importantly, the relative risk is not only increased compared to the majority population of the host country, but also relative to the same ethnic group in the country of origin [116, 117], suggesting that genetic factors alone cannot explain the heterogeneity in psychosis risk. Furthermore, early assumptions that the selective migration of vulnerable individuals explains higher rates of psychosis have been refuted [118]. However, it has to be noted that higher incidence of psychotic disorders is not found in every setting, with a recent meta-analysis reporting heterogeneity in psychosis risk for example in Asian migrants in England [112] and a Canadian study reporting somewhat lower 12-month prevalence rates of mood and anxiety disorders in overseas-born people [119]. Nevertheless, the evidence for higher rates of a range of disorders, most notably psychotic disorders, in ethnic minority groups remains strong and may not so much depend on migration than on factors relating to minority group status.

While migration clearly poses a significant stressor that may affect psychosis risk, particularly during sensitive periods of neurodevelopment [114], the observation that the risk persists into the second generation [114, 120] suggests that it is not simply the experience of migration itself that drives the increased mental health risk. Rather, being in a minority position may expose people to various stressors associated with poor mental health outcomes and may render them more vulnerable to their consequences. If migration itself and genetic factors do not sufficiently explain the higher rates of mental disorders in minority groups, post-migratory factors and social determinants more generally have to be considered as they may affect migrant and First Nations groups alike.

Despite higher rates for mental disorders in Aboriginal and Torres Strait Islander people and other First Nations groups being documented in national survey data and smaller community studies, few studies have directly examined factors that determine vulnerability for psychiatric risk in this population. Social determinants of health are thought to contribute substantially to the existing health disparities between Aboriginal and Torres Strait Islander people and non-Indigenous people living in Australia. Social determinants of health are the *“conditions in which people are born, grow, work, live and age, and the wider set of forces and systems shaping the conditions of daily life”*

[121]. These commonly include education and employment, adequate housing conditions, culturally appropriate access to health care and health literacy [121], but in a wider sense also freedom from discrimination, control over life circumstances and social inclusion [122]. There is empirical evidence that rates of poor mental health between different Aboriginal and Torres Strait Islander communities depend heavily on socioeconomic factors [123].

The pathways through which social determinants contribute to poor mental health outcomes in minority groups at heightened risk for mental ill-health are manifold but only partially understood [124, 125]. In addition to effects on health behaviour (e.g. diet, self-medication) and service use (e.g. barriers to access to culturally appropriate health care), biological pathways are though to contributing to health inequalities. For example, there is evidence pointing to a converging pathway on stress processing, resulting in sequalae of chronic stress that likely impact on the risk mental illness. Chronic stressors commonly experienced by minority groups such as racial discrimination are though to lead to heightened allostatic load (see 1.3.1) and impact on brain regions relevant to stress processing such as the anterior cingulate cortex (ACC) (reviewed elsewhere by the author [126]). Chronic stress elicits differential responses to an acute stressor in the perigenual ACC, a key region of the limbic stress regulatory system, in ethnic minority groups in Germany, which correlated with perceived racial discrimination, suggesting that social stressors may at least partially lead to higher risk for mental ill-health through effects on this brain region [127]. We have recently shown that perceived racial discrimination is similarly associated with the cortisol response to stress in Aboriginal and Torres Strait Islander university students and that chronic self-reported stress is differentially associated with blunted cortisol awakening response (CAR in Aboriginal and Torres Strait Islander and non-Indigenous students [128]. Altered reactivity to acute stress as well as elevated levels of cortisol, the body's main stress mediator and a powerful glucocorticoid, are frequently found in subsets of patients with mood [129, 130] and psychotic disorders [131-133] and might also indicate risk for these disorders [94, 95]. However, the hypothesised relevance of the biological effects of stress in risk for mental ill-health remains unclear in the Aboriginal and Torres Strait Islander population as there is a lack of empirical research.

1.3 Risk biomarkers

1.3.1 Allostatic load

Allostatis has been described as a concept characterised by physiological recalibration to changing demands and complementary to that of homeostasis [134]. More specifically, the term allostasis describes dynamic biological processes that maintain the stability of physiological systems through adjustment of (homeostatic) states in response to environmental stimuli such as stress [134]. Allostatic load (AL) is the result of sustained allostatic states, characterised by excess or imbalance of allostatic mediators, the role of which is normally to maintain stability in an organism [135]. A commonly used example for an allostatic process is the regulation of blood glucose levels in the case of type-2 diabetes mellitus. Glucose levels are primarily maintained by insulin and its functional antagonist glucagon in response to dietary intake of nutrients and dynamic changes in energy demand. In a homeostatic state, postprandial blood glucose levels rise in response to energy intake before returning back to baseline levels through glucose uptake facilitated by the release of insulin. However, excessive exposure to glucose may cause insulin resistance and subsequently lead to chronically elevated blood glucose levels. This may be seen as an allostatic state in which homeostasis has been replaced by allostasis.

Primary mediators of AL that are released in response to stress, such as the glucocorticoid cortisol, pro-inflammatory immune mediators or the sympathetic nervous system, lead to *primary effects* such as oxidative stress or a chronic low-grade inflammatory state. This is then believed to contribute to *primary outcomes* such as cellular dysfunction, epigenetic alterations or effects on neurotransmission, and finally *secondary* and *tertiary outcomes* such as cardiovascular disease, diabetes mellitus, cognitive decline or accelerated ageing [136]. This framework offers an opportunity to measure AL at the level of primary mediators in the hope to detect allostatic processes leading to adverse health outcomes.

The mediators of AL generally consist of neuroendocrine, metabolic, immune and cardiovascular biomarkers that collectively represent a multisystem approach to detect subclinical disturbances [137]. Biomarkers that have been used and evaluated in previous studies include neuroendocrine markers, most importantly cortisol (e.g., 12hr urinary free cortisol), cortisol's endogenous functional antagonist dehydroepiandrosterone (DHEA) and adrenaline/noradrenaline. Immune markers include c-reactive protein (CRP), interleukin 6 (IL6), tumor necrosis factor-α (TNF-α)

and fibrinogen. Metabolic markers including total cholesterol, high and low density lipoproteins (HDL/LDL), glycosylated haemoglobin (HbA1c) as well as body mass index (BMI) and waist-to-hip ratio [137]. Cardiovascular functioning is assessed with resting blood pressure or more recently heart rate variability [138]. The majority of studies have used an AL index consisting of 10 biomarkers first used by Seeman et al. [139], though the authors noted that *"[…] the measure of AL used in these analyses should be seen as initial".*

Many of these biomarkers are commonly used in daily clinical practice and if elevated are diagnostic markers for specific diseases. In the AL model, however, chronic sub-thresholds of numerous biomarkers taken together is seen as a mirror image of metabolic disturbances with strong predictive value for health outcomes. AL also allows studying the underling interacting systems in the same individual, e.g. interactions between chronic low-grade inflammation and exaggerated stress systems, both of which are highly relevant to several mental disorders but are often studied separately. Cluster analysis of the biomarkers used in AL indices demonstrates that the totality of all markers predict health outcomes significantly better than single markers such as waist-to-hip ratio or HbA1c and also significantly better than systemic clusters, for instance, of metabolic parameters [137].

The term AL is used throughout this thesis to refer a state of physiological imbalance characterised by excessive release of primary AL mediators. This thesis takes a pragmatic standpoint in viewing AL as a state of multisystem dysregulation that may have detrimental effects on mental health. For this purpose, AL is operationalised as a composite index of multiple AL primary mediators (further discussed in Chapters 3, 4 and 7).

One application of the AL framework in the past has been to measure the excess biological risk observed in disadvantaged groups of society. Increased AL is often found in impoverished and marginalised populations and is thought to partially mediate the effect of low socioeconomic status on health. Activation of the endogenous stress systems, most notably the HPA axis, is a core feature of the body's response to social stress and central to the concept of allostasis [140]. Low socioeconomic status early in life has been identified as a predictor of such HPA-axis alterations later in life in a large birth cohort study [141]. Similar associations have also been found as a result of discrimination [128]. AL is predicted by several factors associated with socioeconomic disadvantage such as household overcrowding [142] and adverse childhood experiences

[143]. Social support, sense of coherence and positive parental bonding on the other hand have been found to be associated with lower AL [137], indicating a role for social factors that protect against chronic stress. Given the findings that AL is high in disadvantaged populations and strongly associated with low socioeconomic status, AL may be an important factor in health inequalities.

Social stress does not necessarily leave the same traces on the body at every developmental stage. Both animal and human studies have demonstrated that several different critical windows of vulnerability exist whereby the individual is particularly sensitive to environmental stressors [144] and such observations have been integrated into life-course models of health and disease [145]. These vulnerable phases are tightly linked to neurodevelopment and may explain why children are especially vulnerable to the long-lasting effects of an adverse environment.

Importantly, AL may be highly relevant for psychiatric disorders. While the effects of AL on the brain have been central to the concepts since its inception [140, 146, 147] few studies to date have directly investigated AL with relevance to psychiatric outcomes or in psychiatric populations. Seeman and colleagues were among the first to conduct a systematic longitudinal investigation of AL in a large cohort of older adults [139]. In 1118 individuals (age range: 70-79 years), AL was measured and participants were followed-up for 7 years (median). This study found increased risk for overall mortality in individuals with the highest AL (OR=1.23) independent of pre-existing conditions or other relevant potential confounders. However, the strongest association was found for cognitive decline, with individuals with the highest AL being at significantly higher odds for cognitive decline. Early evidence for a link between AL and depressive symptoms comes from the Taiwanese Social Environmental and Biomarkers of Ageing Study (SEBAS), a large cohort of Taiwanese adults aged 50 years and older, which demonstrated associations of AL biomarkers with depressive symptoms [148]. However, while the 10-item AL index was associated with poor cognitive performance, no such relationship was found with depressive symptoms (measured with the CES-D) [149]. Juster and colleagues reported that AL was prospectively associated with depressive symptoms three years later in a cohort of 58 healthy older adults (mean age: 67 years), although the authors note that the power of the analysis was low (0.6) and the effect marginally significant when age and gender were entered as covariates. Similarly, Kobrosly and colleagues reported associations of a multisystem index conceptually similar to AL with depressive symptoms (measured with the PHQ-9) in a large cohort of healthy older adults, with associations with somatic symptoms being

greater than those with affective symptoms [150]. A secondary analysis of a clinical trial in 125 adults aged 65 years and older subsequently published by the same group investigated AL found associations of a simplified AL index with more severe depressive symptoms (PHQ-9) in a dose-response type fashion [151]. In contrast to these studies, a recent study measured AL in a large and ethnically diverse cohort (n=12272) and found no effect of AL on depressive symptoms, irrespective of ethnicity. The differences in methodology and study population preclude conclusions about the reasons why some studies find elevated AL in depressed individuals or associations with depressive symptoms while others do not. However, age emerges as an important confounding factor from these reports.

While the relevance of the AL concept for psychotic disorders has been postulated and discussed in several review articles [56, 152, 153], it has not been applied to clinical populations until very recently. The first published account of AL in patients with schizophrenia reported elevated AL that was associated with more severe psychotic symptoms and decreased psychosocial functioning [154]. The same group also measured AL in structural and functional magnetic resonance imaging (fMRI) studies, demonstrating that AL was associated with reduced global cortisol thickness [155] and reduced functional connectivity in the fornix [156] in patients with schizophrenia, suggesting that AL is related to grey matter loss and white matter integrity.

1.3.2 Polyunsaturated fatty acids

Long-chain PUFA (LCPUFA) play important roles in neuronal function and are key regulators of cell membrane properties [157, 158]. Membrane lipids are part of the lipid bilayer assembly that forms the cellular membrane in mammalian cells, separating the cytosol from the extracellular space [159]. PUFA are commonly found in phospholipids, where they are attached to glycerol through phosphodiester linkages. Phospholipids found in human cell membranes include phosphatidylcholines, phosphatidylethanolamines, phospohatidylserines and phosphatidylinsositols, with PUFA found in each of these sub-types [159]. However, it should be noted that PUFA levels are commonly (but not exclusively) quantified from the phosphatidylethanolamine fraction, which is located at the inner side of the lipid bilayer and contains a high abundance of PUFA [160]. PUFA concentrations can also be quantified in plasma, for example using a dried blood spot test and mass spectrometric analysis [161]. The main types of PUFA relevant to this thesis are omega ω-3 and ω-6

PUFA, referring to the location of the first double bond between the third and fourth (ω-3) and sixth and seventh (ω-6) carbon atom, counted from the omega (-CH_3) end.

Both ω-3 and ω-6 PUFA are considered to be essential fatty acids [159]. That means they cannot be synthesised by mammalian cells and must be obtained from the diet. The most important primary sources of long-chain ω-3 PUFA, particularly EPA and DHA, are phytoplankton and marine algae [162]. PUFA from these sources accumulate in the food chain and can be found in high abundance in fatty fish including sardines and salmon [162]. Other animal based sources include eggs and meat from animals fed with ω-3 rich diets. Common sources of plant oils abundant in ω-3 PUFA include walnuts, flaxseed and other seeds [162]. In addition to natural sources, supplementation with ω-3 PUFA ("fish oil") is becoming increasingly popular. However, both ω-3 and 6 PUFA undergo conversion from short-chain to long-chain PUFA via elongation and desaturation in humans [163]. However, this process is only moderately efficient [164], meaning that short-chain PUFA are only partially metabolised to the longer-chain PUFA thought to be relevant for psychiatric disorders. Both ω-3 and 6 PUFA compete for the same enzymes, namely Δ-6-Desaturase, Elongase and Δ-5-Desaturase. The metabolism of PUFA in humans also includes downstream metabolic products including eicosanoids and docosanoids, which possess pro- and anti-inflammatory properties [165, 166].

Several lines of evidence point to a link between the consumption of ω-3 PUFA from dietary sources, their composition in cell membranes and several psychiatric disorders. First, there is evidence that fish consumption is inversely related to the prevalence of psychiatric disorders, including mood disorders and psychotic disorders. The first study to suggest a link between dietary intake and depression was published two decades ago and reported a strong negative correlation (r=-0.84) between national fish consumption and depression prevalence across several European, American and East Asian countries [167]. The observation that depression rates are higher in those countries with lower fish consumption sparked interest in the role of PUFA in depression. Subsequent studies have replicated this finding using person-level data, for example in a longitudinal study in Australia [168] and one in Japan [169]. By contrast, 'Western' diets high in saturated fats and low in ω-3 PUFA are known to be associated with higher prevalence of depression [170, 171]. In addition to mood disorders, associations of fish consumption have also been reported for psychotic-like symptoms and suicide risk.

Hedelin and colleagues conducted a study among 33623 woman in Sweden and reported that the intake of ω-3 PUFA was associated with a decreased relative risk for psychotic-like symptoms, taking into account relevant confounding variables [172]. The evidence for a relationship between fish consumption and suicide risk is conflicting. A Japanese cohort study (n=256118) reported lower risk for suicide in individuals with daily fish consumption (OR=0.81) compared to ate fish less often [173]. However, a more recent analysis of three American cohort studies (pooled n=205357) failed to replicate this observation, identifying no relationship between self-reported intake of ω-3 PUFA, ω-6 PUFA and subsequent suicide [174].

Second, increased turnover of PUFA may be present in patients with schizophrenia and depression. A key family of enzymes involved in the metabolism of PUFA are phospholipases A_2 (PLA_2), a group of housekeeping enzymes that catalyse the cleavage of fatty acids and lysophospholipids from PUFA. Additional to this function, PLA_2 is also believed to be involved in the regulation of apoptosis [175, 176], oxidative stress and inflammation [177]. Increased activity of PLA_2 has been reported in patients with schizophrenia relative to both controls and non-psychotic patients [178-181] where it is associated with illness severity [182] and brain structural changes [182], and reduced by antipsychotic treatment [180]. In people at UHR for psychosis, intracellular PLA_2 levels correlate with ω-3 and ω-6 PUFA levels and are normalised by supplementation with ω-3 PUFA [160]. The evidence for the role of PLA_2 in depression is less clear and clinical data are limited, but suggestive of higher PLA_2 activity in patients with MDD [181]. However, the *Ban*I polymorphism in the cytosolic PLA_2 gene - associated with higher transcription of PLA_2 - was associated with MDD in a case-control study [183]. Moreover, PLA_2 appears to modulate the risk for depression in patients treated with interferon alpha through modulation of plasma PUFA levels [184]. Similar to schizophrenia, supplementation with the ω-3 PUFA EPA and DHA also increased cytosolic concentrations of PLA_2 in patients with MDD in one study [185].

Third, low blood levels of ω-3 PUFA have been reported in patients with MDD [186], anxiety disorders [187], schizophrenia [188] and at-risk mental states [189]. The balance between ω-6 and ω-3 PUFA is similarly associated with depressive symptoms [190] and with psychotic symptoms in people at UHR for psychosis [191]. While these observations are cross-sectional and preclude causal inferences, they provide additional support for

the notion that decreased intake or increased depletion of ω-3 PUFA may be relevant to these conditions.

Finally, clinical trials have provided evidence for the efficacy of supplementation with ω-3 PUFA at improving symptoms and clinical outcomes in mood disorders and anxiety disorders. Meta-analyses of clinical trials in patients with MDD have revealed heterogeneous findings but demonstrated that EPA-dominant formulations are effective while DHA-dominant formulations are not [192-195]. One study provides evidence of the efficacy of ω-3 PUFA supplementation for anxiety symptoms in healthy volunteers, which was related to increases in the ω-6/3 PUFA ratio [196]. Ω-3 PUFA have also been evaluated in youth at UHR for psychosis. In these trials, transition to psychosis was the primary outcome. The first trial demonstrated superiority of ω-3 PUFA over placebo for the prevention or delay of psychosis transition with large effect size [197], which persisted at the 7-year follow-up [78]. However, two subsequent replication trials did not confirm the efficacy of ω-3 PUFA for transition to psychosis or other clinical or functional outcomes [64, 198].

Collectively, the studies discussed above strongly suggest that PUFA play a role in several psychiatric disorders and potentially in psychosis risk. The implications of the involvement of ω-3 PUFA in the pathophysiology of psychiatric disorders include that supplementation represents a potential treatment approach with few clinically relevant side effects, that their dietary intake may be a potentially modifiable risk factor and that plasma/erythrocyte levels of ω-3 PUFA have potential as biomarkers to inform risk and treatment response. However, the latter hypothesis has not been empirically tested.

1.4 Aims

If our growing understanding of the pathophysiology of mental disorders is to improve prognosis and treatment, identifying and testing potential biomarkers is a crucial step. Cross-sectional and longitudinal studies in populations at risk for or at the transition from sub-clinical distressing symptoms to mental disorders offer a unique opportunity to study the biology and corresponding biomarkers.

The overall aim of the studies presented in this thesis was to examine associations of AL and lipid biology with clinical and functional outcomes in three distinct populations.

These include (a) patients with chronic schizophrenia (SCZ) and FEP, (b) people at UHR for psychosis and (c) Aboriginal and Torres Strait Islander people. To achieve this aim, this thesis employs meta-analysis of existing studies (Chapter 2), cross-sectional (Chapter 3) and longitudinal (Chapters 4 and 5) clinical studies and cross-sectional population studies (Chapters 6 and 7).

1.5 Thesis outline

Chapter 1 outlined the background, rationale and objectives of the work presented in this thesis. The seven chapters that follow consist of six original research articles (Chapters 2 - 7), each containing a separate abstract, introduction, a description of the methods, results and discussion. Finally, Chapter 8 concludes the thesis, draws together key conclusions of the research presented in this thesis and highlights avenues for future research. A list of references is given at the end of each chapter. The six original research articles are organised as follows:

Chapter 2 investigates cortisol awakening response (CAR), a cortisol biomarker standardised to the circadian rhythm or cortisol excretion, in patients with psychosis. This is achieved through meta-analysis of observational studies in patients with SCZ, FEP and people at UHR for psychosis. The qualitative analysis in this chapter also evaluates the existing evidence for association with relevant outcomes (e.g., psychosis transition or treatment response) in these studies.

Chapter 3 reports a study of AL in patients with SZC and FEP. In this study, an AL index was calculated from neuroendocrine, cardiovascular, immune and metabolic biomarkers and contrasted between patients and healthy controls. Associations with clinical symptoms and global functioning are reported and the temporal dynamics of AL are explored at two follow-up time points.

The study reported in **Chapter 4** extends the data reported in the preceding chapter by applying the AL index to a cohort of youth at UHR for psychosis. Six and 12-month follow-up assessments provided an opportunity to examine associations of baseline AL and clinical and functional outcomes in this sample.

Chapter 5 explores the role of selected ω-3 and ω-6 PUFA with relevance to clinical outcomes in youth at UHR for psychosis. By drawing on long-term follow-up data from the Vienna ω-3 study, a placebo-controlled RCT of fish oil for UHR for psychosis, this

chapter reports longitudinal associations with PUFA with Axis I diagnoses 7 years later.

Chapter 6 reports a cross-sectional observational study of Aboriginal and Torres Strait Islander adolescents and adults attending health screens in three communities. The aim of the study reported in this chapter was to examine the relationship between hair cortisol, AL and depressive symptoms.

Chapter 7 is an analysis of the study reported in the preceding chapter. This chapter focuses on the nature of the association between fish/seafood consumption, plasma levels of ω-3 and ω-6 PUFA and depressive symptoms in communities with a diet that is traditionally rich in fish and seafood and low in processed foods.

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Part 1

2 Cortisol Awakening Response in Patients with Psychosis: Systematic Review and Meta-Analysis

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Preface

Cortisol is the end product of the HPA axis and an important mediator of the body's response to stress. While cortisol been investigated in schizophrenia for decades, much of the existing research has focused on single morning cortisol samples and little is known about cortisol in the early stages of psychosis.

Chapter 2 explores cortisol abnormalities across the postulated stages of psychotic disorders (UHR – FEP – SCZ) in a systematic review and meta-analysis of existing literature. In doing this, it fills an important gap in the literature by focusing on CAR as a standardised cortisol readout and by taking into account the different stages mentioned above. Given the lack of direct evidence of AL in psychosis at the time this chapter was written, it was a necessary first step to examine a primary mediator of the AL framework.

Abstract

Neuroendocrine abnormalities are common in patients with schizophrenia and are thought to interact with pathophysiological mechanisms of psychosis. The cortisol awakening response (CAR), defined as the increase in cortisol release in response to waking up, shows associations with social and environmental risk factors of schizophrenia and has been studied as a potential biomarker in schizophrenia. Here, we provide a systematic review and meta-analysis of 11 studies and 879 participants focusing on the CAR of patients with schizophrenia, first episode psychosis, and at-risk mental states. Random-effects meta-analysis showed that patients along the psychosis continuum have an attenuated CAR compared to healthy controls (g=-0.426, 95% CI -0.585 to -0.267, p<0.001, 11 between-group comparisons, n=879). Subgroup analysis showed that CAR alterations can be found in patients with schizophrenia (g=-0.556, 95% CI -1.069 to -0.044, p<0.05, 2 between-group comparisons, n=114) and are already present in patients with first episode psychosis (g=-0.544, 95% CI -0.731 to -0.358, p<0.001, 6 between-group comparisons, n=505), but not in individuals with at-risk mental states. These distinctive alterations of the hypothalamic-pituitary-adrenal axis function may have important implications for a role of CAR as a marker for transition risk. However, the lack of objective verification of sampling adherence in these studies may limit the interpretation of the results.

2.1 Introduction

Neuroendocrine abnormalities are a commonly observed feature in patients with schizophrenia and are thought to interact with the multifactorial etiology of the disorder. One of the most consistently reported neuroendocrine findings in schizophrenia is elevation of plasma and saliva cortisol levels [1, 2], an important observation as cortisol is both a marker and mediator of the body's multisystem response to stress [3]. Cortisol is the hormonal endpoint of the HPA axis and a powerful glucocorticoid with multiple effects on brain and body. As such, cortisol interacts with multiple target tissues including immune cells, metabolic organs and brain areas with high density of glucocorticoid receptors (GR), including the prefrontal cortex and limbic circuitry [4, 5]. Consequently, exposure to excessive cortisol can contribute to neurodevelopmental insults and in the context of contemporary frameworks of psychosis onset it is thought to be relevant for onset and progression of psychosis [6]. However, the role of cortisol in schizophrenia pathophysiology from early risk states to chronic schizophrenia remains mostly unclear.

Aberrations in stress processing in patients with schizophrenia that potentially lead to increased cortisol release and, over time, to dysregulations of the HPA axis are thought to explain the hypercortisolemia found in cross-sectional studies [7]. Lower stress threshold in response to a stressful stimulus and prolonged activation of the limbic system have been observed in patients [8]. The neuroendocrine response to laboratory stress on the other hand is blunted in patients with schizophrenia [9] and dexamethasone suppression is reduced, indicating impaired negative feedback mechanisms [10]. Moreover, brain areas with inhibitory function on the HPA-axis such as the hippocampus are commonly found to be reduced in volume. Furthermore, increased volume of the pituitary gland has been observed in patients with schizophrenia [11-13].

Studying proxy markers for HPA-axis dysregulation at different stages of psychosis may help to elucidate the role of cortisol in schizophrenia. The rapid increase in cortisol levels within the first 30-45 minutes after waking, termed cortisol awakening response (CAR), has received increasing attention in the last decade [14-24]. The CAR appears to be a distinct feature of the diurnal cortisol rhythm that is not necessarily related to overall diurnal cortisol levels [25] or acute stress reactivity [26] and represents a standardised variable that is synchronised with awakening. Importantly, several epidemiological risk factors for schizophrenia appear to be associated with alterations

in stress processing generally and with a blunted CAR specifically. Exposure to childhood adversity was associated with flattening of the CAR in two large cohorts [27, 28], an effect that possibly takes place through epigenetic mechanisms [29]. Additionally, urban upbringing, one of the strongest environmental risk factors for schizophrenia [30, 31], is associated with a similarly blunted CAR [32]. Indeed, urban upbringing has previously been shown to be associated with alterations in social stress processing in the perigenual anterior cingulate cortex [33], a region also involved in the regulation of the CAR [34]. Studies on the association of CAR with chronic perceived stress have reported mixed results but meta-analysis shows that general life stress is associated with a stronger CAR [35].

Focusing on CAR eliminates some of the considerable heterogeneity in previous studies on single cortisol samples, as measuring CAR is inherently a standardized method that allows little variation. Additionally, expert consensus guidelines for the assessment of CAR have been published recently and facilitate the critical interpretation of existing studies [36]. Several studies have reported on CAR in patients along the psychosis spectrum over the last few years with heterogeneous results. As the number of studies focusing on individuals at early stages of schizophrenia and in at-risk states increases, this provides an opportunity to compare cortisol alterations at these different stages. In doing this, we can address the question of whether HPA axis alterations represent vulnerability or rather a consequence of mental disorders [37]. Here, we report on a systematic review of the literature and a meta-analysis of CAR in patients along the psychosis continuum. We describe the state of HPA-axis dysregulation in patients with chronic SCZ, patients with FEP and individuals with ARMS, a risk state for psychosis characterized by sub-threshold psychotic symptoms and/or genetic susceptibility, and discuss the implications of these findings on future research.

2.2 Methods

2.2.1 Study Selection

Original research investigating CAR in patients with SCZ, FEP and ARMS was systematically identified by two authors (MB, ZS) by searching the databases MEDLINE, Scopus, PsycINFO and Web of Science in July 2015. The search strategy included the search terms “schizophrenia” OR “psychosis” OR “psychotic disorder” OR “clinical high risk” OR “CHR” OR “ultra high risk” OR “UHR” OR “first episode” OR “prodromal” AND “morning cortisol” OR “cortisol awakening response” OR “daily

cortisol" OR "diurnal cortisol" OR "circadian cortisol". Hand search of relevant journals and reference lists of included articles was performed in addition to the systematic literature search.

2.2.2 Inclusion and Exclusion Criteria

Included studies reported CAR or used a sampling procedure that allowed computation of CAR from patients with SCZ, FEP, or ARMS, and a healthy control group without psychiatric diagnosis. Eligible articles defined SCZ according to DSM or ICD criteria. Patients with FEP had to be defined as presenting with psychotic illness for the first time. Eligible studies with ARMS individuals included participants of any of the three high-risk groups defined by the ARMS criteria [38, 39]: (1) BLIPS, defined as transient psychotic symptoms with spontaneous remission within one week, (2) attenuated positive symptoms, defined as sub-threshold psychotic symptoms present for more than one week in the past twelve months, and (3) individuals with genetic susceptibility paired with a >30% decline in global functioning. We included cross sectional studies and longitudinal studies if they provided baseline data on CAR. Eligible articles collected samples upon awakening and 30-45 minutes later, as this is the time frame where the highest cortisol concentration is expected to occur [25, 40, 41]. We included peer reviewed full text articles published in English, German, French, Spanish or Hungarian, languages spoken by one or more of the authors. Date of publication was not limited. Exclusion criteria were: (1) Studies without a control group, (2) studies that either did not report CAR or used a sampling schedule that does not allow computation of CAR (e.g. fixed times or single sample), (3) studies that analysed cortisol from specimen that does not allow detection of short term cortisol fluctuations (i.e. urine, hair), (4) studies that reported cortisol measures only after an intervention and (5) studies that did not satisfy minimum quality requirements. Furthermore, we included studies reporting on individuals under the age of 15 only if they had a control group matched for age, as data on changes in diurnal cortisol rhythms with age in the pubertal period is inconsistent but suggests ongoing maturation of the HPA axis until early adulthood [25, 42]. All abstracts identified through the primary search and hand search were screened for relevance in duplicates by four authors (MB, AK, DR, PM) and selected full text articles were screened for inclusion based on the above criteria in duplicates by two authors (MB and AK). (Figure 2.1)

2.2.3 Inclusion of Primary Studies with Subgroup Analyses

Four studies included in this meta-analysis reported data for two subgroups separately [15, 18, 19, 21]. In all four studies, both patient subgroups were eligible for inclusion as patients were included irrespectively of the *a posteriori* subgroup status; hence we included both subgroups pooled together in the quantitative analysis as recommended by the Cochrane Handbook for Systematic Reviews and Meta-Analyses [43]. These were: FEP patients who responded to first-line treatment and non-responders [19], ARMS individuals who developed FEP during follow-up and ARMS individuals who did not transition [18], children with clinical high risk (CHR) and children with family history (FHx) [15], and patients with SCZ with and without cannabis exposure [21]. In order to include these studies, the combined means were computed by weighting the means of both groups.

$$\bar{X}_1 = \frac{n_1\bar{X}_1 + n_2\bar{X}_2}{n_1 + n_2}$$

And the combined standard deviation was computed as

$$S = \sqrt{\frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2 + \frac{n_1 n_2}{n_1 + n_2}(\bar{X}_1 - \bar{X}_2)^2}{n_1 + n_2 - 1}}$$

Where $\bar{X}_1, \bar{X}_2$ are the subgroup means, n_1, $n_2$are the subgroup sample sizes and $S_1, S_2$the subgroup standard deviations.

2.2.4 Correction of Primary Study Sample Overlap

We adjusted the weighting of the study according to the overlap in samples for those studies that reported sample overlap with other studies included in the meta-analysis. We adopted a procedure previously described [44] to avoid bias from excluding studies with overlapping samples. Briefly, if samples between two studies had 100% overlap, each study was weighted only 50%. Consequently, every subject was included only once into the meta-analysis.

2.2.5 Data Extraction

Data were extracted independently by two authors (MB and AK). We extracted (1) means and standard deviations of CAR, defined as the area under the curve with respect to increase (AUCi) between wake-up and peak post wake-up cortisol levels or, where available, CAR, wake-up cortisol and +30 min post wake-up cortisol, (2) clinical status (illness stage, medication status and comorbid ICD and DSM diagnoses), (3) number of individuals in each group and (4) socio-demographic information (including age, gender and socio-economic status). We contacted the authors of those studies

where a suitable data collection schedule was reported but CAR could not be calculated from published data to request additional data.

2.2.6 Assessment of Study Quality

The quality of the included studies was assessed by two authors (MB and AK) using the Newcastle Ottawa Scale (NOS) for non-randomized studies [45] as recommended by the Cochrane Collaboration [43]. Additionally, the methodological quality if the included studies was assessed based on the recently published expert consensus guidelines for the assessment of CAR [36]. As quality assessment in meta-analysis of observational studies remains controversial and clear consensus is lacking, we also used sensitivity analysis to determine the effects of lower quality studies on the overall model [46].

2.2.7 Data Analysis

We used the software Open Meta-Analyst for the quantitative analysis (Tufts, Boston, MA) [47]. Standardised mean difference estimates of differences in CAR between patients and healthy controls were calculated using Hedge's *g*, a measure of effect size that is suited for small sample sizes [48]. Confidence intervals (CI) were also computed. Heterogeneity between studies was assessed using Higgins' I^2 statistic [49]. A p-value of 0.05 was considered significant. Separate subset meta-analyses were performed for ARMS vs. controls, FEP vs. controls and SCZ vs. controls. We expected systematic differences between studies based on the assumption that studies would differ in patient characteristics and therefore chose a random effects model [50]. Finally, leave-one-out meta-analysis was performed to assess the contribution of each study to the overall model.

2.3 Results

2.3.1 Search Results

The initial database search identified 326 records after removal of duplicates (Figure 2.1). Of these, 315 had an abstract and were screened for inclusion. We excluded 248 studies during this step on the basis of their title and abstract and for the remaining 67 and one additional abstract identified through hand search, full-text versions were retrieved and again screened for inclusion. Full texts could not be obtained for 18 abstracts, nine articles did not focus on patients with psychosis, three articles had no control group and 26 used a sampling schedule that was not suitable for calculation of

the CAR. Finally, 11 studies were included into the qualitative and quantitative analysis.

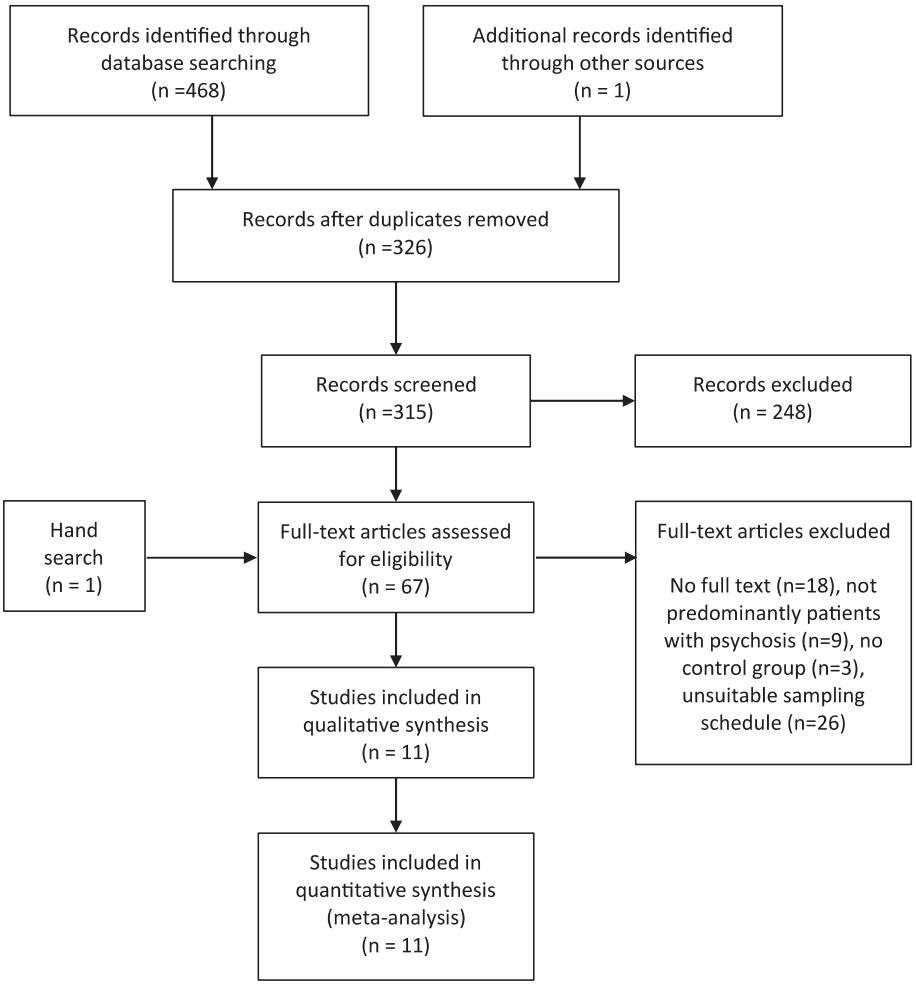

Figure 2.1 Flow diagram of literature search and study inclusion

2.3.2 Study Characteristics

Of the 11 full text articles identified through our literature search and screening procedure, two studied patients with SCZ, six studied patients with FEP and three studied ARMS individuals, all defined as CHR with one study also having a small subgroup with FHx. Consequently, our initial aim to include studies with ARMS more

generally was narrowed down to CHR criteria. ARMS criteria were assessed with the CAARMS [16, 18] and a community screening instrument for psychotic-like experiences [15].

Table 2.1 Characteristics of included studies

Study	Patient characteristics	Cases : controls (n)[a]	Cases : controls (f)	Cases : controls (age)	Study quality
Cullen et al. 2014	AMRS	33 : 40	10 : 23	12.8 ± 0.2 : 13.1 ± 0.2	NOS 8.0; CAR assessed on 2 days; 0, +15, +30, +60 min; no OVSA
Day et al. 2014	ARMS	43 : 38	20 : 15	22.9 ± 0.6 : 24.3 ± 0.7	NOS 6.0; CAR assessed on 1 day; 0, +30, +60 min; no OVSA , negative CAR excluded
Labad et al. 2015	ARMS	29 : 44	7 : 15	22.3 ± 4.6 : 23.2 ± 4.4	NOS 7.0; CAR assessed on 1 day; 0, +30, +60 min; no OVSA
Pruessner et al. 2008	FEP	27 : 40	11 : 20	22.0 ± 3.2 : 22.3 ± 2.6* 24.0 ± 4.0 : 23.1 ± 2.4**	NOS 6.0; 0, +30, +60 min; no OVSA
Mondelli et al. 2010	FEP	50 : 36	18 :10	29.2 ± 1.1 : 27.3 ± 0.8	NOS 6.0; CAR assessed on 2 days[b]; 0, +15, +30, +60 min; no OVSA
Aas et al 2011	FEP	30 : 26	10 : 8	30.1 ± 7.2 : 27.5 ± 4.8	NOS 7.0; CAR assessed on 2 days[b]; 0, +15, +30, +60 min; no OVSA
Pruessner et al. 2013	FEP	56 : 30	19 : 17	23.5 ± 3.6 : 22.7 ± 4.5	NOS 7.0; 0, +30, +60 min; no OVSA
Pruessner et al. 2015	FEP	58 : 27	11 : 12	23.9 ± 3.7 : 22.3 ± 3.6	NOS 7.0; 0, +30, +60 min; no OVSA
Mondelli et al. 2015	FEP	30 : 57	12 : 21	29.2 ± 1.4 : 26.8 ± 0.6	NOS 6.0; CAR assessed on 1 day; 0, +15, +30, +60 min; no OVSA
Hempel et al 2010	SCZ	26 : 45	7 : 12	22.0 ± 3.4 : 23.1 ± 5.3	NOS 5.0; CAR assessed on 1 day; 0, +30 min; no OVSA
Moteleone et al. 2014	SCZ	12 : 15	6 : 3	41.0 ± 7.3 : 37.6 ± 6.9	NOS 5.0; CAR assessed on 2 days[b]; 0, +15, +30, +60 min; no OVSA

[a] Unadjusted sample size; sample size was adjusted in the random-effects meta-analysis to account for sample overlap between studies. [b] CAR was assessed on two days in a subset of the sample * female participants, ** male participants; ± represents standard deviation; ARMS = at-risk mental state, FEP = first episode psychosis, SCZ = schizophrenia, NOS = Newcastle Ottawa Scale; OVSA = objective verification of sample adherence (e.g. electronic monitoring systems or time-stamped photographs of collected samples as recommended by Stalder et al., 2016).

2.3.3 Study Quality

The mean NOS score was 6.4 (range 5-8). All studies satisfied the minimum quality requirements defined *a priori* as satisfying at least one criterion in each category of the NOS, having a control group and assessing cortisol at awakening and 30 minutes thereafter. Ten studies used community controls whereas one study [21] recruited controls from hospital staff. Eight studies collected saliva samples on one day only while three studies collected them on two days. Seven studies provided details about samples that were not returned, and overall there was no significant difference between patients (315/351 = 89.74%) and controls (242/285 = 84.91%) (p=0.6359, χ^2). However, none of the included studies used objective verification of sampling adherence (e.g. electronic monitoring systems or time-stamped photographs of collected samples) as recommended by the recent consensus report on CAR [36].

2.3.4 Overall Model

Overall, random-effects between-group meta-analysis demonstrated a lower CAR in patients compared to healthy controls (g=-0.426, 95% CI -0.585 to -0.267, p<0.001, 11 between-group comparisons, n=879). Heterogeneity between studies was not significant (I^2=24%, p=0.213), indicating that differences between the 11 studies included in the overall model were not explained by any factor other than group status (Figure 2.2).

2.3.5 Subgroup Analysis

We performed sub group meta-analyses for patients with SCZ, FEP and ARMS to assess if blunting of the CAR was present at earlier stages of the psychosis continuum. This subgroup-analysis revealed similar effects in patients SCZ (g=-0.556, 95% CI -1.069 to -0.044, p<0.05, 2 between-group comparisons, n=114) and FEP (g=-0.544, 95% CI -0.731 to -0.358, p<0.001, 6 between-group comparisons, n=505), but no significant effect was found in ARMS individuals (g=-0.170, 95% CI -0.435 to -0.095, p=0.207, 3 between-group comparisons, n=259) (Figure 2.2).

Our meta-analysis included two longitudinal studies that studied the predictive value of CAR for clinical outcomes. One study found that ARMS individuals who transitioned to psychosis within one year had a significantly higher CAR at baseline after controlling for several covariates including awakening time and BMI [18]; however, the authors

mention that this is based on a small sub-sample (n=10) and that 38% of participants did not return their saliva samples The other study grouped FEP patients based on their treatment response to antipsychotic medication over 12 weeks and found that those who did not respond with reduction in symptoms had a significantly more blunted CAR at baseline compared to responders and healthy controls [19].

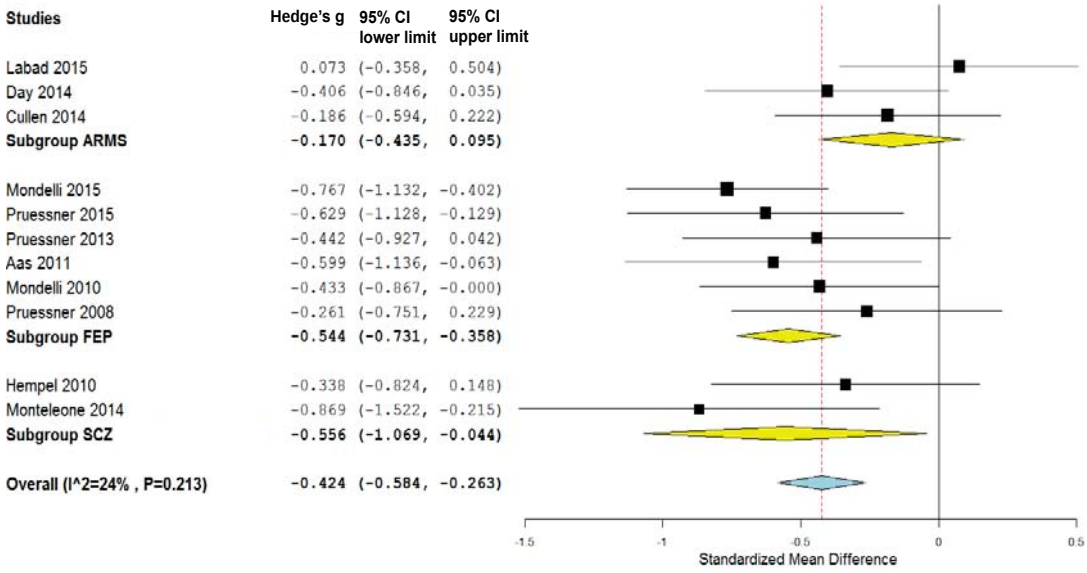

Figure 2.2 Forest plot for random effects between-group meta-analysis comparing cortisol awakening response of individuals at high risk for psychosis (ARMS), patients with first episode psychosis (FEP) and patients with schizophrenia (SCZ) to controls.

2.3.6 Meta-regression

We performed a meta-regression of medication status in the three groups to examine if medication status had an effect on CAR. For this, we collected data on how many study participants were receiving antipsychotic medication when the CAR was assessed and included the percentage of medicated patients as a covariate. Antipsychotic medication has previously been shown to affect cortisol levels [20, 51]. The random-effects meta-regression model found a small but significant influence of antipsychotic medication on CAR when all three groups were included (R^2=-0.004, 95%CI -.008 to -.001, p<0.05) but this effect became non-significant when the analysis was limited to FEP and SCZ studies (R^2=-0.006, 95%CI -0.036 to 0.025, p=0.712) (Figure 2.3A).

Across all studies, meta-regression of sample size revealed no significant effect of sample size on CAR (R^2=-0.006, 95%CI -0.016 to 0.004, p=0.236) (Figure 3.3B). For publication date, no effect was found when all studies were included (R^2=-0.014, 95%CI -0.081 to 0.053, p=0.679) (Figure 2.3C); however, a modest but non-significant

effect was found in FEP and SCZ samples (R^2=-0.065, 95%CI -0.131 to 0.002, *p*=0.057), indicating greater effects in more recent publications.

Meta regression for age demonstrated a significant effect of age on CAR when all studies were included such that older samples had a more blunted CAR (R^2=-0.030, 95%CI -0.052 to -0.007, *p*<0.05) (Figure 2.3D), but this effect became non-significant when the analysis was restricted to FEP and SCZ samples (R^2=-0.024, 95%CI -0.061 to -0.012, *p*=0.191).

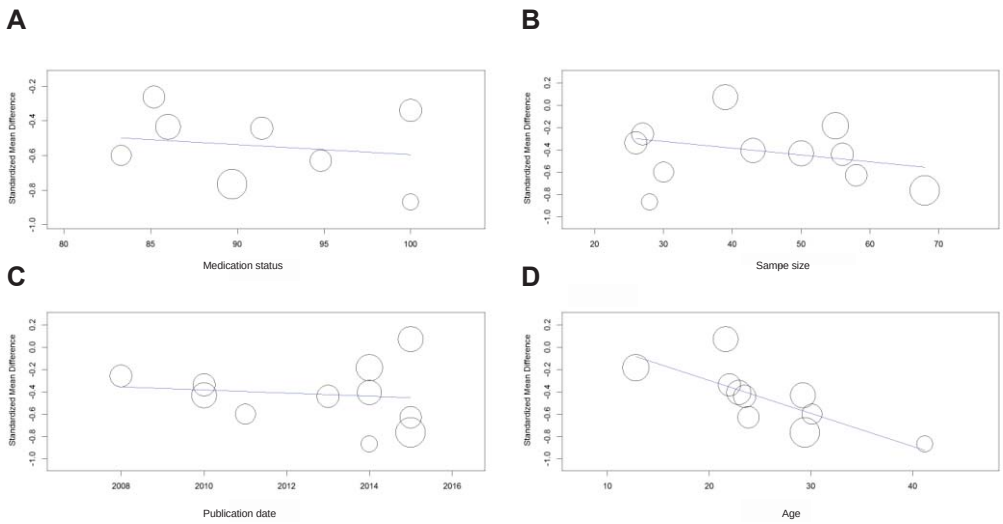

Figure 2.3 Meta-regression on the effects of medication status (A), sample size (B), publication date (C) and age (D) on CAR in patients with FEP and SCZ (A) and patients with ARMS, FEP and SCZ (B,C,D). Circle size reflects the weight of studies in the random-effects meta-regression.

2.3.7 Sensitivity analysis

No study affected the overall estimate by more than 12%. Included studies had sample sizes ranging from 26 to 68, and consequently relative weights differed only moderately between studies. Removing the study with the lowest quality score decreased the estimate by only 6% (Fig 2.4).

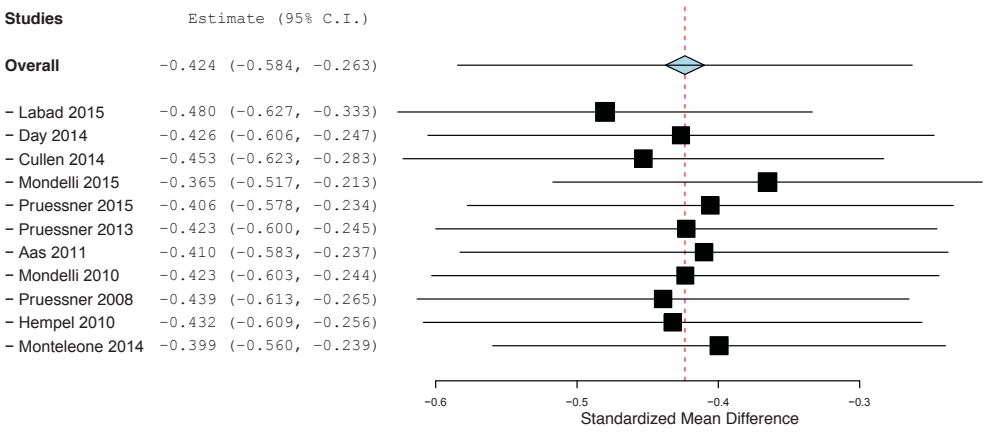

Figure 2.4 Leave-one-out meta-analysis.

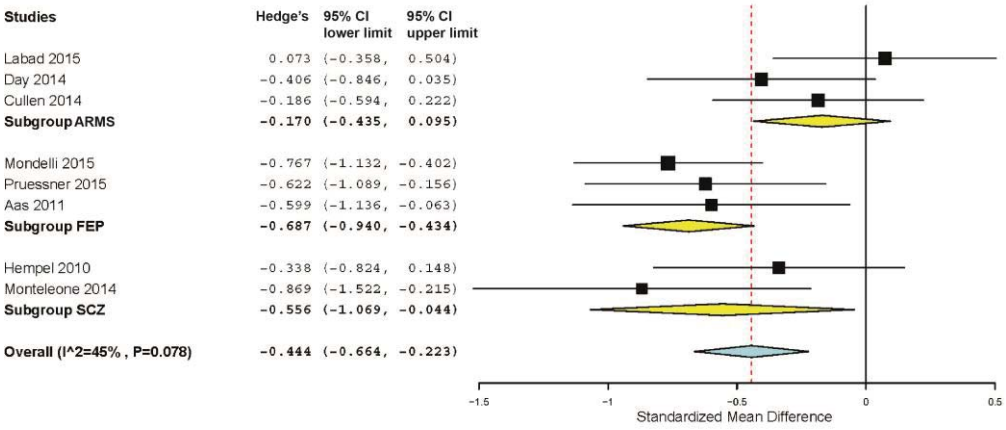

Figure S2.1 Random effects between-group meta-analysis comparing cortisol awakening response of individuals at high risk for psychosis (ARMS), patients with first episode psychosis (FEP) and patients with schizophrenia (SCZ) to controls. Studies that had overlapping samples with another study included in the quantitative analysis were excluded in this model.

2.4 Discussion

Our meta-analysis found that patients along the psychosis-continuum have a blunted CAR compared to healthy controls. Subgroup analysis revealed that this difference is only present in patients with FEP and SCZ, but not in individuals at high risk for psychosis, who did not show a blunted CAR compared to controls. This effect remained significant in a secondary analysis with studies with overlapping samples excluded (S2.1).

Previous meta-analysis of cortisol levels in patients with SCZ showed elevated morning cortisol compared to controls [1] and altered HPA-axis response to psychosocial stress that was characterized by lower anticipation and peak cortisol levels [9]. Together with our analysis of existing studies on CAR, this represents strong evidence for dysregulated HPA-axis activity in patients with SCZ, characterised by a flat diurnal cortisol curve with overall high cortisol levels, and alterations in HPA-axis reactivity to laboratory-induced psychosocial stress.

The increasing number of studies in at-risk individuals and medication-naive FEP patients provides important insight into HPA-axis activity in samples that are not affected by the potentially confounding effects of antipsychotic medication and long illness duration. Our analysis of existing studies in these groups showed that CAR is blunted in FEP patients to a similar extent as in patients with SCZ, but is normal overall in ARMS individuals, with one study finding a blunted CAR in medication free ARMS individuals [16]. Two explanations seem plausible to reconcile this finding: Firstly, changes in HPA axis function may occur only after the onset of a full psychotic episode. Following this logic, HPA-axis dysregulation may be regarded as an epiphenomenon of psychosis, or may be attributable to the stress of experiencing psychosis. While the data synthesized here may support this view, another recent meta-analysis on morning cortisol in high risk individuals found significant elevations of cortisol in ARMS individuals [52]. The other possible explanation is that within ARMS samples, only those who eventually transition to psychosis (or any other mental disorder) have a blunted CAR before a diagnosis is made. Generally, one third of ARMS individuals will go on to develop psychosis within 3 years [53], but the remaining two thirds are also at risk for developing other mental disorders including anxiety and mood disorders [54]. This within-group heterogeneity would be masked in case-control designs and the question of whether some particularly vulnerable individuals present with a blunted CAR can only be clarified with longitudinal studies or retrospective subgroup analysis. One study in our meta-analysis found significantly higher CAR in ARMS individuals who transitioned to psychosis (ARMS-P) compared to those who did not (ARMS-NP) and compared to healthy controls after adjusting for awakening time [18]. This suggests differential HPA-axis activity between ARMS-P and ARMS-NP, but seems difficult to reconcile with the finding of lower rather than higher CAR in patients with psychosis in the present meta-analysis. However, previous longitudinal research has shown that ARMS-P are characterized by higher cortisol than ARMS-NP in a large sample [55], and in the same cohort cortisol was found to have significant predictive value for transition from prodromal states to FEP [56]. A recent meta-analysis, however,

did not confirm this finding [52]. Moreover, there is evidence that pituitary volume, a proxy measure of HPA axis function that underlies dynamic changes, is increased in ARMS-P, adding to the line of evidence demonstrating that HPA axis alterations are present before the onset of psychosis [57]. Consistent with this, another study found larger hippocampal volume in at-risk individuals who transitioned to psychosis [58]. Taken together, these findings provide strong evidence for a role of stress hormones in psychosis and discourage the view that cortisol aberrations are merely caused by the stress of experiencing a mental disorder.

Meta-regression of medication status, age, publication data and sample size found that age and medication status were associated with flatter CAR across all studies. Studies with older subjects in this meta-analysis tended to include chronic patients rather than high-risk individuals, and subjects with longer illness duration. However, CAR may also generally decrease with age. Previous studies have shown that the HPA-axis does indeed change with puberty during adolescence and children generally have different diurnal cortisol rhythms compared to adults [41], but less is known about effects of age on the CAR [25, 35] and age differences within adult samples. Illness duration (years lived with psychosis) may also moderate this relationship as older samples tended to have progressed further compared to younger samples. The latter explanation appears most plausible, as the effect of age became non-significant when the analysis was restricted to FEP and SCZ patients. We also examined effects of medication status on CAR as antipsychotic medication was shown to be associated with lower morning cortisol in patients with SCZ [1]. Meta-regression showed that medicated patients had flatter CAR across all studies, but this effect disappeared when only FEP and SCZ studies were taken into account, where significant between-group differences were observed in the meta-analysis. While we cannot exclude the possibility that antipsychotic medication has a causal effect in flattening CAR, this seems unlikely given that one longitudinal study found no changes in CAR in response to antipsychotic treatment over 12 weeks in medication naïve FEP patients [19].

Evidence suggesting a mechanistic link between blunted CAR and schizophrenia pathophysiology comes from previous human studies showing that individuals with blunted CAR have reduced grey matter volume and increased stress-related activity in brain areas involved in the regulation of the HPA-axis, including the pACC [34]. HPA axis dysregulation is furthermore associated with disruption of the functional connectivity between subACC and hypothalamus in patients with psychotic depression [59]. Chronic dysregulation of the HPA axis and excessive cortisol signaling affect brain regions with

high GR density through genomic and non-genomic mechanisms, thereby contributing to cognitive deficits [60] and possibly to progressive decline in cognitive functioning in patients with psychosis. Over time, chronic exposure to heightened levels of glucocorticoids as a consequence of a dysregulated HPA axis can alter cortical and limbic function through changes in gene expression, neural plasticity and neuroendocrine function [61, 62]. More severe symptoms are associated with worse cognitive functions in patients with a flat CAR [14, 63]. Furthermore, neuroimaging data show that changes in dopamine signaling induced by a standardized stress test correspond with cortisol response [64]. Antipsychotic medication in turn decreases cortisol levels in patients with SCZ [65]. Moreover, one study showed similar increases in the dopamine metabolite homovanillic acid and cortisol in response to metabolic stress induced by administration of the glycolysis inhibitor 2-deoxy-D-glucose in patients with SCZ [66]. Collectively, these results suggest that CAR may play a functional role in linking stress and cognitive processes.

Finally, bi-directional interactions between HPA-axis and the immune system may contribute to mutually sustained neuroendocrine dysregulations and chronic low-grade inflammation (see [67] for a review). Chronic low-grade inflammation is common in patients with SCZ and is already present in ARMS [68] and such pro-inflammatory states are thought to be a risk factor for the development of psychosis [69], although more recent studies show that only a subgroup of patients are characterized by elevations in peripheral cytokines and central microglial activation [70, 71]. It is well known that glucocorticoids can trigger inflammatory processes in the CNS including stress-dependent sensitization of microglia [72, 73], and that exposure to high levels of glucocorticoids can exacerbate the immune system's response to lipopolysaccharide (LPS) [74]. Importantly, healthy individuals with flat CAR indeed have higher levels of IL6 [75] and glucocorticoids can furthermore increase the production of the IL6 receptor in human epithelial cells [76]. Similarly, pro-inflammatory cytokines directly interact with GR signaling on different levels, including transcription of the GR and translocation of the GR into the nucleus [77, 78]. Finally, administration of interferon alpha (INF-α) for 12 weeks led to flattening of the diurnal cortisol curve, directly indicating that cytokines alter HPA-axis function [79]. Understanding such interactions in clinical samples could help to differentiate between patients with certain risk profiles and clarify further how neuroendocrine and immune systems work together in psychosis.

One of the strengths of this meta-analysis is that we were able to compare ARMS, FEP and SCZ, thus providing insight into changes in HPA axis function across

diagnostic categories. While longitudinal studies are clearly needed, this allows some analysis of putative changes in HPA axis function during different stages of psychotic illness. Furthermore, sensitivity analysis showed that no single study disproportionally affected the overall effect.

However, some limitations need to be acknowledged when interpreting these results. Firstly, our subgroup analyses for ARMS and SCZ included only a small number of studies and a relatively small total sample. There is currently limited knowledge on CAR in ARMS and in chronic patients with treatment resistance. Secondly, some of the studies also included subjects with psychiatric comorbidities, or a diagnosis of bipolar I or schizoaffective disorder. Thirdly, while the included studies were consistent in their methodology, none of them employed objective verification of saliva sampling. A recent consensus paper has highlighted objective sampling verification as a crucial step in ensuring validity of CAR data, as even small delays in collection of saliva samples can result in inaccurate CAR estimates [36]. If one group in case-control designs is less adherent to the sampling schedule than the other, this could consequently introduce systematic bias. As none of the included studies objectively verified adherence to saliva sampling, we tested the percentage of participants who were initially recruited but had missing cortisol data in the included studies and found that this rate was not significantly different between patients and controls (89.74% vs. 84.91% respectively, p=0.636), suggesting that return rates were not lower among patients or controls across the included studies. However, in the absence of objective measures to verify adherence to sampling schedules, the potential for bias remains and the results of this meta-analysis need to be interpreted with caution. Future studies should use these quality control measures. Finally, we were not able to examine associations of several covariates of interest with CAR, e.g. symptom severity, BMI or cognitive function as only few of the included studies assessed these covariates and different scales are used to assess symptom scores in ARMS individuals, FEP and SCZ patients. Adjusting the results for BMI would be important to rule out any confounding effect of body weight on HPA-axis function, and associations with symptom and functioning scores could provide important insights into potential mechanisms that link HPA-axis dysregulation to clinical outcomes.

In conclusion, the present meta-analysis found flattened CAR in patients with SCZ and FEP, but not in ARMS. Future studies on cortisol alterations in patients with psychosis should use longitudinal designs to further determine the potential use of CAR as a biomarker with predictive value. Additionally, focusing on functional outcomes of

interest in the context of HPA-activity, and studying other markers such as inflammatory and metabolic markers together with neuroendocrine systems may help to elucidate their interactions. Ultimately, these findings may have clinical implications as the limited evidence today suggests that cortisol dysregulations can implicate treatment resistance and higher risk for transition from ARMS to FEP.

Conflict of Interest

The authors report no biomedical financial interests or potential conflicts of interest.

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3 Allostatic load is associated with psychotic symptoms and decreases with antipsychotic treatment in patients with schizophrenia and first-episode psychosis

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Preface

As we have seen in Chapter 2, there is evidence to suggest that the production of cortisol follows a different pattern in patients with SCZ and FEP compared to healthy controls, and some evidence to suggest a possible link to transition to psychosis and treatment response. Importantly, cortisol is not only a marker of the biological stress response but also a powerful glucocorticoid with downstream effects on multiple tissues including immune cells, neurons and metabolism.

The AL concept has received increasing attention recently as it relates to complex interactions between glucocorticoids, the immune system and the brain. Despite growing interest in the role of AL in psychiatric disorders, few studies to date have measured AL in psychiatric populations. Consequently, there is a strong need to identify whether patients with psychotic disorders have elevated AL, which is hypothesised to indicate risk for adverse physical health outcomes, and to test if high AL is indicative of adverse psychiatric outcomes. The primary aim of Chapter 3 was to quantify AL in patients with SCZ and FEP and to examine associations with symptom domains, treatment response and remission. We reasoned that AL, if elevated in patients relative to controls, would be associated with illness severity and poor outcomes. The study reported in Chapter 3 was conducted in collaboration with the Department of Psychiatry at the University of Magdeburg, Germany, and is published in *Psychoneuroendocrinology*.

Abstract

Current pathophysiological models of schizophrenia suggest that stress contributes to the etiology and trajectory of the disorder. We investigated if AL, an integrative index of neuroendocrine, immune and metabolic dysregulation in response to chronic stress, is elevated in patients with SCZ and FEP and related to psychotic symptoms and social and occupational functioning. Additionally, we assessed the temporal dynamics of AL in response to treatment with second-generation antipsychotics. AL, psychotic symptoms and psychosocial functioning were assessed in a longitudinal design in patients with SCZ (n=28), FEP (n=28), and healthy controls (n=53) at baseline and 6 and 12 weeks after commencement of antipsychotic therapy. AL at baseline was higher in patients with SCZ and FEP relative to controls, but not different between patients with SCZ and FEP. Adjusting for age and smoking, we found that positive symptoms were positively correlated with AL and psychosocial functioning was negatively correlated with AL at trend level. Linear mixed model analysis demonstrated that AL decreased after treatment was commenced in patients with SCZ and FEP between the baseline assessment and the 6 and 12-week follow-up. AL was not predictive of treatment response or symptomatic remission. Our data provide evidence for cumulative physiological dysregulation in patients with SCZ and FEP that is linked to the experience of current positive psychotic symptoms. AL could be a useful tool to monitor biological signatures related to chronic stress and unhealthy behaviors in schizophrenia.

3.1 Introduction

A large body of studies supports the notion that chronic experiences of chronic stress and malfunctioning of the biological stress response are associated with the onset and progression of psychotic disorders [1, 2]. This is evidenced by findings of dysregulations of the stress hormone cortisol in patients with psychotic disorders [3, 4]. Observations that stressful life events can trigger and/or worsen psychotic symptoms lend support to a causative role of stress processes [5]. The stressful experience of psychosis itself in turn can activate the body's stress systems, thus contributing further to physiological dysregulation. Recently, these observations have reshaped pathophysiological theories of the disorder and have contributed to contemporary reconceptualizations of psychosis [6, 7].

The AL model is a framework aimed at understanding stress pathophysiology by encompassing a range of physiological systems [8]. AL is measured by indexing neuroendocrine, immune, metabolic and cardiovascular dysregulation. AL algorithms characterize subtle pathogenic deviations of peripheral biomarkers involved in chronic adaptation to situational demands [9, 10]. Unlike homeostasis, which describes precise regulation of physiological parameters within a narrow range, allostasis refers to dynamic adaptation in response to external stimuli such as stressful life events. Central to these processes is the brain that mediates biobehavioral adaptations [10]. Within the AL framework, environmental stressors induce the release of primary mediators such as cortisol that over time lead to primary effects including elevations of pro-inflammatory cytokines, oxidative stress or mitochondrial dysfunction [11, 12]. These then contribute to damaging effects to the CNS and other organs, including altered gene expression, telomere shortening and ultimately affect blood pressure, lipid metabolism, cognition and neurodegeneration [11, 12]. Thus, chronic or repeated exposure to stress is believed to cause elevated AL in the long term and elevated AL in turn has been shown to predict adverse health indicators. For example, longitudinal studies demonstrate that high AL is associated with all-cause mortality and cognitive decline [13] and reductions in AL are conversely associated with reductions in mortality [14].

Elevated levels of AL primary mediators and primary effects are frequently observed in patients with psychotic disorders [3, 15-19]. This suggests that AL may be elevated in patients with SCZ. In accordance, a recent study found increased AL in a small sample of patients with chronic SCZ that was related to positive symptoms and impaired

functional capacity [20]. AL may contribute to the excess mortality observed in patients with SCZ [21] and possibly to the pathophysiology of the disorder, given that AL entails heightened levels of pro-inflammatory cytokines, glucocorticoids and oxidative and metabolic stress [10, 11].

Collectively, pathogenic AL processes are known to have damaging effects on the brain, including regions that are important to the etiopathogenesis of psychotic disorders, such as the hippocampus and the prefrontal cortex [10, 22-25]. Psychotic disorders on the other hand may contribute to heightened allostatic load through stress-mediated release of glucocorticoids and sympathetic activation, but also through unhealthy lifestyle factors associated with psychotic disorders such as smoking and through the metabolic side effects of antipsychotic medication. While it is unknown if AL is a risk for or the consequence of psychosis, AL and psychopathology may mutually sustain each other in a bi-directional fashion through the above mechanisms.

Given its associations with adverse health outcomes, the AL model may be helpful in detecting multisystem dysfunction of and risk for physiological comorbidities in severe mental illness that commonly accompany psychotic disorders. Beyond associations of AL with adverse physical health outcomes, the multisystem dysregulation encompassed by the AL framework might also contribute to the progression of severe mental illnesses [26, 27]. This is relevant, as biomarkers are urgently needed for current attempts at staging in psychiatry [28]. This concept proposes that it is possible to differentiate illness stages based on clinical characteristics and biological phenotypes. Although no study to date has directly addressed the question of whether AL is related to the staging and neuroprogression frameworks, one study in a small sample of patients with bipolar disorder used a ‘systemic toxicity index’ that was conceptually similar to AL and observed higher systemic toxicity in patients with acute mania or dysthymia but not in euthymic patients [29].

The aims of the present study were twofold. First, we aimed to test whether AL is elevated in unmedicated patients with SCZ relative to healthy controls and predictive of clinically relevant outcomes. Second, we aimed to study dynamic changes in AL in patients with SCZ. We hypothesized that AL would be associated with disease severity in patients with psychosis. Specifically, AL would be higher in patients with SCZ relative to patients with FEP and healthy controls and elevations in AL would be related to the duration of psychosis. We also hypothesized that AL would be

associated with clinical symptoms and psychosocial functioning, predictive of treatment response to second-generation antipsychotic medication and associated with symptomatic remission.

3.2 Methods

3.2.1 Study design

Data for this study came from a blood bank of patients with schizophrenia, first-episode psychosis and healthy controls at the Department of Psychiatry, University of Magdeburg, Germany [30]. The biospecimens used in this study were collected in a naturalistic study of acutely ill in-patients who were un-medicated for at least 6 weeks prior to inclusion into the study. AL and relevant psychometric variables were assessed at baseline and 6 and 12 weeks later. All patients received second-generation antipsychotic medication after the baseline assessment.
Exclusion criteria were substance abuse disorder and/or psychosis induced by another medical condition. Patients with a history of immune diseases, immunomodulatory treatment, cancer, chronic terminal disease, cardiovascular disorders including hypertension, dyslipidemia, diabetes mellitus, substance abuse, severe trauma or clinical/paraclinical findings indicative of these disorders were excluded. Controls were screened for personal or family history of neuropsychiatric disorders. The study was approved by the institutional review board and written informed consent was obtained from all participants. Details concerning the study design have been published previously [30].

3.2.2 Sample

Participants included 28 patients with schizophrenia, 28 patients with first-episode psychosis (26 with a final diagnosis of schizophrenia and 2 with a final diagnosis of schizoaffective disorder; mean duration of untreated psychosis = 7.3 months, standard deviation = 8.9 months) and 53 healthy controls were recruited into the present study (Table 1). All patients were acutely ill in-patients who were un-medicated for at least 6 weeks prior to inclusion into the study, and received one of the second-generation antipsychotics risperidone, olanzapine and quetiapine after inclusion into the study. Patients were recruited from all eligible consecutive admissions to the psychiatric inpatient unit between February 2008 and March 2010 who fulfilled all inclusion criteria and none of the exclusion criteria. Healthy controls were recruited from the community and consisted mainly of blood donors, tertiary students and hospital staff/their family

members. Controls were screened for personal or family history of neuropsychiatric disorders using the Mini-International Neuropsychiatric Interview [31].

Psychometric assessments

Diagnoses were ascertained according to DSM-IV criteria with the Structured Clinical Interview (SCID-I). The PANSS was used to assess psychotic symptoms [32]. The Global Assessment of Functioning (GAF) scale was used to measure social, occupational and psychological functioning [33].

3.2.3 Allostatic load

Biomarkers for the AL index were selected based on (i) representation of several physiological systems including, neuroendocrine, immune, metabolic and cardiovascular parameters, (ii) use in previous AL research [13, 34], and (iii) associations with disease risk. Cardiovascular markers included heart rate, systolic blood pressure, diastolic blood pressure and creatinine kinase. Neuroendocrine markers included cortisol, copeptin, which is derived from a pre-pro-hormone containing arginine-vasopressin and is a surrogate marker for arginine-vasopressin release [35] and the urinary adrenalin and noradrenalin metabolites metanephrine and normetanephrine. Immune markers were IL-6, TNFα, CRP, and e-selectin. Metabolic markers included triglycerides, HDL, LDL, total cholesterol, insulin, fasting glucose, HbA1c, the ligand for the receptor for advanced glycation end products (enrage), waist-to-hip ratio and body mass index.

Fasting blood samples were collected between 8am and 9am. The Human DiscoveryMAP™ multiplex immunoassay platform was used to measure the serum concentrations of cortisol, TNFα, IL-6R, CRP, E-selectin, insulin, enRAGE and CK as described previously [36]. Urinary levels of metanephrine and normetanephrine were measured simultaneously by a commercially available high-performance liquid chromatography (HPLC) method with electrochemical detection (Chromsystems Instruments & Chemicals GmbH, Munich, Germany). An internal standard (3-hydroxy-2-methylbutanoic acid) was used as a comparison to estimate both analyte concentrations. The limits of quantification for metanephrine and normetanephrine were 10 and 30 µg/L, respectively. The ratio of metanephrines to creatinine for each urine sample was calculated to correct for internal dilution. Measurement of creatinine was carried out using standard methods on the Modular platform (Roche Diagnostics, Penzberg, Germany). Copeptin was measured using an ultrasensitive method on a BRAHMS Kryptor compact plus (B.R.A.H.M.S.). The lower limit of sensitivity was 0.9

pmol/L. The concentrations of triglycerides, LDL, HDL and cholesterol were determined by commercial enzymatic methods in a random-access analyzer (Hitachi 911, Roche Diagnostics, Germany). HbA1c was determined in EDTA blood by HPLC (Variant II, Bio-Rad, Munich, Germany). Both intra-assay as well as inter-assay coefficients of variation were <5% for all laboratory assays.

For the computation of the AL index, the 75th percentile (25th percentile for HDL) was determined based on the distribution in the healthy matched control group. We then used a 'scaling' approach previously described to calculate the AL index [37]. Briefly, for each individual, every marker with values above the cut-off (below for HDL) was defined as '1', and the sum of all markers in each systemic category (cardiovascular, immune, neuroendocrine, metabolic) was divided by the number of markers in each category to allow for equal weighting of the four systemic categories. For those markers where the distribution differed between male and female controls, sex-specific cut-offs were calculated and used to compute the AL index as previously done [38]. The rationale for using the 'scaling' approach is included as Supplementary Material.

3.2.4 Statistical analysis

As an alternative to the conservative Kolmogorov–Smirnov test, normality of the data was ascertained by assessing the skewness of data distribution and the associated standard errors. Normality was assumed if skewness divided by the standard error of skewness was between -3 and +3. For the exploration of longitudinal associations of the AL index with psychopathology, we used standardized PANSS scores to compute percent change [39].

To determine if patients with symptomatic remission after 6 and 12 weeks had lower or higher AL at baseline, we adopted the remission criteria by Andreasen, Carpenter [40], defined as a score of 3 or lower in the PANSS items P1 (delusions), P2 (conceptual disorganization), P3 (hallucinatory behavior), N1 (blunted affect), N4 (passive/apathetic social withdrawal), N6 (lack of spontaneity and flow of conversation), G5 (mannerism and posturing) and G9 (unusual thought content). Associations between AL and responses to antipsychotic treatment were examined using the treatment response criteria by Leucht, Davis [41], defined as a 50% reduction in PANSS scores.

Unadjusted and adjusted differences between patients with SCZ, FEP and controls in demographic variables and AL were tested with ANOVA or ANCOVA, and X^2 tests or Fisher's exact test for continuous and categorical variables respectively. Partial correlations adjusting for covariates were used to test associations between AL and psychological variables with adjustment for multiple comparisons using the Bonferroni-Holm method [42]. We estimated the 95% confidence intervals (CI) using a bootstrap estimation approach with 10000 samples. Effect sizes (Cohen's d) were estimated for the comparison of AL between patients with SCZ, FEP and controls at baseline.
We considered age, sex, smoking, cannabis, and illicit drug consumption as covariates because these variables are potential confounders and are common covariates in the AL literature [11]. As AL was not different between participants who consumed cannabis and participants who did not consume cannabis (p=0.881) – or between participants who did or did not consume any other illicit drugs (p=0.427) – we included age, sex and smoking as covariates in subsequent analyses.

Longitudinal associations of AL with psychological variables and differences between the three second-generation antipsychotics used in this study were tested with repeated measures linear mixed model analysis (LMMRM) with random intercept using a Toeplitz covariance structure to account for heterogeneous variances. Binominal logistic regression analysis was used to examine longitudinal associations of AL at baseline (independent variable) and treatment response and symptomatic remission after 12 weeks (dependent variables). Linear regression models were used to test the relationship between AL at baseline (independent variable) and symptom scores after 6 and 12 weeks to delineate associations of baseline AL with clinical symptoms at these follow-up assessments separately. All regression models were fitted as unadjusted models and as models adjusted for baseline PANSS scores, age, sex and smoking, which correlated with the AL index and are common covariates in the AL literature. The Hosmer-Lemeshow test was used to test model calibration. All tests were 2-tailed and a p-value of <0.05 was considered significant. SPSS version 24 was used for all analyses.

3.3 Results

3.3.1 Demographic results

Relative to controls, patients with SCZ and FEP were more likely to smoke and to consume cannabis or other illicit drugs (Table 3.1).

Table 3.1 Demographic and psychometric characteristics.

	Patients		Controls	*p*-value
	Schizophrenia (n=28)	First-episode (n=28)	(n=53)	
Sex female, n (%)	9 (32.1)	13 (46.4)	17 (32.1)	.395[1]
Age at baseline, mean (SD)	40.07 (10.12)	32.96 (11.49)	36.34 (11.49)	.063[2]
Cannabis, yes, n (%)	6 (26.1)	4 (14.3)	1 (1.9)	.003[3]
Smoking, yes, n (%)	19 (67.9)	20 (71.4)	20 (37.7)	.004[1]
Any illicit drug, n (%)	8 (34.8)	5 (19.2)	0 (0)	< .001[3]
Antipsychotic naïve n (%)	0 (0)	24 (85.7)		< .000[1]
PANSS score, baseline	91.30 (17.13)	86.07 (19.18)		.292[2]
PANSS positive, mean (SD)	25.78 (7.00)	22.68 (6.27)		.089[2]
PANSS negative, mean (SD)	20.56 (9.05)	19.04 (8.99)		.535[2]
PANSS general, mean (SD)	44.96 (9.44)	44.36 (10.27)		.821[2]
PANSS score, week 6	58.89 (22.08)	55.14 (16.78)		.477[2]
PANSS positive, mean (SD)	14.82 (6.03)	11.61 (3.97)		.022[2]
PANSS negative, mean (SD)	13.43 (7.48)	15.39 (6.64)		.303[2]
PANSS general, mean (SD)	30.64 (10.78)	28.14 (8.76)		.341[2]
PANSS score, week 12	44.40 (13.17)[4]	51.44 (15.56)[5]		.186[2]
PANSS positive, mean (SD)	10.07 (3.06)[4]	11.13 (4.15)[5]		.427[2]
PANSS negative, mean (SD)	9.40 (3.09)[4]	13.69 (6.64)[5]		.030[2]
PANSS general, mean (SD)	24.93 (8.92)[4]	26.63 (7.69)[5]		.573[2]
GAF score baseline, mean (SD)	28.36 (8.07)[4]	35.43 (13.43)[5]		.020[2]
GAF score, week 6, mean (SD)	53.70 (15.41)[4]	61.46 (14.52)[5]		.060[2]
GAF score, week 12, mean (SD)	67.67 (14.92)[4]	63.94 (12.63)[5]		.063[2]

PANSS = positive and negative symptom scale, GAF = global assessment of functioning, [1] Chi[2], [2] One-way ANOVA, [3]Fisher's exact, [4]n=15, [5]n=16

3.3.2 Cross-sectional results

Allostatic load

A one-way ANCOVA adjusted for age, sex and smoking demonstrated significant between-group differences in AL at baseline between patients with SCZ, FEP and controls ($F_{(4, 106)}$=11.288, p<0.001; Figure 3.1). Post-hoc tests with Bonferroni correction showed significantly higher AL in patients with SCZ compared to controls (4.91 (95%CI 4.23 – 5.61) ± 1.89 (95%CI 1.43 – 2.23) vs. 2.87 (95%CI 2.44 – 3.31) ± 1.62 (95%CI 1.34 – 1.85), Cohen's d=1.16, p<0.001), patients with FEP compared to controls (3.80 (95%CI 3.19 – 4.41) ± 1.66 (95%CI 1.43 – 2.23) vs. 2.87 (95%CI 2.44 – 3.31) ± 1.62 (95%CI 1.34 – 1.85), Cohen's d=0.57, p=0.011), but not between patients with SCZ and patients with FEP (p=0.136). We repeated our analyses using a traditional AL index [13] and confirmed the main findings (Appendix A).

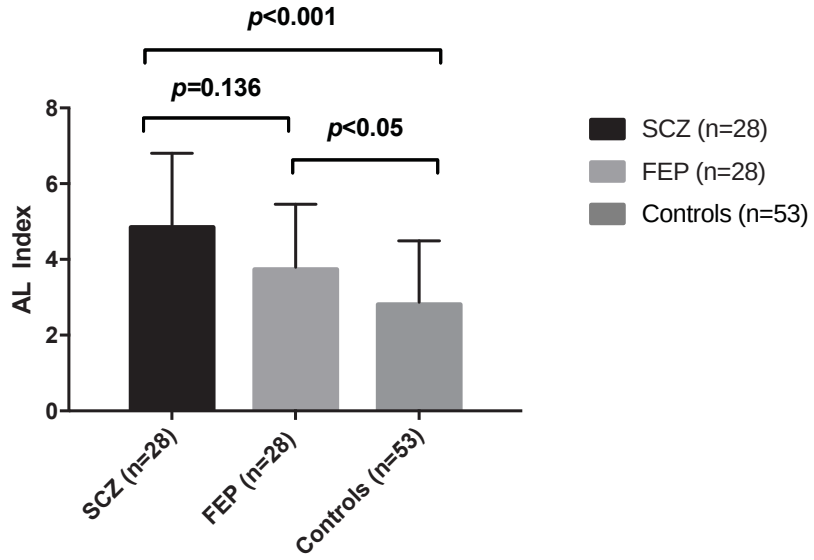

Figure 3.1 Allostatic load (AL) at baseline in patients with schizophrenia (SCZ), first-episode psychosis (FEP) and controls. Error bars represent standard deviations.

Associations between allostatic load with other variables

We next examined associations between AL and psychometric variables at baseline using partial correlations to test the relationship of AL with psychotic symptoms and social and occupational functioning (Figure 3.2A, B). Adjusting for age, sex and smoking, we found that positive psychotic symptoms (PANSS positive subscale) were positively correlated with AL across all patients with a psychotic disorder (adjusted R = 0.510 (95%CI 0.247 – 0.715), p<0.001) and GAF scores were negatively correlated with AL at trend level (adjusted R = -0.224 (95%CI -0.441 – 0.016), p=0.103). No significant associations were found for negative symptoms and the general

psychopathology subscale of the PANSS (p>0.582). Subgroup analysis revealed that positive symptoms were significantly positively associated with AL in patients with SCZ (adjusted R = 0.506 (95%CI 0.076 – 0.789), p=0.012) and in patients with FEP at trend level (adjusted R = 0.445 (95%CI -0.103 – 0.713), p=0.086).

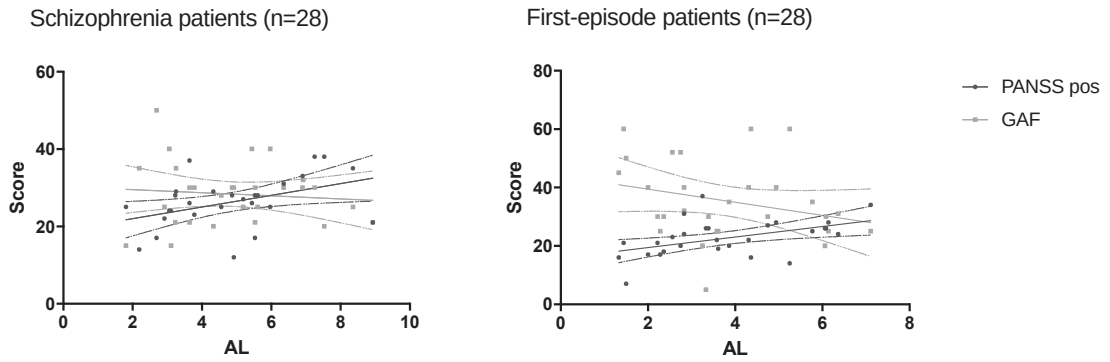

Figure 3.2 Pearson correlations between allostatic load (AL) and positive symptoms and social and occupational functioning in patients with schizophrenia (A) and first-episode psychosis (B) at baseline.

We examined if AL was associated with duration of illness, controlling for age, sex and smoking, and found no significant associations in patients with SCZ (p=0.707).
Lastly for descriptive purposes, we examined group means and standard deviations for all markers that were included in the AL index as well as their correlations with the AL index to examine the contribution of each marker to the overall index (Table 3.2).

3.3.3 Longitudinal results

Predictive value of allostatic load for functional improvement and symptomatic remission

LMMRM analysis did not show an effect of AL at baseline on PANSS scores at the follow-up assessments (week 6 and 12) in FEP (p=0.184) or SCZ (p=0.159). Linear regression analysis testing the association between AL and symptom scores at the time of the two follow-up assessments individually, however, showed that AL was associated with positive symptoms at 12 weeks (β=-1.182, 95%CI -2.262 - -0.103, p=0.034) but not at 6 weeks after the baseline assessment in patients with FEP. The effect at 12 weeks remained significant when the model controlled for age, sex, smoking, baseline PANSS positive score and medication dosage (chlorpromazine equivalent) (β=-1.944, 95%CI -3.438 - -0.451, p=0.016). No significant associations were found for negative symptoms or GAF scores at follow-up.

In patients with SCZ, AL at baseline was not associated with positive symptoms at 6 or12 weeks. AL, however, was associated with GAF scores at 12 weeks (β=3.899, 95%CI -0.816 – 8.616, p=0.097) at trend level. Regression analysis with symptomatic

Table 3.2 Individual biomarkers included in the allostatic load index at baseline.

Biomarker	Psychosis		Controls (*n*=53)	Group difference	Correlation with AL index
	Schizophrenia (*n*=28) mean (SD)	First-episode (*n*=28) mean (SD)	mean (SD)	*p*-value	*Pearson-R*
Allostatic Load	4.91 (1.89)	3.80 (1.66)	2.87 (1.62)	<.001	
Neuroendocrine					
Cortisol	243.79 (87.95)	285.36 (141.84)	217.63 (66.69)	.014	.062
Metanephrine	122.74 (102.04)	93.35 (71.77)	56.79 (36.20)	<.001	.439***
Normetanephrine	321.32 (423.54)	170.14 (130.11)	111.89 (72.96)	.001	.323***
Copeptin	6.94 (4.42)	11.82 (14.73)	5.70 (3.68)	.007	.032
Immune					
TNFα	6.36 (5.50)	4.79 (3.29)	6.17 (7.11)	.535	.237**
IL-6R	25.42 (12.52)	28.33 (10.81)	24.74 (8.27)	.317	.197*
CRP	4.27 (9.77)	2.62 (5.24)	1.86 (4.33)	.274	.225**
E-Selectin	10.07 (5.05)	9.66 (4.86)	10.22 (3.46)	.855	.243**
Lipids					
Triglycerides	1.18 (0.53)	1.17 (0.66)	1.41 (0.91)	.279	.256**
LDL	3.26 (1.35)	2.57 (1.27)	2.90 (0.84)	.073	.221**
HDL	1.45 (0.37)	1.53 (0.38)	1.51 (0.37)	.678	-.085
Cholesterol	5.32 (1.68)	4.64 (1.33)	5.07 (1.02)	.141	.235**
Glucose					
Insulin	3.80 (6.37)	5.78 (6.81)	2.65 (1.66)	.026	.176
HbA1c	5.33 (0.39)	5.31 (0.87)	5.24 (0.40)	.721	.345***
Glucose	5.12 (1.44)	5.33 (0.98)	4.79 (0.53)	.044	.326***
enRAGE	142.93 (138.58)	130.48 (98.36)	66.45 (56.29)	.001	.331***
Cardiovascular					
CK	2.99 (3.97)	2.01 (2.78)	1.48 (1.02)	.045	.401***
HR	88.00 (12.21)	80.11 (12.54)	67.77 (8.68)	<.001	.482***
BPsys	131.68 (15.23)	125.54 (17.50)	120.45 (14.31)	.009	.585***
BPdia	84.39 (10.75)	76.79 (13.35)	74.06 (8.66)	<.001	.511***
Anthropometric					
W/H ratio	0.91 (0.06)	0.86 (0.06)	0.88 (0.07)	.037	.346***
BMI	26.08 (4.72)	23.99 (4.11)	25.58 (3.73)	.131	.434***

SD = standard deviation, TNFα = tumor necrosis factor alpha, IL-6r= soluble interleukin-6 receptor, CRP = c-reactive protein, LDL = low-density lipoprotein, HDL = high density lipoprotein, HbA1c = glycosylated haemoglobin, enrage = receptor for advanced glycation endproducts, CK = creatinine kinase, HR = heart rate, BPsys = systolic blood pressure, BPSdia = diastolic blood pressure, W/H ratio = waist-to-hip ratio, BMI = body mass index; *p<0.05, **p<0.01, ***p<0.001

remission according to Andreasen, Carpenter [40] as the dependent variable demonstrated no significant association with AL in patients with FEP or SCZ (OR=1.36 (95%CI 0.78 – 2.36), p=0.275; n=28/54 (51.9%) in remission). And yet, change in AL between baseline and 6 weeks and change in positive symptoms between baseline and 6 weeks were significantly correlated (R=0.434, p<0.001).

Predictive value of allostatic load for treatment response

We tested if AL predicted treatment response according to the criteria by Leucht [41] and found no relationship between AL at baseline and treatment response (OR=1.04 (95%CI 0.62 – 1.76), p=0.884). Moreover, we found no significant difference in AL at the baseline assessment ($F_{(4, 80)}$=1.977, p=0.578) or change in AL between baseline and follow-up assessments ($F_{(4, 81)}$=0.365, p=0.812) by treatment response.

Effect of antipsychotic medication on allostatic load

AL decreased significantly between the baseline and 6 and 12-week follow-up assessments in patients with SCZ and FEP (both p<0.001; Figure 3.3A). LMMRM analysis revealed no statistically significant differences in change in AL index between patients receiving either risperidone (n=26), quetiapine (n=14) or olanzapine (n=18) (p=0.569) (Figure 3B).

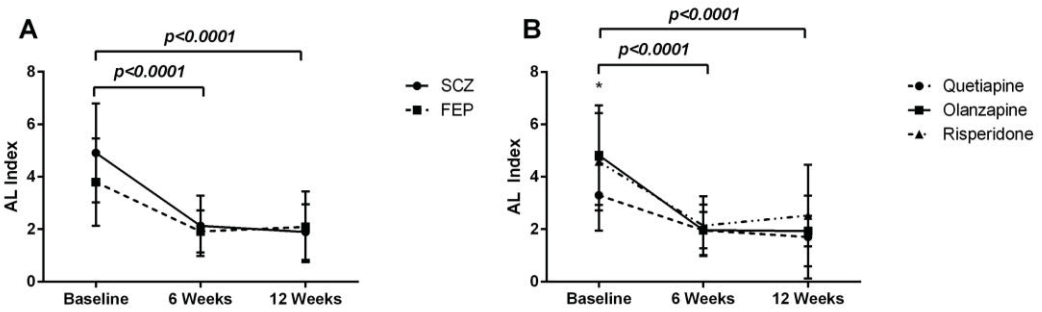

Figure 3.3 Temporal dynamics of allostatic load (AL) in patients with schizophrenia (SCZ) and first-episode psychosis (FEP). (A) AL decreased significantly in both groups between baseline and the 6 and 12 week follow-up (both p<0.0001). (B) A significant decrease in AL was observed in patients receiving olanzapine, quetiapine and risperidone (all p<0.009).

3.4 Discussion

This study examined AL in patients with FEP and SCZ at baseline and 6 and 12 weeks after commencing treatment. We found that (i) AL is elevated in patients with SCZ and FEP relative to healthy controls, (ii) AL is associated with more positive symptoms and lower social and occupational functioning, and (iii) AL decreases over 12 weeks in

acutely psychotic patients who receive second-generation antipsychotics, but is not predictive of treatment response or functional remission. Our data thus confirm the results of previous cross-sectional reports of AL in patients with SCZ [20, 43, 44] by showing elevated AL and associations of positive symptoms and functioning in patients with SCZ. Furthermore, we extend existing knowledge by including drug-naïve FEP patients and by assessing the temporal dynamics of AL in a longitudinal design that demonstrated dynamic changes in AL in patients with psychosis. These short-term changes may be attributable to reductions in symptom severity or therapeutic interventions.

AL is a composite measure of the chronic 'wear and tear' on the body and brain that is thought to be a consequence of chronic or repeated exposure to stress. The high AL in patients with SCZ and FEP in our study may reflect the multisystem dysregulation accompanying and/or leading to acute psychosis. For example, increased metabolic stress [12] may be indicative of stress-related changes in hippocampal volume, which are similarly linked to positive symptoms [24]. While we did not measure perceived chronic stress, stress exposure in the preceding weeks has been shown to be unrelated to AL [20] and may thus be unrelated to the stressfulness of experiencing a psychotic episode.

Importantly, AL is associated with a range of adverse health outcomes including cardiovascular disease and cognitive decline [13], and with proxies of poor health outcomes and ageing such as telomere attrition [45]. In light of these observations and the high prevalence of other cardiovascular risk factors in patients with SCZ [46], the increased AL observed in our study might have implications for the prediction of pathophysiology in SCZ. We thus propose that the elevated AL found in our study represents a primary biological pathway for multisystem damage in patients with SCZ. While our study only followed-up for 12 weeks and was thus not suited to investigate longer-term metabolic and cardiovascular outcomes, the high AL speaks to the need for investigation of the relationship with long-term physical diseases in patients with psychotic disorders, given their elevated risk of multimorbidity and comorbidity.

A critical finding of this study is that AL was increased in patients with FEP and SCZ relative to controls and not associated with duration of disease. Contrary to our hypothesis and recent conceptualizations of neuroprogression in bipolar disorder and SCZ [26], our findings suggest that AL may not increase with longer disease duration or higher illness *stage* [47]. Rather, AL was associated with positive symptoms and poor

functional capacity in our sample and decreased markedly within 6 weeks of treatment. A possible conclusion from this observation is that AL is high in acutely ill patients but not in patients who are treated with anti-psychotic medication. Studies that follow remediated patients over longer time periods are necessary to confirm this.
In the present study, FEP patients with the highest AL also showed the largest improvement in PANSS scores. Our finding that the magnitude of decrease in AL over the 12 week follow-up correlated significantly with the decrease in positive symptoms further supports this hypothesis. Following this line of argumentation, AL may be a state marker or a marker of disease acuity rather than a trait (diagnosis) or stage marker [48]. Additional support for this notion comes from studies showing that elevations in cytokines (e.g., IL-1beta and IL-6) that are commonly observed in at least a subset of patients with SCZ [49] are present predominantly in acute disease states [50]. While the AL index included a variety of markers, some of which may only change slowly, it might similarly reflect acute disease states rather than diagnostic entities. However, care must be taken in interpreting these findings as our study only included acutely psychotic patients at baseline and did not include patients in longer-term remission.

An important question is how the increases of AL observed in schizophrenia relate to the well documented brain structural alterations also related to positive symptoms [24]. To answer this question it is critical to understand whether the hippocampal volume loss that is not related to illness duration reflects a steadily ongoing decline or rather a highly flexible temporal course of the disease as might be indicated by our findings regarding AL assessing peripheral biomarkers. Two recent studies directly addressed the relationship of AL with brain structures and showed that elevated AL in SCZ is related to reduced global cortical thickness [43] and negatively correlated with white matter fractional anisotropy in the fornix [44]. Future studies that combine repeated measures of brain imaging and AL throughout the disease course would be useful in addressing this.

Previous studies investigating AL have not taken the dynamic changes of AL in response to therapeutic interventions into account. Our observation that AL decreased over 12 weeks of combined antipsychotic and psychosocial therapy raises the question of whether antipsychotic medication dampens AL. Two explanations seem plausible. Firstly, in light of the above findings that AL was associated with positive symptoms and the observation that AL decreased across all patients within the follow-up period, one may speculate that AL is associated with acute psychosis. An

alternative explanation would be that antipsychotic drugs actually decrease AL, which conflicts with the view that antipsychotics may worsen AL though their adverse metabolic side effects [18, 51]. However, our 12-week observation time frame might not be long enough to see adverse changes in AL.

Limitations of this study include the small sample size and the naturalistic design. This makes it difficult to delineate the contribution of antipsychotic medication to reductions in AL over a short time frame (12 weeks). Moreover, excluding patients with hypertension and dyslipidemia reduces bias in the relationship between caseness and AL on one hand but may not be representative of the SCZ population more generally. Another limitation is that we did not assess recent chronic perceived stress, which may impact on AL. Similarly, we only took into account smoking as a lifestyle factor with potential relevance for AL. Psychometrically, the GAF is not ideal if intended as a pure measure of social functioning as symptom severity contributes to high GAF scores. Finally, a limitation inherent to all current AL research is that the AL index is not standardized across studies, making it difficult to draw conclusions from multiple studies. Notwithstanding, a recent study demonstrated that the overall AL index is not sensitive to the addition or removal of individual biomarkers [52]. To ameliorate this problem, we confirmed our results with a simplified AL index similar to the one used by other studies [13]. Strengths of our study are the inclusion of patients with SCZ, FEP and controls, the well characterized sample which allowed us to exclude confounding factors (for example, diabetes or other chronic diseases), the use of standardized psychometric instruments, and the 6 and 12 week follow-up investigations that made a dynamic assessment of AL possible.

In conclusion, our data highlight the temporal dynamics of multisystem dysregulation indexed by AL and suggest that AL correlates with positive symptom severity in patients with psychosis. The challenges in the study of AL across illness stages in association with symptom domains and antipsychotic treatment highlight the importance of longitudinal studies, which are pertinent to clarify the questions raised by our data. Future studies should explore the temporality of AL beyond 12 weeks and address the question if AL is a cause or consequence of schizophrenia, clarify in larger samples if AL is a trait or state marker in patients who are not acutely ill but in remission, and confirm if AL is already elevated in at-risk individuals or in those who make a transition to psychosis.

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Conflict of Interest

SB is a director of Psynova Neurotech Ltd and PsyOmics Ltd. The other authors declare that there is no conflict of interest.

Role of Funding Source

The study sponsors had no role in the design of the study, analysis of the data or writing of the article.

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4 Relationship between allostatic load and clinical outcomes in youth at ultra-high risk for psychosis in the NEURAPRO study.

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Preface

The observation that multisystem dysregulation is present in patients with SCZ and even in patients with FEP and related to illness severity posits the question if the mediators of AL precede the onset of psychosis and are indicative of psychosis risk in those who have not yet developed the full-blown disorder. Longitudinal studies in people at UHR psychosis can reveal important insight into the pathophysiological changes during the critical prodromal phase. While limited evidence for cortisol abnormalities and other individual primary mediators of AL in UHR exists, comprehensive studies of biomarkers remain scarce and it is unclear if AL represents a risk factor for psychosis in this group.

The objective of Chapter 4 was to apply to the AL framework to a cohort of people at UHR for psychosis and to examine whether the relationships of AL with symptom dimensions observed in Chapter 3 extend to the UHR phenotype. First, cross-sectional associations of AL with symptom scores are examined. Next, the longitudinal design of the study enabled us to extend the examination of the predictive role of allostatic load as a risk biomarker to a diverse range of clinical outcomes. Being the first study of allostatic load in this population, Chapter 4 affords important insight into multisystem dysregulation as it affects young people with sub-threshold clinical symptoms.
This study used data from the NEUARPRO study, a multi-centre RCT of fish oil versus placebo, and was conducted during the a research visit at Orygen, The National Centre for Excellence in Youth Mental Health.

Abstract

Individuals at UHR for psychosis have an elevated risk of developing psychosis and other psychiatric outcomes. Risk biomarkers can assist in delineating individual risk and allow better prediction of longer-term outcomes. The aim of the present study was to examine if AL, a multisystem index of neuroendocrine, cardiovascular, immune and metabolic dysregulation, is associated with clinical outcomes in youth at UHR for psychosis.

AL was measured in 106 participants of the NEURAPRO study, a multicentre randomized-controlled trial of long-chain omega-3 polyunsaturated fatty acids versus placebo in people at UHR for psychosis. Psychiatric symptoms and social and occupational functioning were assessed at baseline and 6 and 12 months after study intake. Multivariate linear and logistic regression models were used to test the relationship between AL and clinical outcomes.

High AL at baseline was associated with poor social and occupational functioning at 6 months (β =-0.224, p=0.025) and with more severe manic symptoms at 6 months (β=0.207, p=0.026), taking into account relevant covariates including age and smoking status. No significant associations were observed at the 12-month follow-up assessment or with any other clinical outcome measures.

Our data provide initial evidence for a link between AL and impaired functioning in individuals at UHR for psychosis. Further studies are needed to evaluate AL as a potential predictor of early treatment response.

4.1 Introduction

People who seek help for distressing psychiatric symptoms and meet UHR for psychosis criteria have an elevated risk of developing psychotic disorders of approximately 20% (95%CI 17-25%) within two years [1]. In addition to the increased risk for psychosis, impaired psychosocial functioning and comorbid mental disorders are often associated with the UHR phenotype [2, 3]. Over the last two decades, substantial efforts have been made toward the early identification of people at UHR for psychosis and for the provision of stage-specific treatments. However, it is becoming increasingly clear that psychosis risk in the UHR group is heterogeneous and may depend on symptom severity at presentation, as well as on psychosocial and biological risk factors [4, 5]. Identifying biomarkers and endophenotypes can assist in determining who will benefit most from treatment, and to better estimate the risk for psychosis transition and other adverse outcomes [6, 7]. To date, several studies have examined candidate biomarkers in UHR groups including markers of oxidative defence [8], membrane fatty acids [9, 10], cortisol [11, 12], niacin skin sensitivity [13] and multi-analyte indices [14].

AL, the cumulative adverse effects of chronic stress and maladaptation, has been linked to somatic comorbidity in psychiatric populations and may be implicated in the pathophysiology of psychotic disorders [15]. AL characterises subtle elevations in peripheral neuroendocrine, immune, cardiovascular and metabolic biomarkers and, as such, represents a multisystem biomarker index [16]. In the AL framework, chronic or repeated exposure to stress and adverse events triggers the release of stress mediators, which subsequently lead to immune activation and metabolic dysregulation [17]. There is mounting evidence to suggest that AL is implicated in the pathophysiology of psychosis, with two recent studies showing that AL is elevated in patients with schizophrenia and first-episode psychosis relative to healthy matched controls, and related to positive symptoms and reduced functioning [18, 19]. Recent studies also suggest that AL is associated with reduced hippocampal volume and impaired cognition in non-psychiatric populations [20], and with reduced global cortical thickness in patients with schizophrenia [21]. AL has traditionally been viewed as the long-term consequence of exposure to stressful circumstances and several studies demonstrated that it is elevated as a consequence of early life trauma and socioeconomic disadvantage and predictive of adverse health outcomes [20, 22, 23].

Indeed, perceived stress, childhood trauma, unemployment and low educational attainment are also risk factors for psychosis in the UHR group, as evidenced by a recent meta-analysis [5]. Consequently, allostatic load may be relevant for the pathophysiology of psychotic disorders but it is unclear if high AL predisposes to enhanced psychosis risk. However, despite the growing interest in the AL concept in psychiatry [15, 24], no study to date has applied it to a UHR group. As such, further investigation of AL is warranted.

This study aims to investigate the relationship between AL and clinical outcomes in individuals at UHR for psychosis. Specifically, it was hypothesised that AL would be associated with more severe positive symptoms and poor functional outcomes, as previously observed in patients with schizophrenia and first-episode psychosis (Berger et al., 2018).

4.2 Methods

4.2.1 Study Design and Participants

The present study is an analysis of a subgroup of participants (n=106) aged 15 to 24 years of the NEURAPRO study, who provided consent for additional biomarker analyses. The NEURAPRO study is a double-blind placebo-controlled randomized clinical trial (RCT) (ANZCTR Identifier: 12608000475347) of 1.4g ω-3 PUFA (n=53) or placebo (n=53), in addition to 6-20 sessions of cognitive behavioural case management (CBCM) over the 6-month treatment period. This 6-month treatment period was followed by a 6-month follow-up, during which CBCM was provided on an as-need basis. The study protocol was approved by the Melbourne Health Human Research Ethics Committee (MH-HREC 2008.628). Details of this RCT including trial design, randomization, interventions, and inclusion and exclusion criteria are published elsewhere [25, 26].

All participants were recruited from Orygen Youth Health (Orygen), Parkville, Australia and headspace, Sunshine, Australia between March 2010 and September 2014. To be eligible for the present analysis, participants had to provide complete biomarker data in addition to fulfilling the inclusion criteria for the RCT, i.e. being aged 15 to 24 years, ability to provide informed consent, having been identified as a UHR for psychosis person and having low levels of functioning (SOFAS score lower than 50) for at least one year or having experienced a decrease in functioning in the past year (reduction in SOFAS score of at least 30%). The exclusion criteria were past history of treated or

untreated psychotic episode or past treatment with antipsychotic medication equivalent to a lifetime dose of >50mg haloperidol, organic brain disease, current treatment with a mood stabiliser, a diagnosis of a developmental disorder or a history of developmental delay or intellectual disability, acute suicidality, self-harm or aggression toward others, pregnancy, or regular supplementation with n-3 PUFAs. All participants provided written informed consent (parent/legal guardian consent for participants aged 17 or younger) for their participation in the RCT and for the analyses of biomarker data.

4.2.2 Data collection

Participants underwent a series of assessments over the course of the study, consisting of research interviews, self-report measures, and the collection of blood and urine samples. For the present study, data collected at baseline, end of the experimental intervention (Month 6) and one year after baseline (Month 12) were used. Rating scales included the Brief Psychiatric Rating Scale (BPRS), the Scale for the Assessment of Negative Symptoms (SANS), the Social and Occupational Functioning Assessment Scale (SOFAS), the Montgomery-Asberg Depression Rating Scale (MADRS), the Young Mania Rating Scale (YMRS), the Clinical Global Impression (CGI) scale and the Global Functioning Social (GF-S) and Role (GF-R) scales.

4.2.3 Biomarker Assessment/Allostatic Load

The AL index was derived from biomarkers representing cardiovascular, neuroendocrine, immune and metabolic systems. Cardiovascular markers included heart rate (HR), systolic blood pressure (BPsys) and diastolic blood pressure (BPdia). Neuroendocrine markers included morning saliva cortisol. Immune markers included interleukin-6 (IL-6), interleukin-12 (IL-12) and CRP. Metabolic markers included triglycerides, total cholesterol and BMI. These markers were selected based on (i) representation of several physiological systems including, cardiovascular, neuroendocrine, immune, and metabolic parameters, (ii) use in previous AL research [18, 23, 27], and (iii) associations with disease risk.

Study participants provided fasting blood samples at baseline assessment and, following blood collection, plasma was separated from blood cells by centrifugation at 4˚C and at 1800g for 15 mins. Samples were stored at -80C until analysis. CRP, IL-6 and IL-12 were analysed at the University of Adelaide using cytometric bead arrays. Triglycerides, lipoproteins and cholesterol were analysed at Melbourne Health Shared Pathology. Saliva samples were collected at awakening and cortisol was analysed at

Melbourne Health Shared Pathology using an electrochemiluminescense assay (Roche Diagnostics, Indianapolis, IN, USA).

For the computation of the AL index, the 75th percentile (25th percentile for HDL) of each biomarker was determined based on the distribution in the study sample. We then used a 'scaling' approach previously described by others and adapted by our group to calculate the AL index [18, 28]. Briefly, for each individual, every marker with values above the 75th percentile was defined as '1', and the sum of all markers in each category (cardiovascular, immune, neuroendocrine and metabolic) was divided by the number of markers in each category to allow for equal weighting of the four categories. Finally, the scores from the four categories were added and a final AL index calculated. For markers where the distribution differed between male and female controls, gender-specific cut-offs were calculated and used to compute the index [29].

4.2.4 Statistical Analysis

Prior to conducting statistical analyses, data for continuous variables were assessed for outliers and distributional assumptions. Normality of the data was ascertained by assessing the skewness of data distribution and the associated standard errors. Normality was assumed if skewness divided by the standard error of skewness was between -3 and +3. Scores on the CGI severity and improvement subscales were dichotomised as not ill/borderline ill vs. mildly ill or worse, and very much improved/much improved vs. minimally improved or less, respectively. Differences in continuous variables between the baseline and follow-up assessments were determined with paired-sample t-tests. Differences in dichotomous data between the baseline and follow-up assessments were determined with McNemar's test. To test whether allostatic load was associated with change in symptom scores between baseline and Month 6 and baseline and Month 12, we fitted linear and logistic regression models with allostatic load as the predictor variable for continuous and binominal outcome variables, respectively, to estimate standardised coefficients and odds ratios and their 95% confidence intervals. Potential confounding variables were considered as covariates based on hypothesised association with dependent and independent variables, and previous use in the literature. These included age and smoking status as determined from a general health questionnaire administered to participants as well as treatment group. The Hosmer-Lemeshow goodness-of-fit test was used to assess model fit. All analyses were carried out using STATA 13.1 (Stata Corp., College Station, TX, USA).

4.3 Results

A total of 106 participants were included in the analysis. Demographic and clinical characteristics of patients at baseline, Month 6 and Month 12 are reported in Table 4.1. The mean age of study participants was 17.21 (SD 2.37) years at study intake. Mean scores of all rating instruments improved significantly during the study with the exception of GF-S and GF-R scores.

Table 4.1 Demographic and clinical characteristics of participants at baseline, 6 and 12 months

	Baseline (n=106)		6 Months (n=90)		12 Months (n=74)		6 Months vs. Baseline	12 Months vs. Baseline
	N	%	N	%	N	%	p-value	p-value
Gender (f)	70	66.04	-	-	-	-	-	-
Smoking	38	36.19	-	-	-	-	-	-
	Mean	SD	Mean	SD	Mean	SD		
Age	17.21	2.37	-	-	-	-	-	-
BPRS								
Total	43.43	8.89	37.19	8.93	35.74	7.61	<0.001[1]	<0.001[1]
Psychosis subscale	9.09	2.67	6.93	2.86	6.49	2.41	<0.001[1]	<0.001[1]
SANS	17.99	9.86	14.27	11.15	12.96	10.91	<0.001[1]	<0.001[1]
YMRS	4.39	3.14	3.04	2.86	2.67	2.39	0.008[1]	<0.001[1]
MADRS	22.86	8.69	14.18	9.88	13.06	9.79	<0.001[1]	<0.001[1]
SOFAS	55.70	10.93	62.57	13.28	63.22	15.87	<0.001[1]	<0.001[1]
GF-S	6.61	1.19	6.81	1.28	6.83	1.45	0.037[1]	0.098[1]
GF-R	6.21	1.54	6.58	1.81	6.69	1.80	0.122[1]	0.052[1]
	N	%	N	%	N	%		
CGI								
Severity subscale							<0.001[2]	<0.001[2]
Not ill/borderline ill	3	2.83	25	30.86	26	40.62		
Mildly ill or worse	103	97.17	56	69.14	38	59.38		
Improvement subscale							-	-
Very much improved/much improved	-	-	17	20.99	18	28.12		
Minimally improved or less	-	-	64	79.01	46	71.88		

[1]Paired samples t-test, [2] Wilcoxon Signed-Rank test, BPRS=Brief Psychiatric Rating Scale, SANS=Scale for the Assessment of Negative Symptoms, YMRS=Young Mania Rating Scale, MADRS=Montgomery-Asberg Depression Rating Scale, SOFAS=Social and Occupational Functioning Assessment Scale, GF-S=Global Functioning Social Scale, GF-R=Global Functioning Role Scale, CGI=Clinical Global Impression Scale

Mean and standard deviation of the AL index and the various biomarker levels are reported in Table 4.2.

Table 4.2 Allostatic load index and biomarkers at baseline

	M	SD
AL-Index	3.16	1.75
Heart rate (bpm)	72.04	11.31
Blood pressure, systolic (mmHg)	115.12	14.34
Blood pressure, diastolic (mmHg)	73.15	10.19
Cortisol (ng/ml)	16.25	8.52
CRP (mg/L)	1.61	2.42
IL-6 (pg/ml ?)	1693.78	11.639.16
IL-12 (pg/ml ?)	4149.59	11028.4
Total cholesterol (mmol/L)	4.36	0.91
Triglycerides (mmol/L)	1.14	0.78
BMI	25.69	6.46

AL=allostatic load, CRP=c-reactive protein, IL-6=interleukin-6, IL-12=interleukin-12, BMI=body mass index

The AL index was not correlated with any of the rating scales at the baseline assessment (Table 4.3).

Table 4.3 Partial correlations of the Allostatic Load Index with clinical variables at study intake

	Pearson R (adj)	p-value	N
BPRS			
Total	0.089	0.367	106
Psychosis subscale	0.098	0.319	106
SANS	0.133	0.175	106
YMRS	0.079	0.421	106
MADRS	0.058	0.556	106
SOFAS	-0.004	0.966	106
GF-S	-0.039	0.691	106
GF-R	0.022	0.828	106
CGI			
Severity subscale	-0.443	0.746	106
Improvement subscale	-	-	-

BPRS=Brief Psychiatric Rating Scale, SANS=Scale for the Assessment of Negative Symptoms, YMRS=Young Mania Rating Scale, MADRS=Montgomery-Asberg Depression Rating Scale, SOFAS=Social and Occupational Functioning Assessment Scale, GF-S=Global Functioning Social Scale, GF-R=Global Functioning Role Scale, CGI=Clinical Global Impression Scale, OR=odds rato, CI=confidence interval

Multivariate linear and logistic regression taking into account age, smoking, treatment group and baseline scores showed that AL was associated with change in SOFAS scores between baseline and Month 6 (β =-0.224, p=0.025) but not between baseline and Month 12 (β =-0.094, p=0.277). Similarly, AL was associated with change in GF-S scores between baseline and Month 6 (β =-0.252, p=0.013) and Month 12 at trend

level (β =-0.222, p=0.052) and with change in GF-R scores at Month 6 (β =-0.192, p=0.046) but not at Month 12 (β =-0.151, p=0.161). AL was significantly associated with change in YMRS scores at Month 6 (β=0.207, p=0.026) but not at Month 12 (β=0.069, p=0.496). No significant associations were observed with any of the other outcomes measures. Detailed results are reported in Table 4.4.

Table 4.4 Linear and logistic regression analysis of allostatic load as predictor of change in symptom scores between baseline and month 6 and 12

	Coefficient	Beta	p-value	N
BPRS[1]				
Total				
Month 6	0.187	0.041	0.685	89
Month 12	0.133	0.028	0.780	73
Psychosis subscale				
Month 6	0.158	0.094	0.336	90
Month 12	0.131	0.071	0.428	77
SANS[1]				
Month 6	0.313	0.074	0.502	89
Month 12	-0.020	-0.003	0.997	73
YMRS[1]				
Month 6	**0.404**	**0.207**	**0.026**	**88**
Month 12	0.109	0.069	0.496	71
MADRS[1]				
Month 6	0.370	0.067	0.484	89
Month 12	0.201	0.036	0.704	73
SOFAS[1]				
Month 6	**-1.737**	**-0.224**	**0.025**	**90**
Month 12	-1.13	-0.125	0.277	77
GF-S[1]				
Month 6	**-0.159**	**-0.252**	**0.013**	**90**
Month 12	-0.159	-0.222	0.052	77
GF-R[1]				
Month 6	**0.209**	**-0.192**	**0.046**	**90**
Month 12	-0.168	-0.151	0.161	77

[1]Adjusted for age, smoking and baseline scores of the respective outcome variable, [2] Adjusted for age and smoking; BPRS=Brief Psychiatric Rating Scale, SANS=Scale for the Assessment of Negative Symptoms, YMRS=Young Mania Rating Scale, MADRS=Montgomery-Asberg Depression Rating Scale, SOFAS=Social and Occupational Functioning Assessment Scale, GF-S=Global Functioning Social Scale, GF-R=Global Functioning Role Scale, CGI=Clinical Global Impression Scale, OR=odds rato, CI=confidence interval; significant results are bold

4.4 Discussion

The aim of the present study was to examine if AL is associated with clinical outcomes in youth at UHR for psychosis. Our data demonstrate that high AL is related to poorer functional capacity in this group, at least in the short term (6 months), even when confounding variables such as age and smoking are taken into account. In addition, we observed an association of higher AL with increase in manic symptoms after 6 months. However, other hallmarks of psychosis such as positive psychotic symptoms

were not related to AL in the present study. To our knowledge, this is the first study to investigate allostatic mechanisms in individuals at UHR for psychosis.

Our observations add to a growing body of research examining AL in patients with psychotic disorders. Previous studies on AL in patients with schizophrenia and drug-naïve patients with first-episode psychosis have shown elevations of AL in these patients relative to healthy controls and strong associations with positive psychotic symptoms [18, 19], reduced functioning [18, 19], reduced global cortical thickness [21] and white matter integrity [30]. Collectively, these studies provide empirical evidence in support of the hypothesis that AL is related to pathophysiological processes characterising psychotic disorders, and raise the question of whether AL can serve as a biomarker for psychotic disorders. Notably, few studies to date have investigated the prospective association of AL with longer-term outcomes. The present study aimed to fill this gap by applying the allostasis framework to a group of individuals at UHR for psychosis, who represent a viable target group for early intervention.

The results of the linear regression analysis suggest that high AL is indicative of poorer functional capacity in the short term (6 months), both on the SOFAS scale and the two GF scales. Deficits in psychosocial functioning are a core feature of psychotic disorders and are well documented in individuals at UHR for psychosis [3, 26, 31]. In addition to transition to psychosis as the paradigmatic outcome of interest in this group, functioning is gaining interest as it relates to quality of life, personal and vocational recovery, including in those who do not develop psychosis [2]. Moreover, impaired functioning itself is indicative of higher risk for psychosis transition [3, 32-36]. Although this is the first study to assess associations between AL and functioning in people at UHR for psychosis, other biomarker studies support the notion that pathological mechanisms relevant to AL relate to outcomes in UHR. For example, a large proportion of individuals with UHR phenotypes experience distress related to their condition, which is associated with adverse longer-term outcomes in UHR [36] and sub-threshold psychotic symptoms and aberrant salience attribution [37]. Accordingly, aberrations in the diurnal cortisol secretion have been found to be indicative of higher transition risk in individuals with UHR phenotypes [11, 12]. However, not all studies support detrimental effects of distress on functional outcomes [36]. Reduced grey matter volume in frontal and limbic regions is associated with poor functional outcomes in UHR [38]. Notably, several markers of immune activation, including IL-6 and TNF-α, are highly correlated with the AL index in patients with schizophrenia and first-episode psychosis [18]. In clinical trials of ω-3 PUFA, increases in the ω-3 PUFA EPA are

related to lower transition risk [34], which is likely in part due to their anti-inflammatory properties, including their role as a precursor of EPA-derived anti-inflammatory molecules such as resolvins or their ability to downregulate the NFkappaB pathway [39, 40].

An interesting observation of our study is that AL is associated with more severe manic symptoms after 6 months. While it has been hypothesised that allostatic mechanisms contribute to bipolar disorder in a similar fashion as discussed above for psychotic disorders [41-43], few studies to date have directly investigated AL in bipolar disorder. Kapczinski and colleagues [44] reported a systemic toxicity index that bears similarity to AL indices and applied it to patients with bipolar I, finding that this index was increased in depressed and manic patients but not in euthymic patients.

Importantly, our results support a relationship of AL with functioning and manic symptoms only within 6 months but not at the 12-month follow-up assessment. The effect sizes were similar at 12 months compared to those at 6 months, suggesting that the negative finding may be due to lack of power, it has to be considered that AL might not be suitable to inform prognosis over the longer term. Similarly, AL did not predict overall clinical improvement in the present study.

Few studies to date have provided evidence for single biomarkers with strong predictive potential. Multianalyte indices may overcome limitations of single biomarkers in psychiatry, as these are unlikely to explain complex disorders with multifactorial aetiology [45]. Further studies testing potentially predictive biomarkers in longitudinal designs should consider including the AL index to further evaluate its predictive capacity.

The results of our investigation need to be interpreted with several limitations in mind. Firstly, our study did not include a control group, which precludes conclusions about group differences in AL between people at UHR for psychosis and healthy individuals. Secondly, we did not test prospective associations with psychosis transition due to a small number of participants who converted to psychosis in this subgroup of the NEURAPRO trial (n=12). Strengths of this study include the follow-up study design; the well characterised and comparatively large sample size; the observation that AL was prospectively associated with all functioning rating scales in this trial, which allows greater confidence in our findings; and that none of our study participants received antipsychotic medication, preventing any confounding effects on AL.

In conclusion, the short-term effects of AL on functional outcomes and mania symptoms in this UHR cohort highlight the potential role of allostatic mechanisms in mediating risk for poor clinical outcomes and are broadly consistent with recent investigations of AL in first-episode psychosis. AL may be a potential predictor of early treatment response and warrants further investigation. To increase the confidence in our findings, these data will need to be replicated in an independent sample. Ultimately, peripheral biomarker signatures might assist risk predictions and complement clinical characteristics.

4.5 Acknowledgements

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4.6 Conflict of Interest

The authors declare that there is no biomedical or financial conflict of interest in relation to this study.

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5 Omega-6 to omega-3 polyunsaturated fatty acid ratio and subsequent mood disorders in young people with at-risk mental states: a 7-year longitudinal study

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Preface

In addition to immune activation and neuroendocrine dysregulation, fatty acid metabolism is thought to be involved in the pathophysiology of mood and psychotic disorders. Long-chain ω-3 PUFA are potential candidate biomarkers for psychiatric disorders given their key function in regulating cell membrane properties and due to their role as precursors of anti-inflammatory and antioxidant molecules. To date, several observational studies support depletion of these PUFAs in MDD and in SCZ, and clinical trials report superiority of PUFA supplementation over placebo for MDD. In light of these observations, membrane fatty acids may be promising candidate biomarkers. Surprisingly, while most studies have focused on chronic patients, there is a lack of research in patients at early stages, in particular individuals at UHR for psychosis, who have a high risk for psychosis conversion and frequent comorbid mood disorders and represent a viable target group for indicated prevention strategies.

Chapter 5 uses data from the first placebo-controlled RCT of ω-3 PUFA versus placebo in individuals at UHR for psychosis ('Vienna n-3 Study') to test associations of erythrocyte PUFA levels at baseline with clinical outcomes at follow-up. This study was uniquely qualified to answer this question given the well-characterised UHR sample and the long follow-up time of 7 years. Furthermore, the wide range of clinical outcome measures allowed us to test associations with other relevant outcomes in addition to depressive symptoms and MDD. This paper was published in *Translational Psychiatry*.

Abstract

While cross-sectional studies suggest that patients with mood disorders have a higher ratio of ω-6 to ω-3 PUFA and lower levels of omega-3 PUFAs, it is unknown if a high ω-6/3 ratio indicates vulnerability for depression. We tested this hypothesis in a 7-year follow-up study of young individuals with an UHR phenotype. We conducted a secondary analysis of the Vienna Omega-3 study, a longitudinal study of ω-3 PUFAs in individuals at UHR for psychosis (n=69). Levels of ω-6 and ω-3 PUFAs were measured in the phosphatidylethanolamine fraction of erythrocyte membranes at intake into the study. Mood disorder diagnosis was ascertained with the Structured Clinical Interview for DSM-IV-TR and confirmed by review of medical records and interviews of caregivers. A higher ω-6/3 PUFA ratio at baseline predicted mood disorders in UHR individuals over a 7-year (median) follow-up (odds ratio=1.89, 95%CI=1.075–3.338, *p*=0.03). This association remained significant after adjustment for age, gender, smoking, severity of depressive symptoms at baseline, and n-3 supplementation. Consistent results were obtained for individual PUFAs, including lower levels of EPA acid and docosahexaenoic acid (DHA). The predictive capacity of these findings was specific to mood disorders as no associations were found for any other psychiatric disorder. Our data provide the first prospective evidence that the ω-6/3 PUFA ratio is associated with an increased risk for mood disorders in young people exhibiting an UHR phenotype. These findings may have important implications for treatment and risk stratification beyond clinical characteristics.

5.1 Introduction

Long-chain polyunsaturated fatty acids (PUFAs) have important physiological functions and are key regulators of cell membrane properties. As such, they play important structural and functional roles in the human brain and affect monoaminergic neurotransmission, dendritic arborisation, synapse formation, and ion channel function [1, 2]. Ω-3 PUFA have been shown to possess anti-inflammatory [3] and antioxidant[4] properties, while ω-6 PUFAs are generally seen as pro-inflammatory, and a high ω-6/3 PUFA ratio is thought to have adverse health effects [5].

Epidemiological studies show that the dietary intake of ω-3 PUFAs through fish consumption is inversely correlated with the prevalence of depression [6, 7], thus suggesting that ω-3 PUFAs have a protective effect. While the traditional human diet has a ratio of ω-6/3 PUFA of approximately 1:1, contemporary Western diets are characterized by a ratio of around 15:1, reflecting deficient intake of n-3 fatty acids and excessive intake of n-6 fatty acids [8]. Importantly, adherence to a Western diet is associated with a higher prevalence of depression [9]. This has given rise to the hypothesis that the long-term changes in dietary patterns that have occurred in industrialized countries over the last centuries have led to an increase in the ω-6/3 PUFA ratio [5], which may be causally linked to an increase in the incidence of mood disorders. Indeed, case-control studies have suggested that individuals with depression have lower levels of ω-3 PUFAs and higher ω-6/3 PUFA ratios compared to healthy controls [10]. Recent meta-analyses of ω-3 PUFAs supplementation trials in patients with depression have resulted in inconsistent conclusions. Some meta-analyses have concluded that ω-3 PUFA supplementation does not have a significant benefit on depressive symptoms [11], while others have found significant beneficial effects [12-14], which appeared to be related to higher doses of the ω-3 PUFA EPA [12, 15, 16]. Although lower levels of ω-3 PUFAs and higher ω-6/3 PUFA ratios have been reported in adults with depression, little is known about PUFA levels in youth, nor whether abnormalities in membrane lipid composition precede the onset of depression in this group. This is salient because even prenatal and early childhood diet appears to play a role in risk for mood disorders, and as such represents a potential intervention target [17].

An important mechanistic question is whether individuals with low levels of ω-3 PUFAs or an increased ω-6/3 PUFA ratio are at elevated risk of developing mental disorders.

We have recently demonstrated that higher levels of α-linolenic acid, the 'parent' essential fatty acid of the ω-3 family, predicted short-term functional improvement among individuals at ultra-high risk (UHR) of developing psychosis who receive n-3 PUFAs supplementation [18], while decreased levels of nervonic acid, a monounsaturated very long chain fatty acid involved in myelin synthesis, predicted transition to first-episode psychosis in the same study [19]. In contrast, longitudinal studies investigating associations of ω-3 PUFAs and depressive symptoms in general adult population samples have not reported significant associations [20, 21].

To address the hypothesis that low levels of ω-3 PUFAs or an increased ω-6/3 PUFA ratio precedes the onset of mood disorders in at-risk groups, we conducted a secondary analysis of the Vienna Omega-3 study, a 12-week (RCT) of ω-3 PUFAs or placebo in UHR patients [22], to test whether erythrocyte membrane levels of PUFAs predict mood disorders during a (median) 7-year follow-up in young individuals with UHR for psychosis.

5.2 Methods and Materials

5.2.1 Study Design

The present study is a secondary analysis of a double-blind RCT of n-3 PUFAs for indicated prevention of psychosis transition in individuals at UHR for psychosis (trial registration: clinical trials.gov identifier: NCT00396643). Details of this RCT, including the trial design, interventions, randomization, inclusion and exclusion criteria, blinding, and study measures have been published elsewhere [22]. Briefly, a 12-week intervention with 1.2 g per day ω-3 PUFAs (700mg EPA, 480mg DHA) or placebo was conducted with follow-up at 1 year and 7 years (median) after baseline. The study was approved by the Medical University of Vienna Ethics Committee, and written informed consent was obtained from all participants or participants and their legal guardians for participants aged <18 years.

5.2.2 Participants

Eighty-one treatment-seeking individuals were enrolled in the original study. All participants were consecutive admissions to a specialized psychosis detection and treatment unit at the Department of Child and Adolescent Psychiatry, Medical University Vienna, between 2004 and 2006. Participants were aged 13–25 years at first presentation and met criteria for one or more of the three operationally defined and well-validated risk factors for psychosis proposed by Yung et al. [23]: attenuated

positive psychotic symptoms; transient psychosis; and genetic risk plus a decrease in functioning. The present study sample consists of 69 of the 81 individuals enrolled in the trial (85.2%; 47 female, 22 male; mean age 16.38 years, SD 1.83) who had information on diagnostic outcomes 7 years (median) after study entry.

5.2.3 Psychometric Assessments

The primary outcome measure used for this study was any occurring diagnosis of mood disorder according to the Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV) criteria within the seven-year follow-up period, ascertained in a personal interview with the participant using the Structured Clinical Interview for DSM-IV-TR Axis I disorders (SCID-I/P) at the longer-term follow-up assessment (median 7 years after enrolment into the study). If a participant was unavailable for an interview, we aimed to establish psychiatric diagnoses using next of kin and/or medical record information. The Global Assessment of Functioning (GAF) scale was used to measure social, occupational, and psychological functioning at baseline and follow-up. The MADRS was used to assess depressive symptoms. Inter-rater reliability estimates were high (intra-class correlation coefficients >0.92). Cronbach's alpha for the MADRS in this study was 0.89. Inter-rater reliability was maintained by using videotaped interviews every 3 months across the initial 12 months of the RCT and before longer-term follow-up.

5.2.4 Analysis of Erythrocyte Membrane PUFA Composition

Erythrocyte membrane phospholipid composition closely reflects that of neuronal membranes and provides an easily accessible indicator of brain phospholipids [24]. Erythrocytes were separated from whole blood samples and the PUFA composition of phospholipids of the phosphatidylethanolamine fraction was analyzed from erythrocyte membranes. Phosphatidylethanolamine was chosen because it contains higher levels of PUFAs relative to other phospholipids. We used gas chromatography to determine values for PUFAs and included the following into our analysis: 18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6, 22:2n-6, 22:4n-6, 18:3n-3, 20:5n-3, 22:5n-3, 22:6n-3. The detailed methods and rationale are published in previous reports [25].

5.2.5 Statistical Analysis

We included all study participants who did not meet the case definition (i.e., being diagnosed with a mood disorder within the follow-up period, n=43, see Table 1) as the comparison group, which thus consisted of individuals with no psychiatric disorder, but

also individuals with psychotic disorders, anxiety disorder and/or other psychiatric disorders.

As an alternative to the conservative Kolmogorov–Smirnov test with Lilliefors correction, normality of data was ascertained by assessing the skewness of data distribution and the associated standard errors. Skewed variables were log transformed, and a range of <3 was used as the cut-off for the use of parametric tests. Unadjusted between-group differences in demographic variables and fatty acids (FAs) were tested with independent samples t-tests and χ^2 tests or Fisher's exact test for continuous and categorical variables respectively, and group mean values were transformed into effect size measures (Cohen's d) for comparison of FAs between diagnostic categories. We used repeated measures ANOVA to examine changes in scores between and within groups. Logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI) for mood disorder diagnosis within the 7-year follow-up period. PUFAs (z-transformed values) and their composite indices were used as independent variables and ORs were calculated per SD increase in PUFA levels. All regression models were fitted as unadjusted models, models adjusted for age, gender and smoking, and models adjusted for age, gender, smoking, baseline MADRS score, and ω-3 PUFA/placebo group status. We conducted sensitivity analyses excluding individuals with psychotic disorder outcome from both case and comparison group for all tests. The Hosmer-Lemeshow test was used to test model calibration. All tests were 2-sided, a *p*-value of <0.05 was considered significant. SPSS version 22 was used for all analyses.

5.3 Results

5.3.1 Demographics and psychometric measures

The median follow-up duration was 7.14 years (IQR=1.13 years). The sources of information to determine diagnostic outcome were interviews (61 with participants, 4 with next of kin) for 94.2% (65/69) and medical records for 5.8% (4/69) of participants. Of these 69 participants, 26 (37.7%) received a mood disorder diagnosis during the follow-up period (24 major depressive disorder; 2 bipolar II disorder), 20 converted to a psychotic disorder, 19 received an anxiety disorder and 7 a substance use disorder diagnosis. No significant differences in any of the demographic variables were found between participants with and without mood disorder (Table 5.1). While GAF scores were not significantly different between the two groups at the baseline assessment, we

found a significant time by group interaction ($F_{1.000}$=4.50, *p*=0.04), indicating that GAF scores changed differentially between individuals with and without a mood disorder diagnosis. Post-hoc tests revealed that GAF scores did not change significantly during the follow-up period in individuals with mood disorders (baseline 56.9 ± 11.1 vs. follow-up 58.1 ± 13.2, *p*=0.62), whereas individuals with no mood disorders improved

Table 5.1 Demographic characteristics

	All individuals with follow-up data (n=69)		Mood disorder at follow-up (n=26)		No mood disorder at follow-up (n=43)		*p*-value
	mean	SD	mean	SD	mean	SD	
Age at baseline (years)	16.3	1.8	16.3	1.7	16.4	1.8	0.879[b]
	N	%	N	%	N	%	
Sex (female)	47	68.1	17	65.4)	30	69.8	0.705[a]
Alcohol							0.577[c]
Less than weekly	40	58.0	16	61.5	24	55.8	
1-6 drinks per week	18	25.7	5	18.5	13	30.2	
Daily	11	15.7	5	18.5	6	14.0	
Cannabis							0.060[c]
No	58	84.1	21	80.8	37	86.0	
<2g per week	7	10.0	5	18.5	2	4.7	
>2g per week	4	5.7	0	0.0	4	9.3	
Smoking	35	50.7	16	61.5	19	44.2	0.162[a]
Any illicit drug	13	18.6	5	7.1	8	11.4	0.599[c]
	mean	SD	mean	SD	mean	SD	
MADRS score baseline	18.0	8.9	20.5	8.6	16.7	8.9	0.093[b]
MADRS score long term follow-up	14.4	11.0	17.2	9.8	12.9	11.5	0.164[b]
GAF score baseline	60.2	12.9	56.9	11.1	62.2	13.6	0.099[b]
GAF score long term follow-up	66.2	16.7	58.1	13.2	71.1	16.9	0.001[b]

Abbreviations: MADRS, Montgomery–Asberg Depression Rating Scale; GAF, Global Assessment of Functioning; SD, standard deviation
[a]Chi-Square; [b]Independent samples t-test; [c]Fisher's Exact

significantly (baseline 62.2 ± 13.6 vs. follow-up 71.1 ± 16.9, $p<0.001$). No significant time by group interaction and no between-group differences were found in MADRS scores at baseline and follow-up.

5.3.2 Between-group differences in PUFAs

Three ω-6 PUFAs, two ω-3 PUFAs, the sum of ω-3 PUFAs, and the ω-6/3 PUFA ratio were differentially elevated or decreased at baseline in patients with subsequent mood disorders relative to the comparison group (Table 5.2). This effect was specific for mood disorders; no group differences were found for psychotic disorders, anxiety disorders or any other psychiatric disorder. Patients with mood disorders at follow-up had lower baseline levels of linolenic acid (18:2n-6), higher baseline levels of γ -linolenic acid (18:3n-6) and docosadienoic acid (22:2n-6), lower levels of EPA (20:5n-3), lower levels of DHA (22:6n-3), lower sum of ω-3 PUFAs, and a higher ω-6/3 PUFA ratio. No differences were found between patients with mood disorders at follow-up with previous mood disorder episodes relative to patients with no history of mood disorders (all PUFAs $p>0.14$). Sensitivity analyses excluding participants who converted to psychosis (n=20) showed that EPA ($p=0.009$) and the sum of ω-3 PUFAs ($p=0.04$) remained significantly lower in individuals with mood disorder compared to individuals without mood disorder, while linolenic acid (18:2n-6), γ-linolenic acid (18:3n-6), docosadienoic acid (22:2n-6), DHA (22:6n-3), and the ω-6/3 PUFA ratio consistently remained at trend level. To exclude the possibility of selection bias, we also tested for differences in PUFA concentrations between participants with and without follow-up data and found that their membrane PUFA profiles did not differ (all PUFAs $p>0.277$).

5.3.3 Regression analysis of PUFAs for mood disorder

Binary logistic regression analysis with ω-6/3 PUFA ratio as the independent variable demonstrated that a higher basal ω-6/3 PUFA ratio predicted mood disorder over the 7-year follow-up period (Table 5.3). The effect remained significant when we adjusted the model for gender, age, baseline MADRS score and ω-3/placebo group status. This relationship appeared to be driven by a lower sum of ω-3 PUFAs, which was independently predictive of mood disorder diagnosis. A series of logistic regression analyses revealed that linolenic acid (18:2n-6), γ-linolenic acid (18:3n-6), docosadienoic acid (22:2n-6), EPA (20:5n-3), and DHA (22:6n-3) individually predicted mood disorders (Table 3). Sensitivity analyses excluding participants who converted to a psychotic disorder (n=20) showed that EPA (20:5n-3), DHA (22:6n-3) (trend level),

and the ω-6/3 PUFA ratio (trend level), remained predictive of mood disorders and were thus consistent with our main findings. The significant findings also persisted when the two participants with a Bipolar II diagnosis at follow-up were excluded.

Table 5.2 Comparison of erythrocyte membrane phosphatidylethanolamine lipid levels at baseline in patients with mood disorder, psychosis, anxity disorder or other psychiatric disorders after 7 years.

	Mood vs. no mood (n=26 vs. n=43)		Psychosis vs. no psychosis (n=20 vs. n=49)		Anxiety vs. no anxiety (n=19 vs. n=50)		Other vs. no other (n=11 vs. n=58)	
	Effect size (Cohen's d)	p-value	Effect size (Cohen's d)	p-value	Effect size (Cohen's d)	p-value	Effect size (Cohen's d)	p-value
LC ω -6 Fatty Acids								
Linolenic acid (18:2n-6)[a]	-0.553	0.035	0.139	0.543	0.003	0.990	-0.178	0.610
γ-Linolenic acid (18:3n-6)	0.582	0.023	-0.172	0.509	0.260	0.346	0.161	0.603
Dihomo-γ-linolenic acid (20:3n-6)	0.299	0.229	-0.114	0.683	-0.127	0.645	0.264	0.458
Arachidonic acid (20:4n-6)	-0.191	0.447	-0.316	0.213	-0.211	0.410	-0.275	0.371
Docosadienoic acid (22:2n-6)	0.612	0.017	-0.213	0.420	0.015	0.954	-0.188	0.579
Adrenic acid (22:4n-6)	0.243	0.327	-0.242	0.344	-0.148	0.592	-0.014	0.965
LC ω -3 Fatty Acids								
a-Linolenic acid (18:3n-3)[a]	-0.073	0.775	0.106	0.696	-0.307	0.272	0.157	0.650
Eicosapentaenoic acid (20:5n-3)[a]	-0.729	0.003	-0.134	0.612	-0.467	0.077	-0.233	0.497
Docosapentaenoic acid (22:5n-3)	-0.443	0.076	0.018	0.942	-0.134	0.616	-0.058	0.877
Docosahexaenoic acid (22:6n-3)	-0.618	0.010	-0.092	0.749	-0.486	0.090	-0.625	0.087
Sums and Ratios								
Sum of LC ω-6 fatty acids[b]	-0.199	0.413	-0.237	0.349	-0.149	0.565	-0.235	0.541
Sum of LC ω -3 fatty acids[b]	-0.699	0.004	-0.069	0.911	-0.436	0.111	-0.472	0.197
LC ω -6 to LC ω -3 fatty acids ratio	0.610	0.012	0.008	0.975	0.278	0.335	0.283	0.435

[a]Log transformed; [b]Sum comprises long-chain fatty acids ≥20 carbon atoms

Table 5.3 Odds ratios for mood disorder at 7 years follow-up for erythrocyte membrane phosphatidylethanolamine lipid levels at baseline in patients with mood disorders.

	Unadjusted		Model 1[a]		Model 2[b]	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
ω -6 Fatty Acids						
Linolenic acid (18:2n-6)	0.324 (0.111 – 0.942)	0.038	0.254 (0.078 - 0.827)	0.023	0.287 (0.086 – 0.950)	0.041
r-Linolenic acid (18:3n-6)	1.835 (1.069 – 3.150)	0.028	1.881 (1.083 – 3.268)	0.025	1.800 (1.028 – 3.150)	0.040
Docosadienoic acid (22:2n-6)	1.856 (1.095 – 3.145)	0.022	1.810 (1.051 – 3.117)	0.033	1.776 (1.029 – 3.065)	0.039
ω -3 Fatty Acids						
Eicosapentaenoic acid (20:5n-3)	0.383 (0.185 – 0.793)	0.010	0.372 (0.178 – 0.781)	0.009	0.390 (0.172 – 0.883)	0.024
Docosahexaenoic acid (22:6n-3)	0.506 (0.276 – 0.928)	0.028	0.517 (0.279 – 0.960)	0.037	0.488 (0.261 – 0.913)	0.025
Sums and Ratios						
Sum of ω -3 fatty acids	0.453 (0.236 – 0.869)	0.017	0.446 (0.228 – 0.874)	0.019	0.436 (0.200 – 0.950)	0.037
ω -6 to ω -3 fatty acids ratio	1.894 (1.075 – 3.338)	0.027	1.843 (1.035 – 3.284)	0.038	1.815 (1.024 – 3.289)	0.041

[a] Adjusted for age, sex and smoking at baseline; [b] Adjusted for age, sex, smoking, MADRS score and ω -3 PUFA/placebo group status at baseline

5.3.4 Effect modification by gender

We found a gender difference in docosapentaenoic acid (22:5n-3), such that females had lower levels compared to males (2.09±0.42 vs. 2.34±0.39, p=0.01). However, gender did not affect the relationship between any of the PUFAs and mood disorder in the logistic regression models (all p>0.48).

5.4 Discussion

In the present study we tested the hypothesis that ω-6/3 PUFA ratio is a risk biomarker [26] for mood disorders and found that a higher ω-6/3 PUFA ratio in erythrocyte membranes predicts incident mood disorders in young people exhibiting an UHR phenotype. This predictive capacity of ω-6/3 PUFA ratio was specific for mood disorders in this cohort, i.e., the ω-6/3 PUFA ratio did not influence the risk of developing any other psychiatric disorder and persisted after adjusting our model for several confounders, including age, gender, smoking, baseline depressive symptoms, and ω-3 supplementation. Cross-sectional studies suggest that people with depression have higher ω-6/3 PUFA ratios compared to healthy controls, characterized by overall lower ω-3 levels, but normal n-6 levels [10]. These observations are in line with the hypothesis that the changes in diet over the past 150 years may have caused an increase in the incidence of depression through increased ω-6/3 PUFA ratios. To the best of our knowledge, this study is the first to test the hypothesis that higher ω-6/3 PUFA ratios posit a risk for future depression in a longitudinal design with a long (7-year median) follow-up in at-risk individuals.

Ω-6 PUFAs, ω-3 PUFAs, and the balance between the two are important to maintain physiological membrane properties [27]. The n-3 PUFA content in the lipid bilayer of mammalian cells determines the physiological functions of the cellular membrane, including membrane fluidity and the function of ion channels and membrane receptors [27, 28]. As such, ω-3 PUFAs are thought to interact with several pathophysiological mechanisms in depression, including serotonergic neurotransmission [29] and decreased neurogenesis [30]. Additionally, depression is characterized by oxidative stress, which preferentially impacts lipids, increasing the turnover of, and hence the demand for, critical lipids, thus aggravating this imbalance [31]. Data from rodent studies show that dietary restriction of DHA, for instance, appears to predominantly affect DHA levels in the grey matter of cortical areas, the hippocampus, and the striatum [32, 33], and

conversely, supplementation of DHA may have a neuroprotective effect on these brain areas [34].

A noteworthy finding is that individuals with a mood disorder during the 7-year follow-up period had significantly lower EPA and DHA levels at baseline, and these two n-3 PUFAs individually predicted mood disorder in our logistic regression models. This finding is in line with evidence from studies showing that EPA and DHA in particular are decreased in patients with depression [10]. DHA is highly abundant in the human brain and constitutes up to 40% of the total brain FAs, while EPA accounts only for 1% [35]. EPA and DHA are believed to be the main active ingredients in n-3 PUFA supplementation, and importantly, meta-analysis suggests a dose-response relationship between EPA content and clinical efficacy and only supplements with a high EPA content appear to be effective in patients with depression [12, 16].

Converging evidence from recent proteomic and transcriptomic studies suggest a modulatory effect of ω-3 PUFAs on immune function, including downregulation of transcripts for IL-1beta, IL-6, and TNF-α, and the NFkappaB pathway [36, 37]. Consequently, low levels of ω-3 PUFAs are thought to be associated with a pro-inflammatory phenotype [3]. This view is supported by population-based studies demonstrating that a high ω-6/3 PUFA ratio is associated with increased pro-inflammatory cytokines [38]. Data from pre-clinical studies show that a high ω-6/3 PUFA ratio predisposes mice to an excessive immune reaction when confronted with a lipopolysaccharide immune challenge [39]. Moreover, transgenic mice that express ω-3 PUFA desaturase, an enzyme that catalyses the conversion from ω-6 to ω-3 PUFAs that is not naturally expressed in mammalian cells, produce less pro-inflammatory cytokines IL-1β, IL-6, and TNF-α in response to a 24-hour lipopolysaccharide challenge, show a higher proportion of more neuroprotective MHC II-positive microglial cells, and are protected from spatial memory deficits [40]. One clinical study demonstrated that a high ω-6/3 PUFA ratio markedly increased production of IL-6 and TNF-α and patients with depressive symptoms [41]. The relevance of these findings is supported by a recent study demonstrating that only patients with depression and high levels of several pro-inflammatory cytokines respond to ω-3 PUFA supplementation, while those patients with low inflammation do not respond [42]. Collectively, these data suggest that the high ω-6/3 PUFA ratio and the low levels of EPA and DHA found in our study may exert their risk for depression partially through immune mechanisms. Mood disorders were the most common psychiatric diagnosis at follow-up in the Vienna Omega-3 study [43] and in other samples of individuals at-risk for psychosis [44],

affecting up to 42% of individuals who present with an UHR phenotype. Recent research on the frequency of the UHR phenotype in the general population also demonstrated a prevalence of 1.3% and 9.9% for any UHR symptom [45]. Furthermore, based on cumulative findings from long-term follow-up studies, the UHR concept has become more explicitly trans-diagnostic or pluripotent (43). This highlights the importance of studying UHR individuals in regard to mood disorder outcomes. Unlike general population samples [20, 21], UHR individuals represent a viable target group for risk stratification using risk biomarkers [26], which adds clinical relevance to our findings. However, the ω-3 PUFA intervention raises the question as to whether it had an influence on psychiatric outcomes or if it altered the relationship between membrane PUFAs and psychopathology. We have previously shown that the ω-3 PUFA intervention, while effective in preventing psychosis, did not reduce the risk for mood disorders or MADRS scores [43]. Patients who received ω-3 PUFA and patients who received placebo did not differ in their PUFA profiles at baseline [22]. Finally, adjusting our regression models for treatment group did not change the main result. We thus believe that the 12-week intervention does not have implications for the interpretation of the findings of the present study.

Several limitations need to be considered when interpreting the findings of this study. Most importantly, the participants in this study were recruited at a specialized service for early psychosis according to UHR criteria, and thus represent a specific group of young help-seeking individuals. The results of this study might not generalize beyond this setting. Secondly, the relatively small sample size increases the risk of missing smaller effects in some of the variables and warrants replication of our findings. Thirdly, we did not measure dietary intake of ω-3 PUFA during the trial and we thus cannot rule out the possibility that the differences in membrane PUFA composition at baseline in participants who later developed a mood disorder were caused by dietary intake. Strengths of the study include the long follow-up period of 7 years, the use of standardized assessment instruments, the robustness of the findings in the sensitivity analysis, and finally the consecutive sample of UHR individuals allowed a comparison group that consisted of individuals with a variety of psychiatric diagnoses and thus showed that our findings are specific for mood disorders. Additionally, the finding of reduced EPA and DHA levels in individuals who later developed a mood disorder adds face and biological validity, as both of these PUFAs have been implicated in the pathophysiology of depression. Our findings are clinically relevant since mood disorders are the most frequent psychiatric condition observed in UHR patients. Together with meta-analytic evidence showing the efficacy of supplementation with

EPA-predominant formulations in depression, our findings provide a rationale for longer-term n-3 supplementation in UHR individuals.

In conclusion, our results highlight the importance of lipid biology for the identification of risk biomarkers among individuals with UHR states by demonstrating a longitudinal association between membrane PUFA levels and mood disorders 7 years later. These findings support the hypothesis that higher levels of ω-6 PUFAs and lower levels of n-3 PUFAs, possibly due to long-term changes in dietary intake or differential usage, pose a risk for depression. In the context of young people at risk for mental disorders, these findings may have important implications for risk stratification beyond clinical characteristics.

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Conflict of interest

Michael Berk has received grant/research support from the NIH, Cooperative Research Centre, Simons Autism Foundation, Cancer Council of Victoria, Stanley Medical Research Foundation, MBF, NHMRC, Beyond Blue, Rotary Health, Geelong Medical Research Foundation, Bristol Myers Squibb, Eli Lilly, Glaxo SmithKline, Meat and Livestock Board, Organon, Novartis, Mayne Pharma, Servier, and Woolworths, and has been a speaker for Astra Zeneca, Bristol Myers Squibb, Eli Lilly, Glaxo SmithKline, Janssen Cilag, Lundbeck, Merck, Pfizer, Sanofi Synthelabo, Servier, Solvay, and Wyeth, and served as a consultant to Allergan, Astra Zeneca, Bioadvantex, Bionomics, Collaborative Medicinal Development, Eli Lilly, Glaxo

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Part 2

6 Hair cortisol, allostatic load, and depressive symptoms in Australian Aboriginal and Torres Strait Islander people

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Preface

Aboriginal and Torres Strait Islander Australians and other First Nations people are often confronted with social challenges that can affect their mental health. The last two decades have seen a growing body of research supporting the notion that complex adversity converges on stress. Over time, chronic exposure to stress leads to altered release patterns of glucocorticoids and altered cross-talk between the HPA-axis and the immune system. While a main focus of the literature has been on social determinants and health and relationships between stress mediators and health have been examined in North American Indigenous and ethnic minority groups, biological stress mechanisms have not been investigated in the Australian context. Therefore, there is a strong need to explore the role of cortisol, immune activation and allostatic load among Aboriginal and Torres Strait Islander Australians, who are at two-fold higher risk of experiencing depression at population level.

Chapter 7 is based on a cross-sectional community-based study of risk factors, biomarkers and depressive symptoms. This study was conducted as part of an annual health screening (Young Persons Check/Well Persons Health Check) in three Aboriginal and Torres Strait Islander communities in north Queensland in 2016. The aim of Chapter 7 was to examine associations between depressive symptoms and biomarkers of neuroendocrine dysregulation and immune activation.

Abstract

Chronic stress and adversity are associated with poor mental health and are thought to contribute to the existing mental health gap between Aboriginal and Torres Strait Islander people and other Australians. Hair cortisol and allostatic load are indices of sustained stress and may be mediators of the effects of stress on health. The aim of this study was to examine the relationship between hair cortisol, allostatic load and depressive symptoms. This cross-sectional study comprised 329 adolescent and adult participants recruited at two health screening programs operating in three communities in north Queensland. We measured hair cortisol and calculated an allostatic load index from 10 biomarkers. Depressive symptoms were assessed with a version of the Patient Health Questionnaire-9 adapted for Aboriginal and Torres Strait Islander people (aPHQ-9). We found differences in cortisol and allostatic load between the screening programs and communities, which were not explained by depressive symptoms. Overall aPHQ-9 scores were unrelated to hair cortisol ($p=0.246$ and $p=0.938$) and allostatic load ($p=0.295$ and $p=0.883$) when age, gender and smoking were taken into account. However, anhedonia and insomnia sub-scores were each significantly associated with allostatic load in one study site. Our present data did not demonstrate overall associations of stress biomarkers and multisystem dysregulation with depressive symptoms, which suggests that the relationship between cumulative stress and depression may be better explained by other factors in this population. The specific association between anhedonia and insomnia with allostatic load indicates that chronic multisystem dysregulation plays a role in these features of depression in this population.

6.1 Introduction

Chronic or repeated stress are among the best-established risk factors for depression and are thought to mediate in part the effects of social determinants and environmental adversity on mental health. For example, adverse events during childhood such as childhood trauma and socioeconomic disadvantage increase the vulnerability for mood disorders in adulthood [1-3]. Moreover, individuals with a history of childhood adversity are more likely to develop depression when confronted with severe stress than those who did not experience adverse events during childhood [4, 5]. The HPA axis is considered a key system in mediating the link between stress and depression through the excessive release of glucocorticoids [6]. Over time, sustained exposure to adversity and high levels of glucocorticoids is thought to adversely affect areas of the developing brain relevant to depression [7, 8]. The effects of stress may be particularly relevant in the context of mental health disparities affecting indigenous people across the globe, who are often confronted with social challenges that likely converge on stress and may contribute to existing health disparities [9, 10].

Australian Aboriginal and Torres Strait Islander people suffer from a multitude of diseases at higher rates compared to the non-Indigenous population, including common and severe mental disorders [11, 12]. While prevalence rate estimates vary, depression is thought to affect approximately 13-23% of Aboriginal and Torres Strait Islander people [13, 14], 1.5 – 2.3 times more than in the general population. Recently, Harriss and colleagues (Harriss et a. 2018, in press) noted high rates of depressive symptoms in a community based study of Aboriginal youth with 18% experiencing moderate or severe depressive symptoms while another study in the Torres Strait found comparatively low depression rates [15]. Similarly, self reported psychological distress is common, with approximately a third of Aboriginal and Torres Strait Islander adolescents and adults reporting high or very high levels of distress [11, 16] and hospitalisation rates for mental illnesses are approximately twice as high than in the non-Indigenous population [11]. The lasting effects of colonialisation and dispossession, including racial discrimination, trauma and other sequelae, contribute to these health inequalities, likely through chronic effects of stress [9, 10, 17]. We have recently shown that chronic self reported distress and discrimination are associated with an altered pattern of diurnal cortisol production in Aboriginal young adults and depression is associated with the biological response to acute stress [18]. Similar observations of altered stress processing have been made in other minority groups, suggesting that this mechanism

may be suited to explain health disparities in different contexts [19]. Consequently, stress hormones and other mediators of chronic exposure to stress might contribute to depression rates in high-risk groups.

Over time, stress and its biological mediators contribute to detrimental physiological processes known as allostasis, a concept that recognises the long-term consequences of stress and maladaptation [20]. Sustained allostatic states may lead to increased AL, characterised by immune, endocrine and metabolic dysregulation and indexed by a set of routinely used markers including glycosylated haemoglobin, cortisol, CRP, IL6, triglycerides, cholesterol and blood pressure [21, 22]. This set of markers has significant predictive value for adverse health outcomes later in life [23, 24] and together these markers appear to reflect metabolic changes and future risk better than individual risk biomarkers [22]. Several studies also support a link between elevated AL and depression [25-27], suggesting that heightened AL might contribute to depression risk. However, not all studies support this hypothesis [28]. Importantly, ethnic minority status might be a mediator in the link between inflammation/immune activation and depression [29]. Consequently, AL might be well suited to investigate the higher rates of depression in this population [9, 10].

The aim of the present study was to explore the relationship between hair cortisol as well as the AL index and depressive symptoms in three Indigenous communities. We hypothesised that higher levels of cortisol and elevated AL are associated with more severe depressive symptoms.

6.2 Methods

6.2.1 Setting and study design

The present study is based on two health screening programs delivered to Aboriginal and Torres Strait Islander communities in north Queensland and two Torres Strait islands by local health services in collaboration with James Cook University (JCU). The first program is known as the Well Persons Health Check (WPHC) and operates in the communities of Waiben and Mer Island. This program is part of the Zenedath Kes Health Partnership between the Torres and Cape Hospital and Health Service and JCU. The second program is the Young Persons Check (YPC), delivered by Gurriny Yealamucka Health Services Aboriginal Corporation (GYHSAC) and JCU in Yarrabah, a community situated 52km south-east of Cairns. Briefly, the aims of these programs are to provide screening for a range of chronic and communicable disease annually to

all eligible members of the communities. In 2016, a brief depression screening tool was added to the health assessments to estimate the prevalence of moderate to severe depressive symptoms in the communities and to identify participants to whom further mental health care should be offered. Using short screening tools in primary health care settings is aimed at improving the recognition of depression with subsequent stepped care for individuals with a need for further assessment or care [30, 31]. The project was endorsed by the GYHSAC Board of Directors, the TCHHS and the local Community Councils and was approved by the Human Research Ethics Committee of James Cook University (H6404) and the Far North Queensland Human Research Ethics Committee (HREC/16/QCH/70-1059).

6.2.2 Participants

Study participants were recruited from all individuals aged 15-24 years who attended the YPC in March/April 2016 and all individuals aged 15 years or older who attended the WPHC in Waiben and Mer Island in October/November/December 2016, representing approximately 54% (Yarrabah), 6.5% (Waiben) and 29.3% (Mer Island) of the local Aboriginal and/or Torres Strait Islander population in the age range targeted by the health checks, respectively. Participants were recruited via announcements in local media, posters and peer recruiting. For the present analysis, participants were considered eligible if they identified as Aboriginal and/or Torres Strait Islander, were aged 15-24 years (YPC) or 15 years or older (WPHC) and provided written informed consent or verbal consent (n=1) if written consent could not be obtained (participant and parent/guardian consent for participants aged 17 years or younger). In Yarrabah, 350 members of the community were initially approached and 122 completed the depression screening and consented to their participation in the present research. In Waiben and Mer Island, a total of 314 individuals were approached and 207 participants from Waiben and 100 from Mer Island consented and were included in the present analysis.

6.2.3 Data collection

After providing written informed consent, each participant was asked to undergo a series of questionnaires and assessments in addition to the routine health screening. Variables relevant to the present analysis included demographic information (gender, age), diet and other health behaviours (smoking), depressive symptoms, a hair sample for hair cortisol analysis, and blood samples for several routine and research blood tests.

Depressive symptoms were measured with the adapted Patient Health Questionnaire 9 (aPHQ-9), a screening instrument designed to measure depressive symptoms in primary care patients that has been adapted for Aboriginal people in central Australia [32] and is currently undergoing validation [33]. The aPHQ-9 measures depressive symptoms as a score, ranging from 0 (absence of depressive symptoms) to 27 (severe depressive symptoms). A score of 10 or higher in the original PHQ-9 had a sensitivity of 88% and specificity of 88% for a DSM-IV diagnosis of major depression in a validation study of over 6000 primary care patients [34]. This cut-off has been adapted for the present study to distinguish between 'no/mild' and 'moderate/severe' depressive symptoms.

6.2.4 Biomarker Assessment/Allostatic Load

Cortisol was analysed from 3cm hair sections, which were cut as closely to the scalp as possible from the posterior vertex region to assess cumulative cortisol concentrations of the past three months. Sample preparation and analysis was based on the protocol published by Stalder et al. [35] with minor modifications. Briefly, the hair samples were washed 3 times for 3 minutes with 2.5ml isopropanol and left to dry for 12 hrs. The hair samples were then weighed, frozen and subsequently ground in a bead beater with 3.2mm zirconium oxide beads in stainless steel micro vials for 2 minutes. The samples were then incubated with 1.5ml methanol at room temperature for 24 hrs prior to centrifugation at 10000rpm for 2 mins. One ml supernatant was transferred into a new micro vial and evaporated under a constant stream of nitrogen at 50C until completely dry. Then, 0.4ml phosphate-buffered saline was added, the samples were vortexed for 15 s and stored at -20C before being analysed with a commercial immunoassay (Salimetrics, Carlsbad, CA, USA). Inter- and intra-assay coefficients were below 3% for all assays. The analysis was carried out at our laboratory (James Cook University).

Cytokines were analysed using a Procarta 8-plex panel (Procarta Biosystems, Norwich, UK) and CRP was measured with a high-sensitivity assay (Procarta Biosystems, Norwich, UK) at JCU. Glucose, triglycerides, lipoproteins and cholesterol were analysed at a commercial pathology service (Sullivan Nicolaides, Cairns, Australia). Biomarkers for the AL index were selected based on (i) representation of several physiological systems including, neuroendocrine, immune, metabolic and cardiovascular parameters, (ii) use in previous AL research [21, 25], and (iii) associations

with disease risk. Cardiovascular markers included heart rate, systolic blood pressure and diastolic blood pressure. Neuroendocrine markers included hair cortisol. Immune markers were IL-6, TNFα and CRP. Metabolic markers included triglycerides, high-density lipoprotein, low-density lipoprotein, total cholesterol, glucose, HbA1c and BMI.

For the computation of the AL index, the risk cut-off for each individual biomarker was determined based on clinical reference ranges where available and based 75th percentile as determined based on the distribution in each study sample for all other markers. We then used a 'scaling' approach previously described by our group and others to calculate the AL index [36, 37]. Briefly, for each individual, every marker with values above the cut-off (below for HDL) was defined as '1', and the sum of all markers in each category (cardiovascular, immune, neuroendocrine, metabolic) was divided by the number of markers in each category to allow for equal weighting of the four categories. For those markers where the distribution differed between male and female controls, gender-specific cut-offs were calculated and used to compute the index [38].

6.3 Statistical Analysis

Prior to statistical analysis, the distribution of the data was assessed for normality assumptions and outliers. Normality of the data was ascertained by assessing the skewness of the data distribution and the associated standard errors. A range of <3 was used as the cut-off for the use of parametric tests. Variables with greater skewness (cortisol) were log-transformed. Cross-sectional differences in demographic characteristics, biological variables and depression scores were tested with independent-sample t-tests and rank-sum tests for parametric and non-parametric continuous data, and with Chi2 tests for categorical data. aPHQ-9 scores were dichotomised using a cut-off of 10 and the strata moderate/severe and no/mild depressive symptoms were created to group study participants for subsequent analyses. To test the association of biomarker levels and allostatic load with depressive symptoms, logistic regression models and ordered logistic regression models using hair cortisol and allostatic load as independent variables and depressive symptoms as dependent variable were fitted. All regression models were adjusted for potential confounding variables including age, gender and smoking, which correlated with the AL index and are common covariates in the AL literature [22]. The Hosmer-Lemeshow test was used to test model calibration. All tests were 2-sided, a *p*-value of <0.05 was considered statistically significant. Ordered logistic regression models testing associations between independent variables and the individual items of the aPHQ-9 were adjusted for multiple comparisons with the Benjamini-Hochberg method. STATA 13.1 (Stata Corp., College Station, TX, USA) was used for all analyses.

6.4 Results

6.4.1 Demographic results

Descriptive results for demographic variables and PHQ-9 scores across the three sites are presented in Table 6.1. A total of 122 people participating in the YPC and 207 people participating in the WPHC were included in the present analysis. Twenty-two (18%) participants of the YPC had aPHQ-9 scores above the cut-off for probable depression compared to 19 (10.1%) in the WPHC. The mean age of YPC participants was 19.1 years for participants above the cut-off for probable depression and 19.4 years for those below. A larger proportion of participants of with YPC with probable depression were female, though this was not significant. Participants of the YPC above the cut-off were more likely to smoke although this did not reach statistical significance ($p=0.085$).

6.4.2 Hair cortisol

Hair cortisol levels were significantly higher among participants of the WPHC relative to participants of the YPC ($p<0.001$). Hair cortisol was correlated with age ($r=0.243$, $p<0.001$) but was not different between male and female participants of the YPC ($p=0.671$) or WPHC ($p=0.746$). Hair cortisol was not different between participants above and below the cut-off for depression in participants of the YPC ($p=0.914$) and the WPHC ($p=0.960$; Table 6.2).

6.4.3 Allostatic load

AL was significantly higher in participants of the WPHC compared to participants of the YPC ($p<0.001$). AL was correlated with age among participants of the WPHC ($r=0.276$, $p<0.001$) but not among participants of the YPC ($p=0.706$). AL was not different between male and female participants of the YPC ($p=0.847$) or WPHC ($p=0.377$). Of the AL biomarkers, heart rate was higher in participants above the cut-off for moderate

Table 6.1 Demographic characteristics and depression scores of 122 participants of the Young Persons Check (YPC) and 207 participants of the Well Persons Health Check (WPHC)

	YPC (n=122)				WPHC (n=207)			
	no/mild depressive symptoms (n=100)	moderate/severe depressive symptoms (n=22)	Total (n=122)	p-value	no/mild depressive symptoms (n=188)	moderate/severe depressive symptoms (n=19)	Total (n=207)	p-value
Age								
mean (SD)	19.44 (3.22)	19.18 (2.30)	19.39 (3.07)	0.722[1]	41.14 (17.22)	32.00 (10.68)	40.30 (16.92)	0.024[1]
Age group				N/A				0.075[2]
12-25 years	100	22	122		46	6	52	
26-45 years	0	0	0		67	11	68	
46-65 years	0	0	0		59	2	61	
66-85 years	0	0	0		16	0	16	
Gender				0.224[2]				0.788[3]
Female, n (%)	54	15	69		105	10	115	
Male, n (%)	46	7	53		83	9	82	
Smoking, dichotomized				0.085[3]				0.479[3]
Yes, n (%)	62	18	80		83	10	93	
Household size		s		N/A				0.557[2]
Number of people, median (IQR)	N/A	N/A			4 (2 – 6)	4.5 (3 – 8)	4 (2 – 6)	
Education								0.269[2]
Some primary	N/A	N/A			16	0	13	
Some secondary	N/A	N/A			55	5	60	
Completed secondary	N/A	N/A			60	6	66	
Completed tertiary	N/A	N/A			56	7	63	
BMI, mean (SD)	24.89 (6.60)	26.39 (8.21)	25.16 (6.91)	0.361[1]	31.73 (7.19)	29.65 (7.15)	31.54 (7.19)	0.229[1]
aPHQ-9								
Total score, mean (SD)	4.18 (2.82)	16 (4.34)	5.95 (4.91)	<0.001[1]	2.76 (2.56)	14.36 (3.93)	3.82 (4.31)	<0.001[1]
Total score, median (IQR)	4 (2 – 7)	12.5 (10 – 16)	5 (2 – 9)	<0.001[2]	2 (0 – 4)	14 (11 – 15)	3 (1 – 5)	<0.001[2]

[1] Independent samples t-test, [2] Mann-Whitney-U-Test [,3] Chi2-Test, aPHQ-9=adapted Patient Health Questionnaire-9, BMI=body mass index, IQR=interquartile range, SD=standard deviation

Table 6.2 Allostatic load biomarkers of 122 participants of the Young Persons Check (YPC) and 207 participants of the Well Persons Health Check (WPHC)

	YPC (n=122)				WPHC (n=207)			
	no/mild depressive symptoms (n=100)	moderate/severe depressive symptoms (n=22)	Total (n=122)	p-value	no/mild depressive symptoms (n=188)	moderate/severe depressive symptoms (n=19)	Total (n=207)	p-value
Heart rate (bpm)	79.16 (13.52)	85.77 (17.62)	80.35 (14.49)	0.052	71.61 (11.60)	70.42 (10.05)	71.50 (11.45)	0.666
Systolic blood pressure (mmHg)	117.26 (12.60)	118.27 (12.37)	117.44 (12.51)	0.732	124.55 (18.01)	122.05 (19.70)	124.32 (18.14)	0.567
Diastolic blood pressure (mmHg)	74.03 (7.76)	75.27 (10.78)	74.25 (8.35)	0.529	78.10 (12.47)	79.73 (10.96)	78.25 (12.32)	0.584
Hair cortisol (ng/mg)	7.79 (9.61)	7.91 (7.40)	7.81 (9.26)	0.963	14.04 (28.48)	15.74 (21.84)	14.21 (27.83)	0.818
CRP (µg/ml), mean (SD)	0.56 (0.54)	0.44 (0.29)	0.54 (0.51)	0.355	0.97 (0.89)	0.58 (0.44)	0.94 (0.87)	0.059
IL-6 (pg/ml), mean (SD)	9.18 (10.36)	6.86 (4.01)	8.79 (9.62)	0.339	4.25 (6.53)	3.70 (2.08)	4.20 (6.25)	0.716
TNFa (pg/ml), mean (SD)	1.31 (2.16)	1.13 (0.65)	1.28 (1.99)	0.711	0.61 (2.12)	0.41 (0.79)	0.59 (2.03)	0.680
Glucose (mmol/L)	4.95 (1.09)	4.61 (0.62)	4.89 (1.02)	0.181	6.21 (3.34)	5.18 (2.26)	6.10 (3.26)	0.194
HbA1c (%)	5.21 (0.69)	5.11 (0.20)	5.19 (0.63)	0.509	6.08 (1.51)	5.75 (1.59)	6.05 (1.52)	0.364
Triglycerides (mmol/L), mean (SD)	1.60 (1.33)	1.67 (0.84)	1.61 (1.25)	0.803	1.97 (1.08)	2.21 (2.44)	1.99 (1.28)	0.448
Total cholesterol (mmol/L), mean (SD)	4.13 (0.85)	4.19 (1.11)	4.14 (0.90)	0.761	4.74 (1.06)	4.92 (4.40)	4.76 (1.06)	0.497
HDL (mmol/L), mean (SD)	1.08 (0.24)	1.04 (0.26)	1.07 (0.24)	0.473	1.17 (0.36)	1.16 (0.27)	1.17 (0.35)	0.927
LDL (mmol/L), mean (SD)	2.33 (0.65)	2.38 (0.95)	2.34 (0.71)	0.771	2.66 (0.86)	2.86 (0.75)	2.68 (0.85)	0.351
Allostatic load index	2.52 (1.95)	2.74 (1.62)	2.56 (1.89)	0.628	3.42 (1.95)	2.80 (1.84)	3.36 (1.94)	0.185

[1] Independent samples t-test, CRP=c-reactive protein, HbA1c=glycosylated haemoglobin, HDL=high density lipoprotein, LDL=low density lipoprotein, IL-6=interleukin-6, SD=standard deviation

to severe depressive symptoms in the YPC at trend level (p=0.052) but not in the WPHC. CRP was lower among participants above the cut-off in the WPHC at trend level (p=0.059). Levels of the other AL biomarkers did not differ between participants above or below the cut-off in the YPC (all p>0.473) and the WPHC (all p>0.185).

6.4.4 Associations between biomarkers/AL and depressive symptoms

Adjusted for age, gender and smoking, hair cortisol was not associated with increased odds for moderate/severe depressive symptoms in the YPC (p=0.160) or WPHC (p=0.779; Table 6.3). Similarly, AL was not associated with moderate/severe depressive symptoms in the YPC (p=0.778) or WPHC (p=0.883).

Next, we investigated if AL and hair cortisol are associated with individual sub-domains of the aPHQ-9. Adjusted for age, gender and smoking, AL was significantly associated with item 1 (anhedonia) of the aPHQ-9 in participants of the WPHC (adj. Beta=0.312 (95%CI=0.085 – 0.539), p<0.007) and with item 3 (insomnia) in Yarrabah (adj. Beta=0.312 (95%CI=0.085 – 0.539), p<0.007; Figure 6.1). In participants of the YPC, a trend-level association with item 8 (psychomotor retardation) was found (adj. Beta=0.301 (95%CI=-0.003 – 0.607), p=0.053). No associations were found of cortisol with any of the sub-domains.

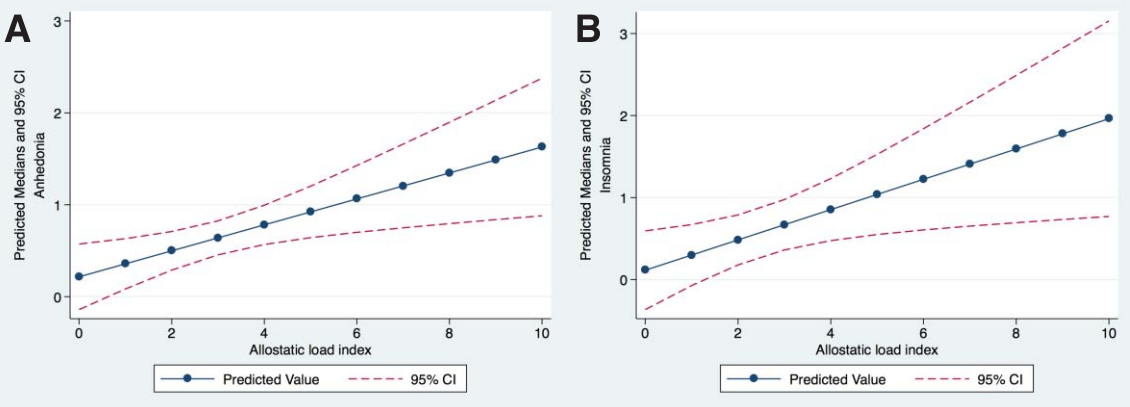

Figure 6.1 Ordered logistic regression analysis of allostatic load and anhedonia in participants of the Well Persons Health Check (WPHC) (A) and of allostatic load and insomnia in participants of the Young Persons Check (YPC) (B). Regression analyses are adjusted for age, gender and smoking.

6.5 Discussion

The aim of the present study was to explore associations of hair cortisol and AL with depressive symptoms in two cohorts of Aboriginal and Torres Strait Islander people taking part in an annual community health check. Contrary to our hypothesis, no association was seen between hair cortisol levels, AL and aPHQ-9 scores. However,

Table 6.3 Logistic regression analysis for moderate/severe depressive symptoms in 122 participants of the Young Persons Check (YPC) and 207 participants of the Well Persons Health Check (WPHC)

	Unadjusted		Model 1[a]		Model 2[b]	
	OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
All participants (n=329)						
Hair cortisol[c]	0.93 (0.66 – 1.30)	0.699	1.31 (0.80 – 2.13)	0.278	1.10 (0.54 – 2.24)	0.779
Allostatic load	0.91 (0.76 – 1.08)	0.296	0.98 (0.82 – 1.18)	0.912	1.06 (0.82 – 1.37)	0.624
Age	0.96 (0.93 – 0.98)	0.004				
Gender	1.26 (0.64 – 2.47)	0.487				
Smoking	1.00 (0.96 – 1.05)	0.685				
YPC (n=122)						
Hair cortisol[c]	0.97 (0.56 – 1.65)	0.913	1.57 (0.77 – 3.21)	0.210	1.74 (0.80 – 3.78)	0.160
Allostatic load	1.06 (0.83 – 1.34)	0.625	1.16 (0.87 – 1.54)	0.295	1.04 (0.74 – 1.48)	0.788
Age	0.97 (0.83 – 1.13)	0.720				
Gender	1.82 (0.68 – 4.86)	0.229				
Smoking	0.99 (0.92 – 1.07)	0.892				
WPHC (n=207)						
Hair cortisol[c]	0.98 (0.62 – 1.57)	0.960	1.05 (0.64 – 1.72)	0.829	1.10 (0.54 – 2.24)	0.779
Allostatic load	0.83 (0.63 – 1.09)	0.187	0.90 (0.68 – 1.19)	0.492	1.02 (0.69 – 1.52)	0.883
Age	0.96 (0.93 – 0.99)	0.029				
Gender	0.87 (0.34 – 2.26)	0.788				
Smoking	1.04 (0.97 – 1.11)	0.207				

[a]Adjusted for age and gender, [b] adjusted for age, gender, and smoking, [c] log transformed

anhedonia and insomnia were each significantly positively correlated with AL in one of the study sites. Cortisol measured from hair was unrelated to depressive symptoms in our study. Cortisol is believed to be relevant to the pathological mechanisms seen in depression for several reasons. For example, a plethora of studies provided evidence for altered cortisol levels in patients with MDD, although the findings are inconsistent. One of the largest studies to date to address this question found a significant but modest elevation of cortisol in patients with MDD irrespective of symptom severity [45]. Other evidence includes findings of down regulation of glucocorticoid receptors, believed to be secondary to chronic hypercortisolism, and decreased glucocorticoid receptor function [46]. While some studies found hair cortisol to be higher in patients with depression [47], more recent studies investigating hair cortisol do not support consistently elevated levels in patients with depression [48], including in children and adolescents [49-51]. However, there is evidence for higher hair cortisol in individuals experiencing chronic stress [48] and low socio economic status [52], suggesting that hair cortisol levels are related to key risk factors for depression.

An important observation was that cortisol was higher in the participants of the WPHC compared to those of the YPC. Clearly, age is a potential confounder in addition to other systematic differences between the communities [48]. The relationship between age and hair cortisol is heterogeneous though, particularly in adolescents [53], and age was not correlated with cortisol levels in our study. In addition, BMI is known to be positively correlated with hair cortisol and might explain differences in hair cortisol between the screening programs [48, 53].

To our knowledge, this is the first investigation of AL in relation to a mental health outcome in an Aboriginal and Torres Strait Islander population and one of few studies to investigate a potential biological mediator of the effects of stress on mental health. AL is commonly conceptualised as a multisystem indicator of the cumulative effects of chronic stress and adversity. In a population that has both high rates of mental ill-health including depression and suicide and a high prevalence of trauma [39], biological stress mediators and AL may contribute to sustain health inequalities. Populations living under high levels of stress – in particular children - are at increased risk for poor mental health and are consequently a priority in mental health research [40]. Our data support a relationship between AL and two features of depression in this group. Previous studies in other populations have provided mixed results concerning the relationship between AL and depression. Juster and colleagues [25] conducted a follow-up study in 58 adults (mean age = 67 years) and observed prospective associations of

increased AL with depressive symptoms in the same year and three years later, although the latter was reduced to a trend when sex and age were included as covariates. Similarly, another study reported associations of depressive symptoms with a multisystem index similar to the AL used here in a Taiwanese cohort (n=958) [41]. In contrast, a recent large (n=12272) cross-sectional study investigated AL and depressive symptoms in an ethnically diverse sample and found no significant associations irrespective of ethnicity [28]. A question raised by these reports is if AL is relevant only in older populations. However, our data neither support a role for AL in older adult or adolescent participants.

The association of AL with anhedonia and sleep disturbances may suggest that AL is selectively related to specific aspects of depression, in particular motivation, psychomotor drive and sleep. Recently, data from the National Health and Nutrition Survey (NHANES) demonstrated selective and robust associations of elevated levels of CRP with fatigue, sleep disturbance and altered appetite [29]. There is preclinical and clinical evidence to suggest that inflammation and oxidative stress – as seen in allostatic states – impair motivation through effects on striatal dopaminergic reward processing [42]. Two recent studies support that peripheral inflammation is selectively associated with anhaedonia in adult patients with depression [43] and across diagnostic categories in young people with psychiatric disorders [44]. Additionally, the link between immune activation and depressive symptoms was missing in some but not all ethnic groups in this study, suggesting that ethnicity may be a moderator of the hypothesised effects of inflammation on depression [29]. Together, these data provide support for the notion that AL may be related to deficits in motivation, psychomotor drive and sleep disturbance.

There are several limitations to this study. First, our study was cross-sectional and inferences cannot be made about the temporal dynamic of hair cortisol or AL and depressive symptoms. Second, several health behaviours and social determinants relevant to AL have not been taken into account comprehensively. Our study was thus unable to investigate which factors determine AL. Third, while AL is conceptualised as the biological traces or chronic stress, perceived stress itself was not assessed. Finally, it is possible that severely depressed individuals were less likely to attend the health check and underreporting of depression may consequently affect our results. The study setting in a community health check setting may also imply a perceived lack of privacy and some study participants may have perceived this as a barrier to disclosing depressive symptoms due to the perceived associated stigma. The study

sample may thus not be representative of the wider community. Strengths of the study include the large sample, the diverse age range and the use of hair cortisol, which is not sensitive to the diurnal variation and sampling bias that can be problematic when measuring cortisol from saliva or plasma.

These shortcomings highlight the need for future research in several domains. For example, it seems necessary to elucidate to what extent the previously found role of AL in depression depends on illness severity, study setting (population-based vs. clinical) or ethnicity. Similarly, inconsistencies in the operationalisation of the AL index open up the possibility that different findings are attributable to the weighting of the AL markers. Finally, conditions characterised by chronic low-grade inflammation such as obesity or type-2-diabetes mellitus are more common in patients with depression and this relationship may be partially attributable to allostatic mechanisms.

In conclusion, our findings add to the few studies on the relationship between hair cortisol, AL and depressive symptoms in a population affected by considerable social and environmental stressors and putatively higher rates of depression. The relationship between these factors and anhedonia and psychomotor drive may indicate that chronic multisystem dysregulation plays a role in specific features of depression in this population.

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Conflict of Interest

The authors report no conflict of interest in relation to this study.

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7 Cross-sectional association of seafood consumption, polyunsaturated fatty acids and depressive symptoms in two communities in the Torres Strait.

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Preface

Besides supplementation and increased depletion, dietary intake is the main determinant of membrane ω-3 PUFA concentrations. Fatty fish and seafood are the main sources of the essential ω-3 PUFA EPA, which has been shown to be the key PUFA responsible for the antidepressive effect in RCTs. Consequently, a diet rich in natural EPA from marine sources may have protective effects against mood disorders. Two decades ago, Hibbeln first showed in a cross-national comparison that the average fish consumption correlated with the prevalence of depression. This observation has since been replicated in several western societies, but little research has focused on populations with a traditional fish-based diet and high rates of distress and few studies measured membrane levels of ω-3 PUFAs. The Torres Strait Islander people of Australia have and continue to rely on a traditional diet characterised by a high content of wild caught fish. Recent reports of comparatively low rates of depression in this population as well as the association of low ω-3 PUFA levels and depressive symptoms observed in Chapter 5 and other studies form the rationale for Chapter 7.

Chapter 7 reports a cross-sectional analysis examining associations between diet, membrane PUFAs and depressive symptoms in two communities in the Torres Strait with differential access to seafood as well as processed food from fast-food outlets. This study refers to the same sample as reported in Chapter 6; however, the study sample was restricted to two sites as levels of PUFAs were not measured in the third site. These two communities in the Torres Strait have differential access to seafood as well as processed food from fast-food outlets.

Abstract

Background Dietary intake of long-chain ω-3 PUFA represents a putative modifiable risk factor for depression, and a high ratio of ω-6 to ω-3 PUFA is frequently observed in patients with major depressive disorder. Recent reports suggest that the availability of fish and seafood may be associated with lower depression rates. The aim of this study was to investigate associations of fish consumption and PUFA levels with depressive symptoms.

Methods Participants for this cross-sectional study (n=206) were recruited at a community screening program in two Torres Strait Islander communities (Mer and Waiben). Depressive symptoms were assessed with the adapted Patient Health Questionnaire-9 (aPHQ-9) and diet with a structured questionnaire. PUFA concentrations were measured with a capillary dried blood spot system (PUFAcoat). Logistic and quantile regression modelling was used to test the relationship between seafood consumption, membrane PUFAs and depression scores.

Results A higher blood ω-6/3 PUFA ratio was associated with moderate/severe depression scores across both study sites (OR = 1.59 (95%CI 1.09 – 2.34), p=0.017). Seafood consumption was higher and the proportion of participants with aPHQ-9 scores above the cut-off for depression was lower on Mer (n=100) compared with Waiben (n=106). Higher seafood consumption was associated with lower depression scores on Waiben (B=-0.57 (95%CI -0.98 – -0.16), p=0.006) but not on Mer.

Conclusions Our findings support an association of ω-3 PUFA from natural sources with depressive symptoms. The availability of fresh seafood in the local diet may represent a protective factor for depression in this setting.

7.1 Introduction

The notion that dietary consumption of fish and seafood is linked to depression prevalence has sparked interest in the role of ω-3 PUFA in the aetiology of depression. ω-3 PUFA – fatty acids that have more than one double bond between carbon atoms with the first double bond at the third carbon atom – are key components of the cellular membrane and regulate cellular functions relevant to depression pathophysiology, including membrane fluidity, ion channel function and receptor signalling [1]. In addition, these PUFA are precursors of metabolites that are thought to have anti-inflammatory [2, 3] and anti-oxidant [4, 5] properties. Ω-6 PUFA in contrast are thought to be pro-inflammatory and are highly abundant in contemporary Western diets [6].

Fish and seafood are the most important sources of several essential ω-3 PUFA, in particular EPA and DHA. Low levels of these PUFA are a robust biological finding in patients with major depression [7-10]. An early study provided epidemiological evidence for a correlation between ω-3 PUFA intake through fish consumption and depression prevalence, giving rise to the hypothesis that dietary intake of ω-3 PUFA may protect against depression [11]. Importantly, contemporary Western diets are characterised by an abundance of ω-6 PUFA and their precursors and a relative lack of ω-3 PUFA[12]. Consequently, the ω-6/3 PUFA ratio is typically high in industrialised countries at 20:1, compared with the ω-6/3 PUFA ratio of people adhering to more traditional diets at 1:1. The high ω-6/3 PUFA ratio is believed to have resulted in increased production of pro-inflammatory eicosanoids, downstream metabolic products of ω-6 PUFA, which are thought to be partially responsible for the rising incidence in chronic disease associated with adherence to Western diets [12]. This hypothesis is supported by observational studies showing lower levels of ω-3 PUFA and a higher ω-6/3 PUFA ratio in patients with major depressive disorder as well as by a recent meta-analysis demonstrating lower risk for depression with higher fish-consumption [13]. Moreover, meta-analyses of interventional trials [14-17] of ω-3 PUFA in adult patients with depression show small but significant beneficial effects of ω-3 PUFA supplementation on depressive symptoms, which appear to be related to higher doses of EPA, and studies in youth are currently underway (ACTRN12613001352796). We have recently shown that a high ω-6/3 PUFA ratio represents a risk factor for mood disorders in a 7-year longitudinal study of youth with at-risk mental states [18]. Consequently, dietary

intake of ω-3 PUFA from fish, seafood and supplements may serve as a potential modifiable risk factor for depression.

Depression is among the largest contributors to the loss of Disability Adjusted Life Years, affecting approximately one in seven people at some point in their lives [19, 20]. The Aboriginal and Torres Strait Islander people of Australia are disproportionately affected by psychological distress and mental ill-heath compared with the general population, which contributes to the existing health gap [21, 22]. While national survey data indicate that Aboriginal and Torres Strait Islander people suffer from depression at higher rates than the general population [22], the prevalence varies between regions and communities and recent reports suggest lower rates in Torres Strait Islander communities [23]. Although these health disparities are attributed to a complex set of social and environmental health determinants, access to fresh food and seafood is problematic in many rural and remote areas and may affect mental health through the above mechanisms.

Data from non-clinical populations with varying levels of seafood consumption are scarce, and little is known about the role of ω-3 PUFA from traditional fish-based diets in Torres Strait Islander people. Consequently, the aim of the current study was to investigate association of ω-3 PUFA intake from fish and seafood, as well as takeaway food consumption, with PUFA levels and depressive symptoms in two Torres Strait Islander communities, with putatively low rates of depression. Based on the background and rationale above, we hypothesised (1) that differences in seafood and take-away food consumption would be reflected in the ratio between ω-6 to ω-3 PUFA, and (2) that there is an association between ω-6/3 PUFA ratio in blood and depressive symptoms.

7.2 Methods

7.2.1 Setting

Data for this study were collected at a community-based screening program (Well Persons Health Check) in two Australian island communities situated in the waters of the Torres Strait between the northern most tip of Queensland and the Western Province of Papua New Guinea. The Well Persons Health Check is a comprehensive health promotion and screening program conducted by the Torres and Cape Hospital and Health Service (TCHHS). In 2016, the Well Persons Health Check was conducted as a collaboration between the TCHHS and James Cook University (Zenadth Kes

Health Partnership). This model of preventative health care was first introduced in 1998 as a health screen for sexually transmitted and chronic cardiovascular and metabolic conditions and takes place annually in several communities in north Queensland [24]. The detailed methodology including the rationale for the health check is reported in [25, 26]. Participation in depression and suicidal ideation screening and use of health data for research purposes were optional and offered in addition to the usual health check.

Waiben Island is a 3.5 square kilometre landmass approximately 39 kilometres north of the Australian mainland. It is the main administrative centre in the Torres Strait region with an estimated population of 2,609, of which 64.5% (n=1,684) are Indigenous Torres Strait Islanders. Travel to the Australian mainland is by sea, or daily direct commercial flights. Mer Island is situated in the eastern Torres Strait region, 210km northeast of Waiben and 340km west of Port Moresby. Mer is approximately 4.3 square kilometres with a population of 365, of which 93.4% (n=341) are Torres Strait Islanders. Mer is accessible by light aircraft from Waiben. Commercial food supply is limited to one convenience store and there are no fast food outlets on Mer.
The study received written support from the local Community Council, Primary Heath Care Service and TCHHS. Ethical approval was granted by the Far North Queensland Human Research Ethics Committee (HREC/16/QCH/70-1059),

7.2.2 Participants

Study participants were recruited from all health check attendees who identified as Aboriginal and/or Torres Strait Islander and participated in October, November and December 2016. The health checks were promoted with posters, radio advertisements and word of mouth. Participants were aged 15 years and older and provided written informed consent (n=205) or verbal consent (n=1) if written informed consent could not be obtained. Participant and parent/legal guardian consent were obtained for participants aged <18 years. A total of 214 community members were screened; 106 participants from Waiben and 100 from Mer had satisfactory data and were included in the present analysis. The study sample represented approximately 6.5% and 29.3% of the local Aboriginal and/or Torres Strait Islander population, respectively.

7.2.3 Data collection

Diet, demographic and psychometric data

Depressive symptoms were measured using the adapted Patient Health Questionnaire (aPHQ-9), a version of the original PHQ-9 specifically adapted for use with Aboriginal people in central Australian communities that has been found to be culturally acceptable [27]. The aPHQ-9 is currently undergoing validation [28]. The aPHQ-9 measures depressive symptoms in the previous two weeks using a Likert scale, ranging from 0 (absence of depressive symptoms) to 27 (severe depressive symptoms). In a validation study of the original PHQ-9 with over 6000 people who presented for primary health care, a score of 10 or higher had a sensitivity of 88% and specificity of 88% for a DSM-IV diagnosis of major depression [29]. This cut-off has been adapted for the present study to distinguish between 'no/mild' and 'moderate/severe' depressive symptoms. Participants completed a food questionnaire as part of the study. Fish and seafood consumption and the intake of take-away food consumed in the week preceding their health check were assessed with non-leading questions. Self-reported seafood included fish, octopus, crayfish, crab, prawns, oysters and clams.

7.2.4 Fatty acid analysis

Whole blood was collected on a validated dried blood spot system and fatty acid composition was analysed by capillary gas chromatography [30]. Values for the following classes of PUFAs were obtained and included in the present analysis: alpha-Linolenic acid (18:3n-3), EPA (20:5n-3), Docosapentaenoic acid (22:5n-3), DHA (22:6n-3), Linolenic acid (18:2n-6), Gamma-Linolenic acid (18:3n-6), Eicosadienoic acid (20:2n-6), Dihomo-gamma-linolenic acid (20:3n-6), Arachidonic acid (20:4n-6), Docosadienoic acid (22:4n-6). The relative abundance (wt%) of these PUFAs was used for all analyses. We included the proportion of total ω-3 PUFAs, the proportion of total n-6 PUFA, the ratio between ω-6 and ω-3 PUFA, and EPA and DHA separately due to their relevance to depression pathophysiology. Lipids were analysed according to standard protocols at the Pathology Queensland Laboratory of Cairns Base Hospital.

7.2.5 Statistical Analysis

Raw scores from the aPHQ-9 and dichotomised scores according to the cut-off published by Kroenke, Spitzer [29] were used to measure depression severity. Kruskal-Wallis tests were performed to assess differences in depression frequency between age groups. Differences in continuous variables between sites and between participants above and below the threshold for depression were tested with t-tests and

with Mann-Whitney-U tests for variables that were not normally distributed. Normality of the data was ascertained by visual inspection of the distribution, the skewness and the associated standard errors. Normal distribution of the data was assumed if skewness divided by the standard error of skewness was between -3 and +3.
To test whether dietary seafood intake was associated with blood PUFA composition, we used linear regression modelling with seafood consumption as the independent variable and the above PUFA parameters as dependent variables. To test the relationship between seafood intake, blood fatty acids and probable depression across both study sites and for each site separately, we fitted logistic regression models using dietary seafood intake, PUFA levels and their summary scores and ratios as independent variables, and probable depression, dichotomised using a cut-off of 10 on the aPHQ-9, as the dependent variable. As the number of individuals above the cut-off for depression was small, we also fitted quantile regression models using the same variables and raw aPHQ-9 scores as the dependent variable. Quantile regression was chosen as the dependent variable and the residuals for this dependent variable were not normally distributed in linear regression models. To estimate 95% confidence intervals for the quantile regression models, bootstrap resampling was used with 1000 repetitions. Effect sizes are reported as odds ratios (OR) and beta coefficients (B). Based on the literature on ω-3 PUFAs and depression, we considered the following parameters as covariates, as they may affect both the blood fatty acid profile and risk for depression: age, gender, BMI and smoking. Covariates were included in the regression models if they correlated with one of the independent variables and the dependent variable ($p<0.2$). All regression models were fitted as unadjusted models, and as models adjusted for the above covariates. The Hosmer-Lemeshow goodness-of-fit test was used to test the model calibration for all models. A p-value <0.05 was considered significant. Our study was powered ($1-\beta=0.8$) to detect $OR>1.46$ or $OR<0.68$ based on the distribution of the independent variables (PUFA) and our samples size, and $OR>1.30$ or <0.76 at $1-\beta=0.5$. The power analysis was conducted with G*Power for Mac OS. STATA 13.1 (Stata Corp, College Station, Texas, USA) for Mac OS was used for all other analyses.

7.3 Results

7.3.1 Demographic results and diet

Demographic and psychometric results and fatty acid concentrations are reported in Tables 1 and 2. The study included a total of 206 participants of whom 106 resided on Waiben and 100 on Mer Island (Table 1). No significant differences in age and gender

distribution were observed between participants at the two study sites. Study participants on Mer reported significantly higher seafood consumption and lower take-away food consumption compared with participants on Waiben. Similarly, a higher percentage of participants on Mer Island consumed seafood twice weekly or more as recommended by the WHO [31], although this did not reach statistical significance. Of the 206 participants, 19 were identified as having moderate to severe depressive symptoms using the aPHQ-9 (Table 2). Of these, 3 resided on Mer and 16 on Waiben. Consistent with this, mean aPHQ-9 scores were lower on Mer. Participants with aPHQ-9 scores above the cut-off for major depression were younger and had higher take away food consumption at trend level.

7.3.2 Blood polyunsaturated fatty acid levels

The relative abundance of PUFAs was different between the two study sites (Table 1), such that participants on Mer had higher levels of EPA, DHA and total ω-3 PUFA, and lower levels of total n-6 PUFA at trend level. This was also reflected in a significantly lower ω-6/3 PUFA ratio on Mer. Levels of LDL were lower on Mer at trend level, whereas triglycerides, HDL and total cholesterol did not differ between the study sites. Participants with aPHQ-9 scores above the cut-off for depression had a significantly higher ω-6/3 PUFA ratio, and a trend for higher ω-6 PUFA, lower ω-3 PUFA and lower levels of EPA and DHA (Table 2). Levels of lipids did not differ between participants with aPHQ-9 scores below and above the cut-off.

7.3.3 Covariates

Of the covariates considered for the analysis, age was negatively correlated with the ω-6/3 ratio ($r=-0.519$, $p<0.001$) and with aPHQ-9 scores (rho=-0.269, $p<0.001$). aPHQ-9 scores were higher among female compared with male participants although this was not statistically significant (3 (IQR 1 – 6) vs. 2 (IQR 0 – 4), $p=0.103$); similarly the n-6/3 ratio was higher among male participants (6.18 (95%CI 5.93 – 6.44) vs. 5.83 (95%CI 5.57 – 6.07), $p=0.047$). BMI was negatively correlated with the n-6/3 ratio ($r=-0.153$, $p=0.027$) but BMI was not associated with aPHQ-9 scores (rho=0.008, $p=0.900$). Smoking rates were not different between participants with no/mild depressive symptoms and participants with moderate/severe depressive symptoms ($chi2 = 0.538$, $p=0.463$) and the number of cigarettes smoked per day was not different between participants with no/mild depressive symptoms and participants with moderate/severe depressive symptoms (14.9 (95%CI 9.54 – 20.25) vs. 11.08 (95%CI 9.06 – 13.10), $p=0.210$).

Table 7.1 Demographic characteristics and PUFA levels of 206 people attending the 2016 Zenadth Kes Health Partnership Health Check

	Waiben (n=106)		Mer (n=100)		
					p-value
Age, mean (SD)	38.69	(15.06)	42.19	(18.58)	0.139[1]
Gender					0.817[2]
Female, n (%)	60	(56.60)	55	(55)	
Male, n (%)	46	(43.40)	45	(45)	
Smoking, dichotomized					0.924[2]
Yes, n (%)	47	(44.33)	45	(45)	
BMI, mean (SD)	31.29	(7.30)	31.82	(7.15)	0.600[1]
Depressive symptoms					
aPHQ-9, mean (SD)	4.45	(5.07)	3.19	(3.24)	0.035[1]
a PHQ-9, median (IQR)	3	(0 – 7)	3	(1 – 4)	0.437[3]
aPHQ-9 score, dichotomized[#]					0.003[4]
Moderate/severe, n (%)	16	(16.7)	3	(3)	
No/mild, n (%)	90	(83.3)	97	(97)	
Takeaway (weekly)					
Mean (SD)	1.13	(1.41)	0.54	(0.96)	<0.001[1]
Median (IQR)	1	(0 - 2)	0	(0 – 1)	<0.001[3]
Seafood (weekly)					
Mean (SD)	2.06	(1.75)	3.03	(2.85)	0.003[1]
Median (IQR)	2	(1 – 3)	2	(1 – 5)	0.014[3]
Dichotomized					0.078[2]
<2/week, n (%)	74	(68.51)	57	(57)	
>=2/week, n (%)	33	(31.49)	43	(43)	
Fatty Acids					
EPA (wt%), mean (SD)	0.57	(0.23)	0.65	(0.25)	0.021[1]
DHA (wt%), mean (SD)	2.35	(0.51)	3.00	(0.69)	<0.001[1]
Total n-3 (wt%), mean (SD)	4.60	(0.80)	5.43	(1.01)	<0.001[1]
Total n-6 (wt%), mean (SD)	29.40	(3.37)	28.65	(2.91)	0.087[1]
n6/3 ratio, mean (SD)	6.56	(1.22)	5.45	(1.15)	<0.001[1]
Lipids					
Triglycerides, mean (SD)	2.02[§]	(1.53)	1.95[&]	(0.99)	0.700[1]
LDL, mean (SD)	2.79[§]	(0.92)	2.56[&]	(0.77)	0.065[1]
HDL, mean (SD)	1.17[§]	(0.36)	1.17[&]	(0.35)	0.988[1]
Total cholesterol, mean (SD)	4.89[§]	(1.15)	4.62[&]	(0.96)	0.091[1]

[1] Independent samples t-test, [2] Chi2-Test, [3] Mann-Whitney-U-Test, [4]Fisher's Exact, [§]n=90, [&]n=94 [#]no/mild=aPHQ-9 score <10, moderate/severe=aPHQ-9 score >=10; BMI=body mass index, EPA=eicosapentaenoic acid, DHA=docosahexaenoic acid, HDL=high density lipoprotein, IQR=interquartile range, LDL=low density lipoprotein, aPHQ-9=Patient Health Questionnaire-9, SD=standard deviation

Table 7.2 Demographic characteristics and PUFA levels of 206 people attending the 2016 Zenadth Kes Health Partnership Health Check by depressive symptom score

	Moderate/severe (n=19)		No/mild (n=187)		
					p-value
Age, mean (SD)	32.00	(10.68)	41.24	(17.21)	0.022[1]
Gender					0.807[2]
Female, n (%)	10	(52.63)	105	(56.14)	
Male, n (%)	9	(47.37)	82	(43.86)	
Smoking, dichotomized					0.466[2]
Yes, n (%)	10	(52.63)	82	(43.85)	
BMI, mean (SD)	29.65	(7.15)	31.74	(7.21)	0.229[1]
Depressive symptoms					
aPHQ-9, mean (SD)	14.36	(3.93)	2.77	(2.56)	<0.001[1]
aPHQ-9, median (IQR)	14	(11-15)	2	(0 – 4)	<0.001[3]
Takeaway (weekly)					
Mean (SD)	1.36	(1.73)	0.79	(1.18)	<0.053[1]
Median (IQR)	1	(0 - 2)	0	(0 – 1)	<0.204[3]
Seafood (weekly)					
Mean (SD)	2.31	(2.26)	2.55	(2.41)	0.674[1]
Median (IQR)	2	(1 – 4)	2	(1 – 3.5)	0.529[3]
Dichotomized					0.614[2]
<2/week, n (%)	13	(68.42)	117	(62.56)	
>=2/week, n (%)	6	(31.58)	70	(37.44)	
Fatty Acids					
n6/3 ratio, mean (SD)	6.72	(1.06)	5.95	(1.32)	0.014[1]
Total n-6 (mol%), mean (SD)	30.04	(2.91)	28.93	(3.18)	0.148[1]
Total n-3 (mol%), mean (SD)	4.59	(0.92)	5.04	(1.00)	0.057[1]
EPA (mol%), mean (SD)	0.52	(0.22)	0.62	(0.24)	0.124[1]
DHA (mol%), mean (SD)	2.38	(0.63)	2.69	(0.68)	0.055[1]
Lipids					
Triglycerides, mean (SD)	2.21	(2.44)	1.96[§]	(1.08)	0.435[1]
LDL, mean (SD)	2.86	(0.75)	2.65[§]	(0.86)	0.346[1]
HDL, mean (SD)	1.16	(0.27)	1.17[§]	(0.36)	0.918[1]
Total cholesterol, mean (SD)	4.92	(1.06)	4.74[§]	(1.06)	0.487[1]

[1] Independent samples t-test, [2] Chi2-Test, [3] Mann-Whitney-U-Test, [4]Fisher's Exact, [§]n=165; BMI=body mass index, EPA=eicosapentaenoic acid, DHA=docosahexaenoic acid, HDL=high density lipoprotein, IQR=interquartile range, LDL=low density lipoprotein, aPHQ-9=Patient Health Questionnaire-9, SD=standard deviation

7.3.4 Relationship between diet and blood fatty acids

Linear regression modelling using seafood intake and takeaway food consumption as independent variables and total ω-3 PUFA as the dependent variable was significant ($F_{(2, 204)}$=7.84, p<0.001; adjusted R2 = 0.062) and showed small but significant effects for takeaway food consumption (B=-0.21, p=0.002) and for seafood intake at trend level (B=0.13, p=0.050) across both study sites and all participants. Consistent results were obtained for EPA and DHA, and an inverse association was observed with total ω-3 PUFAs (data not shown).

7.3.5 Relationship between diet, blood fatty acids and depressive symptoms

Across both sites, being above the cut-off for depression was associated with a higher ω-6/3 ratio (OR = 1.58 (95%CI 1.09 – 2.34), p=0.017; Table 3). This association remained significant when the analysis was adjusted for gender and BMI, and remained consistent at trend level when age was included as a covariate. There was a trend for an association of lower DHA and depressive symptoms (OR = 0.46 (95%CI 0.21 – 1.02), p=0.057) as well as lower total ω-3 PUFAs and depressive symptoms (OR = 0.58 (95%CI 0.33 -1.01), p=0.058). Similarly, we observed a trend for an association between takeaway food consumption and depressive symptoms (OR = 1.33 (95%CI 0.98 – 1.80), p=0.064). No significant association was found between seafood intake and depressive symptoms (p=0.673). The directionality of these associations was consistent when study participants were stratified by site. None of the lipid markers were associated with depressive symptoms.

For study participants on Waiben, higher seafood intake was associated with lower levels of depressive symptoms, which persisted at trend level when age, gender and BMI were included as covariates (Table 4) whereas, higher takeaway food consumption was significantly associated with higher levels of depressive symptoms. No significant association was found between membrane PUFAs and depressive symptoms. In study participants on Mer, a higher ω-6/3 ratio and lower levels of EPA were significantly associated with depressive symptoms but no significant associations were found between diet (seafood, takeaway) and depressive symptoms. These associations remained at trend level when the analysis was adjusted for gender and BMI, but did not persist when age was taken into account. However, no association was observed between seafood intake and depressive symptoms.

Table 7.3 Odds ratios (95%CI) for the association between seafood intake, blood polyunsaturated fatty acid levels and moderate/high depressive symptoms for 206 people attending the 2016 Zenadth Kes Health Partnership Health Check

	Unadjusted		Model 1[a]		Model 2[b]	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
All participants (n=206)						
Dietary intake						
Seafood	0.95 (0.76 – 1.18)	0.673	0.95 (0.76 – 1.19)	0.715	0.95 (0.74 – 1.21)	0.696
Takeaway	1.33 (0.98 – 1.80)	0.064	1.33 (0.98 – 1.80)	0.063	1.26 (0.93 – 1.71)	0.132
ω -3 Fatty Acids						
Eicosapentaenoic acid (20:5n-3)	0.13 (0.01 – 1.72)	0.123	0.14 (0.01 – 1.84)	0.136	0.35 (0.02 – 5.46)	0.457
Docosahexaenoic acid (22:6n-3)	0.46 (0.21 – 1.02)	0.057	0.48 (0.22 – 1.08)	0.079	0.58 (0.24 – 1.39)	0.229
Sums and Ratios						
Sum of ω -3 fatty acids	0.58 (0.33 – 1.01)	0.058	0.59 (0.33 – 1.04)	0.072	0.68 (0.36 – 1.28)	0.241
Sum of ω -6 fatty acids	1.12 (0.95 – 1.31)	0.149	1.12 (0.95 – 1.31)	0.163	1.05 (0.88 – 1.25)	0.530
ω -6 to ω -3 fatty acids ratio	1.58 (1.09 – 2.34)	0.017	1.62 (1.08 – 2.43)	0.020	1.45 (0.92 – 2.30)	0.106
Waiben (n=106)						
Dietary intake						
Seafood	0.79 (0.55 – 1.14)	0.218	0.79 (0.55 – 1.14)	0.222	0.80 (0.54 – 1.17)	0.253
Takeaway	1.20 (0.86 – 1.68)	0.266	1.20 (0.85 – 1.68)	0.280	1.15 (0.82 – 1.62)	0.404
ω -3 Fatty Acids						
Eicosapentaenoic acid (20:5n-3)	0.39 (0.02 – 5.25)	0.481	0.35 (0.23 – 5.34)	0.454	0.63 (0.03 – 10.67)	0.750
Docosahexaenoic acid (22:6n-3)	0.67 (0.23 – 1.96)	0.475	0.69 (0.23 – 2.03)	0.512	0.77 (0.25 – 2.40)	0.663
Sums and Ratios						
Sum of ω -3 fatty acids	0.82 (0.41 – 1.63)	0.578	0.80 (0.40 – 1.62)	0.548	0.88 (0.42 – 1.85)	0.755
Sum of ω -6 fatty acids	1.04 (0.88 – 1.22)	0.629	1.06 (0.89 – 1.25)	0.491	1.02 (0.84 – 1.22)	0.830
ω -6 to ω -3 fatty acids ratio	1.22 (0.78 – 1.91)	0.370	1.32 (0.80 – 2.15)	0.265	1.20 (0.70 – 2.06)	0.494
Mer (n=100)						
Dietary intake						
Seafood	1.22 (0.97 – 1.54)	0.083	1.19 (0.95 – 1.49)	0.132	1.92 (0.97 – 3.82)	0.062
Takeaway	1.14 (0.39 – 3.30)	0.816	1.07 (0.33 – 3.45)	0.906	1.18 (0.36 – 3.93)	0.778
ω -3 Fatty Acids						
Eicosapentaenoic acid (20:5n-3)	0.01 (0.01 – 29.44)	0.258	0.01 (0.01 – 26.04)	0.244	0.14 (0.01 – 997.33)	0.665
Docosahexaenoic acid (22:6n-3)	0.95 (0.18 – 5.07)	0.953	0.85 (0.15 – 4.75)	0.855	3.71 (0.28 – 49.70)	0.323
Sums and Ratios						
Sum of ω -3 fatty acids	0.66 (0.18 – 2.45)	0.539	0.66 (0.18 – 2.50)	0.544	1.81 (0.29 – 11.18)	0.523
Sum of ω -6 fatty acids	1.48 (0.92 – 2.37)	0.102	1.49 (0.89 – 2.51)	0.130	1.28 (0.74 – 2.21)	0.375
ω -6 to ω -3 fatty acids ratio	1.79 (0.66 – 4.87)	0.255	1.67 (0.64 – 4.35)	0.297	0.87 (0.22 – 3.46)	0.846

[a] adjusted for gender and BMI, [b] adjusted for age, gender and BMI; OR=odds ratio, CI=confidence interval

Table 7.4 Quantile regression models for the association between seafood intake, blood polyunsaturated fatty acid levels and moderate/high depressive symptoms for 206 people attending the 2016 Zenadth Kes Health Partnership Health Check

	Unadjusted		Model 1[a]		Model 2[b]	
	B (95% CI)	p-value	B (95% CI)	p-value	B (95% CI)	p-value
All participants (n=206)						
Dietary intake						
Seafood	-0.15 (-0.35 – 0.04)	0.122	-0.16 (-0.36 – 0.42)	0.119	-0.08 (-0.30 – 0.13)	0.442
Takeaway	1.00 (0.58 – 1.41)	<0.001	0.84 (0.16 – 1.52)	0.015	0.75 (0.11 – 1.40)	0.022
ω -3 Fatty Acids						
Eicosapentaenoic acid (20:5n-3)	0 (-3.50 – 3.50)	1.000	0 (-2.07 – 2.07)	1.000	2.10 (0.02 – 4.19)	0.047
Docosahexaenoic acid (22:6n-3)	0 (-1.04 – 1.04)	1.000	0 (-0.87 – 0.87)	1.000	0.35 (-0.48 – 1.19)	0.411
Sums and Ratios						
Sum of ω -3 fatty acids	0 (-0.73 – 0.73)	1.000	0 (-0.58 – 0.58)	1.000	0.26 (-0.25 – 0.77)	0.320
Sum of ω -6 fatty acids	0.12 (-0.01 – 0.26)	0.084	0.15 (-0.02 – 0.32)	0.085	0.04 (-0.06 – 0.14)	0.430
ω -6 to ω -3 fatty acids ratio	0 (-0.62 – 0.62)	1.000	0 (-0.55 – 0.55)	1.000	-0.10 (-0.51 – 0.30)	0.606
Waiben (n=106)						
Dietary intake						
Seafood	-0.50 (-0.95 – -0.04)	0.031	-0.32 (-0.82 – 0.17)	0.200	-0.31 (-0.79 – 0.15)	0.190
Takeaway	1.00 (0.41 – 1.58)	0.001	1.12 (0374 – 1.86)	0.003	1.09 (-0.50 – 1.68)	<0.001
ω -3 Fatty Acids						
Eicosapentaenoic acid (20:5n-3)	2.72 (-2.29 – 7.75)	0.284	1.52 (-3.98 – 7.02)	0.585	3.80 (-2.11 – 9.71)	0.205
Docosahexaenoic acid (22:6n-3)	0 (-2.91 – 2.91)	1.000	0 (-2.44 – 2.44)	1.000	0.49 (-2.07 – 3.07)	0.702
Sums and Ratios						
Sum of ω -3 fatty acids	0 (-1.75 – 1.75)	1.000	0 (-1.67 – 1.67)	1.000	0.21 (-1.21 – 1.65)	0.762
Sum of ω -6 fatty acids	0 (-0.33 – 0.33)	1.000	0 (-0.28 – 0.28)	1.000	-0.01 (-0.29 – 0.27)	0.935
ω -6 to ω -3 fatty acids ratio	0 (-0.87 – 0.87)	1.000	0 (-0.97 – 0.97)	1.000	-0.09 (-1.04 – 0.86)	0.851
Mer (n=100)						
Dietary intake						
Seafood	0.20 (-0.39 – 0.39)	1.000	-0.14 (-0.45 – 0.16)	0.362	-0.04 (-0.27 – 0.18)	0.712
Takeaway	0 (-1.31 – 1.31)	1.000	0.49 (-0.68 – 1.67)	0.407	0.12 (-1.13 – 1.38)	0.846
ω -3 Fatty Acids						
Eicosapentaenoic acid (20:5n-3)	-2.31 (-4.47 - -0.14)	0.037	-1.51 (-3.77 – 0.74)	0.185	0.36 (-1.81 – 2.55)	0.740
Docosahexaenoic acid (22:6n-3)	0 (-1.12 – 1.12)	1.000	-0.39 (-1.14 – 0.36)	0.307	0.32 (-0.46 – 1.11)	0.413
Sums and Ratios						
Sum of ω -3 fatty acids	0 (-0.85 – 0.85)	1.000	-0.35 (-0.96 – 0.25)	0.252	0.20 (-0.28 – 0.69)	0.402
Sum of ω -6 fatty acids	0.17 (-0.04 – 0.39)	0.119	0.28 (0.13 – 0.43)	<0.001	0.18 (0.02 – 0.39)	0.076
ω -6 to ω -3 fatty acids ratio	0.86 (0.10 – 1.63)	0.026	0.62 (-0.08 – 1.33)	0.084	0.11 (-0.70 – 0.94)	0.777

[a] adjusted for gender and BMI, [b] adjusted for age, gender and BMI, CI=confidence interval

7.4 Discussion

In this community-based cross-sectional study we tested the hypothesis that fish consumption and the consumption of takeaway food rich in ω-6 PUFA are inversely associated with blood PUFA status and depressive symptoms. We found significant positive associations of fish and seafood consumption with levels of ω-3 PUFA, a negative association of takeaway food consumption with ω-3 PUFA, and an association of the ω-6/3 PUFA ratio and low levels of ω-3 PUFA with depressive symptoms. To the best of our knowledge, this is the first study to address this question in a community study of Torres Strait Islander people with a diet consisting of a high proportion of local fish and seafood.

Our findings are consistent with a meta-analysis [8] and subsequent observational studies [7, 32-34] of ω-3 PUFA in patients with major depressive disorder, dietary interventions with ω-3 PUFA [35] and with studies in other clinical populations [36]. Collectively, these studies support the notion that depression is accompanied by low levels of ω-3 PUFA levels. Moreover, population-based studies observed an association of low ω-3 PUFA levels with depressive symptoms, including a large prospective cohort study of elderly people in Japan [37] and the National Health and Nutrition Examination Survey in the United States [38]. The Netherlands Study of Depression and Anxiety (NESDA) reported depleted levels of ω-3 PUFA in acute but not in remitted depression, which were correlated with depression severity [39], suggesting a dose-response relationship. Conversely, supplementation with ω-3 PUFAs has been found to be effective for major depressive disorder, with higher doses of EPA having greater effects [14-16], suggesting that ω-3 PUFA administration can alleviate depressive symptoms.

Contrary to our initial hypothesis, we did not observe a consistent direct association between dietary intake of fish and seafood and depressive symptoms. This may seem surprising, given that fish and seafood are the primary sources of EPA and DHA. However, the lack of association is consistent with previous large-scale studies that failed to observe associations of dietary fish intake and depression while showing significant associations of ω-3 PUFA levels with depression [37, 38, 40]. Several reasons might explain the lack of association in our study. Besides a true absence of association, the dietary questionnaire used in the present study only assessed fish and seafood intake in the past week. Consequently, the longer-term effects of dietary

habits may not be adequately represented in our study. Secondly, ω-3 PUFA from other dietary sources as well as supplementation may additionally confound the effect of dietary fish intake. Finally, it is conceivable that a ceiling effect is present at least in the community with higher fish consumption, beyond which an effect of dietary intake on the risk for depression cannot be observed. The observation of a significant relationship between the ω-6/3 PUFA ratio and depression in the quantile regression model in the study site with lower seafood intake could potentially indicate a ceiling effect, whereby a relationship between ω-3 PUFA and depressive symptomatology exists only below a certain level of dietary ω-3 PUFA intake. The fact that the results from the quantile regression models are only partially consistent with the logistic regression models may indicate that the observed relationship between membrane PUFAs and depression might only apply to more severe depression, but not to depressive symptoms below the threshold for major depression.

While ω-3 PUFA have been recognised as a specific dietary component linked to depression and other conditions, the role of foods that are high in saturated and n-6 fatty acids is less clear. Our results indicate that the consumption of take-away food is associated with higher aPHQ-9 scores on Waiben where takeaway food was more readily available and consumption significantly higher. Similarly, takeaway food consumption was also associated with lower ω-3 PUFA levels and a higher ω-6/3 ratio. This suggests that foods high in ω-6 PUFA (for example, linolenic acid) have opposing effects on the levels of ω-3 PUFA but also directly on depression. This is in line with the view that 'Western' diets high in ω-6 PUFA and low in ω-3 PUFA are associated with higher rates of depression [12, 41]. Collectively, these findings support the emerging notion that diet is a modifiable risk factor for depression [42].

The question whether low levels of ω-3 PUFA precede or indicate risk for depression cannot be answered in our cross-sectional study. Indeed, it is possible that depression predisposes to an unhealthy diet and low intake of fresh seafood and reverse causation or a bi-directional relationship are possible. However, we have previously shown in a longitudinal study that low levels of ω-3 PUFA and a high ω-6/3 PUFA ratio are risk factors for mood disorders in young people with at-risk mental states, suggesting that low membrane PUFA levels precede the onset of depression in this group [18]. While at-risk mental states are characterised by ongoing sub-clinical psychopathology and differ from the community sample in the present study, the specificity of the observed association for mood disorders in this previous study [18] suggests that low levels of ω-3 PUFA indeed represent a risk factor for depression.

Aboriginal and Torres Strait Islander Australians continue to experience depression and other mental disorders at higher rates relative to the general population. While the social and environmental determinants of the excess psychiatric morbidity are complex and no single intervention is likely to ameliorate all determinants of mental health, our data suggest that a diet that is rich in ω-3 PUFA and low in ω-6 PUFA may be beneficial. While longitudinal studies are needed to establish whether diet can have a lasting impact on depression risk and it is premature to draw firm conclusions from the currently available data, sustainable access to healthy food in rural and remote communities should be a priority and may be beneficial not only to physical health but also to mental health and wellbeing.

Several limitations warrant consideration in the interpretation of these findings. First, as mentioned above, we did not take ω-3 PUFA supplementation into account, which limits the conclusions that can be drawn from the association of dietary intake of fish and seafood with PUFA levels and depression. Second, a multitude of risk factors for mood disorders are known to interact with the complex aetiology of the disorder, many of which were not taken into account in the present study. Third, the study setting opens up the possibility for underreporting of depression, since it is plausible that severely depressed individuals were less likely to attend. Participants may have been less likely to disclose symptoms in this setting due to a perceived lack of privacy in a community health check setting, and due to perceived associated stigma. The study sample may thus not be representative of the wider community. Fourth, the sub-group of study participants with aPHQ-9 scores above the cut-off for probable depression was small (n=18) and the results of our logistic regression analysis is limited by this fact. Finally, although the PHQ-9 is an often used and well validated instrument to assess depression in primary care settings and is a sensible choice as a brief depression screener in health checks [43], the validity of the adapted instrument used in our study remains unknown. The version adapted for Central Australian Aboriginal people used in our study is currently undergoing validation [28].

In conclusion, the association of dietary intake of fish/seafood and takeaway food, ω-3 PUFA levels and depressive symptoms in our cross-sectional study in two Torres Strait Islander communities highlights the potential role of traditional fish-based diet as a protective factor for depression. A growing body of literature supports the notion that diet is an important modifiable risk factor for depression and our findings align with this view and represent a potential explanatory model for epidemiological differences in depression prevalence in Indigenous populations.

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Declaration of Interest

The authors report no medical or financial conflict of interest. The funding bodies had no role in the design of the study or analysis of the data.

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8 General Discussion

The overarching aim of this thesis was to examine potential biomarkers in two distinct high-risk populations. Specifically, the hypotheses of this thesis were that AL would be elevated in patients with mental disorders and concomitantly related to illness severity and progression; and that similarly ω-3 PUFA are indicative of poor outcomes in depression. By combining evidence from meta-analysis, secondary analyses of clinical trials and a cross-sectional study conducted in three Aboriginal and Torres Strait Islander communities, this thesis tested the above hypotheses and contributes novel knowledge relevant to high-risk groups.

The findings of this thesis demonstrate unique associations of AL with psychotic disorders. The main findings relevant to AL are dysregulation of primary AL mediators in psychotic disorders (**Chapter 2**), elevated AL in psychosis that is related to illness severity and functioning (**Chapter 3**) and indicative of poor functioning in people at UHR for psychosis (**Chapter 4**). No consistent evidence was found for the role of AL in depression (**Chapter 6**). Low levels of ω-3 PUFA as well as a high ω-6/3 PUFA ratio were found to be indicative of a higher risk for mood disorders in people at UHR for psychosis (**Chapter 5**) and related to depressive symptoms in otherwise healthy individuals (**Chapter 7**). Collectively, these findings address current knowledge gaps and add to a growing body of research aimed at identifying risk biomarkers in psychiatry.

In this final chapter I will synthesise and discuss the main findings of this thesis and place them in the context of existing and emerging literature. Strengths and limitations of the individual chapters will be discussed in the wider context of this body of work and related research. Finally, this chapter concludes with recommendations for future research and a discussion of clinical implications for the work presented in this thesis.

Chapters 3 and 4 provided novel insights into the role of AL in patients with psychotic disorders and young people at UHR for psychosis. In the last decade several authors have argued that the AL framework is highly relevant to psychiatric populations [1-6]. The main arguments that emerged in the literature can be broadly grouped into those that propose AL as a tool to understand and predict cardiovascular and other prevalent physical comorbidities in people living with severe mental illnesses [1, 6], and those that discuss AL as a framework to understand the pathophysiology of mental disorders [2-4]. A third important are of research is the uncertainty surrounding the suitability of AL

determine risk for illness progression, treatment resistance and other clinical outcomes of interest. The research presented in Chapters 3 and 4 directly addresses this third research concern by measuring AL in clinical populations and by testing associations with symptoms (Chapter 3) and prospective associations with clinical outcomes (Chapter 4).

The main finding of Chapter 3 was that the AL index is elevated in patients relative to health controls matched for age and gender, with a gradual increase in AL from controls to first-episode patients and then to patients with SCZ. Chapter 3 also showed that AL is related to the severity of positive psychotic symptoms and impaired functional capacity. These observations suggest AL is related to illness severity in acutely ill patients. The observation that AL decreased with six weeks treatment also supports this notion. An important aim of this chapter was to test if AL could be predictive of treatment response and remission criteria. This hypothesis, however, was not supported by the data, which may be attributable to the relatively small sample size of this study and the consequent lack of power to detect small effects in subsets of the sample.

The study undertaken in Chapter 3 confirms and extends the only previous investigation of AL in SCZ and healthy controls [7]. During the same time period when our study was under investigation, another study reporting AL in patients with SCZ was published [7]. The authors of this study measured AL in 30 patients with SCZ (mean age: 32.5 years) and 20 healthy controls and found elevated AL in patients compared to controls and significant correlations with both positive psychotic symptoms (Brief Psychiatric Rating Scale) and impaired functional capacity (UCSD Performance-Based Skills Assessment-2). Similarly to the data reported in Chapter 3, no associations were observed with other psychiatric symptoms. The authors also assessed self-reported stress in the past month (Perceived Stress Scale) and found it to be unrelated to AL, suggesting that the stressful experience of psychosis itself does not sufficiently explain heightened AL. This latter observation is also consistent with a recent study in which we reported that insulin resistance in first-episode patients is unrelated to chronic stress and cortisol levels [8]. Chapter 3 thus confirms the only other direct measurement of AL in patients with SCZ and adds important knowledge by including (drug-naïve) patients with FEP and by assessing AL at three time points.

An important question raised by Chapter 3 involved determining if AL precedes psychosis and can be used to inform risk prediction (e.g. for poor outcomes) in

individuals at UHR for psychosis. This question is salient because heightened AL may contribute to the underpinnings of the pathophysiology observed in psychotic disorders and, if present before the onset, might be an indicator of risk for psychosis transition or other associated outcomes. This question informed the study reported in Chapter 4, which found that AL was indicative of poor functioning (assessed with SOFAS, GF-S and GF-R). This was the first study to examine prospective associations of AL with clinical outcomes in people at UHR for psychosis and confirms the importance of multisystem dysregulation for functional outcomes. While no associations were observed with psychotic symptoms, the relationship between AL and functioning indicates that AL may not only be associated with poor functioning in acutely psychotic patients, but also indicative of poor functioning in people at UHR for psychosis, who have a 20% (95%CI 17-25%) risk of developing psychosis within two years [9]. This is a highly relevant finding, as everyday functioning is an important outcome for patients with psychosis [10]. Together, Chapters 3 and 4 address a critical gap in knowledge and provide the first evidence of the role of AL before and at the onset of psychosis.

Chapter 6 demonstrated no association of AL with depressive symptoms (measured with the aPHQ-9) in adolescents or adults. The evidence for elevated AL in patients with clinical depression as well as for a relationship with depressive symptoms in otherwise healthy adults is somewhat inconsistent. While some studies found AL to be increased [11] and related to future depressive symptoms [12], other studies did not confirm this with a recent large cross-sectional study (n=12272) reporting no association of depressive symptoms (measured with the PHQ-9) with AL [13]. An important question that arises from the data presented in Chapters 3, 4 and 6 is if elevated AL is specific to psychotic disorders. Clearly, the differences in study population and methodology do not allow direct comparisons between the studies. Moreover, given the limited number of studies particularly in patients with psychosis, it seems premature to attempt to answer this question. While it is possible that AL is involved in psychotic disorders to a higher degree than in depression, alternative explanations are that studies in psychosis tended to recruit more severely ill individuals while most studies in depressed individuals relied on population-based cohorts. This question should be further addressed in future studies.

A theoretical advantage of the AL index, when applied in psychiatric populations, is that it acknowledges the multitude of pathomechanisms that contribute to many psychiatric disorders. This may have advantages over individual biomarkers, which may be of limited benefit in complex disorders characterised by heterogeneous

pathophysiology. The AL index used in several chapters of this thesis might help to overcome this problem as it clusters and reduces the dimensionality of the information contained in the multiple biomarkers. Moreover, the theoretical framework of allostasis provides a biological rationale for this approach that may apply transdiagnostically. In contrast to such an approach, over the past decade, discovery-driven approaches have seen a tremendous rise in popularity within psychiatric research. These include biological tools ("-omics" techniques) but also statistical and mathematical modelling approaches such as machine learning or joint modelling [14, 15].

One aim of this thesis was to examine whether lipid biology is related to clinical outcomes has been explored in Chapter 5. Using data from the Vienna Omega-3 Study [16], we were able to examine prospective associations of several classes of PUFAs with clinically relevant outcomes. At the time of publication, these were some of the longest follow-up data in UHR cohorts available and allowed important insights into the role of PUFAs, which were measured at the baseline assessment of this study. Chapter 5 reported strong associations (OR=1.89, 95%CI 1.07 – 3.33) of the balance between ω-6 and ω-3 PUFA with mood disorders at the 7-year assessment. Mood disorders were prevalent in this study and in other UHR studies [17] with 37% of participants being diagnosed with a mood disorder diagnosis (major depressive disorder or bipolar I disorder) at some point during the follow up. Somewhat surprisingly, no significant associations were observed with other outcomes, including psychotic disorders.

A key observation of Chapter 5 was that low levels of EPA and DHA were similarly predictive of mood disorders. This is relevant as only interventions with a high content of EPA appear to be effective in patients with major depressive disorder [18, 19]. In contrast, for people at UHR for psychosis, fish oil (840mg EPA and 560mg DHA per day) was found to be ineffective at reducing depressive symptoms in the NEURAPRO study [20]. However, the primary analysis of this study focused on depressive symptoms (MADRS) as a secondary outcome but not on an Axis I diagnosis. It is conceivable that study participants with MDD could have been in remission at the primary outcome assessment and that consequently, a SCID diagnosis may be a more appropriate outcome compared to a momentary assessment of severity scores for this particular question. Moreover, if low levels of EPA and DHA as well as the ω-6/3 PUFA ratio are indicative of the risk for mood disorders, it is plausible that only individuals at UHR for psychosis with low levels of these ω-3 PUFA would benefit from fish oil. Consequently,

pre-treatment levels of EPA or other n-3 PUFA should be considered as part of the inclusion criteria in future studies.

Chapter 7 examined the role of PUFAs for depression from a different perspective in a sub-group of participants from the WPC study. A major aim of the WPC was to identify risk and protective factors for depressive symptoms. Motivated by the results of Chapter 5, we investigated whether blood PUFA levels, fish consumption, and depressive symptoms were associated in a healthy population sample. Indeed, the ω-6/3 PUFA ratio was associated with being above the cut-off for depression on the aPHQ-9 (unadjusted OR=1.58, 95%CI 1.09 – 2.34), although this was reduced to a trend when age, sex and BMI were included as covariates. The power of this analysis was limited by the small group of participants with aPHQ-9 scores above this (n=19). Consistent with Chapter 5, EPA and DHA were independently associated with depressive symptoms at trend level (unadjusted OR=0.13, 95%CI 0.01 – 1.72 and OR=0.46, 95%CI 0.21 – 1.02, respectively), highlighting their relevance for depression. Importantly, the two Torres Strait Islander communities participating in this study represent a unique and distinct cohort due to their geographical location, their access to fresh seafood and the limited availability of fast food in one of the study sites. The findings of this study, together with other recent evidence supported by our data that the prevalence of depression is comparatively low in this population [21], raise the question if the high fish and seafood consumption could be a protective factor for depression. This is especially relevant as prevalence estimates for several mental disorders including MDD are higher for Aboriginal and Torres Strait Islander people than for the general population [22-25]. Caution is warranted if these findings are to be directly generalised to other populations. While the mechanisms by which ω-3 PUFA are thought to reduce depression risk may be universal, a multitude of social health determinants are known to influence the risk for mental health and these have not been comprehensively addressed in this study.

The cross-sectional design of this study precludes conclusions about the directionality of these associations or assumptions about causation. However, in light of the evidence from longitudinal studies, including in people at UHR for psychosis as presented in Chapter 5, it seems reasonable to propose that ω-3 PUFA from diet and other sources modify the risk for depression in clinical cohorts and for depressive symptoms in otherwise healthy individuals at population level. The growing understanding of the biology of PUFA and their role in modulating key cellular functions relevant to psychiatric disorders adds biological validity to this hypothesis.

However, a bi-directional relationship, whereby depressed individuals are less likely to consume adequate amounts of these essential fatty acids, is possible but cannot be confirmed based on the data presented in this thesis. Loss or increase of appetite are both diagnostic criteria of major depressive disorder according to the DSM-V [26] and ICD-10 [27], and several studies support the notion that healthy diets are generally associated with lower depression risk [28-30].

Ultimately, a biomarker or risk index that is useful for clinical practice will have to (1) relate to defined outcomes of interest (e.g. psychosis transition or treatment response), (2) significantly exceed the predictive capacity of clinical characteristics alone, (3) be predictive in at least a well-defined sub-group of patients, (4) be cost-effective and feasible to collect in practice. While a common criticism of AL is that the multitude of relevant biomarkers is expensive and difficult to collect routinely, the 10-biomarker AL index used in this thesis and in similar constellation in other studies [7, 31] is collected relatively easily, as some elements are routinely measured in clinical practice (e.g. blood pressure, lipids, HbA1c, CRP) while most others can be quantified from a blood test (e.g. IL-6). Only cortisol requires special attention, as plasma cortisol is subject to the diurnal rhythm of the HPA-axis, which may be overcome by measuring hair cortisol from hair (see Chapter 6). Similarly, fatty acids (Chapters 5 and 7) can be measured from a dried blood spot with a method similar to a simple blood glucose test [32].

The findings reported in this thesis suggest several avenues for future research:

- The association of AL with psychosis (Chapters 3 and 4) indicates a need for future investigation to determine if lowering AL can ameliorate the illness. While the effects of lower AL for mental illness have not been directly studied to date, there is evidence to suggest that lower AL is associated with reduced mortality in older adults [33]. Few studies to date have directly addressed potential avenues to reduce AL. Given that commonly described determinants of AL include potentially modifiable risk factors such as low childhood SES [34], adverse childhood experiences [35], or discrimination and associated social gradients in health [36, 37], addressing these might provide opportunities to change the trajectory of allostasis in vulnerable populations. For example, direct interventions addressing the effects of chronic stress have been evaluated for example in school-aged children [38] and future studies may with to investigate AL in this population.

- The observation that low levels of ω-3 PUFA and a high ω-6/3 PUFA ratio are associated with depressive symptoms (Chapter 7) and that these indices place youth at UHR for psychosis at higher risk for developing mood disorders (Chapter 5) strengthen the rationale for biomarker-guided intervention studies. In this context, using low levels of ω-3 PUFA as an inclusion criterion for RCTs of fish oil may be more appropriate than including participants irrespective of their PUFA status. In fact, a very recent finding of the NEURAPRO clinical trial was that participants with a n-3 index[ii] <3% showed greater rates of favourable overall clinical improvement compared to those with higher ω-3 indices, and that fish oil was significantly more beneficial than placebo when only participants with low n-3 indices were included in the analysis [39]. This is particularly relevant as the primary intention-to-treat analysis showed no superiority of fish oil over placebo [20], likely due to an effective background intervention, low adherence and ω-3 intake from other sources in the placebo group [40].
- While there is already rapidly growing interest surrounding the role of nutrition on mental health in the scientific community (e.g. ref [41]), Chapter 7 adds evidence in support of the notion that diet is a modifiable risk factor for depression. While this study was cross-sectional and does not permit inferences about the causality of the relationship between dietary intake of n-3 PUFA and depressive symptoms, the robustness of this observation and the biological validity of the relationship strongly suggest that a carefully improved diet might be beneficial for patients with MDD and possibly to reduce the risk for depression before the onset.
- While inception cohort studies such as birth cohort studies are particularly suited to identify risk factors and would overcome many of the limitations of the research presented in this thesis, there are several difficulties in applying such designs to biomarker studies, including the cost of measuring a range of biomarkers in very large samples and the relatively low incidence of UHR states. Adequately powered clinical cohort studies recruiting help-seeking individuals with ARMS may be more appropriate for risk prediction.
- Ultimately, future studies should aim to combine information from various biomarkers to evaluate their predictive capacity in longitudinal studies, particularly also in relation to individual biomarkers. Risk calculators [42, 43] are one such approach and combine different parameters into a single risk index,

[ii] The n-3 index is calculated as EPA + DHA.

yet biomarker information has not been considered so far. The aim of such undertakings would be to assist in developing personalised approaches of developing individualised approaches with a decision support system.

Taken together, the studies presented in this thesis provide novel insights into two candidate risk mechanisms for mental disorders and contribute to the understanding of AL and lipid biology with relevance to people with at-risk mental states and Indigenous mental health. Applying the AL framework to a clinical population has revealed clinical correlates of multisystem dysregulation in patients with psychotic disorders and provided evidence for AL as an indicator of poor functional outcomes in youth at UHR for psychosis. Similarly, demonstrating that the balance of ω-6 to ω-3 PUFA indicates risk for mood disorders over a long follow-up period is critical as the ω-6/3 PUFA ratio is highly modifiable and as such provides a rationale for biomarker-guided interventions. These insights highlight new candidate risk biomarkers and provide scope to further evaluate them in clinical populations. While conversion to psychosis has been the primary outcome of interest in this group, mood disorders are common and will need to be addressed in future studies. Identifying candidate risk biomarkers is not only an essential prerequisite for better prediction of risk and need for care but also add to our understanding of the potentially modifiable social and biological risk factors and their effects on the developing brain [44]. Ultimately, this may lead to the development of novel benign treatments and personalised treatment strategies appropriate for young people.

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Appendix

Appendix A - Supplementary Materials for Chapter 3

Methods

Alternative allostatic load index

We replicated our main results using a traditional allostatic load index in order to allow better comparisons to original validation studies (Seeman et al., 2001). This index consisted of heart rate, systolic blood pressure, diastolic blood pressure, waist-to-hip ratio, cholesterol, high-density lipoprotein, low-density lipoprotein, glycosylated hemoglobin, cortisol, metanephrine and normetanephrine.

'Scaled' allostatic load index

The scaling approach may have advantages over more 'traditional' count-based calculations of allostatic load as it weighs the contribution of individual biomarkers in relation to the number of included biomarkers. Consequently, each 'system' (e.g. cardiovascular, immune, lipid and glucose metabolism) contributes equally to the allostatic load index. Our approach of including a larger number of parameters also reduces the contribution of individual ones and thus avoids maximizing differences of individual biomarkers that are differentially elevated or decreased (e.g. blood pressure as an indicator of acute/fluctuating stress activation) and those of outliers.

Additional Results

Simplified allostatic load index

Adjusting for age and smoking, allostatic load was significantly different between the three groups ($F_{(2, 95)}$=11.78, p<0.001). Allostatic load was highest in patients with schizophrenia (6.10 ± 2.88), followed by patients with first-episode psychosis (4.38 ± 2.14) and controls (3.35 ± 1.86). Across all patients, allostatic load was significantly positively correlated with positive psychotic symptoms (r=0.312, p=0.042) but no clear association was found with GAF scores (r=-114, p=0.46).

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