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UNIVERSITY OF TARTU
Press

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Dissertation was accepted for the commencement of the degree of Doctor of Philosophy in Neurosciences on February 12th, 2020, by the Joint Council for the Curriculum of Neurosciences

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Commencement: May 22th, 2020

This research was supported by the grants from the Estonian Research Foundation (IUT 20-41, IUT 20-42, PUT PRG685), and by the European Union through the European Regional Development Fund (Project no. 2014-2020.4.01.15-0012).

ISSN 1736-2792
ISBN 978-9949-03-325-6 (print)
ISBN 978-9949-03-326-3 (pdf)

University of Tartu Press
www.tyk.ee

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, referred to in the text by Roman numerals I–III.

I. **Balotšev R**, Haring L, Koido K, Leping V, Kriisa K, Zilmer M, Vasar V, Piir A, Lang A, & Vasar E (2017). Antipsychotic treatment is associated with inflammatory and metabolic biomarkers alterations among first-episode psychosis patients: a 7-month follow-up study. Early Intervention in Psychiatry 0:1–9.

II. Kriisa K, Leppik L, **Balõtšev R**, Ottas A, Soomets U, Koido K, Volke V, Innos J, Haring L, Vasar E, & Zilmer M (2017). Profiling of acylcarnitines in first episode psychosis before and after antipsychotic treatment. Journal of Proteome Research 16(10), 3558−3566.

III. **Balõtšev R**, Koido K, Vasar V, Janno S, Kriisa K, Mahlapuu R, Ljubajev U, Parksepp M, Veiksar P, Volke V, Lang A, Haring L, Zilmer M, & Vasar E (2017). Inflammatory, cardio-metabolic and diabetic profiling of chronic schizophrenia. European Psychiatry 39, 1–10.

Contribution of the author:

I. The author was involved in the design of the study, collection and analysis of the data, and writing of the manuscript and handled the publication process.
II. The author was involved in the analysis of the data and writing the manuscript.
III. The author was involved in the design of the study, collection and analysis of the data, and writing of the manuscript and handled the publication process.

ABBREVIATIONS

AC(s)	Acylcarnitine(s)
AP(s)	antipsychotic(s)
acetyl-CoA	acetyl-coenzyme A
acyl-CoA	acyl-coenzyme A
BBB	blood brain barrier
BMI	body mass index [weight (kg)/ height $(m)^2$]
CACNA1I	calcium voltage-gated channel subunit alpha 1 I
CACNA1C	calcium voltage-gated channel subunit alpha 1 C
CACNB2	calcium voltage-gated channel auxiliary subunit beta 2
CADM2	cell adhesion molecule 2
CARN	carnitine
CI	confidence interval
CNS	central nervous system
COMT	catechol-O-methyltransferase
CPT 1	carnitine palmitoyltransferase 1
CPZ	chlorpromazine
CRP	C-reactive protein
CS(s)	control subject(s)
DRD2	dopamin receptor D2
EGF	epidermal growth factor
ERV	endemic reference value
FAO	fatty acid β-oxidation
FEP	first-episode psychosis
GLM	general linear model
GWAS	genome-wide association study
GRIA1	glutamate ionotropic receptor AMPA type subunit 1
GRIN2A	glutamate ionotropic receptor NMDA type subunit 2A
GRM3	glutamate metabotropic receptor 3
HbA1c	glycated hemoglobin
HDL-c	high-density lipoprotein cholesterol
ICAM	intracellular adhesion molecule
ICD-10	International Classification of Diseases, tenth revision
IDO	Indoleamine-2,3-dioxygenase
IGF	insuline-like growth factor
IL(s)	interleukin(s)
INF-γ	interferon gamma
KYNA	kynurenic acid
LCFA	long-chain fatty acid
LCAC	long-chain acylcarnitine
LDL-c	low-density lipoprotein cholesterol
LLOQ	lower level of quantification
LOD	level of detection

LTA4H	leukotriene A4 hydrolase
LTC4S	leukotriene C4 synthase
MCP-1	monocyte chemoattractant protein-1
MetS	metabolic syndrome
MHC	major histocompatibility complex
NF-kB	nuclear factor-kappa B
NMDA	N-methyl-D-aspartate
OR	odds ratio
OxS	oxidative stress
PAI-1	plasminogen-activator inhibitor-1
PANSS	Positive and Negative Syndrome Scale
SCAC	short-chain acylcarnitine
SCH	schizophrenia
sIL2R	soluble interleukin-2-receptor
SRR	serine racemase (protein coding gene)
TGs	triglycerides
Th1	type 1 T helper cells
Th2	type 2 T helper cells
TLRs	toll-like retseptor
TNF-α	tumor necrosis factor alfa
T2DM	type 2 diabetes mellitus
VEGF	vascular endothelial growth factor
WAT	white adipose tissue
UHR	ultra-high risk
ULOQ	upper limit of quantification
WHO	World Health Organization
η^2	eta-squared
5-HT	5-hydroxytryptamine

1. INTRODUCTION

Schizophrenia (SCH) spectrum disorders are severe and chronic recurring psychotic illnesses that have a strong biological basis (Tamminga & Medoff, 2000). The illness usually manifests in young adulthood, lasts one's whole life (Pagsberg, 2013), and is characterized by significant impairments in reality testing, behavior, and functioning (Heckers et al., 2013). The clinical deterioration that occurs in SCH may, in effect, begin before the first-episode of psychosis (FEP), thus early disease identification and intervention may favorably alter the course and outcome of SCH (Lieberman et al., 2001). To strengthen the effectiveness of interventions, a deeper scientific effort is needed to properly identify and characterize the entire course of the disease. However, despite extensive achievements in recent years, the pathogenesis of the SCH spectrum disorders has remained poorly understood, mainly because their pathophysiology is not directly apparent in molecular or histopathologic analyses of the human brain (Hayashi-Takagi et al., 2014). Notwithstanding the limitations, peripheral blood samples have been used for decades in psychiatric research as substitutes for central nervous system (CNS) samples (Hayashi-Takagi et al., 2014). Immune dysregulation in at least a subgroup of SCH patients has been found in numerous studies comparing patients to healthy controls, and results have indicated that patients with FEP or chronic stage of the psychotic disorder, on a group level, show an imbalance in pro- and anti-inflammatory cytokines (interleukin (IL)-1β, interferon (INF)-γ, and IL-6, IL-4, IL-10 among others) as well as innate immunity, including the monocyte/macrophage system (Miller et al., 2011; Upthegrove et al., 2014; Rodrigues-Amorim et al., 2018; Pillinger et al., 2019b; Fraguas et al., 2019). There is a strong influence of pro-inflammatory cytokines on tryptophan/kynurenine metabolism which influences the serotonergic and glutamatergic neurotransmission via neuroactive metabolites such as kynurenic acid (Leonard et al., 2012; Plitman et al., 2017; Cervenka et al., 2017).

Activation of the immune system in SCH occurs not only in the periphery but also in the brain (Khandaker et al., 2015; Müller, 2018). The periphery and CNS are connected through immune regulators such as the cytokines and also through the endocrine and metabolic systems. Furthermore, it has become increasingly evident that the gut microbiota and the brain communicate in a bidirectional manner (Lazar et al., 2019) via various routes including the immune system, tryptophan metabolism, the vagus nerve and the enteric nervous system, involving microbial metabolites such as short-chain fatty acids, and branched-chain amino acids (Cryan et al., 2019).

Also, studies have extensively documented the metabolic and bioenergetic dysfunctions in SCH and confirmed that the disease might have an intrinsic link between obesity, prediabetic markers, in particular impaired glucose tolerance and insulin resistance, and type 2 diabetes mellitus (T2DM) (Pillinger et al., 2019a). Moreover, the adipocytes are producing various signal molecules, such

as hormones (adiponectin, leptin, resistin), chemokines, such as monocyte chemoattractant protein-1 (MCP-1) and proinflammatory cytokines, including tumor necrosis factor (TNF)-α, IL-1β and IL-6, all culminating in a low-grade inflammation (Carillo et al., 2018). The inflammation may also be triggered by oxidative stress (OxS) (Fraguas et al., 2019), which in turn may lead to mitochondrial oxidation dysfunction (Miller et al., 2009; Rollins et al., 2009; Zuccoli et al., 2017). Acylcarnitines (ACs) are produced as products of incomplete mitochondrial fatty acid oxidation. Bioenergetic alterations defined by ACs imbalance have been detected in case of several conditions, including, but not limited to, obesity, cardiometabolic disorders and SCH (Schönfeld et al., 2016; Lopaschuk, 2016; Cao et al., 2019).

Previous results indicate that the peripheral markers may change at different stages among patients with chronic psychotic disorder, but together suggest that the peripheral immune system is over-activated both in individuals undergoing their FEP and in people with a chronic stage of the SCH spectrum disorders (Goldsmith et al., 2016; Pillinger et al., 2019b).

Importantly, it should be noted that antipsychotic (AP) drugs can modulate components of the metabolic and inflammatory-related pathways (Wang et al., 2018a).

Based on the above studies from a wide range of authors, the primary objective of this dissertation was to examine the profiles of inflammatory and metabolic biomarkers in FEP patients before and after AP treatment over a 7-month observation, as well as in patients with a chronic stage of the SCH spectrum disorders, and to study interactions between the immune system and metabolic shifts in patients with different stages of SCH spectrum disorders.

2. REVIEW OF LITERATURE

2.1. Common characteristics and the course of SCH spectrum disorders

SCH and other psychotic disorders differ in terms of clinical characteristics, the course and severity of the disease from person to person – some only have one frank psychotic break during their lifetime while others may experience several full-blown psychotic episodes with little recovery between these episodes and a deepening decline in functioning (Weinberger & Harrison, 2011). The SCH spectrum disorders are described in general by fundamental and characteristic distortions of thinking and perception, and by inappropriate or blunted affect. Clear consciousness and intellectual capacity are usually maintained although certain cognitive deficits may evolve with time (WHO, 2019).

Psychosis is a syndrome – that is a set of symptoms in which it is hard for someone to think clearly, make advisable judgments, understand reality, communicate effectively, as well as respond emotionally and behave appropriately (Tamminga & Medoff, 2000).

The combination of psychotic symptoms varies, depending on the individual. However, these symptoms could be classified into three dimensions:

- Positive symptoms – also known as psychotic symptoms (e.g. delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behavior, agitation) (WHO, 2019).
- Negative symptoms are thoughts, feelings, or behaviors normally present but are absent or diminished in an individual with a psychotic disorder. Examples of negative symptoms are social withdrawal, apathy, poverty of speech, blunted or flat affect, limited emotional expression, anhedonia (i.e., inability to experience pleasure), as well as lack of motivation and defects in attention control (WHO, 2019).
- Cognitive symptoms emphasize an "executive dysfunction", which includes problems representing and maintaining goals, allocating attentional resources, evaluating and monitoring performance, and utilizing these skills to solve problems (WHO, 2019).

It has been hypothesized that the SCH spectrum disorders have four stages: (i) ultra-high risk (UHR) for psychosis, (ii) prodromal period, (iii) FEP, and (4) chronic stage or chronic disability from the disease.

UHR state of psychosis is based on a combination of moderate psychotic symptoms, help-seeking behavior, genetic risk, and social/occupational degradation (Bhavsar et al., 2018). There is evidence of cognitive impairment (deficiency) during the UHR period in adolescents and young people with a family history of psychosis or SCH (Bora et al., 2014; Kahn et al., 2015). The appearance of psychosis is usually preceded by a period (weeks, months, 2–5 years) (Häfner & Maurer, 2006) of mental and behavioral anomalies, including violations of the cognitive and emotional spheres, the perception of reality, inter-

action with society, motivation and sleep. *FEP* has been defined as a condition that meets at least the following criteria: (i) an individual is having a first treatment contact due to psychotic symptoms; (ii) defined duration of antipsychotic medication use; (iii) and/or duration of psychosis is specified (Breitborde et al., 2009). *The chronic stage of the SCH spectrum disorders* is characterized by persistent or repeated exacerbations of psychotic symptoms after a short- or long-term remission. The chronic stage may cause disability, complications of drug therapy, and lead to unemployment and inability to live independently. About 60% of FEP cases become chronic SCH, and approximately 25% will recover within the first five or six years (an der Heiden & Häfner, 2000). Meta-analyses have repeatedly confirmed that SCH is associated with higher-than-average mortality rates: two or three times higher than in the general population (Laursen et al., 2014; Simon et al., 2018). Data obtained from studies investigating life years lost among patients with SCH indicate a reduction of 13.5 years in life-span for men and 11.4 years for women (Laursen et al., 2018). The causes of death range from suicide and cancer to cardiovascular and respiratory diseases, as well as other natural and unnatural causes (Auquier et al., 2007). However, nowadays, more and more attention is paid to diabetes, metabolic syndrome (MetS) and cardiovascular complications that are widespread in patients with SCH and are risk factors for premature mortality (Azad et al., 2016; Schmitt et al., 2018; Simon et al., 2018).

2.2. Epidemiology of SCH spectrum disorders

The incidence for FEP is estimated at 34 new cases per 100,000 person-years, the median age-at-referral for men is 22.5 years and for women 23.4 years, whereas the incidence rates are highest for both men and women before 20 years of age (Kirkbride et al., 2017). The lifetime prevalence of psychotic disorders varies widely across studies and is from 2.5% to 3% of all psychotic disorders, from 0.9% to 1.2% for SCH, and 0.3% for schizoaffective disorder (Perälä et al., 2007; Chang et al., 2017). In addition, point prevalence of SCH on adults range from 0.9 to 17.4 per 1000 of the population (Warner & De Girolamo, 1995) and the median value per 1000 persons (10-90% quantiles) for the distributions for lifetime prevalence is 4.0 (1.6-12.1) (Saha et al., 2005).

2.3. Etiopathogenesis of SCH spectrum disorders

The risk of developing SCH spectrum disorders is associated with a genetic predisposition and environmental impact (Walder et al., 2014). Complex interactions between several external factors that affect the key periods in the development of the nervous system may result in a pronounced state of the disease (Howes & McCutcheon, 2017; Prata et al., 2017).

More than hundred years of research into SCH has not revealed specific etiologic factors for the development of the disorder. In modern psychiatry, it is considered that SCH is a heterogeneous multifactorial disorder with unknown single etiological cause, which is based on a predisposition model to the effect of stress (Häfner, 2014; Klengel & Binder, 2015; Howes & McCutcheon, 2017; Bolhuis et al., 2019). Impairments in cortical and subcortical circuitry, including disturbances in several brain neurochemical and immunological systems, and molecular mechanisms, appear to be core components of the neurobiological basis of SCH (Meyer & Feldon, 2009; Nakazawa et al., 2012; Schifani et al., 2017). People with SCH spectrum disorders show abnormalities in several organ systems in addition to the CNS (i.e., the immune and cardiometabolic systems, and hypothalamic-pituitary-adrenal system) (Pillinger et al., 2019a).

2.3.1. Biological factors

2.3.1.1. Genetic factors

Genetic factors and gene-environment interactions together contribute to over 80% of the probability for developing SCH (Sullivan et al., 2003; Tandon et al., 2008). If one of the parents or siblings has the illness, the probability of developing the disease is 9%. If both parents have SCH, then the probability of getting the disease is 36%. Twin studies of SCH show consistent evidence of genetic effect in monozygotic than dizygotic twins, 48% and 17%, respectively (Tamminga & Medoff, 2000).

The summation of data obtained from studies using new genomic technologies confirms that SCH is determined by the interaction of risk genes (Wang et al., 2018b). To date, genome-wide association studies (GWAS) of the entire genome have revealed 128 associations covering more than 100 different genetic loci (Ripke et al., 2014). It also revealed rare, but repetitive, 11 copy number variants (CNV) loci, which individually determine the relatively high risk of SCH (Malhotra & Sebat, 2012).

In SCH, 75% of the 108 identified associations covering certain loci are protein-coding genes (Ripke et al., 2014). There are associations of the expression of some genes in the brain that support the currently available hypotheses related to the dopamine receptor D_2 (*DRD_2*), glutamatergic neurotransmission (e.g., *GRM3, GRIN2A, SRR, GRIA1*) and synaptic plasticity, as well as coding for calcium channel subunits (*CACNA1C, CACNB2,* and *CACNA1I*) (Heyes et al., 2015; Fernández-Montoya et al., 2016; Devor et al., 2017). Furthermore, GWAS studies of SCH describe the statistically significant association with many highly correlated variants in the major histocompatibility complex (MHC), which plays an important role in the immune system and the immune system development. This process presupposes a relationship between SCH spectrum disorders and the immune system (Ripke et al., 2014). What is more, SCH spectrum disorders have been shown to share common risk alleles with

other psychiatric disorders, such as bipolar disorder, major depressive disorder, autism spectrum disorders and attention deficit hyperactivity disorder (Rees et al., 2015).

At the same time, the studies have confirmed that SCH is highly pleiotropic (i.e., multiple effects are produced by a single gene) (Lam et al., 2019; Schrode et al., 2019). Epidemiological, clinical and genetic studies suggest high comorbidity between SCH spectrum disorders and cardiovascular risk factors (e.g. levels of triglycerides (TGs), low- and high-density lipoprotein cholesterols (LDL-c and HDL-c)) and MetS, as well as the relationship between body mass index (BMI) and immunological parameters (Cheng et al., 2012; Liou et al., 2012; El-Hadidy et al., 2014; Misiak et al., 2016; Kalelioglu et al., 2017).

2.3.1.2. Neurochemical theory of psychotic disorder

Neurochemical theory suggests that specific abnormalities in the brain neurotransmitter systems may cause different kinds of psychotic symptoms. The key role belongs to the excitatory glutamatergic system (Stahl, 2008), the neurons of which use more than two-thirds of the energy released during the oxidation of glucose in the brain (Rothman et al., 2003). N-methyl-d-aspartate (NMDA) receptors are the major subtype of glutamate receptors that participate in rapid excitatory synaptic transmission. In addition to binding glutamate, the NMDA receptor requires glycine as an endogenous co-agonist for its activation and maximizing its conductance (Blanke & VanDongen, 2009). NMDA receptors are located in brain circuits that regulate the release of dopamine (Javitt, 2010) and dysfunction of the glutamatergic system leads to an imbalance of the dopaminergic activity (Javitt, 2010; Rubio et al., 2012), causing alterations in the function of other neurotransmitter systems (e.g., serotonin, gamma-aminobutyric acid (GABA), norepinephrine, acetylcholine) and in the levels of various neuropeptides (Brisch et al., 2014). NMDA hypofunction is often seen as the basis for positive symptoms, negative symptoms, and cognitive impairment in SCH (Lee & Green, 2016). Hyperfunction of dopaminergic neurotransmission in the mesolimbic pathway leads to positive symptoms and hypofunction in the mesocortical pathway causes negative symptoms and cognitive decline (Stahl, 2008; Owen et al., 2016).

2.3.1.3. The role of inflammation in SCH spectrum disorders

Alterations in the communication pathways between the immune and the nervous system play a crucial role in the initiation and progression of psychotic disorders (Khandaker et al., 2014a; Howes & McCutcheon, 2017). The evidence accumulated over the past two decades has shown that there are several ways in which systemic inflammation can have a profound effect on brain function, leading to changes in mood, cognition, and behavior (Khandaker &

Dantzer, 2016). Autoimmune diseases are associated with an increased risk of developing SCH and other psychiatric disorders (Khandaker et al., 2014b; Wang et al., 2018a).

There is an imbalance between pro-inflammatory and anti-inflammatory cytokines in SCH (Khandaker et al., 2015). Cytokines are signaling molecules that synchronize innate and adaptive immunity, affecting many different cells, including neurons of the CNS (Upthegrove et al., 2014), taking part in the synaptic plasticity of neurons, tissue repair, neurogenesis, and synaptogenesis (Altamura et al., 2014; Kakar, 2015). Under normal physiological conditions, the peripheral immune system is separated from the CNS by the blood-brain barrier (BBB), which is formed by vascular cells (i.e., endothelial cells, pericytes, and smooth muscle cells), glia (i.e., astrocytes, oligodendroglia, and microglia) and neurons (Zlokovic, 2011). However, under certain conditions, the permeability of the BBB increases and cytokines produced by chronically activated macrophages and T-lymphocytes penetrate into the CNS (Prat et al., 2005; Duarte-Delgado et al., 2019). Researchers have identified altered levels of several growth factors, pro- and anti-inflammatory biomarkers, including cytokines, such as IL-6, IL-1β, IL-8, IL-4, IL-10, and interferon (INF)–γ in both the brain and peripheral blood in SCH and FEP patients (Watanabe et al., 2010; Miller et al., 2011; de Witte et al., 2014; Prata et al., 2017).

Ligands for ErbB receptors are members of the molecular superfamilies represented by epidermal growth factor (EGF) and neuregulins, and are implicated in the etiopathology of SCH (Futamura et al., 2002; Stefansson et al., 2002). EGF has a neurotransmitter-like or neuromodulatory role in the CNS (Yamada et al., 1997). EGF enhances NMDA receptor-mediated increase of the intracellular Ca^{2+} concentration in cultured rat hippocampal neurons (Abe & Saito, 1992), protects against the glutamate toxicity-induced death of dopaminergic neurons in culture, induces an increase of glutamine synthetase activity in astrocytes in vitro, and exhibits a neurotrophic influence on dopaminergic neurons (Yamada et al., 1997). There is an evidence that serum EGF levels are decreased in patients with SCH (the sample consisted of drug-naïve patients n=4 and treated chronic patients n=45) (Futamura et al., 2002). Contrary, Hashimoto et al. (2005) found that serum levels of drug-naïve patients (n=15) or medicated patients (n=25) with SCH did not differ from those of CSs (n=40). However, the EGF level was correlated with the severity of symptoms in patients with SCH (Hashimoto et al., 2005). Growth factors have an impact on cytokine production and are related to inflammatory responses. The preclinical studies suggest that there is an association of EGF with pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α, and the anti-inflammatory cytokine IL-10 (Islam et al., 2016).

Increased blood levels of IL-6 are one of the most consistently confirmed immunological features associated with SCH (Goldsmith et al., 2016; Pillinger et al., 2019b; Fraguas et al., 2019). IL-6 has pro- and anti-inflammatory properties which are context-dependent (Hunter & Jones, 2015). The elevated levels of IL-6 are related to the duration of the disorder, the resistance of

patients to AP treatment, and can be taken as state markers for acute disease exacerbation (Zalcman et al., 1994; Miller et al., 2011). Moreover, there is evidence that IL-6 modulates dopaminergic metabolism and symptomatology in SCH (Kim et al., 2000). Furthermore, IL-6 also has a non-immunological and non-neurochemical role, such as inducing obesity-related metabolic disorders in SCH (Borovcanin et al., 2017).

IL-1 is a pro-inflammatory cytokine that has neurodegenerative and neuroprotective properties, and it is involved in the modulation of synaptic plasticity as well as stress responses in the brain (Sapolsky, 1987; Giles et al., 2015). Although IL-1α and IL-β are encoded by separate genes, they elicit similar biological activity (Shaftel et al., 2008). This cytokine strongly modulates the release and processing of EGF and neuregulin 1, and exhibits association with ErbB signaling (Higashiyama et al., 2008). There are inconsistencies between studies concerning IL-1 levels in patients with SCH (Miller et al., 2011; Upthegrowe et al., 2014; Goldsmith et al., 2016).

IL-2 influences various lymphocyte subsets during differentiation, immune responses, and homeostasis (Boyman & Sprent, 2012). Furthermore, it is a potent modulator of dopamine activity in the mesocorticolimbic and mesostriatal systems, and it is also associated with increased motor activity and psychopathological outcomes of SCH, which at least partly reflect aberrations in central dopaminergic transmission (Zalcman, 2002). Several studies have reported altered peripheral levels of IL-2 in SCH (Ganguli et al., 1989; Potvin et al., 2008).

IL-8 (or chemokine C-X-C motif ligand 8) is a chemokine produced mainly by macrophages. IL-8 production is increased by OxS, which in turn causes the recruitment of inflammatory cells and induces a further increase in OxS mediators, making it a key parameter in localized inflammation (Vlahopoulos et al., 1999). IL-8 was also shown to be associated with an increase in BMI (Sharabiani et al., 2011). In several studies, it has been found that IL-8 levels were higher in patients with SCH than those of CSs (Kaminska et al., 2001; Zhang et al., 2002).

IL-10 is a regulatory cytokine, which maintains the balance between pro- and anti-inflammatory cytokines (Murray, 2006). Evidence has demonstrated that IL-10 is associated with SCH. A meta-analysis of genomic studies demonstrated that in the Asian population, subjects with a single nucleotide polymorphism (rs1800872) and two haplotypes (A-C-A and G-C-C) of IL-10 are vulnerable to SCH (Gao et al., 2014). Furthermore, a significant increase of IL-10 was observed in patients with SCH compared with CSs (Kunz et al., 2011), and it has been demonstrated that blood levels of IL-10 are correlated to the extent of cognitive impairment in patients with SCH (Xiu et al., 2016).

IL-4 is an anti-inflammatory cytokine that contributes to the suppression of the immune and inflammatory response. Previous results have described decreased levels (Kim et al., 2009) or no significant changes in the serum levels of IL-4 in SCH patients (Potvin et al., 2008).

Studies regarding TNF-α levels in individuals with SCH vary from low (Francesconi et al., 2011; Tian et al., 2014) to no difference (Kaminska et al., 2001; Potvin et al., 2008) to higher levels (Miller et al., 2011; Di Nicola et al., 2013; Goldsmith et al., 2016). TNF-α is involved in systemic inflammation and is produced mainly by activated macrophages, natural killer cells, and lymphocytes. Furthermore, TNF-α is synthesized in adipose tissue by adipocytes and data suggests an important role TNF-α holds in the insulin resistance of obesity and T2DM (Hotamisligil & Spiegelman 1994).

Studies suggest that there is a linear relationship of ferritin concentrations and acute-phase proteins (DePalma et al., 2010; Namaste et al., 2017). The increased plasma ferritin concentration, as a marker of increased iron concentrations, is associated observationally and genetically with low-grade inflammation, possibly indicating a causal relationship from increased ferritin to inflammation (Moen et al., 2018).

INF-γ elevations are frequently noted in patients with SCH (Miller et al., 2011; Pillinger et al., 2019a). INF-γ is produced predominantly by T helper cells, macrophages, and natural killer cells as a part of the innate response, and it is also an important activator of MHC molecule expression (Billiau, 1996). Moreover, INF-γ is a pro-inflammatory cytokine involved in the pathology of the neuroinflammatory response and is mostly released from activated microglia (Na et al., 2014).

Activation of the immune system in SCH occurs not only in the periphery but also in the brain (Leonard et al., 2012). Inflammation in the CNS is mediated by pro-inflammatory cytokines, microglial cells, astrocytes, and invading immune cells such as monocytes, macrophages, and T or B lymphocytes (Schwarz, 2003). Furthermore, besides the direct action of cytokines on brain cells, a biochemical link exists between cytokines and the tryptophan-kynurenine pathway (Schwarz, 2003). Tryptophan is the precursor of two distinct metabolism pathways, leading to the end products of either 5-hydroxytryptamine (5-HT) or kynurenine. Enzymes of the kynurenine pathway are expressed in different tissues and cell types throughout the body and are regulated by cues, including inflammatory signals (Cervenka et al., 2017). By the enzymatic action of indoleamine-2,3-dioxygenase (IDO), tryptophan is converted into quinolinic acid, a potent neurotoxin, related to NDMA receptor activation, and tryptophan-2,3-dioxygenase (TDO) enzymatic activity is related to the synthesis of kynurenic acid, an NMDA and nicotine alpha7 receptor antagonist (Macedo et al., 2019). Proinflammatory cytokines (e.g., INF-γ, IL-1, IL-2, IL-6, TNF-α) can induce IDO activity (Carlin et al., 1989; Leonard et al., 2012). Thus, as a consequence of this systemic metabolism integration, peripheral inflammation can facilitate the accumulation of kynurenine in the brain, which has been associated with psychotic symptoms and cognitive deficits among patients with SCH (Javitt et al., 2012; Cervenka et al., 2017). The kynurenine pathway generates tryptophan metabolites with diverse biological activities throughout the body and although mainly studied in relation

to the brain and mental health, the action of kynurenine metabolites on peripheral tissues might be even more meaningful (Cervenka et al., 2017).

However, it has been suggested that there are biological subtypes of SCH spectrum disorders (Miller et al., 2009), and that immune alterations are seen only in a proportion of patients (Pillinger et al., 2019b).

2.3.1.4. Cardiometabolic abnormalities in SCH spectrum disorders

SCH spectrum disorders are associated with a 2- to 3-fold excess mortality (Nielsen et al., 2013; Suvisaari et al., 2013; Termorshuizen et al., 2014) and a 8–20-year life span shortening (Chang et al., 2011; Nordentoft et al., 2013). Poor physical health has traditionally been referred as a result of secondary impacts of the disease itself, in addition to an unhealthy lifestyle and a poor diet, or seen as a consequence of AP treatment (McGreadi et al., 2003; Bressington et al., 2016). However, in recent years, studies in drug-naïve FEP patients have confirmed that dysfunction in the cardiometabolic system is already present at the early stage of the disease (Pillinger et al., 2019a).

It has been repeatedly confirmed that compared with the general population, people with severe mental illness have a higher risk of developing obesity-related problems such as hypertension, stroke, MetS, and T2DM (Foley & Morley, 2011; De Hert et al., 2012; Stubbs et al., 2015). MetS incidence in psychiatric patients with FEP has been shown to range between 6.0% and 9.8% (Fleischhacker et al., 2013; Mitchell et al., 2013). The incidence of MetS among patients with SCH ranges from 24% to 43% in males and from 27% to 52% in females (Lin et al., 2018). MetS is a combination of insulin resistance, impaired glucose regulation, dyslipidemia, hypertension, microalbuminuria, and obesity (Anjum et al., 2018). Obesity occurs when excess energy accumulates in adipocytes and it involves an increase in both the number and the size of fat cells. There are three types of adipose tissue: brown, beige and white (Carrillo et al., 2018). Brown adipose tissue is less abundant and is involved in lipid oxidation and energy balance; beige adipose tissue has the pathway of adaptive thermogenesis, and white adipose tissue is an endocrine organ that secretes different molecules (Carrillo et al., 2018). One of the major features of adipocyte biology is the discovery of its complex secretory activities (Lafontan, 2005). Leptin, adiponectin, proinflammatory cytokines, acute phase reactant proteins, MCP-1, and resistin are of great interest among the growing number of factors found to be secreted by adipocyte (Trayhurn & Beattie, 2001; Havel, 2004; Lafontan, 2005).

The overexpressed pro-inflammatory cytokines, particularly IL-6, are considered as the link between obesity and inflammation (Hotamisligil, 2006). It has been established that higher plasma levels of IL-6 are significantly correlated with an increased amount of adipose tissue and increased BMI (Lee et al., 2017) and it has been shown that one-third of total circulating concentrations of IL-6 originate from adipose tissue (Fontana et al., 2007). Also,

TNF-α has been considered as a key component in the obesity-diabetes link (Tzanavari et al., 2010). TNF-α has been suggested to be involved in the pathogenesis of SCH. However, this relationship remains controversial (Potvin et al., 2008).

Adiponectin or adipocyte-specific protein is involved in the regulation of glucose levels as well as in fatty acid breakdown, and its serum concentrations are reduced in a variety of obese and insulin resistance states (Lafontan, 2005). Comparative meta-analysis has shown that people with SCH treated with second-generation AP treatment have lower plasma adiponectin levels than CSs (Bartoli et al., 2015).

MCP-1 is a potent adipokine. It is considered to be a specific chemo-attractant for monocytes and macrophages, which may also play a role in the development of obesity, diabetes, and cardiovascular diseases (Panee, 2012). The elevated level of MCP-1 and its association with MetS in patients with SCH has been shown (Drexhage et al., 2008).

Biologically active procoagulant molecule plasminogen activator inhibitor-1 (PAI-1) is also produced by adipocytes (Lafontan, 2005). Current evidence suggests that PAI-1 plays the central role played in many age-related subclinical (i.e., inflammation, atherosclerosis, insulin resistance) and clinical (i.e., obesity, comorbidities) conditions (Cesari et al., 2010). Furthermore, there is also emerging literature sugesting the plasminogen pathway in SCH (Hoirisch-Clapauch & Nardi, 2016; Jeffries et al., 2018).

Leptin is a mediator of long-term regulation of energy balance, controlling food intake and thereby inducing weight loss (Klok et al., 2007). Furthermore, leptin is a pleiotropic molecule that may regulate neuroendocrine and immune functions (Margetic et al., 2002; Havel et al., 2004). Positive effectors of leptin production are glucose, insulin, glucocorticoids, and TNF-α, among others (Lafontan, 2005). Leptin and insulin act synergistically to modulate the central regulation of feeding and whole-body energy homeostasis (Niswender & Schwartz, 2003). In humans, both high and low levels of leptin have been associated with psychopathology of mental disorders (Wędrychowicz et al., 2014).

Several studies have identified resistin as the main hormone linking insulin-resistance to obesity, primarily through the activation of Toll-Like Receptor 4 signaling pathways (Benomar & Taouis, 2019). Also, resistin is found to be associated with acute and chronic inflammatory-related diseases (Pang & Lee, 2006). Researchers have indicated that the rates of insulin resistance, impaired glucose tolerance, and hyperinsulinemia are high in AP-naïve FEP patients, patients with a chronic stage of the disease and unaffected relatives of patients (Guest, 2019). Furthermore, Tomasik et al. (2019) recently demonstrated that SCH polygenic risk score was significantly linked to insulin resistance in AP-naïve FEP patients regardless of demographic, lifestyle and clinical factors.

Moreover, it is widely accepted that healthy gut microbiota is essential for human health. Studies demonstrate that the microbiota may impact weight gain and adiposity through several interconnected pathways, such as energy harvest

and production of microbial metabolites, through effects on inflammatory responses and the gut-brain axis (Lazar et al., 2019). Preclinical experiments in gnotobiotic models of mice and rats revealed the effect of an imbalance of the microbiota on the intestinal mucosa and the host immune system (Karlsson et al., 2011; Wrzosek et al., 2013). This causes an increase in various immunological mediators in the intestinal wall, thereby stimulating the body's immune system (Okumura & Takeda, 2017) and implicating the presence of low-grade inflammation (Cani et al., 2008) and metabolic imbalance (Cani et al., 2007). Furthermore, there are significant alterations in the microflora of obese and SCH people (Ley et al., 2006; Clemente et al., 2012; Zheng et al., 2019).

Several mechanisms are responsible for hepatic and muscle insulin resistance (Abel, 2010). These include (i) increased activation of inflammation-mediated signaling cascades; (ii) lipotoxicity, which can be caused by excessive accumulation of TGs and metabolic intermediates, such as diacylglycerols and ceramides, which come from saturated fatty acids (Rutkowsky et al., 2014); (iii) incomplete mitochondrial β-oxidation of long-chain fatty acids (LCFA), due in part to relatively low tricarboxylic acid cycle capacity, which increases tissue accumulation of acetyl-coenzyme A (acetyl-CoA) and generates chain-shortened AC molecules that activate pro-inflammatory pathways implicated in insulin resistance (Adams et al., 2009).

ACs metabolism is a key factor in regulating the balance of intracellular sugar and lipid metabolism (Qu et al., 2016). The metabolism of fatty acids (with a long chain) begins in the cytoplasm with the addition of carnitine (CARN), then the process of oxidation in the mitochondria of the cells takes place according to the β-oxidation (each time the β-carbon atom is oxidized) with the active participation of AC located on the surface of the inner mitochondrial membrane (Muñiz, 2003; Rosenthal & Glew, 2009; Grevengoed et al., 2014). Studies suggest that the long-chain acyl-coenzyme A (acyl-CoA) synthetases may play a role in directing fatty acids either toward complex lipid synthesis and storage or toward oxidation (Coleman et al., 2002). However, blood ACs are indirect indicators of altered β-oxidation of fatty acids (Schooneman et al., 2013).

There is evidence that lipolysis and β-oxidation are activated during SCH, probably as a result of insufficient energy supply to the brain (Yang et al., 2017), which can cause a shift in the profile of ACs and CARN (i.e., the accumulation of species acyl-CoA in mitochondria), causing the CARN shuttle to work in reverse order (Knottnerus et al., 2018). ACs profile analysis is a method for diagnosing disorders of fatty acid oxidation and metabolic disorders of organic acids (Graef et al., 1997; Cao et al., 2019). Thus, mitochondrial dysfunction associated with SCH (Rollins et al., 2009) could be described through the altered serum profiles of ACs (Millington & Stevens, 2011).

2.3.2. Psychosocial factors

Traumatic life events in childhood are a potential risk factor for SCH. According to research data, the risk of developing psychosis in people with a history of childhood trauma is almost 3 times higher than in the CSs, while in the population this risk is 33% (Şahin et al., 2013). Among prodromal patients, 70% have a history of traumatic events (Bechdolf et al., 2010), which are maltreatment, physical, psychological, and sexual abuse as well as loss or divorce of parents in childhood, substance abuse by parents and poverty (Green et al., 2014).

2.3.3. Environmental factors

Ecological theories link the genesis of SCH with pre-, peri- and postnatal damage to the fetus, which promotes the expression of gene combinations that may predispose to SCH (Stilo et al., 2011). The risk of SCH is increased, for example, if during pregnancy, the mother suffered an infection of influenza or rubella, was malnourished, had diabetes mellitus or smoked (Mueser & McGurk, 2004). Those suffering from SCH are more often born in the winter or early spring, have a small birth weight, are born in difficult births or suffered a CNS lesion or a head injury in early childhood (Tandon et al., 2008). One of the alleged pathogenetic mechanisms of SCH is local hypoxia of the brain during critical periods in the migration and maturation of neurons (Weinberger & Harrison, 2011).

It is well known that psychostimulants such as amphetamine and methamphetamine increase synaptic dopamine concentrations. Furthermore, the use of cannabis is a potential risk factor predisposing to SCH. The findings indicate a 40% increase in the likelihood of developing psychosis in cannabis users compared to the control group (Manrique-Garcia et al., 2012; Gage et al., 2016; Di Forti et al., 2019). The use of cannabis leads to the development of psychosis 2.7 years earlier compared to those who developed psychosis not having used cannabis (Degenhardt et al., 2013). In the GWAS, 35 significant genes were identified in 16 regions, whereas 21 of the genes had different levels of expression in humans using cannabis, the largest correlation in the cell adhesion molecule 2 (*CADM2*) gene, which demonstrated evidence for a causal positive impact of SCH on cannabis use (Pasman et al., 2018). Furthermore, the evidence of gene-environment interaction between the catechol-O-methyltransferase (*COMT*) gene and exposure to cannabis has an important role in dopamine metabolism in the prefrontal cortex and other brain regions (Caspi et al., 2005; Tunbridge et al., 2006).

In summary, studies in recent decades have provided ample evidence of the importance of inflammatory and metabolic changes in SCH spectrum disorders, which affect the course of the disease, the development of side effects of the AP treatment, and patients' quality of life. Immune dysregulation and metabolic

alterations have been found in numerous studies comparing AP-naïve or treated FEP patients to healthy controls. Meta-analyses have confirmed that patients with an SCH spectrum disorder, on a group level, demonstrate signs of the low-grade peripheral inflammation and metabolic changes. The identification of quantifiable biological biomarkers and using these biomarkers in clinical practice have great potential for improving diagnostic accuracy, stratifying treatment selection, providing prognostic information, monitoring relapse, detecting and helping to prevent mental health problems, particularly psychotic symptoms before they reach clinical-level symptomatology.

3. AIMS OF THE THESIS

This thesis is based on three empirical articles that explore inflammatory and metabolic abnormalities at the beginning and in the chronic stage of the SCH spectrum disorders.

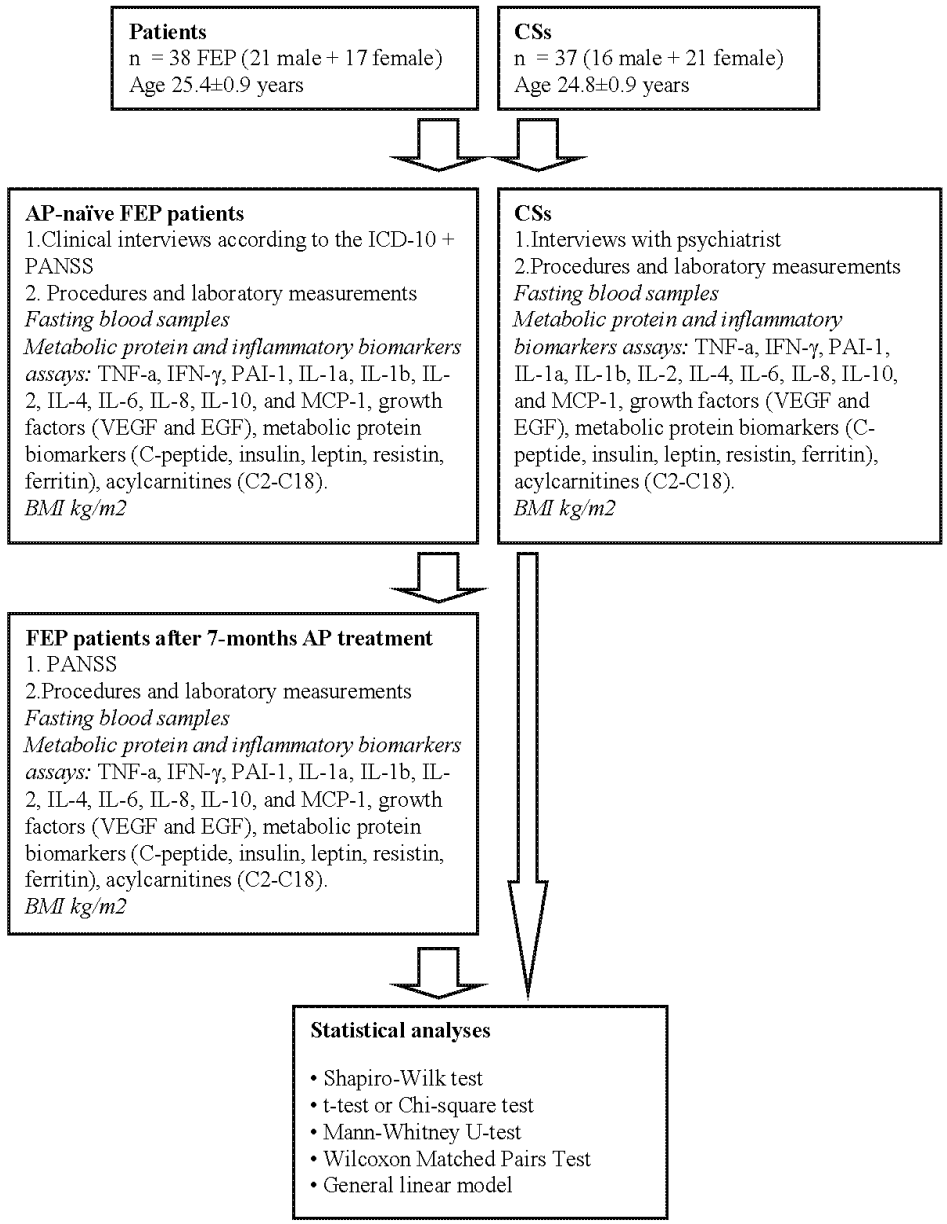
The general thematic research foci were the following:

1. To evaluate the inflammatory and metabolic biomarker level differences in patients with FEP before and after a 7-month treatment with APs as compared to CSs (Paper I).
2. To characterize a bioenergetic dysfunction through a complex interplay between inflammatory and metabolic protein biomarkers as well as ACs profiles in patients with FEP before and after a 7-month AP treatment as compared to CSs (Paper II).
3. To identify low-grade inflammatory and metabolic alterations in patients with SCH spectrum disorders as compared to CSs (Paper III).

4. SUBJECTS AND METHODS

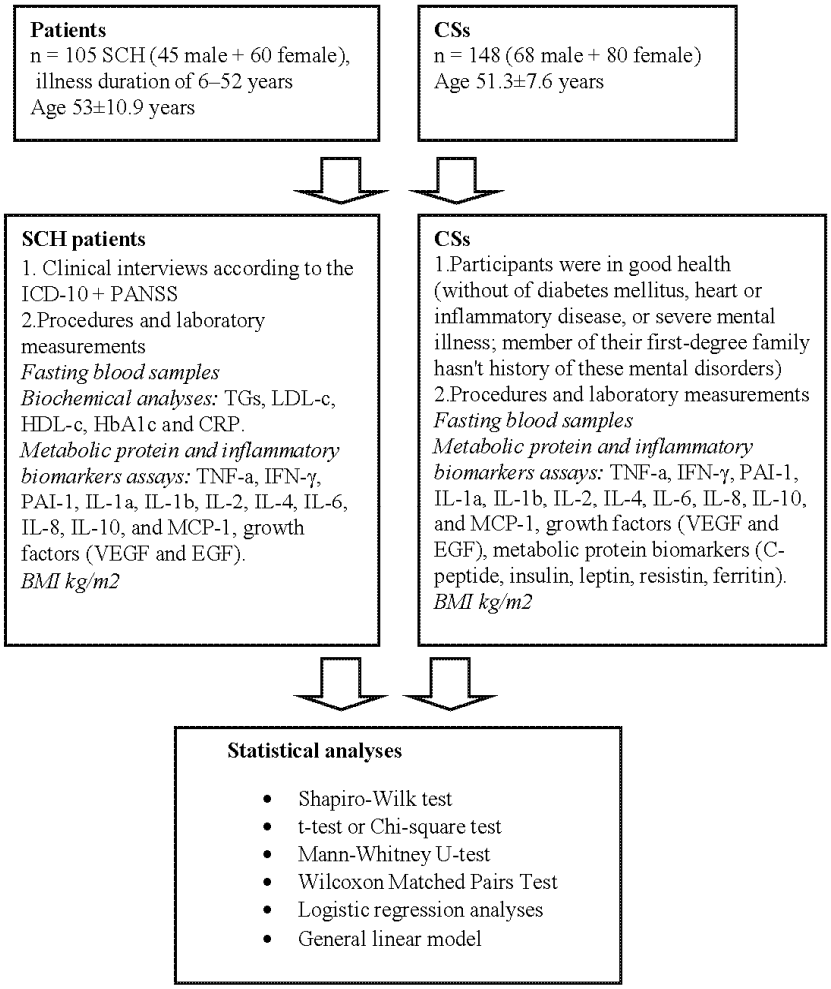
The research project was approved by the Ethics Review Committee on Human Research, the University of Tartu, Estonia (initial approval No 177/T-2 issued on 15 Feb 2008 and follow-up approval No 211/M-22 issued 23 Jan 2012; No96/16 issued 21 Aug 2001 and No 176/T-4 issued 17 Nov 2008). Written informed consent was obtained from all participants.

Below are visual illustrations of the design of studies: Figure 1 comprises participants and methods for FEP patients' studies, and Figure 2 provides an overview of the study on patients with the chronic stage of the disease.



ICD-10 – International Classification of Diseases, PANSS – Positive and Negative Syndrome Scale, FEP – first-episode psychosis, SCH – schizophrenia, CSs – control subjects, AP – antipsychotics, BMI – body mass index, IL – interleukin, CRP – C-reactive protein, TNF-α – tumor necrosis factor-α, INF-γ – interferon-γ, VEGF – vascular endothelial growth factor, EGF – epidermal growth factor, PAI-1 – plasminogen-activator inhibiitor-1, MCP-1 – monocyte chemo-attractant protein-1.

Figure 1. Schematic summary of materials and methods for FEP patients' studies (Paper I, II).



ICD-10 – International Classification of Diseases, PANSS – Positive and Negative Syndrome Scale, FEP – first-episode psychosis, SCH – schizophrenia, CSs – control subjects, AP – antipsychotics, BMI – body mass index, IL – interleukin, CRP – C-reactive protein, TNF-α – tumor necrosis factor-α, INF-γ – interferon-γ, VEGF – vascular endothelial growth factor, EGF – epidermal growth factor, PAI-1 – plasminogen-activator inhibitor-1, MCP-1 – monocyte chemo-attractant protein-1, TGs – triglycerides, LDL-c – low-density lipoprotein cholesterol, HDL-c – high-density lipoprotein cholesterol, HbAc1 – glycated hemoglobin.

Figure 2. Schematic summary of materials and methods for SCH patients' study (Paper III).

4.1. Subjects

The FEP and SCH patients were recruited from the Psychiatric Clinic of the Tartu University Hospital, Estonia (Paper I, II, and III) and partially from the *Võisiku* Psychiatric Nursing Home, a state nursing home in central Estonia (Paper III). Psychiatric diagnoses were based on clinical interviews according to the criteria of the International Classification of Diseases, tenth edition (ICD-10) (WHO, 1992), and the illness history was confirmed by an examination of the patients' medical records and semi-structured psychiatric interviews.

4.1.1. FEP patients and CSs (Paper I, II)

38 FEP patients (21 males and 17 females; mean age 25.4±0.9 years) fulfilled the following inclusion criteria: they were 18–45 years old; had experienced a FEP; duration of their untreated psychosis had been less than 3 years; no AP treatment received before the first contact with medical services for psychosis. Patients were excluded from the study if they had psychotic disorders additionally to any other main health condition or had substance-induced psychosis. Thirty-six FEP patients underwent treatment using AP medication (two refused) and were included in the follow-up analysis. History of AP medication was collected according to reviews of patients' medical charts. Patients were treated with various AP medications according to what was clinically indicated, and treatment options were allowed to change over the course of the 7-month interval. During the follow-up period, patients received either atypical (n=24), typical (n=1) or mixed (n=11) AP medication; the theoretical chlorpromazine (CPZ) daily dose equivalent means (±sd) was 396 (±154) mg (range from 80 to 640 mg). Twenty-eight patients were treated only with APs, but 5 patients additionally needed mood stabilizers and 6 patients also received antidepressants or hypnotics. Psychopathology was measured using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). Data were collected at two time points: on admission and after the follow-up period (mean duration 7.2±0.7 months).

Thirty-seven mentally healthy subjects participated in the study as CSs (16 males and 21 females, mean age 24.8±0.9 years). The CSs sample was recruited by advertising in the same geographical area the FEP patients came from. CSs were interviewed by experienced psychiatric doctors to avoid the inclusion of subjects as controls with mental disorders. Exclusion criteria for the control group also included psychotic disorders among close relatives. Data from CSs were collected cross-sectionally.

4.1.2. Patients with the chronic stage of the disease and CSs (Paper III)

The total number of patients who agreed to take part and met the inclusion criteria was 105 (45 males and 60 females). They were aged 28–74 (53.1±10.9) years, with illness duration of 6–52 years (mean 19.9±9.8). They had a stable AP medication regimen and sustained clinical status for at least one month before entering the study. All the patients were taking AP drugs and some received more than one medication, as was clinically indicated. Psychopathology was measured using the PANSS (Kay et al., 1987). The following AP medications were taken: haloperidol (30 cases), zuclopenthixol (n=29), clozapine (n=24), chlorprothixene (n=17), risperidone (n=13), olanzapine (n=11), aripiprazole (n=6), sertindole (n=6), quetiapine (n=5), sulpiride (n=5), perphenazine (n=3), melperone (n=2), chlorpromazine (n=2) and flupenthixol (n=1). Patients with SCH suffered neither from comorbid psychiatric disorder nor from somatic disease caused primarily by inflammation imbalance.

CSs were recruited by advertisement among hospital staff and the general public of the same geographical area. Inclusion criteria for the control group were the absence of a personal history of diabetes mellitus, cardiac or inflammatory illness, or severe mental disorder (including psychotic, bipolar and major depressive disorder). Exclusion criteria for the control group also included psychotic disorders among close relatives. Datasets were collected cross-sectionally.

4.2. Methods

4.2.1. Procedures and laboratory measurements

All venous blood samples were taken from the antecubital vein after subjects had fasted overnight for a minimum of 12 hours, between the hours of 9am and 10am. Blood (5 ml) was sampled using anticoagulant-free tubes and kept for 1 h at 4 °C (for platelet activation) before the serum was isolated. Blood samples were centrifuged at 2000 rpm for 15 min at 4 °C, and then the serums from FEP patients and their CSs were stored at -20 °C and serums from chronic patients and their CSs were kept at -80 °C until testing.

4.2.2. Biochemical analyses, and measurements of inflammatory, metabolic protein biomarkers, and ACs

4.2.2.1. Biochemical measurements

Biochemical analyses, including TGs, LDL-c, HDL-c, glycated hemoglobin (HbA1c) and C-reactive protein (CRP) were determined by standard clinical laboratory methods using certified assays performed at the Tartu University Hospital's Clinical Laboratory, Estonia.

4.2.2.2. Measurement of inflammatory biomarkers

High-sensitivity biochip array technology (Randox Biochip, RANDOX Laboratories Ltd., Crumlin, UK) was used for simultaneous quantitative detection of multiple analyses. The core technology of the Randox Biochip is a solid-state device that contains an array of discrete test regions of immobilized antibodies specific to different cytokines and growth factors. A sandwiched chemiluminescent immunoassay was employed (according to the manufacturer's protocol) on the cytokine array to measure the serum concentration of cytokines (TNF-α, IFN-γ, PAI-1, IL-1α, IL-1b, IL-2, IL-4, IL-6, IL-8, IL-10, and MCP-1) and growth factors (VEGF and EGF). The results were expressed as picograms per milliliter. The reproducibility of the assay for individual cytokines was determined using the quality controls provided with the kit.

4.2.2.3. Measurement of metabolic protein biomarkers

The following metabolic biomarkers: C-peptide, insulin, leptin, resistin, and ferritin, were measured according to biochip array technology (Randox Biochip, RANDOX Laboratories Ltd, Crumlin, U.K., Metabolic Syndrome Array I for Evidence Investigator™).

Intra-assay and inter-assay precisions for inflammatory and protein metabolic biomarkes are given in Supplementary Table A-5, Table A-6, and Table A-8. Supporting information, units and sensitivity are provided in Supplementary Table A-7, and Table A-9.

The serum concentrations of adiponectin (ng/ml) were analysed by a quantitative sandwich enzyme immunoassay technique, using a commercially available kit (R&D Systems, Minneapolis, MN, USA). Intra-assay and inter-assay presicions are given in Supplementary Table A-10.

4.2.2.4. Measurement of ACs

The serum levels of ACs were determined with the AbsoluteIDQ^{TM} p180 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) using the flow injection analysis tandem mass spectrometry ([FIA]−MS/MS) as well as high-performance liquid chromatography ([HPLC]−MS/MS) technique. All measurements were performed as described in the manufacturer's manual UM-P180. Identification and quantification of the metabolites were achieved using multiple reactions monitoring along with internal standards. From all statistically important changes of ACs in our study, in the discussion, we only used values that were at least 2.3 times higher than the level of detection (LOD) given in the manual of the Biocrates AbsoluteIDQ p180. The calculation of metabolite concentrations was automatically performed by MetIDQ software (BIOCRATES Life Sciences AG). To ensure data quality, they were checked based on the LOD. Average values of all measured ACs are presented in Supplementary Table A-11.

4.3. Statistical analysis

4.3.1. Demographic and clinical variables

Group differences regarding demographic characteristics were analyzed using a t-test or Chi-square test (Paper I, II, and III). An alpha of 0.05 was used as the cutoff for significance.

4.3.2. Inflammatory, metabolic protein biomarkers and ACs variables

The application of Shapiro-Wilk tests indicated that some of the inflammatory, metabolic and growth factor marker values were not normally distributed ($p < 0.05$). A Mann-Whitney U-test was applied to compare these parameters of the SCH patients and CSs (Paper III), and the AP-naïve FEP patients and CSs (Paper I and II). Wilcoxon Matched Pairs Test was used to compare the FEP patients' pre- and post-treatment conditions. Differences between FEP patients and CSs (based on the Mann-Whitney U-test), and differences between the pre- and post-treatment values within the patient's group (based on the Wilcoxon Matched Pairs Test) were considered to be significant at $p < 0.005$ (Paper I) or $p < 0.001$ (Paper II). Additionally, effect sizes (eta-squared, η^2) were computed and interpreted as small, medium and large, with corresponding η^2 ranging from 0.01 to 0.05, from 0.06 to 0.13 and ≥ 0.14, respectively (Cohen, 1988) (Paper I and II). Categorical (disease, gender, smoking status) and continuous (age) covariates were used in a general linear model (GLM) to compare biomarker levels (dependent variables) between groups (Paper I and II). To achieve the best fit of the model, the least significant variable was removed from the model until all variables had *p*-values less than 0.05. F-tests were used to further compare the approaches of linear models and to analyze the significant (disease or treatment) main effects in the final models. Furthermore, partial eta-squared values were established for the final models (Paper I and II). Because GLM analyses required normally distributed data, biomarkers' values were log10-transformed to approximate normality. To establish the treatment main effect (i.e., difference between pre- and post-treatment measurement occasion) on BMI and serum biomarkers levels (dependent variables), within-subjects' analysis (GLM repeated measures, adjusted for gender and smoking status) were utilized.

Also, GLM analysis was utilized to examine the associations between the ratio of TGs to HDL-c (TGs/HDL-c) and biomarkers as well as LDL-c, HbA1c serum levels and their main effects in patients (Paper III).

The statistical analyses were performed using Statistica software (StatSoft Inc., 12th and 13th edition) for Windows.

5. RESULTS

5.1. Inflammatory, metabolic and metabolomic alterations in FEP (Paper I, II)

5.1.1. General description of the study groups

There were no significant differences between FEP patients at admission and CSs in terms of age, gender or BMI values (Table 1). The 7-month AP treatment caused a significant change in BMI, and mean BMI gain at 7-month follow-up was 3.0 (±2.2) kg/m^2. During the 7-month treatment, psychopathology total score decreased significantly (Z=5.2, p <1E-06). Also, the difference in tobacco use (8 patients [21.1%] vs. 7 CSs [18.9%]) was not statistically significant.

Table 1. Demographic variables of FEP patients (n=38) and CSs (n=37) and clinical variables in FEP patients at the baseline and after 7-month AP treatment (n=36).

	FEP patients before AP treatment	FEP patients after AP treatment	CSs	Comparisons p-values
Age, years (mean ± s.d.)	25.4 ± 5.5	-	24.8 ± 5.3	t =0.49 p = 0.62
Gender (male / female)	21/17	-	16/21	$X^2_{(1)}$ = 1.08, p = 0.30
BMI (mean ± s.d.)	22.6 ± 2.9[a]	25.6 ± 4.0[b]	23.0 ± 3.0	$t_{(73)}$ = -0.69, p = 0.49[a] $t_{(35)}$ = -8.07, p <1E-06[b]
Smoking status (male / female)	8 (8/0)	8 (8/0)	7 (3/4)	$X^2_{(1)}$ = 0.05, p = 0.82[a]
CPZ equivalents (mg/day)	-	396 ± 154	-	-
PANSS (total symptom score)	58.9 ± 11.5	36.0 ± 8.3	-	Z = 5.23, p <1E-06

[a] Comparison between FEP patients and CSs;
[b] Comparison between FEP patients at baseline and after 7-month AP treatment.
AP – antipsychotic; BMI – body mass index; CPZ – chlorpromazine; FEP – first-episode psychosis; PANSS – Positive and Negative Syndrome Scale.

5.1.2. Differences in the level of biomarkers between AP-naïve FEP patients and CSs

Regarding measured growth factors as well as inflammatory and metabolic markers, a significant elevation of EGF, IL-4, IL-6, ferritin, resistin, and PAI-1 levels, and decrease in leptin, and IL-1β levels emerged when AP-naïve FEP patients were compared to CSs (Table 2). According to our sample, elevated serum levels of IL-4, IL-6, PAI-1, ferritin and resistin, and decreased levels of IL-1β and leptin were associated with a moderate effect size and EGF level was associated with a large effect size of the disease impact.

As for the measured carnitine, 39 ACs, and hexoses twenty-three ACs and hexoses exhibited shifts in FEP patients compared to CSs (descriptive information about all measured biomarkers is provided in the Appendix Table A-1). Significant differences in seven increased ACs levels (C14:1, C16, C16:1, C16:1-OH, C18:1, C18:2, C6[C4:1-DC]), and one decreased AC (C3) level demonstrated large effect sizes. Additionally, elevated levels of C3-DC(C4-OH), C18, C14:2, and decreased levels of C5, C9 with effect size of 0.10−0.14 occurred when AP-naïve FEP patients were compared with CSs (Table 2).

Table 2. Comparisons of inflammatory and metabolic biomarkers levels (pg/mL) between FEP patients before AP treatment and CSs.

Biomarkers	FEP patients before AP treatment	CSs	Z-value	*p*-value	Effect size η^2
	Median (min–max)	Median (min–max)			
Inflammatory biomarkers and growth factor					
IL (Interleukin)**-1β**[a]	1.29 (0.44 – 3.44)	1.91 (0.45 – 4.47)	-3.02	0.003	0.12
IL-4[a]	1.70 (1.02 – 3.75)	1.30 (0.96 – 2.84)	3.18	0.001	0.13
IL-6[a]	1.07 (0.33 – 5.03)	0.60 (0.13 – 1.93)	3.17	0.002	0.13
PAI (Plasminogen activator inhibitor)**-1**[b]	25.68 (8.92 – 48.58)	21.35 (7.57 – 49.88)	2.75	0.006	0.10
Ferritin[b]	56.0 (4.26 – 238.7)	22.1 (1.22 – 184.7)	2.86	0.004	0.11
EGF (Epidermal growth factor)[a]	17.55 (2.20 – 51.83)	2.05 (0.66 – 7.74)	6.67	<1E-06	0.59
Metabolic protein biomarkers[b]					
Leptin	0.85 (0 – 2.22)	1.41 (0.38 – 6.69)	-2.73	0.006	0.11
Resistin	2.96 (1.69 – 8.58)	2.66 (1.59 – 4.10)	2.79	0.005	0.11

Biomarkers	FEP patients before AP treatment	CSs	Z-value	*p*-value	Effect size η^2
	Median (min–max)	Median (min–max)			
Acylcarnitines[c]					
C14:1 (Tetradecenoylcarnitine)	0.06 (0.03 – 0.15)	0.04 (0.02 – 0.11)	-3.34	0.0008	0.15
C14:2 (Tetradecadienyl-carnitine)	0.02 (0.01 – 0.07)	0.02 (0.01 – 0.05)	-3.10	0.002	0.13
C16 (Hexadecanoylcarnitine)	0.12 (0.06 – 0.20)	0.09 (0.04 – 0.12)	-4.28	0.00002	0.24
C16:1 (Hexadecenoylcarnitine)	0.03 (0.02 – 0.06)	0.02 (0.02 – 0.04)	-4.51	7E-06	0.27
C16:1-OH (Hydroxyhexa-decenoylcarnitine)	0.01 (0.01 – 0.02)	0.01 (0.01 – 0.02)	-3.42	0.0006	0.16
C18 (Octadecanoylcarnitine)	0.05 (0.03 – 0.09)	0.04 (0.02 – 0.07)	-2.92	0.004	0.11
C18:1 (Octadecenoyl-carnitine)	0.13 (0.05 – 0.23)	0.09 (0.05 – 0.15)	-4.86	1E-06	0.32
C18:2 (Octadecadienyl-carnitine)	0.04 (0.02 – 0.07)	0.03 (0.01 – 0.05)	-3.68	0.0002	0.18
C3 (Propionyl-carnitine)	0.23 (0.14 – 0.49)	0.31 (0.13 – 0.61)	3.72	0.0002	0.19
C3-DC(C4-OH) (Malonyl-carnitine (Hydroxybutyrylcarnitine))	0.05 (0.03 – 0.25)	0.04 (0.03 – 0.07)	-2.95	0.003	0.12
C6(C4:1-DC) (Hexanoylcarnitine (Fumarylcarnitine))	0.03 (0.02 – 0.05)	0.03 (0.02 – 0.04)	-3.43	0.0006	0.16
C5 (Valerylcarnitine)	0.14 (0.10 – 0.31)	0.17 (0.10 – 0.29)	2.73	0.006	0.10
C9 (Nonaylcarnitine)	0.05 (0.03 – 0.08)	0.06 (0.03 – 0.12)	2.88	0.004	0.11

Z-adjusted values according to Mann-Whitney U-test (FEP_b compared to CSs).
The table contains between-groups difference data with the effect size ≥ 0.10.
[a] High-sensitive biochip array technology was used to measure levels of cytokines and growth factors.
[b] Metabolic biochip array technology was used to measure metabolic and additional inflammatory markers.
[c] Serum level of ACs was determined using the AbsoluteIDQ™ p180 kit.
AP – antipsychotic; CSs – control subjects; FEP – first-episode psychosis.

In conclusion, AP-naïve FEP patients had a significant profile change of the serum levels of inflammatory, metabolic protein biomarkers, and ACs compared with CSs.

Thereafter a multivariate GLM analysis was performed to confirm a significant main effect of the disease on the measured putative biomarker levels. The final regression model was built from a set of candidate predictors, by removing predictors on *p*-values ($p > 0.05$), in a stepwise manner. The proposed model appeared to fit well in estimating the difference between AP-naïve FEP patients and CSs as measured by 32 biomarkers ($F_{(32,34)} = 6.22$, $p <$ 1E-06, partial $\eta^2 =$ 0.85) (Table 3).

Table 3. Acylcarnitines, growth factors, metabolic and inflammatory biomarker serum levels in FEP patients before treatment with APs compared to CSc.

Biomarkers	β	β (95 % CI)	*t-value*	*p*-value
Inflammatory markers and growth factor				
IL (Interleukin)**-4**[a]	0.38	0.15, 0.61	3.36	0.001
IL-6[a]	0.40	0.18, 0.63	3.53	0.0008
IL-1β[a]	-0.29	-0.52, -0.07	-2.60	0.01
EGF (Epidermal growth factor)[a]	0.77	0.61, 0.93	9.75	<1E-06
Ferritin[b]	0.29	0.11, 0.48	3.14	0.003
PAI (Plasminogen activator inhibiitor)**-1**[b]	0.28	0.06, 0.49	2.53	0.01
Metabolic protein biomarkers[b]				
Resistin	0.30	0.07, 0.54	2.55	0.01
Leptin	-0.29	-0.51, -0.07	-2.65	0.01
Acylcarnitines[c]				
C10 (Decanoylcarnitine)	0.34	0.11, 0.57	2.96	0.004
C12 (Dodecanoylcarnitine)	0.33	0.09, 0.56	2.79	0.007
C12:1 (Dodecenoylcarnitine)	0.30	0.07, 0.53	2.60	0.01
C14 (Tetradecanoylcarnitine)	0.34	0.11, 0.57	2.99	0.004
C14:1 (Tetradecenoylcarnitine)	0.47	0.25, 0.68	4.37	0.00005
C14:1-OH (Hydroxytetradecenoylcarnitine)	0.34	0.10, 0.57	2.87	0.006
C14:2 (Tetradecadienylcarnitine)	0.44	0.22, 0.66	3.95	0.0002
C16 (Hexadecanoylcarnitine)	0.49	0.28, 0.71	4.65	0.00002
C16:1 (Hexadecenoylcarnitine)	0.56	0.36, 0.75	5.73	<1E-06
C16:1-OH (Hydroxyhexadecenoylcarnitine)	0.46	0.24, 0.67	4.27	0.00007
C18 (Octadecanoylcarnitine)	0.34	0.11, 0.57	2.90	0.005
C18:1 (Octadecenoylcarnitine)	0.56	0.36, 0.76	5.56	1E-06
C18:2 (Octadecadienylcarnitine)	0.44	0.23, 0.66	4.08	0.0001

Biomarkers	β	β (95 % CI)	*t-value*	*p*-value
C2 (Acetylcarnitine)	0.25	0.03, 0.47	2.23	0.03
C3 (Propionylcarnitine)	-0.43	-0.64, -0.21	-4.00	0.0002
C3-DC(C4-OH) (Malonylcarnitine (Hydroxybutyrylcarnitine))	0.36	0.14, 0.59	3.20	0.002
C4 (Butyrylcarnitine)	-0.31	-0.53, -0.09	-2.75	0.008
C6(C4:1-DC) (Hexanoylcarnitine (Fumarylcarnitine))	0.45	0.23, 0.67	4.02	0.0002
C5 (Valerylcarnitine)	-0.25	-0.48, -0.02	-2.18	0.03
C5-DC(C6-OH) (Glutarylcarnitine (Hydroxyhexanoylcarnitine))	0.23	0.003, 0.46	2.02	0.047
C7-DC (Pimelylcarnitine)	0.26	0.02, 0.49	2.18	0.03
C8 (Octanoylcarnitine)	0.25	0.01, 0.49	2.10	0.04
C9 (Nonaylcarnitine)	-0.35	-0.58, -0.12	-3.00	0.004
Hexoses	0.27	0.05, 0.50	2.41	0.02

β – regression coefficients, CI – confidence intervals,
p-values (derived from GLM analysis) – significance values of $\log_{10}$-transformed biomarkers serum levels, adjusted for gender, age and smoking status.
[a] High-sensitive biochip array technology was used to measure levels of cytokines.
[b] Metabolic biochip array technology was used to measure metabolic and additional inflammatory markers.
[c] Serum level of ACs and hexose were determined with the AbsoluteIDQ™ p180 kit.
APs – antipsychotics; CSs – control subjects; FEP – first-episode psychosis.

Most notable elevations emerged in the levels of EGF, C16:1, C18:1, C16, C14:1, C16:1-OH, C18:2, C14:2, C6(C4:1-DC), and IL-6 in the patient group. In addition, the level of C3 was significantly reduced in the FEP patients' group as compared with CSs. Thus, our results demonstrate that there is a disease-dependent interaction between ACs, hexoses, metabolic protein biomarkers and inflammatory markers**.**

5.1.3. Effects of AP treatment on BMI, growth factors, inflammatory and metabolic protein biomarkers, and ACs profiles

Several measured biomarker levels were significantly changed after the 7-month AP in FEP patients (Table 4, Appendix Table A-2). The strongest decline was observed for EGF, followed by IL-2, ferritin, and IL-6. By contrast, the levels of C-peptide and leptin had significantly increased after treatment. The number of patients displaying the level of C-peptide higher than 2 pg/ml before treatment was 5 out of 35, whereas after the 7-month AP treatment the respective number of such patients increased to 17. Moreover, continuous

7-month AP treatment resulted in a significant shift in favor of the C-peptide compared to insulin ($Z = 3.29$, $p < 0.001$), and showed a significant shift ($Z = 3.91$, $p = 0.00009$) in favor of leptin compared to adiponectin. Furthermore, significant trends emerged for the 17 measured ACs (Table 4, Appendix Table A-2). However, four of them (C16, C18:1, C18:2, C3) were associated with a large effect size of the treatment impact.

Table 4. Comparisons of growth factors, inflammatory and metabolic protein biomarkers as well as acylcarnitines levels between FEP patients before and after 7-month treatment with APs.

Biomarkers	FEP patients before AP treatment	FEP patients after AP treatment	Z-value	*p*-value	Effect size η^2
	Median (min–max)	Median (min–max)			
Inflammatory biomarkers and growth factor					
IL (Interleukin)**-1α**[b]	0.30 (0 – 1.78)	0.24 (0 – 1.77)	2.47	0.01	0.11
IL-2[a]	3.05 (1.18 – 3.99)	2.06 (1.14 – 3.34)	4.78	2E-06	0.32
IL-4[a]	1.70 (1.02 – 3.75)	1.35 (0.92 – 4.32)	2.88	0.004	0.12
IL-6[a]	1.10 (0.34 – 5.03)	0.60 (0.19 – 2.27)	3.34	0.0008	0.16
INF (Interferon)**-γ**[a]	0.34 (0.20 – 0.77)	0.21 (0.17 – 0.69)	3.21	0.001	0.16
Ferritin[b]	56.0 (4.26 – 238.7)	30.9 (4.75 – 150.0)	4.19	0.00003	0.25
EGF (Epidermal growth factor)[a]	17.55 (2.20 – 51.83)	1.68 (0.49 – 7.74)	5.16	<1E-06	0.38
Metabolic protein biomarkers[b]					
C-peptide	0.80 (0.12 – 4.48)	1.90 (0.28 – 7.71)	3.10	0.002	0.14
Leptin	0.85 (0 – 2.22)	1.21 (0 – 9.29)	3.38	0.0007	0.16
Adiponectin	7335 (2093 – 2334)	5591 (1084 –10223)	2.43	0.02	0.12
Resistin	2.96 (1.69 – 8.58)	2.80 (1.42 – 4.76)	2.61	0.009	0.10

Biomarkers	FEP patients before AP treatment	FEP patients after AP treatment	Z-value	*p*-value	Effect size η^2
	Median (min–max)	Median (min–max)			
Acylcarnitines[c]					
C14:2 (Tetradecadienyl-carnitine)	0.02 (0.01 – 0.07)	0.02 (0.01 – 0.04)	2.75	0.006	0.11
C16 (Hexadecanoylcarnitine)	0.12 (0.06 – 0.20)	0.08 (0.04 – 0.15)	3.40	0.0007	0.16
C16:1 (Hexadecenoyl-carnitine)	0.03 (0.02 – 0.06)	0.02 (0.01 – 0.05)	3.05	0.002	0.13
C16:1-OH (Hydroxyhexa-decenoylcarnitine)	0.01 (0.01 – 0.02)	0.01 (0.01 – 0.02)	2.62	0.009	0.10
C18 (Octadecanoylcarnitine)	0.05 (0.03 – 0.09)	0.04 (0.02 – 0.06)	2.70	0.007	0.10
C18:1 (Octadecenoylcarnitine)	0.13 (0.05 – 0.23)	0.08 (0.04 – 0.17)	4.13	0.00004	0.24
C18:2 (Octadecadienyl-carnitine)	0.04 (0.02 – 0.07)	0.03 (0.02 – 0.05)	3.86	0.0001	0.21
C3 (Propionylcarnitine)	0.23 (0.14 – 0.49)	0.30 (0.14 – 0.62)	3.22	0.001	0.15
C3-DC(C4-OH) (Malonyl-carnitine (Hydroxy-butyrylcarnitine))	0.05 (0.03 – 0.25)	0.04 (0.03 – 0.07)	2.69	0.007	0.10

Z-values according to Wilcoxon Matched Pairs Test (FEP patients, pre- and post-treatment occasions).
The table contains between groups difference data with effect size ≥ 0.10.
[a] High-sensitive biochip array technology was used to measure levels of cytokines.
[b] Metabolic biochip array technology was used to measure metabolic protein and additional inflammatory markers.
[c] Serum level of ACs was determined with the AbsoluteIDQ™ p180 kit.
AP – antipsychotic; CSs – control subjects; FEP – first-episode psychosis.

To provide a more comprehensive framework for the essence of inflammatory as well as metabolic protein biomarkers and ACs profile differences within the groups, we expanded our data analysis. Repeated measure GLM analysis revealed that statistically significant main effects of the seven-month AP treatment included 13 ACs, IL-2, IL-4, INF-γ, EGF, C-peptide, leptin, and BMI (Table 5). During the treatment, the most prominent changes emerged for the levels of EGF, IL-2 and C18:1. The effect size (partial eta-squared) of the final model was 0.83 ($F_{(20.44)} = 10.49$, $p < 1E\text{-}06$).

Table 5. Effects of 7-month treatment with APs on biomarker levels in FEP patients.

Biomarkers	β	β (95 % CI)	*t-value*	*p*-value
Inflammatory biomarkers and growth factor				
IL (Interleukin)**-2**[a]	0.68	0.41, 0.94	5.14	3E-06
IL-4[a]	0.44	0.13, 0.74	2.89	0.005
INF (Interferon)**-γ**[a]	0.35	0.04, 0.66	2.23	0.03
EGF (Epidermal growth factor)[a]	0.75	0.55, 0.96	7.37	<1E-06
Metabolic protein biomarkers[b]				
C-peptide	-0.31	-0.63, 0.00	-2.01	0.048
Leptin	-0.34	-0.62, -0.05	-2.36	0.02
Body mass index (BMI)	-0.52	-0.83, -0.21	-3.36	0.001
Acylcarnitines[c]				
C14 (Tetradecanoylcarnitine)	0.39	0.08, 0.71	2.51	0.01
C14:1 (Tetradecanoylcarnitine)	0.42	0.12, 0.72	2.81	0.007
C14:1-OH (Hydroxytetradecenoylcarnitine)	0.38	0.06, 0.70	2.38	0.02
C14:2 (Tetradecadienylcarnitine)	0.45	0.15, 0.75	2.98	0.004
C16 (Hexadecanoylcarnitine)	0.47	0.18, 0.77	3.19	0.002
C16:1 (Hexadecenoylcarnitine)	0.46	0.18, 0.74	3.25	0.002
C16:1-OH (Hydroxyhexadecenoylcarnitine)	0.42	0.11, 0.72	2.73	0.008
C18 (Octadecanoylcarnitine)	0.41	0.08, 0.73	2.52	0.01
C18:1 (Octadecenoylcarnitine)	0.54	0.27, 0.81	3.98	0.0002
C18:1-OH (Hydroxyoctadecenoylcarnitine)	0.36	0.04, 0.68	2.26	0.03
C18:2 (Octadecadienylcarnitine)	0.45	0.16, 0.74	3.13	0.003
C3 (Propionylcarnitine)	-0.39	-0.70, -0.07	-2.44	0.02
C3-DC(C4-OH) (Malonylcarnitine (Hydroxybutyryl-carnitine))	0.41	0.12, 0.70	2.81	0.007

β – regression coefficients, CI – confidence interval,
p-values (derived from GLM repeated measure) – significance values of $\log_{10}$-transformed biomarkers serum levels in patients' group before treatment compared to biomarkers values measured after the 7-month treatment with APs.
[a] High-sensitive biochip array technology was used to measure levels of cytokines.
[b] Metabolic biochip array technology was used to measure metabolic markers.
[c] Serum level of ACs was determined with the AbsoluteIDQ™ p180 kit.
AP – antipsychotic; CSs – control subjects; FEP – first-episode psychosis.

In conclusion, during the 7-month AP-treatment, EGF serum level demonstrated the strongest decline, followed by IL-2, C18:1, C16, C16:1, and C18:2. Simultaneously, an increased level of C-peptide and leptin and a decreased level

of adiponectin were found among the metabolic protein markers. In addition, continuous AP-treatment was associated with significantly increased BMI in the FEP patients' group.

5.1.4. Comparisons of levels of growth factors, inflammatory and metabolic protein biomarkers, and ACs profiles between FEP patients after 7-month AP treatment and CSs

In the next step, the inflammatory and metabolic status of treated FEP patients was compared with CSs. Importantly, significant elevation emerged in the levels of several cytokines: IL-1α, IL-1β, IL-2, INF-γ (Table 6), and C-peptide (Appendix Table A-3) when treated FEP patients were compared to CSs. By contrast, previously demonstrated elevated levels of EGF, ferritin, IL-6, IL-4, PAI-1, and resistin, as well as the diminished level of leptin (Table 2, Appendix Table A-2), had returned to the level of CSs (Appendix Table A-3). No differences in ACs levels were detected between the post-treatment status of FEP patients and CSs (Appendix Table A-3).

Table 6. Comparisons of inflammatory biomarkers levels (pg/mL) between FEP patients after 7-month AP treatment and CSs.

Biomarkers	FEP patients after AP treatment	CSs	Z-value	*p*-value	Effect size η^2
	Median (min–max)	Median (min–max)			
Inflammatory biomarkers					
IL (Interleukin)**-1α**[b]	0.24 (0.00 – 1.77)	0.32 (0.20 – 1.67)	-2.89	0.004	0.12
IL-1β[a]	1.21 (0.40 – 3.39)	1.91 (0.45 – 4.47)	-3.35	0.0008	0.16
IL-2[a]	2.06 (1.14 – 3.34)	2.81 (1.34 – 4.49)	-4.55	5E-06	0.28
INF (Interferon)**-γ**[a]	0.21 (0.17 – 0.69)	1.30 (1.02 – 3.75)	-3.07	0.002	0.13

Z-adjusted values according to Mann-Whitney U-test (FEP patients after 7-month treatment compared to CSs).
The table contains between groups difference data with effect size ≥ 0.10.
[a] High-sensitive biochip array technology was used to measure levels of cytokines.
[b] Metabolic biochip array technology was used to measure the level of cytokine.
AP – antipsychotic; CSs – control subjects; FEP – first-episode psychosis.

In conclusion, comparing CSs to patients with FEP during the 7-month AP treatment, the levels of certain cytokines (IL-1α, IL-1β, IL-2, INF-γ) and metabolic proteins (C-peptide and leptin levels) had an upward trend, and the ACs had returned to CSs level.

5.2. The difference in the profiles of inflammatory and metabolic protein markers between patients with the chronic stage of SCH spectrum disorders and CSs (Paper III)

5.2.1. General description of the study groups

There were no statistically significant differences between the two groups (patients with SCH spectrum disorders and CSs) in terms of age ($t_{(251)} = 1.56$, ns), and gender ($\chi^2_{(1)} = 0.24$, ns), as seen in Table 7. Patients had a significantly higher ($p = 0.007$) BMI compared to CSs. Among the patients' group, the mean disease duration was 19.9 (±9.8) years and the value of the mean PANSS general psychopathology symptom score was 76.2 (±29.1).

Table 7. Demographic characteristics of the study sample.

	SCH patients	CSs	Comparisons *p*-values
Age, years (mean ± s.d.)	53.1 ± 10.9	51.3 ± 7.57	$t = 1.56$, $p = 0.12$
Gender (male / female)	45/60	68/80	$X^2_{(1)} = 0.24$, $p = 0.63$
BMI (mean ± s.d.)	27.9 ± 6.4	24.5 ± 2.9	$t_{(124)} = 2.76$, $p = 0.007$
PANSS (total symptom score)	76.2 ± 29.1	-	-

BMI – body mass index; CSs – control subjects; PANSS – Positive and Negative Syndrome Scale.

5.2.2. Biochemical measurements for clinical routine blood tests

While biochemical blood test data were available only for patients, we used the endemic reference values (ERV) to assess the prevalence of cardio-metabolic risk factors in the patients' group (Table 8). The CRP, TGs, and LDL-c mean levels were somewhat higher in patients compared to the ERV. The level of TGs of 52 patients fell outside the ERV. The same trend emerged for 48 and 46 patients when ratios of TGs/HDL-c and LDL-c/HDL-c values were compared to ERV. Calculation of the ratio for TGs/HDL-c and LDL/HDL-c revealed the elevation in patients of both genders. The level of HbA1c for the whole patient group was close to the upper threshold of the ERV. In more detail, our results demonstrated that a relatively high number of patients were in a pre-diabetic/ diabetic state. The number of patients in a pre-diabetic state (HbA1c over 6.0%)

was 28 (26.7%) and the number of patients exceeding the threshold for diabetes (HbA1c over 6.5%) was 6 (5.7%). Altogether, 32.4% of the SCH patients were in a pre-diabetic/diabetic state and significantly higher HbA1c values emerged among the female patients ($Z = 2.15$, $p = 0.03$). According to the clinical records, 11 patients (10.5%) had already received a diagnosis of T2DM. Nine of the confirmed diabetic patients were under treatment with anti-diabetic drugs (one of them needed insulin).

Table 8. Biochemical parameters of patients with SCH spectrum disorders compared to the endemic reference value (ERV).

Biochemical parameters ERV	Patients values Mean ± standard error of the mean and range in parentheses	Number (%) of patients whose blood biochemical parameters were out of limit of the ERV
CRP (< 5 mg/L)	5.20 ± 0.86 (0.90 – 66)	30 (28.6%)
TGs (< 1.7 mmol/L)	2.00 ± 0.11 (0.38 – 7.16)	52 (53.3%)
HDL-c (male >1.0 mmol/L, female >1.2 mmol/L)	1.35 ± 0.04 (0.55 – 3.09)	Male: 9 (20.0%) Female: 28 (43.3%)
TGs/HDL-c (male < 1.7, female < 1.4)	1.74 ± 0.14 (0.20 – 11.4)	Male: 20 (44.4%) Female: 28 (46.7%)
LDL-c (< 3.0 mmol/L)	3.84 ± 0.11 (1.65 – 7.40)	77 (73.3%)
LDL-c/HDL-c (male < 3.0, female < 2.5)	3.13 ± 0.13 (0.74 – 6.57)	Male: 19 (42.2%) Female: 27 (45.0%)
HbA1c (< 6.0 %)	5.90 ± 0.10 (4.83 – 13.72)	34 (32.4%)

CRP – C-reactive protein; HbA1c – glycated hemoglobin; HDL-c – high-density lipoprotein cholesterol; TGs – triglycerides; LDL-c – low-density lipoprotein cholesterol; SCH – schizophrenia.

Ratios of TGs/HDL-c and LDL/HDL-c were elevated in patients of both genders and the level of HbA1c for the whole patient group was close to the upper threshold of the ERV.

5.2.3. Biomarker level differences among patients with SCH spectrum disorders and CSs

The differences in the levels of cytokines and growth factors varied between patients and CSs (Table 9, Appendix Table A-4). The most prominent elevations (effect size $\eta^2 \geq 0.13$) were detected in the levels of widely recognised pro-inflammatory markers: IL-2, IL-6, IL-8, IFN-γ, MCP-1 as well as the anti-inflammatory marker IL-10 in patients' group compared to CSs. Similarly, the

ratios of INF-γ/IL-4 and INF-γ/IL-10 (i.e. these ratios were used to evaluate the balance between pro-inflammatory and anti-inflammatory cytokines) were significantly higher in patients compared to CSs. Contrary, the ratio of IL-2/INF-γ (a common feature of autoimmune disorder) was significantly lower in the patients' group. It is also worth emphasizing that the values of some well-known pro-inflammatory markers (IL-1α, IL-1β and TNF-α) were not significantly different between the two study samples.

Table 9. Inflammatory markers values (pg/mL) of patients with SCH spectrum disorders compared to CSs.

	SCH patients	CSs	Z-value	*p*-value	Effect size η^2
	Median (min–max)				
Pro-inflammatory cytokines[a]					
IL (Interleukin)**-2**	2.18 (0.80 – 8.10)	1.21 (0.47 – 2.97)	8.78	<1E-06	0.31
IL-6	1.80 (0.38 – 28.20)	0.89 (0.33 – 2.29)	8.01	<1E-06	0.26
IL-8	9.90 (2.27 – 65.50)	6.63 (3.20 – 13.10)	6.14	<1E-06	0.15
MCP (Monocyte chemo-attractant protein)**-1**	244.8 (42.75 – 741.9)	165.3 (26.90 – 345.2)	6.98	<1E-06	0.19
IFN (Interferon)**-γ**	4.12 (0.41 – 38.87)	1.23 (0.16 – 4.10)	9.93	<1E-06	0.40
Anti-inflammatory cytokines[a]					
IL-10	0.77 (0.21 – 7.46)	0.55 (0.20 – 1.32)	6.27	<1E-06	0.16
Ratio of biomarkers					
INF-γ/IL-4	3.09 (0.38 – 21.24)	0.75 (0.12 – 4.82)	9.63	<1E-06	0.37
INF-γ/IL-10	4.35 (0.34 – 56.23)	2.00 (0.15 – 12.71)	6.26	<1E-06	0.16
IL-2/INF-γ	0.60 (0.09 – 6.24)	1.14 (0.24 – 7.09)	-5.61	<1E-06	0.13

Z-adjusted values according to Mann-Whitney U-test.
The table contains between groups difference data with effect size ≥ 0.10.
[a] High-sensitive biochip array technology was used to measure levels of cytokines.
CSs – control subjects; SCH – schizophrenia.

The logistic regression analysis was performed in order to confirm the results of non-parametric difference test on the two groups. Age, gender, and BMI were used as covariates in the models. First, we conducted a series of univariate logistic regression analyses to select candidate variables for the final model. For that, we used the *p*-value cut-off point of 0.10. Based on to the results of the univariate logistic regression analyses, we found that IL-2 ($W = 53.63$, $p < 1E-06$), INF-γ ($W = 43.99$, $p < 1E-06$), IL-6 ($W = 42.88$, $p < 1E-06$), MCP-1 ($W = 39.33$, $p < 1E-06$), IL-8 ($W = 33.4$, $p < 1E-06$), IL-10 ($W = 25.61$, $p < 1E-06$),

VEGF (W = 11.96, p = 0.0006), EGF (W = 10.25, p = 0.001), and TNF-α (W = 3.09, p = 0.08) showed greater Wald statistic parameters than the other variables (IL-1α, IL-1β, IL-4). Thereafter, a multivariate logistic regression analysis revealed that elevated levels of IL-2, IL-6, IL-10, and INF-γ were associated with the higher risk of belonging to the patient group (odds ratios (OR): 4.55, 4.16, 10.36, 2.62, respectively) (Table 10). The high level of TNF-α was associated with a lower risk (OR = 0.29) of belonging to the SCH patient group. In contrast, IL-8, MCP-1, VEGF, EGF levels, as well as gender and age, were not significant predictors in the multivariate model to differentiate groups. Next, we demonstrated that the disease was associated with pro-inflammatory/anti-inflammatory (INF-γ/IL-4) balance disturbances (OR = 3.68, CI 95% = 1.61 – 8.41, $p < 0.002$), and this INF-γ/IL-4 imbalance was accompanied by older age (OR = 1.06, CI 95% = 1.01 – 1.11, $p < 0.03$), and higher BMI (OR = 1.24, CI 95% = 1.08 – 1.42, $p < 0.002$). Ratios of INF-γ/IL-10 and IL-2/INF-γ were not statistically significant predictors (p = 0.16, and p = 0.15, respectively) in this regression model.

Table 10. Multivariate logistic regression analysis of variables associated with the risk of belonging to the group of patients with SCH spectrum disorders.

Variables	Odds ratio	95 % CI	*p*-value
Pro-inflammatory cytokines[a]			
IL (Interleukin)**-2**	4.55	2.00, 10.34	0.00003
IL-6	4.16	1.48, 11.68	0.007
IL-8	1.24	0.99, 1.55	ns
MCP (Monocyte chemo-attractant protein)**-1**	1.00	0.99, 1.03	ns
TNF (Tumor necrosis factor)**-α**	0.29	0.13, 0.63	0.002
IFN (Interferon)**-γ**	2.62	1.65, 4.17	0.00005
Anti-inflammatory cytokines[a]			
IL-10	10.36	1.43, 78.82	0.02
Growth factors[a]			
VEGF (Vascular endothelial growth factor)	1.00	0.99, 1.01	ns
EGF (Epidermal growth factor)	1.01	0.99, 1.03	ns
Demographic variables			
Gender	0.66	0.21, 2.10	ns
Age	1.02	0.96, 1.08	ns

CI – Confidence intervals.
[a] High-sensitive biochip array technology was used to measure levels of cytokines.
SCH – schizophrenia.

The cytokine profile distinguished patients with SCH spectrum disorders from CSs. In the patient group, a statistically significant increase was found in the levels of pro-inflammatory markers (IL-2, IL-6, TNF-α, IFN-γ, MCP-1) and the

level of anti-inflammatory marker (IL-10). Besides, disturbance of the pro-/anti-inflammatory status caused by SCH spectrum disorders and probably by its treatment was confirmed by the elevated value of the INF-γ/IL-4 ratio in the patients' group.

5.2.4. Relation of biomarker levels with HbA1c in patients' group

Thereafter, GLM analysis was performed, to establish associations between elevated levels of HbA1c and inflammatory biomarkers in the patient group. Gender, age, and BMI were included as covariates in the model. Stepwise selection of parameters was used to achieve the best-fitted model. Outcome of the analysis showed that several biomarkers, INF-γ (β = 0.69, [95% CI 0.55 – 0.84], t = 9.46, p < 1E-06), IL-6 (β = 0.29, [95% CI 0.15 – 0.43], t = 4.21, p < 1E-06), MCP-1 (β = -0.22, [95% CI (-0.37) – (-0.07)], t = -2.94, p = 0.004), and IL-2 (β = 0.18, [95% CI 0.04 – 0.32], t = 2.61, p = 0.01)) were significantly associated with HbA1c serum concentration in patients. The effects of age and BMI were not statistically significant in the final model. The explained variance (R^2_{adj} = 0.60) of IL-2, IL-6, INF-γ, MCP-1 on HbA1c, adjusted for gender, was 60% ($F_{(5)}$ = 28.00, p < 1E-06). The higher levels of INF-γ, IL-6 and IL-2, and lower levels of MCP-1 were significant predictors of elevated HbA1c value in the patient group.

5.2.5. Relation of metabolic and biomarker levels with the ratio of TGs to HDL-c in patients with SCH spectrum disorders

Finally, to evaluate associations between the ratio of TGs/HDL-c (which was considered to indicate a risk for the development of insulin resistance and cardiovascular diseases) and measured serum markers, the GLM analysis was used. Gender, age, and BMI did not make a significant contribution to the regression models and were not included in the final model. The results demonstrated that higher values of INF-γ/IL-10 ratio (β = 0.41, [95% CI 0.13 – 0.68], t = 2.93, p = 0.004), HbA1c (β = 0.37, [95% CI 0.12 – 0.63], t = 2.88, p = 0.005), LDL-c (β = 0.22, [95% CI 0.03 – 0.41], t = 2.31, p = 0.02), and INF-γ/IL-4 (β = 0.39, [95% CI 0.00 – 0.78], t = 1.99, p = 0.049), as well as a lower level of INF-γ (β = -0.68, [95% CI (-1.18) – (-0.18)], t = -2.68, p = 0.009) were the predictors of the elevated value of TGs/HDL-c ratio. The explained variance (R^2_{adj} = 0.19) of these biomarkers for insulin resistance and cardiovascular disease risk parameter (TGs/HDL-c) was 19% ($F_{(5)}$ = 5.51, p < 0.0001).

Together, these results confirmed that the interplay exists between pro-/anti-inflammatory status alteration, insulin resistance and cardiovascular disease risk parameter (expressed as HbA1c and as the ratio between TGs and HDL-c) in the patients with a chronic stage of the psychotic disorder.

6. DISCUSSION

This dissertation has the following main goals: (i) to study the profiles of ACs, inflammatory and metabolic protein biomarkers in FEP patients before and after a 7-months AP-treatment and compare these to CSs, (ii) to characterize a bio-energetic dysfunction through the a complex interplay between inflammatory and metabolic protein biomarkers as well as ACs profiles in patients with FEP before and after a 7-month AP-treatment and compare these to CS, and (iii) to investigate inflammatory and cardio-metabolic status alterations among patients with the chronic stage of the SCH spectrum disorders and compare these to CSs.

The results of the research confirmed that putative biomarkers are altered in the serum of patients with SCH spectrum disorders at different stages of the disease, and suggested that peripheral immune system is over-activated and metabolic status is imbalanced in both the individuals undergoing their FEP and subjects with a chronic stage of the disease. Although the design of the study does not allow for establishing causation, results support the idea that low-grade inflammation, alterations in the lipid profile and insulin resistance play a central role in the pathogenesis of the SCH spectrum disorders, phenomena already seen in the early stage of the disease as well as during the disease progression and continuous AP treatment.

6.1. Differences in the level of biomarkers between FEP patients and CSs

In general, the results of this study confirmed that there are differences between the AP-naïve FEP patients, FEP patients after the 7-month treatment and CSs, in terms of certain biomarkers related to inflammatory and metabolic profiles.

6.1.1. Putative biomarker profile differences between AP-naïve FEP patients and CSs

Concerning the interplay between cytokines, growth factor, metabolic protein biomarkers, and ACs, we found significantly elevated levels of EGF, IL-4, IL-6, ferritin, PAI-1, resistin, 19 ACs and reduced levels of IL-1β, leptin, and 4 ACs in the group of AP-naïve patients compared to CSs.

In comparison to CSs, the AP-naïve FEP patients showed significantly higher level of EGF serum concentrations. EGF exhibits neurotrophic and neuromodulatory effects on various types of neurons in the CNS (Yamada et al., 1997). Preclinical studies suggest that EGF is related to the regulation of dopaminergic neuron activity in the CNS (Eda et al., 2013), and EGF selectively enhances the NMDA receptor-mediated increase of the intracellular calcium concentration in neurons (Abe & Saito, 1992). Inhibitors of EGF

receptors can ameliorate some of the behavioral impairments of an animal model for SCH (Mizuno et al., 2013). Studies have shown a link between the EGF receptor family or the ErbB signaling network and dopamine, GABA, and glutamatergic systems, and have confirmed that ErbB signaling can serve as a new starting point for non-dopaminergic-based drug development of SCH (Golani et al., 2014; Tadmor et al., 2018). Moreover, Golani et al. (2014) demonstrated that the alteration of ErbB signaling in the rat brain during adolescence results in changes to the dopaminergic system that emerges via pathological learning and hedonic behavior in adulthood, and suggests the possible role of the pathway in the development of cognitive skills and motivated behavior. It is speculated that increased levels of EGF in a person's bloodstream may reflect a connection between psychosis and the status of the dopaminergic system. Furthermore, using mouse models, Wang et al. (2017) showed a link between epidermal growth factor receptor and regulation of oxidative stress in macrophages, and that the destruction of that receptor signaling pathways reduces the activity of inflammation. Therefore, one may suggest that increased serum levels of EGF in this study may reflect a connection between psychotic symptoms and the altered dopaminergic, GABAergic and/or glutamatergic neurotransmitter systems.

The elevated level of IL-6 in AP-naïve patients has also been confirmed by multiple meta-analyses (Miller et al., 2011; Di Nicola et al., 2013; Upthegrove et al., 2014; Pillinger et al., 2019b; Fraguas et al., 2019). IL-6 is a soluble mediator with a pleiotropic effect on inflammation, immune response, hematopoiesis, and it is also involved in the regulation of metabolic, regenerative and nervous processes (Scheller et al., 2011). Findings suggest a potential link between an elevated level of IL-6 and vulnerability to OxS, which might be associated with GABAergic dysfunction in the brain of patients with SCH (Watanabe et al., 2010). Furthermore, IL-6 also has a role in minimising the inflammatory response by reducing the production of IL-1β and TNF-α (Schindler et al., 1990) and by inducing the production of anti-inflammatory cytokine IL-10 (Raison et al., 2018). Thus, in addition to EGF, IL-6 also appears to be a clear candidate biomarker of FEP, as confirmed by our study.

In the current study, a moderate increase in IL-4 in AP-naïve FEP patients could probably be explained by its role in the activation of the type 2 T helper cells (Th2) immune response and its consequent anti-inflammatory action (Derecki et al., 2010; Doherty et al., 2018). It is shown in preclinical studies that IL-4 can decrease the integrity of the endothelium and increase the permeability of the BBB (Duarte-Delgado et al., 2019; Małkiewicz et al., 2019). Altered levels of IL-6 and IL-4 may indicate the induction of low-grade inflammation in AP-naïve FEP patients.

Regarding PAI-1, increased levels in the AP-naïve FEP group compared with the CSs were reported by Bocchio-Chiavetto et al. (2018). For decades, tissue plasminogen activator was known as a protein with a main role to modulate coagulation through intravascular fibrin degradation. However, it is also synthesized and released by neurons and glial cells. Tissue plasminogen activa-

tor is involved in synaptic plasticity, it has a role in maintaining the integrity of the BBB as well as to guide neurite outgrowth, cell migration, neurogenesis, and excitotoxic cell death (Melchor & Strikland, 2005; Benarroch, 2007). The PAI-1 is a serine protein inhibitor that functions as an inhibitor of tissue plasminogen activator (Vaughan, 2005). It has been suggested that the loss of that activator function may be associated with the clinical conditions related to SCH, such as hyperhomocysteinemia, insulin resistance, and T2DM (Hoirisch-Clapauch & Nardi, 2013). Furthermore, in the context of AP-naïve FEP patients, an elevated level of PAI-1 may indicate possible sequential changes in the permeability of the BBB. Cesari et al. (2010) have reported that TNF-α and IL-6 are major contributors to the increase of PAI-1 level.

Serum ferritin (i.e. the acute phase protein) is a well-known marker of inflammation, and abnormally elevated serum ferritin is a consequence of cell stress and damage (Kell & Pretorius, 2014; Kim et al., 2018). Ferritin is a primary marker of iron metabolism and plays an important role in maintaining iron metabolism homeostasis and regulating iron content in the brain (Kim et al., 2018). Furthermore, ferritin levels were positively correlated with CNS dopamine levels in Parkinson's disease (Piao et al., 2017). Results from this study are consistent with a published report by Schwarz et al. (2010), describing an assortment of 51 analytes, among which was an elevated level of ferritin that could distinguish patients with SCH from CSs. Thus, the low-grade inflammatory state in drug-naïve FEP patients in this study is characterized by an increase in the level of pro-inflammatory cytokine (IL-6) and acute phase proteins (i.e., ferritin and PAI-1).

In the present study, the level of IL-1β was decreased in AP-naïve FEP patients when compared to CSs, and a similar trend was demonstrated by Zhu et al. (2018). However, these results contradicted with findings from previous studies (Miller et al., 2011; Di Nicola et al., 2013; Trovão et al., 2019). Such discrepancies may be related to different study populations (i.e. patients' age, presentation of the disease or treatment duration), and the disease state.

In this study resistin (i.e. initially described as an adipocyte-specific hormone associated with obesity, insulin resistance, diabetes, and cardiometabolic disease (Kawabe et al., 2015)) was significantly increased in AP-naïve patients with FEP, while leptin (i.e., a hormone secreted by fat cells to regulate energy balance (Klok et al., 2007; Kelesidis et al., 2010)) was reduced. Resistin is a cytokine that induces low-grade inflammation by stimulating monocytes (Lee et al., 2014). No specific receptor has been identified for resistin, although it seems to share signaling pathways with other pro-inflammatory molecules (Bokarewa et al., 2005). The correlation between resistin and inflammatory markers (e.g. IL-6) is particularly noteworthy given the observation that resistin is produced by macrophages in response to inflammation (Filková et al., 2009; Schwartz & Lazar, 2011). Importantly, the resistin itself can contribute to the onset of inflammatory conditions by mediating enhanced activation of cytokines (IL-6, TNF-α) and nuclear kappa B (NF-κB) (Bokarewa et al., 2005; Stofkova, 2010). Thus, the interaction between adipocytes and macrophages can lead to

hyper-resistinemia (Luo et al., 2018). An increase in the above factors can be considered as a possible sign of an inflammatory response during FEP.

It has been hypothesized that leptin plays a role in SCH because of its relationship with weight gain and obesity (Henderson et al., 2015). However, studies evaluating leptin levels in patients with FEP have been inconclusive (Chouinard et al., 2018; Pillinger et al., 2019b; Martorell et al., 2019). Reduced leptin levels in this study can be viewed as a reduced nutritional status of patients, reflecting a slightly reduced amount of fat tissue in AP-naïve FEP patients. In terms of leptin, there are studies indicating its potential interaction with resistin (Kershaw & Flier, 2004; Rajala et al., 2004; Qi et al., 2006). What is more, in rodents, the effect of resistin depends on the presence or absence of leptin, but together they have a cross effect on the regulation of glucose energy and homeostasis (Qi et al., 2006).

There were no significant differences in baseline measurements of fasting plasma insulin, C-peptide and adiponectin levels in AP-naïve patients with FEP compared to CSs, in this study. The results differed from several previous studies that reported higher levels of insulin and C-peptide in patients with FEP compared with CSs (Pillinger et al., 2017) or the absence of significant differences in insulin resistance in AP-naïve FEP patients compared to CSs (Sengupta et al., 2008). A meta-analysis conducted by Pillinger et al. (2017) confirmed that altered glucose homeostasis is an integral part of the disease and appears from the very beginning of the disease. The discrepancy between our results and previous studies can be partly explained by the size of the sample and how patients were involved in the studies.

It is important to note that the difference in BMI values between AP-naïve FEP patients and CSs was not statistically significant.

The present study also focused on the analysis of ACs profile alterations in the early stage of psychosis because subjects with SCH have a risk of metabolic abnormalities, T2DM, and multiple cardiovascular diseases (Vancampfort et al., 2015). ACs are involved in bioenergetic pathways and provide potential biomarker targets for identifying early changes and onset characteristics of the bioenergetic dysfunction. Studies suggest that SCH can be characterized by molecular signatures, thus presenting opportunities to identify metabolic dysregulation even before the initiation of AP treatment. ACs are the intermediate metabolites produced during fatty acids oxidation (FAO). Alterations in blood and tissue ACs (LCACs, ≥14 chain lengths) may occur through many physiological and pathophysiological metabolic events in which pools of acyl-CoA) fatty acid metabolites are converted to ACs via conjugation to CARN by carnitine palmitoyltransferase 1 (CPT) or after mitochondrial translocation, CPT2 converts AC to free CARN and acyl-CoA, which subsequently enters the FAO pathway (Knottnerus et al., 2018). Excessive accumulation of LCACs may occur when there is significant discordance between long-chain fatty acid availability, storage and oxidative utilization (McCoin et al., 2019). Evidence has suggested that LCACs have bioactive properties that may be integrated with multiple pathways involved in inflammation, cell stress, and insulin resistance

(McCoin et al., 2015). We found that levels of several LCACs (C14:1, C16, C16:1, C18:1, C18:3 among others) were increased and levels of SCACs (e.g. C3) were decreased when AP-naïve patients were compared to CSs. It may be hypothesized that the FEP correlates with a downregulated beta-oxidation of fatty acids, which leads predominantly to higher amounts of LCASs. What is more, the alterations of glucose metabolism are especially relevant in the context of increased risk of metabolic syndrome or T2DM in SCH. Although this can often be linked with APs side effects, a recent meta-analysis by Pillinger et al. (2017) confirmed that altered glucose homeostasis is intrinsic to the disease and present from the illness onset. Evidence suggests that FEP is accompanied by disturbances in glucose utilization and energy production. Our study established an increase in hexoses levels in FEP patients compared to CSs which refers to possible shifts in carbohydrate metabolism. All the above-mentioned shifts (LCACs, hexoses) may indicate a certain mitochondrial dysfunction during FEP as mitochondria are fundamental-functional "cross-road" for the metabolism of lipids, glucose and amino acids. It is known that SCACs are tightly linked to amino acid and ketone bodies' metabolism. Furthermore, results from this study demonstrated that there is a disease-dependent interaction between ACs, protein metabolic and low-grade inflammatory biomarkers in AP-naïve patients with FEP.

6.1.2. Biomarker profile differences between AP-naïve FEP patients and FEP patients after 7-month treatment with APs

On the background of AP treatment, the greatest decrease was found for EGF, followed by IL-2, ferritin, IL-6, and IL-4 among patients. According to the results, we may suggest that a 7-month AP treatment can reduce levels of pro-inflammatory biomarkers and diminish low-grade inflammation in the early stage of the chronic psychotic disorder. Previous researches concerning the impact of AP treatment on inflammatory markers, and more precisely on cytokine levels, have so far provided mixed findings. Some studies have found increased levels of inflammatory markers after continuous AP treatment, while others have found a reduction or no change in cytokine levels. A recent study by Juncal-Ruiz et al. (2019) provides additional evidence that AP treatment has an anti-inflammatory effect and could normalize immune balance. Moreover, results suggest that AP drugs may have both direct anti-inflammatory and indirect pro-inflammatory activity, depending on the effect on weight-gain and increased adiposity (Mondelli & Howes, 2014). This emphasizes the importance of measuring metabolic dysfunction in addition to evaluating inflammatory markers.

Several metabolic biomarkers significantly changed after the 7-month AP treatment in FEP patients. AP treatment caused an elevation on the leptin level and a reduction on the resistin level. In patients with SCH spectrum disorders, hyperleptinemia has been described and it is correlated with AP-induced weight

gain and metabolic side effects of the treatment (Potvin et al., 2015), rather than the direct effect of atypical AP drugs on the physiology of leptin (Jin et al., 2008; Potvin et al., 2015). Leptin level increases in conjunction with the amount of fat (Mantzoros et al., 2011). A paradoxical hyperleptinemic state during weight gain is associated with leptin resistance and indicates a loss of leptin's ability to stop overeating behavior (Caro et al., 1996).

Furthermore, treatment significantly increased the serum concentration of C-peptide and indicated the prevalence of insulin resistance among FEP patients who received AP drugs compared to the metabolic state of AP- naïve FEP patients and CSs. According to the results of our work, the number of patients with C-peptide levels above 2 pg / ml increased from 5 to 17 after treatment with atypical APs. There has been a significant shift in favor of C-peptide in comparison with insulin after treatment with APs. Such a shift towards an increase in C-peptide level (C-peptide and insulin are released in equimolar amounts) is most likely due to changes in insulin sensitivity after treatment.

The level of adiponectin in our work was significantly decreased in patients with FEP after 7-months of AP treatment. Similar results have been obtained by Barcones et al. (2018). Adiponectin is a blood protein produced by adipocytes, and it has anti-inflammatory effects and increases insulin sensitivity. The levels of the protein in the circulation decrease with an increasing BMI (Arita et al., 1999). It is important to note that the relationship between adiponectin and leptin shifted in favor of leptin after 7-months of AP treatment, thus this ratio may be the preferable biomarker of the MetS in patients with SCH (Chen et al., 2018).

Additionally, the results revealed that AP drug treatment reduced the levels of LCACs (C16, C16:1, C18:1, C18:2, among others) and this trend was positively correlated with a decrease in the levels of EGF, IL-2, IL-4, and IL-6. Preclinical studies indicate that LCAC C18:1 (oleoyl - l - carnitine) inhibits the activity of the glycine transporter 2 carrier (GlyT2) on inhibitory nerve endings by increasing synaptic glycine, which reduces the excitability in nociceptive chains and provides analgesia in models of neuropathic and inflammatory pain (Carland et al., 2013). The close connection between nociceptive and inflammatory processes is a well-established phenomenon. At the same time, AP treatment was associated with an elevated level of SCAC C3 and this was in turn linked to elevated levels of leptin and C-peptide, as well as weight gain. This finding was confirmed by Cao et al. (2019) and it seems to underline the role of SCACs in the regulation of energy metabolism. Taking into account changes in markers of inflammation and metabolism during AP treatment in patients with FEP, it is likely that LCACs are involved in the formation and maintenance of inflammation, and SCACs are more involved in the development of the metabolic syndrome.

In this study, FEP patients received various generation AP drugs, and the dosages were adjusted or the drugs were replaced with another active substance as it was clinically necessary during the follow-up period. Therefore, it is difficult to draw any serious conclusions about the effect of specific AP drugs.

However, treatment improved the severity of symptoms on the PANSS psychopathology scale, but this positive change was accompanied by a marked increase in BMI, an often observed side effect of AP drugs (Tarricone et al., 2010; McEvoy et al., 2013; Bak et al., 2014). AP drugs should be considered not only as a risk factor for insufficient physical activity and poor physical fitness (Vancampfort et al., 2016) but also as dysregulation factors for hormones that control appetite and food intakes, such as insulin, leptin, and adiponectin (Sentissi et al., 2008). Additionally, they function as factors that cause alterations in biosynthesis and regulation of fatty acids (Polymeropoulos et al., 2009), cellular bioenergetics (Cao et al., 2019), and inflammatory marker levels (Fraguas et al., 2019).

6.1.3. Biomarker profile differences between FEP patients after 7-month treatment with APs and CSs

Simultaneous measurement of inflammatory and metabolic factors after the 7-month AP treatment demonstrated that markers showing an initial increase (i.e., EGF, ferritin, IL-6, IL-4, PAI-1, and resistin) or a decrease (i.e. leptin) returned to CSs level. However, several cytokines – IL-1α, IL-1β, IL-2, INF-γ, and the level of C-peptide – showed an increase levels in treated FEP patients were compared to CSs. A significant number of studies have attempted to determine whether there are differential cytokine profiles in individuals with the psychotic disorder after AP treatment. Elevated levels of INF-γ, IL-1 family cytokines, and IL-2 are frequently noted in meta-analyses when AP treated patients with a psychotic disorder are compared to CSs (Potvin et al., 2008; Miller et al., 2011; Goldsmith et al., 2016). Some studies have suggested that specific biomarker alterations in SCH are associated with psychopathology. Gonzáles-Blanco et al. (2019) suggested that the level of IL-2 is associated with the severity of the motivation and pleasure domain of negative symptoms and the level of IL-1β predicts the severity of the general symptomatology score.

Regarding the effects of the AP drugs on the glucose metabolism, the existing studies consistently report metabolic disturbances which are characterized by a complex association between the elevated level of C-peptide, hyperinsulinemia and insulin resistance.

Additionally, no differences in ACs levels were detected between the post-treatment status of FEP patients and CSs in this study.

6.2. Differences in the level of biomarkers in the chronic phase of the SCH spectrum disorders

The chronic stage of SCH is characterized by a constant or repeated exacerbation of psychotic symptoms after a short-term or long-term disease remission. In a meta-analysis, Vancampfort et al. (2013) found that multi-episodic patients

with SCH had an increased risk of cardio-metabolic disorders (abdominal obesity, hypertension, low level of HDL-c, hypertriglyceridemia, MetS, and diabetes) compared to CSs. Moreover, chronic low-grade inflammation is associated with both mental and cardiovascular disorders (de Rooij et al., 2009).

6.2.1. Changes in inflammatory markers in patients with the chronic phase of the SCH spectrum disorders

In our study, the most prominent elevation in patients was detected in the levels of pro-inflammatory markers IL-2, IL-6, IL-8, IFN-γ, and MCP-1 and the anti-inflammatory marker IL-10 when compared to CSs. Similarly, the INF-γ/IL-4 and INF-γ/IL-10 ratios were significantly higher in SCH patients compared to CSs. Contrary, the ratio of IL-2/INF-γ was significantly lower in the patient group.

Similarly to our results, in several studies increased blood levels of IL-6 have been described as one of the most frequently confirmed immunological characteristics associated with SCH (Miller et al., 2011; Pillinger et al., 2019b), although contradictory findings have also been reported (O'Brien et al., 2008). IL-6 plays a critical role in the pathogenesis of inflammatory disorders and the physiological homeostasis of neural tissue. Preclinical studies have demonstrated that, on the one hand, IL-6 has trophic effects on glial cells (Kahn & Vellis, 1994) and on the other hand, elevated levels of IL-6 disrupt neuronal function by enhancing neurotoxicity via an increase of intracellular calcium level during NMDA receptor activation (Qiu et al., 1998, Nelson et al., 2004). Thus, IL-6 has both neurotrophic and neurotoxic effects on different neural cell types and at different developmental stages. Additionally, IL-6 impacts the hypothalamic-pituitary-adrenal axis and stimulates the release of several hormones from the hypophysis (Spangelo et al., 1989). Evidence suggests that in addition to providing communication between immune cells, specific cytokines play a role in signaling the brain to produce neurochemical, neuroendocrine, neuroimmune, and behavioral changes (Kronfol & Remick, 2000). Studies have confirmed that the interaction between these complex systems is disrupted in patients with SCH (Perkovic et al., 2017).

IL-6 has a close functional relationship with IL-2 and TNF-α (Müller et al., 1999). These biomarkers mediate immune and inflammatory responses and activate cytokines that seem to play a key role in the CNS. They are actively transported into CNS but are also released from the activated astrocytes and microglia cells (Müller et al., 2000). Results from this study confirm the close interplay between elevated levels of INF-γ, IL-2, IL-6, IL-10, and the decreased level of TNF-α. IL-2 has an essential role in the key functions of the immune system, primarily via its direct effect on T cells. Elevated serum levels of IL-2 are consequences of the activated macrophages and T-cells induced by chronic inflammation (Smith, 1991). Furthermore, IL-2 is a potent modulator of dopamine activity in the mesocorticolimbic and mesostriatal systems (Zalcman

et al., 1994). Several studies have pointed out altered peripheral levels of IL-2 in SCH when compared with CSs (Arolt et al., 2000; Zhang et al., 2002; Potvin et al., 2008). Moreover, associations between IL-2 and different groups of symptoms including negative symptoms and cognitive performance of SCH (Asevedo et al., 2014), and associations between elevated cerebrospinal fluid levels of IL-2 and the recurrence of psychotic symptoms have been described (McAllister et al., 1995). Therefore, researchers have suggested that the effect of IL-2 on the central dopaminergic system may contribute to developing psychotic symptoms. However, data suggest that cerebrospinal fluid or blood IL-2 levels are different in varied biological subtypes and stages of SCH.

In this study, serum IL-8 levels were significantly elevated in the patient group. In most studies, it has been found that IL-8 levels were more elevated in patients with a SCH spectrum disorders than in the CSs (Kaminska et al., 2001; Kuloglu et al., 2011). IL-8 is a characteristic member of the chemokine family. It is released from monocytes, macrophages, endothelial cells, and activated T cells. Zhang and colleagues (2005) demonstrated that IL-8 secretion is increased by OxS and suggested that in addition to its known proinflammatory properties, IL-8 might have a tissue-protective activity and a role in tissue remodeling during the resolution of inflammation. Furthermore, IL-8 has been shown to be associated with obesity (Sharabiani et al., 2011).

In this study, levels of TNF-α could not define patients from CSs. However, TNF-α was found to be associated with SCH in the multivariate model. TNF-α is released from primarily stimulated monocytes and macrophages and it is a multifunctional proinflammatory cytokine. It plays an important role in linking the complex events involved in inflammation and immunity (Strieter et al., 1993). Several studies have reported elevated levels of TNF-α (O'Brien et al., 2008; Goldsmith et al., 2016) among patients with SCH, and some studies have demonstrated either decrease (Francesconi et al., 2011) or no change in TNF-α levels (Pedrini et al., 2012; Lv et al., 2015). Low sample sizes, different methods of cytokine measurement, engaging patients with different disease stages and disease duration, and various sociodemographic characteristics of the study populations may be some reasons for the diverging results.

Meta-analyses have found that the blood levels of IFN-γ were elevated in patients with SCH compared to CSs (Miller et al., 2011; Goldsmith et al., 2016). In contrast, Potvin et al. (2008) concluded that the INF-γ level did not change, and other studies have found that INF-γ levels are significantly lower in patients with SCH (Arolt et al., 2000; Na et al., 2007). Inconsistencies in INF-γ levels suggest that immune activation in subgroups of patients may be part of the underlying pathophysiologic process of SCH spectrum disorders. It is known that IFN-γ is mainly secreted by activated T-cells and it can stimulate the activation of macrophages, activate the orchestration of the innate immune system, coordinate the interaction of lymphocytes with endothelium, regulate type 1 T helper/type 2 T helper cells (Th1/Th2) balance and control cell proliferation and apoptosis (Schroder et al., 2004; Mattyasovszky et al., 2017). Furthermore, high levels of IFN-γ may lead to CNS inflammation and damage

to oligodendrocytes, and this may be one of the reasons why there is a switch from cellular to humoral Th2 immunity in patients with SCH (Dimitrov et al., 2014). INF-γ is found in neuronal synapses and it may act at the level of the synapse to influence brain function (Garay & McAllister, 2010). Additionally, this cytokine plays an important role in the kynurenine pathway, as it is considered to be the main inducer of IDO, one of the enzymes that convert tryptophan to kynurenine in one of the first steps of the kynurenine pathway. It is generally suggested that diseases, where microglia are activated, favor the production of 3-hydroxykynurenine and quinolinic acid, whereas suppression of this branch or astrocyte activation may favor kynurenine acid (KYNA) synthesis (Campbell et al., 2014). KYNA acts in the brain as a glycine-site NMDA receptor antagonist, a key in the glutamatergic neurotransmission system, which is thought to be involved in the pathophysiology of SCH (Plitman et al., 2017).

Based on the data from this study, the serum Th1/Th2 ratios (i.e., expressed as ratios of INF-γ/IL-4 and INF-γ/IL-10) examined in subjects with SCH were higher than those of their healthy counterparts. At individual serum cytokine levels, patients with SCH as a whole group had elevated levels of INF-γ and IL-10, but lower average serum levels of IL-4. This reflects the pro-inflammatory activity of the immune system. Our results are consistent with studies in which researchers found an elevation of IL-10 levels in SCH patients (Kunz et al., 2011). IL-10, a pleiotropic Th2 cytokine with immune-regulatory functions, maintains the balance between pro- and anti-inflammatory cytokines (Murray, 2006). Increased levels of IL-10 may reflect a compensatory or counter-regulatory mechanism in response to the elevated levels of proinflammatory cytokine levels (Yilmaz et al., 2014). Previous studies have shown that IL-10 levels were positively correlated to the severity of clinical symptoms of schizophrenia (Xiu et al., 2014). Moreover, a meta-analysis demonstrated that there might be an association between single nucleotide polymorphisms of *IL-10* and susceptibility to SCH (Gao et al., 2014). Also, regardless of IL-10 anti-inflammatory properties, its elevated concentration increases the risk of future cardiovascular events (Lakoski et al., 2008). In our sample, patients with a chronic SCH spectrum disorders were predominantly in a pre-diabetic/diabetic state, which may contribute to the expression of Th1 cytokines (Xia et al., 2017).

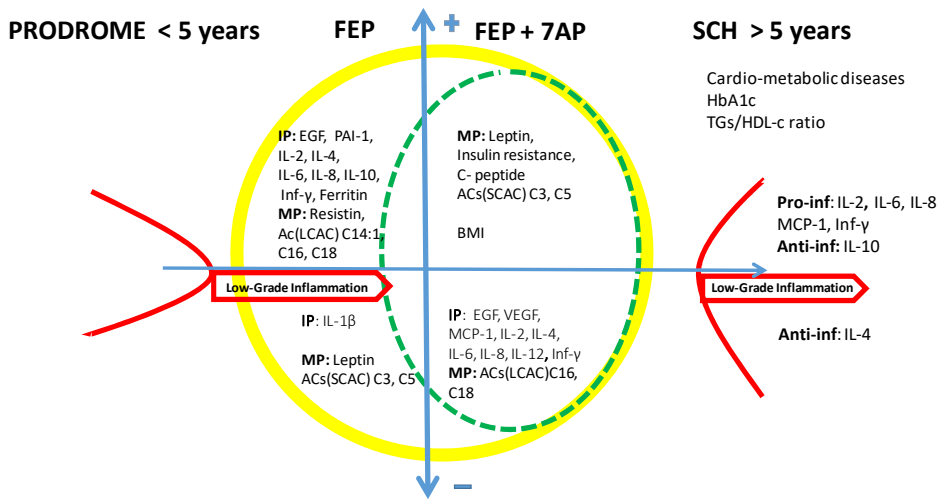
MCP-1 is a well-studied chemokine, but the findings are contradicting. Studies have demonstrated significantly higher (Dimitrov et al., 2013; Lin et al., 2017) or similar (Domenici et al., 2010) results between patients with SCH and CSs. In this study, an increase in the level of MCP-1 in patients with SCH spectrum disorders emerged compared to CSs. MCP-1 is one of the key chemokines that regulate migration and infiltration of monocytes/macrophages during inflammation (Deshmane et al., 2009). Pro-inflammatory substances, including MCP-1, can disrupt the integrity and affect the permeability of the BBB (Yao & Tsirka, 2014). Additionally, blood glucose, OxS, and proinflammatory cytokines, among other biomarkers, may up-regulate the expression of MCP-1 (Tesch, 2008). Increased serum MCP-1 level correlates with markers of

cardiovascular diseases and metabolic disorder including obesity, insulin resistance, and T2DM (Panee, 2012).

6.2.2. Changes in metabolic protein markers in patients with the chronic phase of the SCH spectrum disorders

In this study, patients mean BMI was 27.9. Many factors may contribute to weight gain in patients with a chronic psychotic disorder: sedentary lifestyle, unhealthy food habits, genetic susceptibility and AP treatment are considered the main reasons. Meta-analyses have constantly confirmed that almost all APs cause weight gain (Spertus et al., 2018; Huhn et al., 2019). APs are among the most obesogenic medications and this indirect mechanism is coherent with our understanding of the pathophysiology of the development of MetS and T2DM (Whicher et al., 2018). At the same time, studies have indicated that rates of insulin resistance, impaired glucose tolerance, hyperinsulinemia, and dyslipidemia are also high in patients who have not used APs (Chadda et al., 2013; Pillinger et al., 2017). The prevalence of MetS in patients with SCH was found to be ranging from 3.3% to 68.0% (Chadda et al., 2013). In this study, 32.4% of our patients with chronic psychotic disorder were in a pre-diabetic or diabetic state. According to international clinical guidelines, HbA1c is a reliable marker for assessing both the pre-diabetic and diabetic state of a patient (American Diabetes Association, 2018; Davies et al., 2018). Mean HbA1c level in our sample of patients with SCH spectrum disorders was close (5.90±0.10) to the diabetic state (ERV for HbA1c is above 6.0%). Furthermore, in this study, patients' elevated level of HbA1c was associated with increased levels of INF-γ, IL-2 and IL-6. As a result, the pro-inflammatory state was a significant predictor of the higher HbA1c levels in our patient sample. What is more, in our study, the mean ratio of TGs/HDL-c was higher (1.74±0.14) than ERV (≤1.7 mmol/l) among patients as compared to CSs. Studies have shown that TGs/HDL-c ratio is a highly significant predictor of cardiovascular disease, and it is also independently related to insulin resistance, obesity and MetS (Grundy et al., 2004). Also, compared with ERV, patients had increased dyslipidemic parameters of cardiometabolic risk such as LDL-c and TGs, ratios of LDL-c/HDL-c and TGs/HDL-c. In our study, patients with chronic SCH spectrum disorders were mostly overweight and had a prediabetic/diabetic state and had elevated levels of low-grade inflammatory markers. There is substantial clinical evidence that an increase in proinflammatory mediators in patients with SCH spectrum disorders initiates changes in glucose and lipid metabolism and thereby contributes to insulin resistance (Leonard et al., 2012). Given the high prevalence of the MetS in patients with SCH, the timely identification of cardiometabolic risk factors using candidate biomarkers and subsequent treatment can improve the quality of life of patients and reduce health care costs (Shakeri et al., 2016).

Schematic overview of the main findings of this study is presented in Figure 3, which reflects changes in biomarkers in accordance with a hypothetical separation at the stage of clinical manifestation of SCH spectrum disorders. It is important to note that the results of our studies indicate the presence of changes in the biomarkers of inflammation before and after treatment with AP drugs. Important links between inflammation and MetS are ACs, which determine the pattern of energy metabolism, namely beta-oxidation of fatty acids. During the chronic stage of SCH spectrum disorders, the risk of cardio-metabolic disorders increases, therefore, an algorithm based on regular monitoring of HbA1c, TGs/HDL-c ratio and levels of pro-inflammatory markers could help to prevent or delay diabetes and cardiovascular diseases and thereby reduce premature deaths in patients with chronic psychotic disorder.



FEP – first-episode psychosis; 7AP – 7-month antipsychotic drug treatment; SCH – schizophrenia; **IP** – inflammation profile ; **Anti-inf** – anti-inflammation; **Pro-inf** – pro-inflammation; EGF – epidermal growth factor; VEGF – vascular endothelial growth factor; PAI (plasminogen activator inhibiitor)-1; IL (interleukin)-2, IL-4, IL-6, IL-8, IL-10; Inf-γ – interferon-gamma; **MP** – metabolic profile; ACs – acylcarnitines; LCACs – long-chain acylcarnitines; SCACs – short-chain acylcarnitines; HbA1c – glycated hemoglobiin; BMI – body mass index; TGs/HDL-c – triglycerides/high-density lipoprotein cholesterol ratio.

Figure 3. Schematic summary of the study results.

7. STRENGTHS AND LIMITATIONS

Before summarizing the results of this thesis, the following methodological issues, participant sample characteristics, and limitations should be considered.

The first issue concerns the recruitment of subjects that was based on opportunity rather than random sampling. Subjects in the control group came from a sub-population and the results can therefore not be generalized to the entire Estonian population (Paper I, II, III). Secondly, the recruited patients formed a small cohort due to the rarity of AP-naïve FEP patients. They were heterogeneous in terms of their diagnosis, drug treatment, and duration of illness without treatment – something that was difficult to avoid among any sample of patients with FEP (Paper I, II). Thirdly, it was not possible to control the eventual effects of any particular pharmacological agent on the measured putative biomarkers due to our small sample size and the fact that different types of treatment drugs were used during the observation period. For this reason, we analyzed the main effects of AP treatment at the group level (Paper I, II). Fourthly, we collected data from CSs at a single time point and did not control their health condition or metabolic and inflammatory biomarker levels after the same follow-up period as was done for the FEP patient group (Paper I, II). Fifthly, we did not evaluate either the participant's dietary or physical activity habits. This is worth mentioning, since the chronicity of the disease and continuous AP treatment may adversely affect lifestyle factors in patients with a psychotic disease (Paper I, II, III). Sixthly, the naturalistic and cross-sectional nature of the survey did not permit us to study the temporal relationships between the treatment effects and the inflammatory or cardio-metabolic risk markers in patients with a chronic psychotic disorder (Paper III). Seventhly, we used EVR to establish disturbances in well-known cardiometabolic risk marker levels among SCH patients, because these data were not available for our CSs sample (Paper III).

Although the findings should be interpreted with caution, this study has several strengths.

Firstly, we used naturalistic study design and no restrictions were applied in regard to patients' treatment or other clinically relevant decisions (Paper I, II, III). Second strength is the longitudinal study design (Paper I, and Paper II), which allowed to discover associations between variables that are not related to various FEP patients background characteristics. Thirdly, dropout rate among enrolled FEP patients during the study period was very low (5%), only two patients refused to take APs and they were excluded from the follow-up analysis (Paper I, II). Fourthly, this study represents comprehensive and simultaneous examination of the inflammatory and metabolic biomarkers in patients with SCH spectrum disorders compared to CSs (Paper I, II, III). Fifthly, we used well-known and well-validated techniques to define biomarker levels and laboratory tests were performed following standard procedures and quality controls (Paper I, II, III). Sixthly, we used relevant covariates (i.e., BMI,

smoking status, age, etc.) in statistical analyses, which allowed to account for natural heterogeneity in the population (Paper I, II, III). Finally, results of this study emphasize the value of measurement of dynamic biomarker profiles at different stages of the SCH spectrum disorders.

8. CLINICAL RELEVANCE

According to the results, there is a close relationship between the immune and metabolic processes that influence the development of the MetS in patients with SCH spectrum disorders. Thus, monitoring inflammatory and metabolic biomarkers in patients with FEP (before and during the AP treatment) and in patients with the chronic stage of SCH helps to assess the likelihood of them developing cardio-metabolic disorders and select patients who need interventions, thereby making it possible to improve the quality of life and prolong the years of life of the patients. Despite the proven efficacy of APs to treat psychotic symptoms, these medications have unwanted effects on metabolism. Weight gain may be one of the reasons for poor drug compliance; obesity may induce a prolonged state of low-grade inflammation and thereby lead to increased cardio-metabolic complications and mortality. It can be argued that the imbalance between metabolism and the immune system is a characteristic feature of the endo-phenotype of SCH spectrum disorders, regardless of the stage of the disease. In our studies, altered levels of low-grade inflammatory markers and ACs were identified in AP-naïve patients with FEP and further AP treatment caused unwanted side effects in metabolism. The results of our study and other similar studies highlight the importance of putative biomarker profile assessments and multidisciplinary management of medical and behavioral conditions in patients with SCH spectrum disorders at different stages of the disease. The risks and benefits of individual APs should be carefully considered when making treatment choices.

9. IMPLICATIONS FOR FURTHER RESEARCH

SCH spectrum disorders are etiologically and pathophysiologically heterogeneous diseases, that varies widely in clinical manifestation, course of progression and maybe even biomarker profiles from person to person. Thus, there is need to move toward a more personal approach, which could allow to define treatment-relevant subtypes of the disorder. Biomarker-profiles diagnostic and treatment utility should be better implemented for clinical practice. Identification and characterization of biomarkers are crucial first steps in this process. Future research in larger samples could demonstrate linkage between putative biomarkers and clinical end points.

10. CONCLUSIONS

This dissertation presents a comprehensive investigation into the changes of inflammatory and metabolic candidate biomarkers in SCH spectrum disorders in different stages of the disease.

The conclusions are as follows:

1. Significantly elevated levels of EGF, IL-2, IL-4, IL-6, ferritin, resistin, and PAI-1 as well as decreased levels of leptin, and IL-1β emerged when AP-naïve FEP patients were compared to CSs. A 7-month AP-treatment reduced the shifts in biomarkers mentioned above. The most salient changes emerged in the levels of EGF, ferritin, PAI-1, IL-4, and IL-6. At the same time, AP treatment was accompanied by elevated serum levels of C-peptide, leptin and reduced level of adiponectin.

2. Simultaneously with previously described elevated levels of EGF, IL-4, IL-6, ferritin, resistin, PAI-1, and decreased levels of leptin and IL-1β, drug-naïve FEP patients had elevated serum levels of LCACs (i.e. C14:1, C16, C16:1 and C18:1) and reduced level of SCAC (C3) as compared to CSs. After a 7-month AP treatment of FEP patients, the established shift in EGF, and proinflammatory biomarkers returned to the corresponding levels of CSs. In addition to previously mentioned metabolic protein biomarker shifts, LCACs (C16, C18) levels were diminished and SCACs (C3, C5) levels were increased during the AP treatment. The results demonstrated that AP-naïve patients with FEP had low-grade inflammatory state which was reversed by the 7-month AP treatment. The impact of the treatment was concurrently associated with unwanted side effects on metabolism. Results confirmed that there is a disease stage and AP treatment dependent interplay between inflammatory, metabolic protein biomarkers and ACs in patients with FEP.

3. Patients with a chronic stage of the SCH spectrum disorders displayed the most prominent elevation in the levels of pro-inflammatory markers IL-2, IL-6, IL-8, IFN-γ, MCP-1 as well as the anti-inflammatory marker IL-10 compared to CSs. The INF-γ/IL-4 and INF-γ/IL-10 ratios were significantly higher in SCH patients indicating a significant shift towards a pro-inflammatory state compared to CSs. These results demonstrated that patients with SCH had specific low-grade inflammatory biomarker profiles independent of gender or age. Furthermore, higher levels of IL-2, INF-γ, and IL-6 and lower levels of MCP-1 were significant predictors of increased HbA1c in the group of patients with SCH spectrum disorders. These results confirmed a link between persistent low-grade inflammation and an increase in HbA1c in chronic SCH patients. Moreover, there was a strong relationship between increased value of the ratio of typical cardiometabolic risk marker (TGs/HDL-c) and elevated levels of INF-γ, HbA1c, and LDL-c as well as

reduced levels of IL-4 and IL-10. The results demonstrated that the chronic stage of the psychotic illness is accompanied by low-grade inflammation and patients with SCH spectrum disorders have an increased risk of metabolic and cardiovascular diseases.

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SUMMARY IN ESTONIAN

Immuun- ja metaboolse süsteemi vastastikune mõju skisofreeniaspektri häire erinevates staadiumides

Skisofreeniaspektri häired on rasked ja kroonilised psühhootilised haigused, millel on tugev bioloogiline alus. Haigus avaldub tavaliselt noores täiskasvanueas ja kestab terve elu ning seda iseloomustavad olulised tegelikkuse tunnetamise ja käitumishäired, millega võib kaasneda toimetuleku alanemine.

Epidemioloogilised ja geneetilised uuringud on näidanud metaboolse ja immuunsüsteemi rolli skisofreeniaspektri häirete avaldumise ja kulu etiopatogeneesis. Nii esmast psühhootilist episoodi (EPE) kui ka kroonilist haiguse staadiumi on seostatud kesknärvisüsteemi (KNS) toimimist mõjutava kogu organismi immuunsussüsteemis ilmnevate eripäradega. Haigusega kaasnevat immuunsussüsteemi talitlushäire olemasolu kinnitab mikrogliia aktivatsioon ajus ning tsütokiinide ja muude põletikuliste markerite muutunud tase seljaajuvedelikus ja veres. Meta-analüüsid kinnitavad, et nii ravinaiivsete EPE patsientide kui ka kroonilise haiguse ägenemisfaasis olevate patsientide vereseerumis on põletikku soodustavate tsütokiinide (nt. interleukiin-(IL)1β, IL-6) tasemed tõusnud ning põletikuvastase tsütokiini IL-10 sisaldus on langenud. Lisaks on leitud selliste põletikuliste protsessidega seotud kasvufaktorite nagu veresoonte endoteeli kasvufaktori (VEGF) ja epidermaalse kasvufaktori (EGF) seerumitaseme nihkeid psühhoosihaigetel. Samaaegselt on populatsioonipõhised longitudinaaluuringud näidanud verest mõõdetud IL-6 kõrgenenud taseme seost südamehaigusega ja II tüüpi suhkurtõvega ning skisofreeniaspektri häiretega inimestel esineb sageli kaasuvate haigustena nimetatud eluiga lühendavaid kehalisi haigusi. Tsütokiinid on tihedalt seotud ka oksüdatiivse stressiga kaasneva vabade radikaalide tekkimisega organismis, mis aktiveerivad hüpotalamuse-hüpofüüsineerupealiste telge. See omakorda põhjustab muutusi suhkru ja rasvhapete ainevahetuses, tuues kaasa raku jõujaamade ehk mitokondrite talitlushäireid ja madalaastmelise põletiku püsimise kogu kehas. Kogunenud tõendusmaterjal näitab, et skisofreeniaspektri häired seonduvad rasvhapete häirunud ainevahetusega KNS-is ja metaboolse sündroomi (MetS) kõrge suhtarvuga. Kroonilise psühhootilise häirega patsientide vereseerumis on tuvastatud muutuseid lipiidide, fosfolipiidide, triglütseriidide (TGd) ja kolesterooli tasemetes. Rasvhapped lagundatakse mitokondrites beetaoksüdatisooni tulemusena. Rasvhapete aktiivvormideks on atsüülkarnitiinid (AKd), mille profiili analüüsi kasutatakse beetaoksüdatsioonihäirete tuvastamiseks. Sõltuvalt süsinikuahela pikkusest eristatakse pika-, keskmise- või lühikeseahelalisi AKde. Varasemad uuringud on näidanud, et AKd on seotud insuliintundlikkusega, rasvhapete metabolismiga, põletikulise vastusega, oksüdatiivse stressi ja bioenergeetiliste funktsioonidega. Lisaks on psühhootilise häire erinevates staadiumides leitud teiste hulgas nt. glükoosi, insuliini, C-peptiidi, leptiini, adiponektiini, resistiini ja teiste ainevahetuse toimimist peegeldavate biomarkerite tasemete nihkeid.

Tuginedes varasematele teadustulemustele on põletikuliste ja ainevahetuslike eripärade uurimine skisofreeniaspektri häirete erinevates staadiumides jätkuvalt suure tähtsusega. Sedalaadi uuringud pakuvad lisanduvat teavet haiguse olemuse ja kulu mõistmiseks ning võimaldavad vältida antipsühhootilise (AP) raviga kaasneda võivaid ebasoovitavaid kõrvaltoimeid.

Uurimistöö eesmärgid

Antud doktoritöö keskendus põletiku ja ainevahetuse nihete uurimisele EPE patsientidel enne AP ravi alustamist ja 7-kuulise AP ravi järgselt ning kroonilises haiguse staadiumis olevatel patsientidel AP ravi foonil.

Uurimistöö eesmärgid:

1. Hinnata põletiku ja metabolismimarkerite tasemete erinevuste olemasolu EPE patsientide vereseerumis enne ja pärast 7-kuulist AP ravi võrreldes kontrollgruppi kuulujatega.
2. Iseloomustada põletiku-, metabolismimarkerite ja AKde profiilide koostoimete kaudu seisundist tulenevat bioenergeetilist düsfunktsiooni EPE patsientidel enne ja pärast 7-kuud kestnud AP ravi võrreldes kontrollgruppi kuulujatega.
3. Tuvastada madalaastmelised põletikulised ja ainevahetuslikud nihked kroonilises staadiumis olevate skisofreeniaspektri häirega patsientidel võrreldes kontrollgruppi kuuluvate isikutega.

Uuritavad ja meetodid

Uurimisprojekt kooskõlastati Tartu Ülikooli inimuuringute eetika komiteega. Kõik uuritavad andsid kirjaliku informeeritud nõusoleku uuringus osalemiseks. Patsiendid kaasati Tartu Ülikooli Kliinikumi psühhiaatriakliinikusse ravile pöördunud EPEga patsientide hulgast ning kroonilises staadiumis olevad patsiendid hõlmati Kesk-Eestis, Jõgeva maakonnas asuvas Võisiku hooldekodus viibivate isikute seast. Kontrollgrupi moodustasid eakaaslased, kellel enesel ega lähisugulasel ei esinenud psühhootilist või muud rasket psüühikahäiret või kehalist haigust.

EPEga patsientide valimi moodustasid 38 isikut (21 meest ja 17 naist), kelle keskmine vanus oli 25,4±0,9 aastat, neil oli esmakordselt diagnoositud psühhootilise episoodi avaldumine ning nad polnud varasemalt tarvitanud AP ravi. Isikud arvati uuringust välja, kui psühhootilise häire avaldumise põhjuseks oli psühhoaktiivsete ainete tarvitamine või neil esines kaasuvana raske põletikuline haigus. Psühhiaatrilised diagnoosid põhinesid kliinilisel küsitlusel vastavalt Rahvusvahelise Haiguste Klassifikatsiooni 10. väljaandele (RHK-10). EPEga patsientide AP ravi toimus vastavalt raviarsti valikule ning jälgimisperioodi jooksul võis raviarst raviannused või APi toimeainet kliinilisest seisundist tulenevalt muuta. EPEga patsientide andmeid koguti kahel ajahetkel: uuringusse kaasamise hetkel ja pärast jälgimisperioodi (keskmine kestus 7,2±0,7 kuud). Kontrollgruppi kuulus 37 isikut (16 meest ja 21 naist; keskmise vanusega 24,8±0,9 aastat), kelle kohta andmed koguti ühel ajahetkel.

Kroonilises staadiumis olevate skisofreeniaspektri häirega patsientide valimi moodustasid 105 isikut (45 meest ja 60 naist), kes olid stabiilses kliinilises seisundis ja tarvitasid regulaarselt AP ravi. Uuritavate keskmine vanus oli 53,1±10,9 aastat ja nende haigus oli kestnud 6–52 aastat. Kontrollgruppi kulus 148 inimest (68 meest ja 80 naist keskmise vanusega 51,3±7,6 aastat).

Uuritavatelt koguti hommikul tühjakõhu vereseerumid. Lisaks kaardistati isikute demograafilised taustaandmed, mõõdeti nende pikkus ja kaal.

Biokeemilised markereid määrati standardsete kliiniliste laboratoorsete meetoditega, kasutades Tartu Ülikooli Kliinikumi kliinilise labori sertifitseeritud analüüse. Põletiku-, metabolismi markerite ja AKde määramiseks kasutati tänapäevaseid rahvusvaheliselt aktsepteeritud määramismeetodeid (kõrgrõhuvedelikkromatograafia ja tandem-massispektromeetria kombinatsiooni, *sandwich* kemoluminestsents-immuunmeetodit ja fotokolorimeetria tehnikaid).

Uurimistöö tulemused ja järeldused

Antud uurimistöös hinnati põhjalikult põletiku ja metaboolsete biomarkerite muutusi skisofreeniaspektri häirete erinevates staadiumides.

Uurimistöö tulemusel selgus:

1. AP ravi eelselt olid EPEga patsientidel EGF, IL-2, IL-4, IL-6, ferritiini, resistiini ja PAI-1 sisaldused seerumis tõusnud ning IL-1ß ja leptiini tasemed olid langenud võrreldes kontrollgruppi kuulujatega. 7-kuuline AP ravi kõrvaldas eelnevalt kirjeldatud biomarkerite tasemete nihked ning kõige silmatorkavamad muutused ilmnesid EGFi, ferritiini, PAI-1, IL-4 ja IL-6 tasemete osas. Samas, AP-ravi tõi esile C-peptiidi ja leptiini suurenenud sisalduse ning adiponektiini taseme languse patsientide vereseerumis.

2. Võrreldes kontrollgruppi kuulujatega oli EPE-ga patsientidel AP ravi eelselt ilmnenud EGFi, IL-4, IL-6, ferritiini, resistiini ja PAI-1 seerumsisalduse tõus ja IL-1ß ja leptiini langus seotud samaaegselt esinenud pikaahelaliste AK-de (sh. C14:1, C16, C16:1 ja C18:1) sisalduse tõusu ning lühikeseahelalise AKi (C3) taseme langusega seerumis. 7-kuuline AP ravi soodustas patsientidel EGFi ja põletikuliste markerite tasemete pöördumise kontrollgruppi kuulujatega sarnasele tasemele, kuid tõi kaasa metaboolsete markerite ebasoovitud nihetele lisaks ka lühikeseahelaliste AKde (C3, C5) taseme tõusu ja pikaahelaliste AK-de (sh. C16, C18) taseme languse seerumis.

   Tulemused näitasid, et ravieelses seisundis kaasneb EPEga madala raskusastmega krooniline põletik, mis 7-kuulise AP-ravi foonil taandub, kuid ravi toob samaaegselt kaasa olulised negatiivsed efektid ainevahetuse toimimisele. Tõendust leidis, et põletikuliste ja metaboolsete markerite ning AKde vahel on haigusprotsessist ja AP-raviefektist tulenev koostoime.

3. Skisofreeniaspektri häire kroonilises staadiumis olevate haigete vereseerumis on võrreldes kontrollgruppi kuulujatega kõrgenenud järgnevate põletikku soodustavate: IL-2, IL-6, IL-8, IFN-γ, MCP-1 ja põletikuvastase markeri IL-10 tasemed. Biomarkerite omavaheliste suhete (INF-γ/IL-4 ja INF-γ/IL-

10) keskmised väärtused olid patsientidel märkimisväärselt kõrgemad, mis näitab olulist nihet põletikulise seisundi suunas võrreldes kontrollgruppi kuulujatega. Need tulemused kinnitavad, et skisofreeniaspektri häirega patsientidel esineb kroonilises haiguse staadiumis, sõltumata soost või vanusest, püsiv madala raskusastmega põletik. Lisaks, IL-2, INF-γ, IL-6 kõrgem ning MCP-1 madalam tase olid seotud kõrgenenud HbA1c tasemega, mis pakub tõendust seose olemasolust püsiva madalaraskusastmega põletiku ja glükoosiainevahetushäire vahel kroonilise haigusstaadiumiga patsientide grupis. Patsientide grupis ilmnes kardio-metaboolsete häirete riskimarkeri (ehk suhte TGd/HDL-kolesterool) kõrgenenud väärtus, mis oli seotud põletikku soodustava markeri INF-γ sisalduse tõusuga (võrreldes põletikuvastaste markerite IL-4 ja IL-10 tasemetega) ning kõrgenenud HbA1c ja LDL-kolesterooli väärtustega. Tulemused näitasid, et skisofreeniaspektri häire kroonilises staadiumis olevatel haigetel püsib kehas madalaastmeline kroonilise põletik ning neil esineb kõrgenenud risk haigestuda ainevahetushäiretesse ja südame-veresoonkonna haigustesse.

ACKNOWLEDGEMENTS

This study was carried out at the Psychiatry Clinic of the Tartu University Hospital in collaboration with the Institute of Biomedicine and Translational Medicine, University of Tartu and was financially supported by the Estonian Research Foundation (IUT 20-41, IUT 20-42, PUT PRG685), and the European Regional Development Fund (Project no. 2014-2020.4.01.15-0012).

I would like to express my gratitude to everyone who has guided me during this long work, as it would have not been possible without their support.

My sincerest gratitude and deepest respect goes to my first supervisor Professor Veiko Vasar (16.02.1956-07.03.2015), who encouraged me to start my Ph.D. program. His clear vision and consistent thoughts about biological psychiatry were crucial when I decided which subject area to choose to study.

I am very grateful to my supervisors Professor Eero Vasar, Professor Mihkel Zilmer, Liina Haring (MD, Senior Research Fellow), and Kati Koido (Senior Research Fellow) for the opportunity to complete my thesis. I would like to thank Professor Eero Vasar and Professor Mihkel Zilmer, who provided an excellent scientific advice and find time to discuss the issues particularly related to the biomarker part of my work. I am deeply thankful to Liina Haring for her continuous guidance, support, constructive criticism, and useful comments in the process of manuscript preparation. It was a great pleasure to work with her in the clinical practice which provided me the opportunity to improve my professional skills as well as to grow as an individual. Thanks also to Kati Koido, who kindly help to manage the procedural aspects of the research project.

I am extremely grateful to Professor Pille Taba and Professor Ain Raal for reviewing the thesis and making several helpful suggestions and comments.

My warmest thanks belong to my colleagues in the Psychiatry Clinic of the Tartu University Hospital who helped to link my studies and job duties.

I would like to thank all my friends for the care and support they are providing during these years. Special thanks belong to Kadri and Kärolin.

I am thankful to all the patients and healthy volunteers who agreed to participate in the studies and made this scientific project possible.

And last but not least - my deepest gratitude and respect goes to my family who has always supported me on the self-educational path that I have selected.

APPENDIX

Table A-1. Identification of differences in serum cytokines, growth factor, metabolic biomarkers (pg/mL), and acylcarnitine levels (μmoles/mL) between first-episode psychosis (FEP) patients before antipsychotic (AP) treatment and control subjects (CSs)

Biomarkers	FEP patients before AP treatment	CSs	Z-value[a]	*p*-value[a]	Effect size $(I)^2$
	Median (min–max)	Median (min–max)			
Inflammatory markers and growth factor					
IL (Interleukin)**-1α**[a]	0.20 (0.06 – 1.33)	0.16 (0.06 – 0.60)	0.42	ns	-
IL (Interleukin)**-1α**[b]	0.30 (0 – 1.78)	0.32 (0.20 – 1.67)	-1.29	ns	-
IL-1β[a]	1.29 (0.44 – 3.44)	1.91 (0.45 – 4.47)	-3.02	0.003	0.12
IL-2[a]	3.04 (1.18 – 3.99)	2.81 (1.34 – 4.49)	3.18	0.001	0.13
IL-4[a]	1.70 (1.02 – 3.75)	1.30 (0.96 – 2.84)	3.18	0.001	0.13
IL-6[a]	1.07 (0.33 – 5.03)	0.60 (0.13 – 1.93)	3.17	0.002	0.13
IL-6[b]	0.84 (0.12 – 8.64	0.53 (0.23 – 1.54)	2.53	0.01	0.09
IL-8[a]	5.25 (1.61 – 10.95)	5.29 (1.72 – 17.87)	0.02	ns	-
IL-10[a]	0.45 (0.19 – 1.51)	0.51 (0.19 – 1.35)	0.21	ns	-
INF-γ (Interferon-γ)[a]	0.34 (0.20 – 0.77)	0.30 (0.19 – 0.53)	1.38	ns	-
TNF (Tumor necrosis factor)**- α**[a]	1.88 (1.01 – 7.56)	1.70 (0.86 – 8.70)	0.42	ns	-
TNF (Tumor necrosis factor)**- α**[b]	5.43 (2.92 – 10.73)	5.17 (3.42 – 8.73)	0.13	ns	-
PAI-1 (Plasminogen activator inhibitor-1)[b]	25.68 (8.92 – 48.58)	21.35 (7.57 – 49.88)	2.75	0.006	0.10
Ferritin[b]	56.0 (4.26 – 238.7)	22.1 (1.22 – 184.7)	2.86	0.004	0.11
MCP-1 (monocyte chemoattractant protein -1) [a]	114.22 (23.05 – 364.61)	104.98 (26.02 – 358.9)	0.02	ns	-
EGF (Epidermal growth factor)[a]	17.55 (2.20 – 51.83)	2.05 (0.66 – 7.74)	6.67	<1E-6	0.59
VEGF (Vascular endothelial growth factor) [a]	32.66 (9.7 – 148.52)	42.84 (8.28 – 147.88)	0.87	ns	-

Biomarkers	FEP patients before AP treatment	CSs	Z-value[a]	*p*-value[a]	Effect size (I^2)
	Median (min–max)	Median (min–max)			
Metabolic protein markers[b]					
Insulin	7.30 (3.10 – 60.59)	8.47 (1.80 – 126.9)	-0.55	ns	-
C-peptide	0.80 (0.12 – 4.48)	1.20 (0.37 – 2.28)	-1.29	ns	-
Leptin	0.85 (0 – 2.22)	1.41 (0.38 – 6.69)	-2.73	0.006	0.11
Resistin	2.96 (1.69 – 8.58)	2.66 (1.59 – 4.10)	2.79	0.005	0.11
Adiponectin	7336 (2093 – 12334)	7076 (2382 – 14273)	0.52	ns	-
Acylcarnitines[c]					
C0 (Carnitine)	33.30 (18.30 – 46.60)	32.20 (17.30 – 52.70)	0.17	ns	-
C10 (Decanoylcarnitine)	0.31 (0.17 – 0.87)	0.28 (0.15 – 0.45)	- 2.13	0.03	0.06
C10:1 (Decenoylcarnitine)	0.14 (0.06 – 0.30)	0.13 (0.08 – 0.23)	- 0.96	ns	-
C10:2 (Decadienylcarnitine)	0.06 (0.04 – 0.09)	0.06 (0.04 – 0.10)	0.54	ns	-
C12 (Dodecanoylcarnitine)	0.12 (0.06 – 0.32)	0.11 (0.06 – 0.18)	-2.29	0.02	0.07
C12-DC (Dodecanedioylcarnitine)	0.13 (0.08 – 0.15)	0.12 (0.11 – 0.14)	-0.59	ns	-
C12:1 (Dodecenoylcarnitine)	0.13 (0.07 – 0.29)	0.12 (0.07 – 0.19)	-2.13	0.03	0.06
C14 (Tetradecanoylcarnitine)	0.03 (0.02 – 0.05)	0.03 (0.01 – 0.04)	-2.44	0.02	0.08
C14:1 (Tetradecenoylcarnitine)	0.06 (0.03 – 0.15)	0.04 (0.02 – 0.11)	-3.34	0.0008	0.15
C14:1-OH (Hydroxytetradecenoyl-carnitine)	0.02 (0.01 – 0.03)	0.02 (0.01 – 0.02)	-2.45	0.01	0.08
C14:2 (Tetradecadienylcarnitine)	0.02 (0.01 – 0.07)	0.02 (0.01 – 0.05)	-3.10	0.002	0.13
C14:2-OH (Hydroxytetradecadienyl-carnitine)	0.01 (0.01 – 0.02)	0.01 (0.01 – 0.02)	-0.18	ns	-
C16 (Hexadecanoylcarnitine)	0.12 (0.06 – 0.20)	0.09 (0.04 – 0.12)	-4.28	0.00002	0.24
C16-OH (Hydroxyhexadecanoyl-carnitine)	0.02 (0.01 – 0.03)	0.01 (0.01 – 0.03)	-2.34	0.02	0.07

Biomarkers	FEP patients before AP treatment	CSs	Z-value[a]	*p*-value[a]	Effect size (I^2)
	Median (min–max)	Median (min–max)			
C16:1 (Hexadecenoylcarnitine)	0.03 (0.02 – 0.06)	0.02 (0.02 – 0.04)	-4.51	0.000007	0.27
C16:1-OH (Hydroxyhexadecenoyl-carnitine)	0.01 (0.01 – 0.02)	0.01 (0.01 – 0.02)	-3.42	0.0006	0.16
C16:2 (Hexadecadienylcarnitine)	0.01 (0.01 – 0.02)	0.01 (0.01 – 0.02)	-1.53	ns	-
C16:2-OH (Hydroxyhexadecadienyl-carnitine)	0.03 (0.02 – 0.04)	0.03 (0.02 – 0.04)	1.08	ns	-
C18 (Octadecanoylcarnitine)	0.05 (0.03 – 0.09)	0.04 (0.02 – 0.07)	-2.92	0.004	0.11
C18:1 (Octadecenoylcarnitine)	0.13 (0.05 – 0.23)	0.09 (0.05 – 0.15)	-4.86	0.000001	0.32
C18:1-OH (Hydroxyoctadecenoyl-carnitine)	0.03 (0.02 – 0.05)	0.02 (0.01 – 0.05)	-2.02	0.04	0.05
C18:2 (Octadecadienylcarnitine)	0.04 (0.02 – 0.07)	0.03 (0.01 – 0.05)	-3.68	0.0002	0.18
C2 (Acetylcarnitine)	4.33 (1.73 – 14.00)	3.96 (1.78 – 5.96)	-0.67	ns	-
C3 (Propionylcarnitine)	0.23 (0.14 – 0.49)	0.31 (0.13 – 0.61)	3.72	0.0002	0.19
C3-DC(C4-OH) (Malonylcarnitine (Hydroxy-butyrylcarnitine))	0.05 (0.03 – 0.25)	0.04 (0.03 – 0.07)	-2.95	0.003	0.12
C5-OH(C3-DC-M) (Hydroxyvaleryl-carnitine (Methyl-malonylcarnitine))	0.06 (0.04 – 0.11)	0.06 (0.04 – 0.09)	-0.29	ns	-
C3-OH (Hydroxypropionylcarnitine)	0.04 (0.03 – 0.07)	0.04 (0.03 – 0.05)	0.88	ns	-
C3:1 (Propenoylcarnitine)	0.03 (0.02 – 0.05)	0.03 (0.02 – 0.04)	-0.80	ns	-
C4 (Butyrylcarnitine)	0.17 (0.10 – 0.32)	0.18 (0.12 – 0.30)	2.63	0.008	0.09
C4:1 (Butenylcarnitine)	0.05 (0.03 – 0.06)	0.05 (0.03 – 0.06)	-1.71	ns	-
C6(C4:1-DC) (Hexanoylcarnitine (Fumarylcarnitine))	0.03 (0.02 – 0.05)	0.03 (0.02 – 0.04)	-3.43	0.0006	0.16
C5 (Valerylcarnitine)	0.14 (0.10 – 0.31)	0.17 (0.10 – 0.29)	2.73	0.006	0.10
C5-DC(C6-OH) (Glutarylcarnitine (Hydroxyhexanoylcarnitine))	0.03 (0.02 – 0.04)	0.02 (0.01 – 0.04)	-2.11	0.04	0.06
C5-M-DC (Methylglutarylcarnitine)	0.04 (0.03 – 0.06)	0.04 (0.03 – 0.06)	-0.44	ns	-

Biomarkers	FEP patients before AP treatment	CSs	Z-value[a]	*p*-value[a]	Effect size (I^2)
	Median (min–max)	Median (min–max)			
C5:1 (Tiglylcarnitine)	0.08 (0.05 – 0.11)	0.08 (0.05 – 0.12)	-0.17	ns	-
C5:1-DC (Glutaconylcarnitine)	0.03 (0.02 – 0.03)	0.03 (0.02 – 0.04)	-2.23	0.03	0.07
C6:1 (Hexenoylcarnitine)	0.01 (0.007 – 0.01)	0.009 (0.006 – 0.01)	-1.27	ns	-
C7-DC (Pimelylcarnitine)	0.03 (0.02 – 0.06)	0.03 (0.02 – 0.04)	-1.75	ns	-
C8 (Octanoylcarnitine)	0.14 (0.10 – 0.33)	0.13 (0.09 – 0.20)	-1.48	ns	-
C9 (Nonaylcarnitine)	0.05 (0.03 – 0.08)	0.06 (0.03 – 0.12)	2.88	0.004	0.11
Hexose	4291.50 (2802– 5918)	3907.00 (2335– 5533)	-2.59	0.01	0.09

Z-adjusted values according to Mann-Whitney U-test (FEP_b compared to CS).
[a] High-sensitive biochip array technology was used to measure levels of cytokines.
[b] Metabolic biochip array technology was used to measure metabolic markers.
[c] Serum level of ACs and hexoses were determined with the AbsoluteIDQ™ p180 kit.

Table A-2. Identification of differences in serum cytokines, growth factor, metabolic biomarkers (pg/mL), and acylcarnitine levels (µmoles/mL) between the first-episode psychosis (FEP) patients before antipsychotic (AP) treatment and FEP patients after 7-month AP treatment

Biomarkers	FEP patients before AP treatment	FEP patients after 7-month AP treatment	Z-value[b]	*p*-value[b]	Effect size (I^2)
	Median (min–max)	Median (min–max)			
Inflammatory markers and growth factor					
IL (Interleukin)**-1α**[a]	0.20 (0.06 – 1.33)	0.15 (0.08 – 1.26)	2.01	0.04	0.06
IL (Interleukin)**-1α**[b]	0.30 (0 – 1.78)	0.24 (0 – 1.77)	2.47	0.01	0.11
IL-1β[a]	1.29 (0.44 – 3.44)	1.21 (0.40 – 3.39)	1.24	ns	-
IL-2[a]	3.04 (1.18 – 3.99)	2.06 (1.14 – 3.34)	4.78	2E-6	0.32
IL-4[a]	1.70 (1.02 – 3.75)	1.35 (0.92 – 4.32)	2.88	0.004	0.12
IL-6[a]	1.07 (0.33 – 5.03)	0.60 (0.19 – 2.27)	3.34	0.0008	0.16

Biomarkers	FEP patients before AP treatment	FEP patients after 7-month AP treatment	Z-value[b]	*p*-value[b]	Effect size (η^2)
	Median (min–max)	Median (min–max)			
IL-6[b]	0.84 (0.12 – 8.64	0.75 (0.23 – 1.64)	2.16	0.03	0.07
IL-8[a]	5.29 (1.72 – 17.87)	4.12 (1.25 – 17.36)	2.17	0.03	0.07
IL-10[a]	0.51 (0.19 – 1.35)	0.47 (0.27 – 2.10)	0.34	ns	-
INF-γ (Interferon-γ)[a]	0.34 (0.20 – 0.77)	0.21 (0.17 – 0.69)	3.21	0.001	0.16
TNF (Tumor necrosis factor)**- α**[a]	1.88 (1.01 – 7.56)	1.71 (1.03 – 2.82)	1.46	ns	-
TNF (Tumor necrosis factor)**- α**[b]	5.43 (2.92 – 10.73)	5.10 (2.84 – 9.30)	0.01	ns	-
PAI-1 (Plasminogen activator inhibitor-1)[b]	25.68 (8.92 – 48.58)	22.01 (7.06 – 57.07)	2.24	0.03	0.07
Ferritin[b]	56.0 (4.26 – 238.7)	30.9 (4.75 – 150.0)	4.19	0.00003	0.25
MCP-1 (monocyte chemoattractant protein -1) [a]	104.98 (26.02 – 358.90)	105.91 (10.63 – 339.55)	0.2	ns	-
EGF (Epidermal growth factor)[a]	17.55 (2.20 – 51.83)	1.68 (0.49 – 7.74)	5.16	<1E-6	0.38
VEGF (Vascular endothelial growth factor) [a]	42.84 (8.28 – 147.88)	26.34 (4.77 – 92.29)	4.07	0.00005	0.23
Metabolic protein markers[b]					
Insulin	7.30 (3.10 – 60.61)	10.10 (3.03 – 66.81)	1.26	ns	-
C-peptide	0.80 (0.12 – 4.48)	1.90 (0.28 – 7.71)	3.10	0.002	0.14
Leptin	0.85 (0 – 2.22)	1.21 (0 – 9.29)	3.38	0.0007	0.16
Resistin	2.96 (1.69 – 8.58)	2.80 (1.42 – 4.76)	2.61	0.009	0.10
Adiponectin	7335 (2093 – 2334)	5591 (1084 –10223)	2.43	0.02	0.12
Acylcarnitines[c]					
C0 (Carnitine)	33.30 (18.30 – 46.60)	30.35 (15.80 – 58.40)	0.32	ns	-
C10 (Decanoylcarnitine)	0.31 (0.17 – 0.87)	0.22 (0.12 – 1.08)	1.43	ns	-
C10:1 (Decenoylcarnitine)	0.14 (0.06 – 0.30)	0.13 (0.06 – 0.31)	0.80	ns	-
C10:2 (Decadienylcarnitine)	0.06 (0.04 – 0.09)	0.05 (0.04 – 0.10)	0.72	ns	-

Biomarkers	FEP patients before AP treatment	FEP patients after 7-month AP treatment	Z-value[b]	*p*-value[b]	Effect size (η^2)
	Median (min–max)	Median (min–max)			
C12 (Dodecanoylcarnitine)	0.12 (0.06 – 0.32)	0.09 (0.04 – 0.28)	1.71	ns	-
C12-DC (Dodecanedioylcarnitine)	0.13 (0.08 – 0.15)	0.12 (0.10 – 0.16)	0.45	ns	-
C12:1 (Dodecenoylcarnitine)	0.13 (0.07 – 0.29)	0.12 (0.05 – 0.27)	1.63	ns	-
C14 (Tetradecanoylcarnitine)	0.03 (0.02 – 0.05)	0.02 (0.01 – 0.04)	2.59	0.01	0.09
C14:1 (Tetradecenoylcarnitine)	0.06 (0.03 – 0.15)	0.04 (0.02 – 0.16)	2.53	0.01	0.09
C14:1-OH (Hydroxytetradecenoyl-carnitine)	0.02 (0.01 – 0.03)	0.01 (0.01 – 0.03)	2.29	0.02	0.07
C14:2 (Tetradecadienylcarnitine)	0.02 (0.01 – 0.07)	0.02 (0.01 – 0.04)	2.75	0.006	0.11
C14:2-OH (Hydroxytetradecadienyl-carnitine)	0.01 (0.01 – 0.02)	0.01 (0.01 – 0.02)	1.65	ns	-
C16 (Hexadecanoylcarnitine)	0.12 (0.06 – 0.20)	0.08 (0.04 – 0.15)	3.40	0.0007	0.16
C16-OH (Hydroxyhexadecanoyl-carnitine)	0.02 (0.01 – 0.03)	0.01 (0.01 – 0.03)	1.76	ns	-
C16:1 (Hexadecenoylcarnitine)	0.03 (0.02 – 0.06)	0.02 (0.01 – 0.05)	3.05	0.002	0.13
C16:1-OH (Hydroxyhexadecenoyl-carnitine)	0.01 (0.01 – 0.02)	0.01 (0.01 – 0.02)	2.62	0.009	0.10
C16:2 (Hexadecadienylcarnitine)	0.01 (0.01 – 0.02)	0.01 (0.01 – 0.02)	2.47	0.01	0.09
C16:2-OH (Hydroxyhexadecadienyl-carnitine)	0.03 (0.02 – 0.04)	0.03 (0.02 – 0.04)	0.70	ns	-
C18 (Octadecanoylcarnitine)	0.05 (0.03 – 0.09)	0.04 (0.02 – 0.06)	2.70	0.007	0.10
C18:1 (Octadecenoylcarnitine)	0.13 (0.05 – 0.23)	0.08 (0.04 – 0.17)	4.13	0.00004	0.24
C18:1-OH (Hydroxyoctadecenoyl-carnitine)	0.03 (0.02 – 0.05)	0.02 (0.01 – 0.05)	1.38	ns	-
C18:2 (Octadecadienylcarnitine)	0.04 (0.02 – 0.07)	0.03 (0.02 – 0.05)	3.86	0.0001	0.21
C2 (Acetylcarnitine)	4.33 (1.73 – 14.00)	4.11 (2.24 – 7.72)	0.66	ns	-
C3 (Propionylcarnitine)	0.23 (0.14 – 0.49)	0.30 (0.14 – 0.62)	3.22	0.001	0.15
C3-DC(C4-OH) (Malonylcarnitine (Hydroxybutyrylcarnitine))	0.05 (0.03 – 0.25)	0.04 (0.03 – 0.07)	2.69	0.007	0.10

Biomarkers	FEP patients before AP treatment	FEP patients after 7-month AP treatment	Z-value[b]	*p*-value[b]	Effect size (η^2)
	Median (min–max)	Median (min–max)			
C5-OH(C3-DC-M) (Hydroxy-valerylcarnitine (Methylmalonyl-carnitine))	0.06 (0.04 – 0.11)	0.06 (0.05 – 0.12)	1.11	ns	-
C3-OH (Hydroxypropionyl-carnitine)	0.04 (0.03 – 0.07)	0.04 (0.03 – 0.07)	0.05	ns	-
C3:1 (Propenoylcarnitine)	0.03 (0.02 – 0.05)	0.03 (0.02 – 0.06)	1.02	ns	-
C4 (Butyrylcarnitine)	0.17 (0.10 – 0.32)	0.17 (0.10 – 0.31)	0.78	ns	-
C4:1 (Butenylcarnitine)	0.05 (0.03 – 0.06)	0.05 (0.03 – 0.07)	0.86	ns	-
C6(C4:1-DC) (Hexanoylcarnitine (Fumarylcarnitine))	0.03 (0.02 – 0.05)	0.02 (0.01 – 0.06)	2.22	0.03	0.07
C5 (Valerylcarnitine)	0.14 (0.10 – 0.31)	0.16 (0.10 – 0.39)	2.51	0.01	0.09
C5-DC(C6-OH) (Glutarylcarnitine (Hydroxyhexanoylcarnitine))	0.03 (0.02 – 0.04)	0.02 (0.01 – 0.04)	2.37	0.02	0.09
C5-M-DC (Methylglutarylcarnitine)	0.04 (0.03 – 0.06)	0.04 (0.02 – 0.05)	1.00	ns	-
C5:1 (Tiglylcarnitine)	0.08 (0.05 – 0.11)	0.07 (0.04 – 0.12)	1.05	ns	-
C5:1-DC (Glutaconylcarnitine)	0.03 (0.02 – 0.03)	0.02 (0.01 – 0.04)	0.92	ns	-
C6:1 (Hexenoylcarnitine)	0.01 (0.007 – 0.01)	0.009 (0.006 – 0.01)	1.43	ns	-
C7-DC (Pimelylcarnitine)	0.03 (0.02 – 0.06)	0.03 (0.01 – 0.05)	2.16	0.03	0.07
C8 (Octanoylcarnitine)	0.14 (0.10 – 0.33)	0.12 (0.07 – 0.42)	1.18	ns	-
C9 (Nonaylcarnitine)	0.05 (0.03 – 0.08)	0.06 (0.04 – 0.09)	1.83	ns	-
Hexose	4291.50 (2802 – 5918)	4054.50 (2846 – 7189)	0.93	ns	-

Z-values according to Wilcoxon matched pairs test (FEP patients before AP treatment compared to FEP patients after 7-month AP treatment).
[a] High-sensitive biochip array technology was used to measure levels of cytokines.
[b] Metabolic biochip array technology was used to measure metabolic markers.
[c] Serum level of ACs and hexoses were determined with the AbsoluteIDQ™ p180 kit.

Table A-3. Identification of differences in serum cytokines, growth factor, metabolic biomarkers (pg/mL), and acylcarnitine levels (μmoles) between the first-episode psychosis (FEP) patients after 7-month antipsychotic (AP) treatment and control subjects (CSs).

Biomarkers	FEP patients after 7-month AP treatment	CSs	Z-value[a]	*p*-value[a]	Effect size $(I)^2$
	Median (min–max)	Median (min–max)			
Inflammatory markers and growth factor					
IL (Interleukin)**-1α**[a]	0.15 (0.08 – 1.26)	0.16 (0.06 – 0.60)	-0.65	ns	-
IL (Interleukin)**-1α**[b]	0.24 (0 – 1.77)	0.32 (0.20 – 1.67)	-2.89	0.004	0.12
IL-1β[a]	1.21 (0.40 – 3.39)	1.91 (0.45 – 4.47)	-3.35	0.0008	0.16
IL-2[a]	2.06 (1.14 – 3.34)	2.81 (1.34 – 4.49)	-4.55	5E-6	0.28
IL-4[a]	1.35 (0.92 – 4.32)	1.30 (0.96 – 2.84)	0.33	ns	-
IL-6[a]	0.60 (0.19 – 2.27)	0.60 (0.13 – 1.93)	0.79	ns	-
IL-6[b]	0.75 (0.23 – 1.64)	0.53 (0.23 – 1.54)	1.74	ns	-
INF (Interferon)**-γ**[a]	0.21 (0.17 – 0.69)	0.30 (0.19 – 0.53)	-3.07	0.002	0.13
TNF (Tumor necrosis factor)**- α**[a]	1.71 (1.03 – 2.82)	1.70 (0.86 – 8.70)	-0.31	ns	-
TNF (Tumor necrosis factor)**- α**[b]	5.10 (2.84 – 9.30)	5.17 (3.42 – 8.73)	0.31	ns	-
PAI (Plasminogen activator inhibitor)**-1**[b]	22.01 (7.06 – 57.07)	21.35 (7.57 – 49.88)	0.79	ns	-
Ferritin[b]	30.9 (4.75 – 150.0)	22.1 (1.22 – 184.7)	1.04	ns	-
EGF (Epidermal growth factor)[a]	1.68 (0.49 – 7.74)	2.05 (0.66 – 7.74)	-1.12	ns	-
Metabolic protein markers[b]					
Insulin	10.10 (3.03 – 66.81)	8.47 (1.80 – 126.9)	0.83	ns	-
C-peptide	1.90 (0.28 – 7.71)	1.20 (0.37 – 2.28)	2.32	0.02	0.08
Leptin	1.21 (0 – 9.29)	1.41 (0.38 – 6.69)	-0.07	ns	-
Resistin	2.80 (1.42 – 4.76)	2.66 (1.59 – 4.10)	1.22	ns	-
Adiponectin	5591 (1084 –10223)	7076 (2382 – 14273)	-1.52	ns	-

Biomarkers	FEP patients after 7-month AP treatment	CSs	Z-value[a]	*p*-value[a]	Effect size (I^2)
	Median (min–max)	Median (min–max)			
Acylcarnitines[c]					
C0 (Carnitine)	30.35 (15.80 – 58.40)	32.20 (17.30 – 52.70)	-0.81	ns	-
C10 (Decanoylcarnitine)	0.22 (0.12 – 1.08)	0.28 (0.15 – 0.45)	-1.23	ns	-
C10:1 (Decenoylcarnitine)	0.13 (0.06 – 0.31)	0.134 (0.08 – 0.23)	-0.01	ns	-
C10:2 (Decadienylcarnitine)	0.05 (0.04 – 0.10)	0.06 (0.04 – 0.10)	-1.65	ns	-
C12 (Dodecanoylcarnitine)	0.09 (0.04 – 0.28)	0.11 (0.06 – 0.18)	-1.05	ns	-
C12-DC (Dodecanedioylcarnitine)	0.12 (0.10 – 0.16)	0.12 (0.11 – 0.14)	-0.01	ns	-
C12:1 (Dodecenoylcarnitine)	0.12 (0.05 – 0.27)	0.12 (0.07 – 0.19)	-0.24	ns	-
C14 (Tetradecanoylcarnitine)	0.02 (0.01 – 0.04)	0.03 (0.01 – 0.04)	-1.27	ns	-
C14:1 (Tetradecenoylcarnitine)	0.038 (0.02 – 0.16)	0.04 (0.02 – 0.11)	-0.99	ns	-
C14:1-OH (Hydroxytetradecenoyl-carnitine)	0.01 (0.01 – 0.03)	0.02 (0.01 – 0.02)	-0.80	ns	-
C14:2 (Tetradecadienylcarnitine)	0.02 (0.01 – 0.04)	0.02 (0.01 – 0.05)	-1.36	ns	-
C14:2-OH (Hydroxytetradecadienyl-carnitine)	0.01 (0.01 – 0.02)	0.01 (0.01 – 0.02)	-1.70	ns	-
C16 (Hexadecanoylcarnitine)	0.08 (0.04 – 0.15)	0.09 (0.04 – 0.12)	-0.36	ns	-
C16-OH (Hydroxyhexadecanoyl-carnitine)	0.01 (0.01 – 0.03)	0.01 (0.01 – 0.03)	0.76	ns	-
C16:1 (Hexadecenoylcarnitine)	0.02 (0.01 – 0.05)	0.02 (0.02 – 0.04)	-0.55	ns	-
C16:1-OH (Hydroxyhexa-decenoylcarnitine)	0.01 (0.01 – 0.02)	0.01 (0.01 – 0.02)	0.38	ns	-
C16:2 (Hexadecadienylcarnitine)	0.01 (0.01 – 0.02)	0.01 (0.01 – 0.02)	-1.49	ns	-
C16:2-OH (Hydroxyhexa-decadienylcarnitine)	0.03 (0.02 – 0.04)	0.03 (0.02 – 0.04)	-0.56	ns	-
C18 (Octadecanoylcarnitine)	0.04 (0.02 – 0.06)	0.04 (0.02 – 0.07)	0.26	ns	-
C18:1 (Octadecenoylcarnitine)	0.08 (0.04 – 0.17)	0.09 (0.05 – 0.15)	-0.63	ns	-

Biomarkers	FEP patients after 7-month AP treatment	CSs	Z-value[a]	*p*-value[a]	Effect size (I^2)
	Median (min–max)	Median (min–max)			
C18:1-OH (Hydroxyoctadecenoyl-carnitine)	0.02 (0.01 – 0.05)	0.02 (0.01 – 0.05)	-0.53	ns	-
C18:2 (Octadecadienylcarnitine)	0.03 (0.02 – 0.05)	0.03 (0.01 – 0.05)	-0.39	ns	-
C2 (Acetylcarnitine)	4.11 (2.24 – 7.72)	3.96 (1.78 – 5.96)	0.69	ns	-
C3 (Propionylcarnitine)	0.30 (0.14 – 0.62)	0.31 (0.13 – 0.61)	-0.72	ns	-
C3-DC(C4-OH) (Malonylcarnitine (Hydroxybutyrylcarnitine))	0.04 (0.03 – 0.07)	0.04 (0.03 – 0.07)	-0.14	ns	-
C5-OH(C3-DC-M) (Hydroxy-valerylcarnitine (Methylmalonylcarnitine))	0.06 (0.05 – 0.12)	0.06 (0.04 – 0.09)	-0.19	ns	-
C3-OH (Hydroxypropionyl-carnitine)	0.04 (0.03 – 0.07)	0.04 (0.03 – 0.05)	-1.13	ns	-
C3:1 (Propenoylcarnitine)	0.03 (0.02 – 0.06)	0.03 (0.02 – 0.04)	-0.45	ns	-
C4 (Butyrylcarnitine)	0.17 (0.10 – 0.31)	0.18 (0.12 – 0.30)	-1.71	ns	-
C4:1 (Butenylcarnitine)	0.05 (0.03 – 0.07)	0.05 (0.03 – 0.06)	0.54	ns	-
C6(C4:1-DC) (Hexanoylcarnitine (Fumarylcarnitine))	0.02 (0.01 – 0.06)	0.03 (0.02 – 0.04)	-0.58	ns	-
C5 (Valerylcarnitine)	0.16 (0.10 – 0.39)	0.17 (0.10 – 0.29)	-0.53	ns	-
C5-DC(C6-OH) (Glutarylcarnitine (Hydroxyhexanoylcarnitine))	0.02 (0.01 – 0.04)	0.02 (0.01 – 0.04)	-0.34	ns	-
C5-M-DC (Methylglutaryl-carnitine)	0.04 (0.02 – 0.05)	0.04 (0.03 – 0.06)	-0.64	ns	-
C5:1 (Tiglylcarnitine)	0.07 (0.04 – 0.12)	0.08 (0.05 – 0.12)	-0.83	ns	-
C5:1-DC (Glutaconylcarnitine)	0.02 (0.01 – 0.04)	0.03 (0.02 – 0.04)	-0.08	ns	-
C6:1 (Hexenoylcarnitine)	0.009 (0.006 – 0.01)	0.009 (0.006 – 0.01)	-1.40	ns	-
C7-DC (Pimelylcarnitine)	0.03 (0.01 – 0.05)	0.03 (0.02 – 0.04)	-1.49	ns	-
C8 (Octanoylcarnitine)	0.12 (0.07 –0.42)	0.13 (0.09 – 0.20)	-1.11	ns	-
C9 (Nonaylcarnitine)	0.06 (0.04 – 0.09)	0.06 (0.03 – 0.12)	-1.03	ns	-

Biomarkers	FEP patients after 7-month AP treatment	CSs	Z-value[a]	*p*-value[a]	Effect size (η^2)
	Median (min–max)	Median (min–max)			
Hexose	4054.50 (2846 – 7189)	3907.00 (2335– 5533)	1.68	ns	-

Z-adjusted values according to Mann-Whitney U-test (FEP patients after 7-month AP treatment compared to CSs).
[a] High-sensitive biochip array technology was used to measure levels of cytokines.
[b] Metabolic biochip array technology was used to measure metabolic markers.
[c] Serum level of ACs and hexoses were determined with the AbsoluteIDQ™ p180 kit.

Table A-4. Inflammatory marker values[a] (pg/mL) of patients with schizophrenia (SCH) spectrum disorder compared to control subjects (CSs)

	SCH patients	CSs	Z - value[a]	*p*-value	Effect size η^2
	Median (min–max)				
Pro-inflammatory cytokines					
IL (Interferon)**-1α**	0.07 (0.03 – 1.13)	0.09 (0.04 – 0.35)	-1.46	ns	-
IL-1β	0.57 (0.21 – 11.46)	0.54 (0.34 – 1.67)	-0.26	ns	-
IL-2	2.18 (0.80 – 8.10)	1.21 (0.47 – 2.97)	8.78	<1E-6	0.31
IL-6	1.80 (0.38 – 28.20)	0.89 (0.33 – 2.29)	8.01	<1E-6	0.26
IL-8	9.90 (2.27 – 65.50)	6.63 (3.20 – 13.10)	6.14	<1E-6	0.15
MCP-1	244.8 (42.75 – 741.9)	165.3 (26.90 – 345.2)	6.98	<1E-6	0.19
TNF (Tumor necrosis factor)**-α**	3.23 (1.39 – 8.71)	3.38 (1.30 – 6.54)	-1.67	ns	-
IFN (Interferon)**-γ**	4.12 (0.41 – 38.87)	1.23 (0.16 – 4.10)	9.93	<1E-6	0.40
Anti-inflammatory cytokines					
IL-4	1.25 (0.58 – 5.04)	1.44 (0.46 – 2.83)	-3.18	0.002	0.04
IL-10	0.77 (0.21 – 7.46)	0.55 (0.20 – 1.32)	6.27	<1E-6	0.16
Ratio of biomarkers					
INF-γ/IL-4	3.09 (0.38 – 21.24)	0.75 (0.12 – 4.82)	9.63	<1E-6	0.37
INF-γ/IL-10	4.35 (0.34 – 56.23)	2.00 (0.15 – 12.71)	6.26	<1E-6	0.16
IL-2/INF-γ	0.60 (0.09 – 6.24)	1.14 (0.24 – 7.09)	-5.61	<1E-6	0.13
Growth factors					
VEGF (Vascular endothelial growth factor)	119.4 (12.62 – 739.5)	115.9 (14.14 – 306.8)	2.06	0.04	0.02
EGF (Epidermal growth factor)	55.26 (2.64 – 170.64)	47.60 (3.57 – 101.00)	1.98	0.048	0.02

Z-adjusted values according to Mann-Whitney U-test.
The table contains between groups difference data with effect size ≥ 0.10.
[a] High-sensitive biochip array technology was used to measure levels of cytokines.

2. Quality control

2.1. Cytokines, chemokines, and growth factors quantitation

Table A-5. Intra-assay precision of high-sensitive cytokine array

Analyte	Level 1		Level 2		Level 3	
	Conc pg/ml	%CV	Conc pg/ml	%CV	Conc pg/ml	%CV
IL (Interleukin)**-1α**	2.7	10.9	45.4	9.7	169.7	11.4
IL-1ß	5.1	9.3	63.9	8.7	233.4	7.4
IL-2	16.7	7.8	84.1	5.8	255.8	6.9
IL-4	13.1	9.6	69.9	9.5	232.2	8.4
IL-6	1.4	11.9	10.5	9.8	26.7	7.8
IL-8	11.6	9.4	123.0	9.4	629.0	7.0
IL-10	8.2	6.8	93.9	5.6	286.0	6.1
IFN (Interferon)**-γ**	7.9	10.1	145.7	7.4	620.1	7.7
TNF (Tumor necrosis factor)**-α**	9.4	7.1	111.8	7.2	486.1	12.7
MCP (Plasminogen activator inhibitor)**-1**	27.6	12.2	136.3	8.9	320.8	5.8
VEGF (Vascular endothelial growth factor)	17.3	10.4	146.4	10.8	456.0	7.3
EGF (Epidermal growth factor)	13.0	9.2	36.7	9.5	198.7	9.6

Conc – concentration; CV – coefficient of variation.

Table A-6. Inter-assay precision of high sensitive cytokine array

Analyte	Level 1		Level 2		Level 3	
	Conc pg/ml	%CV	Conc pg/ml	%CV	Conc pg/ml	%CV
IL (Interleukin)**-1α**	2.7	15.5	51.1	8.9	174.9	13.4
IL-1ß	4.2	9.9	66.7	11.8	213.7	8.7
IL-2	16.4	7.8	85.2	6.5	258.9	8.2
IL-4	12.5	11.6	71.4	8.6	206.9	8.8
IL-6	1.5	8.4	10.3	7.4	23.7	8.4
IL-8	11.3	11.1	132.6	9.9	560.8	9.2
IL-10	8.2	6.7	96.8	6.5	239.4	7.5
IFN (Interferon)**-γ**	8.1	11.4	135.4	6.4	542.1	7.6
TNF (Tumor necrosis factor)**-α**	9.9	8.6	116.5	6.7	442.1	7.0
MCP (Plasminogen activator inhibitor)**-1**	27.9	12.8	139.8	10.4	272.4	7.2
VEGF (Vascular endothelial growth factor)	17.4	12.0	135.2	7.2	395.7	10.7
EGF (Epidermal growth factor)	13.1	11.7	38.9	8.5	181.2	9.6

Conc – concentration; CV – coefficient of variation.

Table A-7. Sensitivity of cytokine assay

Analyte	Calibration range (pg/ml)	Sensitivity (pg/ml)
IL (Interleukin)**-1α**	0 – 225	0.19
IL-1ß	0 – 1125	0.26
IL-2	0 – 1200	0.90
IL-4	0 – 1500	2.12
IL-6	0 – 400	0.12
IL-8	0 – 1450	0.38
IL-10	0 – 450	0.37
IFN (Interferon)**-γ**	0 – 500	0.44
TNF (Tumor necrosis factor)**-α**	0 – 600	0.59
MCP (Plasminogen activator inhibitor)**-1**	0 – 500	0.66
VEGF (Vascular endothelial growth factor)	0 – 1000	1.53
EGF (Epidermal growth factor)	0 – 450	1.04

2.2. Metabolic and inflammatory biomarkers quantitation

Table A-8. Intra-assay and inter-assay precision of metabolic and cytokine array.

Analyte	Intra-assay			Inter-assay		
	Level 1 % CV	Level 2 % CV	Level 3 % CV	Level 1 % CV	Level 2 % CV	Level 3 % CV
C-peptide	6.9	5.6	5.4	7.9	8.6	12.2
Ferritin	5.7	8.2	7.2	10.0	5.7	8.2
Insulin	9.4	7.8	7.5	10.3	9.0	14.0
IL (Interleukin)**-1α**	4.3	3.8	3.6	8.5	7.6	10.7
IL-6	6.4	6.1	6.3	4.9	6.3	8.4
Leptin	7.7	4.6	5.3	8.7	6.0	8.2
PAI (Plasminogen activator inhibitor)**-1**	10.5	10.9	10.7	14.0	13.1	14.3
Resistin	12.1	10.1	11.8	5.2	7.1	8.6
TNF (Tumor necrosis factor)**-α**	6.1	6.3	5.5	9.2	7.4	7.9

CV – coefficient of variation.

Table A-9. Units and sensitivity of metabolic and cytokine array.

Analyte	Units	Sensitivity
C-peptide	ng/mL	0.21
Ferritin	ng/mL	3.27
Insulin	IU/mL	2.32
IL (Interleukin)-**1α**	pg/mL	0.77
IL-6	pg/mL	0.73
Leptin	ng/mL	1.10
PAI (Plasminogen activator inhibitor)**-1**	ng/mL	2.34
Resistin	ng/mL	1.06
TNF (Tumor necrosis factor)**-α**	pg/mL	0.66

2.3. Adiponectin quantitation

Table A-10. Intra-assay and inter-assay precision of adiponectin

Analyte	Intra-assay precision			Inter-assay precision		
Sample	1	2	3	1	2	3
Number of samples	20	20	20	40	40	40
Mean (ng/mL)	19.8	69.9	143	20.5	74.4	157
Standard deviation	0.50	2.40	6.76	1.40	4.30	10.8
CV (%)	2.5	3.4	4.7	6.8	5.8	6.9

CV – coefficient of variation.

Intra-assay precision (precision within an assay): three samples of known concentration were tested on one plate to assess intra-assay precision. Inter-assay precision (precision between assays): three samples of known concentration were tested in separate assays to assess inter-assay precision.

Sensitivity. The minimum detectable dose of human adiponectin ranged from 0.079-0.891 ng/mL. The mean minimum detectable dose was 0.246 ng/mL. The minimum detectable dose was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

2.4. Acylcarnitine quantitation

Biocrates Absolute*IDQ*™ p180 kit (Biocrates Life Sciences AG, Innsbruck, Austria) enables the measurement of 188 endogenous metabolites and 45 metabolite ratios using a combination of flow injection analysis and liquid chromatograph tandem mass spectrometry technique. The assay allows simultaneous quantification of 188 metabolites, including 40 ACs. The kit has been validated according to the Food and Drug Administration guidelines and Biocrates company holds an ISO 9001:2008 certification of quality. ACs, and hexose were quantified by their relative intensity over the chosen isotopically labeled internal standard. In addition, 3 quality control standards are measured to ensure the normalization of signal intensities during inter-plate

measurements. The metabolite concentrations were calculated linearly using a combination of Analyst (ABSciex, Framingham, USA) and MetIDQ (Biocrates Life Sciences AG, Innsbruck, Austria) software. Due to lack of isotopic standards for lipids and ACs in the flow-injection analysis mode the results are classified as semi-quantitative.

Limits of detection. The absolute minimum limit of detection (LOD) for a metabolite is largely dependent on the sensitivity of the mass-spectrometer and the ionization of a metabolite. LODs, lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) for selected metabolites are given in Table A-11.

Table A-11. Acylcarnitine quantitation

Analyte		Quality Type (FIA)		Evaluated Quantification		
MetIDQ Short Name	**Biochemical Name**	**Valid**	**Semi**	**LOD (µM)**	**LLOQ (µM)**	**ULOQ (µM)**
C0	Carnitine	X		4	5	120
C10	Decanoylcarnitine	X		0.16	0.3	6
C10:1	Decenoylcarnitine		X	0.12		
C10:2	Decadienylcarnitine		X	0.04		
C12	Dodecanoylcarnitine	X		0.057	0.4	12
C12-DC	Dodecanedioylcarnitine		X	0.2		
C12:1	Dodecenoylcarnitine		X	0.2		
C14	Tetradecanoylcarnitine	X		0.03	0.4	6
C14:1	Tetradecenoylcarnitine		X	0.015		
C14:1-OH	Hydroxytetradecenoylcarnitine		X	0.015		
C14:2	Tetradecadienylcarnitine		X	0.012		
C14:2-OH	Hydroxytetradecadienylcarnitine		X	0.015		
C16	Hexadecanoylcarnitine	X		0.018	0.4	12
C16-OH	Hydroxyhexadecanoylcarnitine		X	0.015		
C16:1	Hexadecenoylcarnitine		X	0.06		
C16:1-OH	Hydroxyhexadecenoylcarnitine		X	0.02		
C16:2	Hexadecadienylcarnitine		X	0.008		
C16:2-OH	Hydroxyhexadecadienylcarnitine		X	0.03		
C18	Octadecanoylcarnitine	X		0.02	0.4	6
C18:1	Octadecenoylcarnitine		X	0.04		
C18:1-OH	Hydroxyoctadecenoylcarnitine		X	0.023		
C18:2	Octadecadienylcarnitine		X	0.009		
C2	Acetylcarnitine	X		0.15	0.4	35
C3	Propionylcarnitine	X		0.08	0.4	15
C3-DC(C4-OH)	Malonylcarnitine (Hydroxybutyrylcarnitine		X	0.09		
C5-	Hydroxyvalerylcarnitine		X	0.1		

Analyte		Quality Type (FIA)		Evaluated Quantification		
MetIDQ Short Name	**Biochemical Name**	**Valid**	**Semi**	**LOD (µM)**	**LLOQ (µM)**	**ULOQ (µM)**
OH(C3-DC-M)	(Methylmalonylcarnitine)					
C3-OH	Hydroxypropionylcarnitine		X	0.05		
C3:1	Propenoylcarnitine		X	0.03		
C4	Butyrylcarnitine	X		0.03	0.4	12
C4:1	Butenylcarnitine		X	0.03		
C6(C4:1-DC)	Hexanoylcarnitine (Fumarylcarnitine)	X		0.08	0.2	6
C5	Valerylcarnitine	X		0.04	0.4	12
C5-DC(C6-OH)	Glutarylcarnitine (Hydroxyhexanoylcarnitine)		X	0.035		
C5-M-DC	Methylglutarylcarnitine		X	0.06		
C5:1	Tiglylcarnitine		X	0.04		
C5:1-DC	Glutaconylcarnitine		X	0.015		
C6:1	Hexenoylcarnitine		X	0.035		
C7-DC	Pimelylcarnitine		X	0.035		
C8	Octanoylcarnitine	X		0.17	0.2	8
C9	Nonaylcarnitine		X	0.04		

PUBLICATIONS

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Liikmelisus: Eesti Psühhiaatrite Selts, liige

Publikatsioonid:

Balotšev R, Haring L, Koido K, Leping V, Kriisa K, Zilmer M, Vasar V, Piir A, Lang A, & Vasar E (2017). Antipsychotic treatment is associated with inflammatory and metabolic biomarkers alterations among first-episode psychosis patients: A 7-month follow-up study. Early Intervention in Psychiatry 0:1–9.

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