# High Circulating MIF Levels Indicate the Association with Atypical Antipsychotic-induced Adverse Metabolic Effects

**By**

**Pingyi Gao**

A Thesis submitted to the Faculty of Graduate Studies of
The University of Manitoba
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

College of Pharmacy
Rady Faculty of Health Sciences
University of Manitoba
Winnipeg

**Abstract**

Atypical antipsychotics (AAPs) are primary medications for schizophrenia (SZ). However, their use is frequently associated with the development of adverse metabolic effects, and the mechanisms behind these negative effects remain inadequately elucidated. To investigate the role of macrophage migration inhibitory factor (MIF) in regulating antipsychotic-induced metabolic abnormalities, between 2017 and 2020, a cross-sectional study was conducted, involving 142 healthy individuals and 388 SZ patients undergoing treatment with either typical antipsychotic (TAP) or AAP medications. Symptoms of SZ patients were evaluated using the Positive and Negative Syndrome Scale (PANSS), and measurements of metabolic indices and plasma MIF levels were performed on all individuals. A significant increase in plasma MIF levels was observed in groups receiving five major AAP monotherapies in comparison to healthy controls (all $p < 0.0001$). There was no such increase shown in the group receiving TAP treatment ($p > 0.05$). Elevated plasma MIF levels displayed a notable correlation with insulin resistance ($\beta = 0.024$, $p = 0.020$), as well as with the levels of triglycerides ($\beta = 0.019$, $p = 0.001$) and total cholesterol ($\beta = 0.012$, $p = 0.038$) in the groups receiving AAPs. However, while the TAP group also displayed certain metabolic dysfunction compared to healthy controls, no significant association was evident with plasma MIF levels (all $p > 0.05$). In conclusion, plasma MIF levels exhibit a distinctive correlation with metabolic abnormalities triggered by AAPs. Hence, there is potential for further development of MIF as a distinctive marker for monitoring adverse metabolic effects induced by AAPs in clinical settings.

**Acknowledgements**

I would like to express my profound gratitude to **Dr. Dake Qi**, my esteemed professor, for granting me the opportunity to join his program and immerse myself in the research experience. His unwavering confidence in my potential has been immensely impactful. Additionally, I wish to extend my heartfelt thanks to **Dr. Jiming Kong** and **Dr. Kaarina Kowalec**, members of my evaluation committee, for their support and invaluable feedback. Their multidisciplinary insights have significantly enhanced the relevance and quality of this project.

I am also deeply grateful to **Xi Chen** for her dedicated time, valuable contributions, and assistance in overcoming the challenging aspects of this research. My sincere thanks go to my mentor, **Yadan Qi**, for her consistent support and guidance. She has always been there to provide essential training, review my proposals and papers, and offer her valuable opinions.

Furthermore, I extend my gratitude to my amazing friends and colleagues, **Liujun Chen**, **Yiheng Huang**, **Lisha Li**, and **Ethan Nicholson**, whose advice, guidance, and invaluable friendship have played a crucial role throughout this academic journey.

**Dedication**

**This thesis and hard work are dedicated to:**

My parents, Jin Gao and Yujie Guo

*For always making their best effort to give me the best education and opportunities, they have my eternal gratitude.*

My esteemed professor
Dr. Yuewen Gong

*For always offering invaluable assistance throughout the challenging academic journey.*

My longtime confidantes
Mike Mckenna, Dr. Zhanqing Yan

*For providing unconditional support during tough times*

**Table of Contents**

**List of Tables**

**List of Figures**

# Chapter I: Introduction

The treatment of psychosis and related mental illnesses has undergone a remarkable evolution over the past century. From the early days of exorcisms to the advent of modern psychopharmacology, the history of psychiatric treatment is a testament to the relentless pursuit of improving patient care.

## 1.1 Overview of Psychiatric Treatments

Throughout history, various approaches have been experimented with and employed to manage symptoms of mental illness. In ancient civilizations, mental illness was often attributed to supernatural causes, leading to treatments such as rituals and prayers. The Middle Ages and Early Modern Ages saw a shift towards more punitive measures, with individuals deemed mentally ill subjected to exorcisms and confinement in asylums. It wasn't until the early 20th century that significant advancements in mental health care emerged, such as the introduction of insulin coma therapy, Metrazol therapy, and lobotomy. The mid-20th century represented a pivotal moment in the treatment of psychosis with the discovery of the first antipsychotic medication, chlorpromazine (CPZ). This breakthrough triggered the era of psychopharmacology, transforming the management of psychiatric disorders, especially schizophrenia (SZ). Subsequent developments, including atypical antipsychotics (AAPs) in the 1990s, further enhanced treatment outcomes by providing better efficacy and reduced adverse effects compared to traditional antipsychotics, thereby dominating the market and becoming the most prevalent medications to date.

### 1.2 Psychiatric Treatments in Ancient Times to the Middle Ages

Within ancient societies such as Mesopotamia, Israel and India, mental illness was often shrouded in mysticism and interpreted through the lens of superstition and mythology [1]. Individuals experiencing mental disorders were believed to be under the influence of supernatural forces, spanning from malicious spirits to divine judgment. Mental afflictions were often seen as spiritual possession, punishment by deities for perceived disrespect or wrongdoing, or as the consequences of curses placed upon the afflicted individuals. In ancient China, individuals with mental disorders were believed to be the result of an imbalance in yin and yang, reflecting an imbalance in the natural order [2]. Similarly, Greek physicians at the time were convinced that mental illness stemmed from the imbalance of four main humors in the body [3]. Consequently, both the Chinese and Greeks understood the significance of preserving a harmonious balance of the system, establishing the foundations for future advancements in medical research and development.

During this period, a variety of treatment approaches were utilized. Ritualistic practices were mainly adopted to align with prevalent cultural and religious ideologies. Exorcisms were conducted, calling upon protective deities to remove perceived malevolent spirits from those afflicted. Patients from China received treatment utilizing traditional Chinese medicine and acupuncture [2]. Due to the uncertainty surrounding the origins of mental illness, ancient Greek physicians opted for more natural therapies, including dietary adjustments and massages, laying the foundation for modern physical therapy [4].

As societies transitioned into the Middle Ages, attitudes towards mental illness became increasingly influenced by religious dogma and fear, especially in Europe. The prevailing belief in divine retribution and spiritual causation led to a more punitive approach to treatment.

Individuals deemed mentally ill were often viewed as possessed by demons or witches and subjected to harsh and often cruel interventions. Thus, despite being perceived as cruel and inhumane, any bodily remedies aimed at expelling demons from patients, such as trephining and whipping, were considered methods of treating patients. Furthermore, exorcisms, torture and imprisonment were prevalently enforced upon patients suffering from mental disorders. Collectively, these approaches reflected a misguided attempt to promote the well-being of patients at the time.

### 1.3 Psychiatric Treatments in the Early Modern Period

The Renaissance era marked a pivotal shift in understanding and psychiatric treatments, driven by a resurgence of interest in rational learning, humanism, and scientific inquiry. During this period, there was a departure from the superstitions and religious dogma of the Middle Ages, as scholars and thinkers endeavored to discover the mysteries of the human mind through observation, reason, and empirical study. This shift caused tension and rifts among the churches and religious communities.

In response to this shifting paradigm, asylums began to emerge as institutions dedicated to housing and caring for individuals with mental illness. In contrast to the bleak and punitive conditions of the Middle Ages, Renaissance asylums were conceived as significantly improved places where individuals could receive treatment, support, and protection from the harsh realities of the outside world [5]. Despite the great intentions at first, due to the overflow of patients, some were treated inhumanely and subjected to being chained up, becoming a spectacle for paying visitors. Moreover, healthcare professionals often applied physical approaches to patients within the asylum. Furthermore, the sanitation conditions were abysmal and concerning.

### 1.4 Psychiatric Treatments in the Early 20th Century

The early 20th century was an important period for significant advancements in mental health care, propelled by the emergence of the Mental Hygiene Movement [6] and improvements in psychiatric treatment methods. As physicians and scientists sought to address mental disorders in the new era, they discovered novel therapeutic approaches, yet their efficacy and controversies persisted simultaneously.

One such treatment method, insulin coma therapy, emerged in 1927 [7] as an approach to treating severe mental illness, including SZ. Physicians induced a low blood sugar coma in patients following insulin injections under the belief that substantial changes in insulin levels could affect brain function. Despite this therapy showed some promise in alleviating symptoms [8], it also carried significant risks, including brain damage, prolonged coma and high mortality rates [9]. Therefore, this therapy was eventually discontinued in clinical practices.

Metrazol therapy, developed by Hungarian physicians in the 1930s, represents another treatment option introduced in the early 20th century. This therapy involved inducing seizures utilizing the stimulant. Initially employed as a therapeutic intervention, Metrazol therapy was linked to various adverse effects, such as vertebral fractures and pulmonary tuberculosis[10], leading to its eventual withdrawal by the FDA in the early 1980s. Despite its dangers, Metrazol therapy paved the way for the development of electroconvulsive therapy (ECT), which remains an effective treatment option for certain mental illnesses, such as treatment-resistant depression [11].

Alongside these experimental therapies, lobotomy gained popularity as a treatment for severe psychiatric disorders during the 1940s and 1950s [12]. This procedure, which involved

surgically disconnecting between the prefrontal cortex and frontal lobes of the brain, was considered as a revolutionary breakthrough in psychiatric treatment and even won the Nobel Prize in Physiology and Medicine in 1949 [13]. However, lobotomy was fraught with serious risks and side effects, including epilepsy and a personality defect [14]. As safer and more effective treatment options emerged in the 1950s, the utilization of lobotomy decreased, ultimately falling out of favor due to its associated dangers and controversies.

Despite the limitations of these early treatment methods, they exemplify the evolving comprehension of mental illness and the continual endeavor to alleviate human suffering through compassionate and evidence-based care.

### 1.5 Psychiatric Treatments in the Mid-20th Century to Present

The 1950s marked a transformative era in the treatment of mental disorders, characterized by the discovery of antipsychotic medications and numerous antidepressants. These developments revolutionized psychiatric care, leading to significantly improved outcomes for individuals with severe mental disorders.

A pivotal moment in psychiatric history occurred with the invention of chlorpromazine in the 1950s, the first antipsychotic medication [15]. CPZ, originally developed as an antihistamine, was believed to have profound effects on mitigating the symptoms of psychosis, including hallucinations, delusions, and disorganized thinking [16]. Its invention represented a paradigm shift in the treatment of SZ and other mental diseases, offering a pharmacological intervention that was more effective than previous treatments.

Subsequent developments in psychopharmacology have significantly transformed the landscape of psychiatric care, particularly in the treatment of psychosis. One of the most notable advancements occurred in the 1990s with the emergence of AAPs, which expanded the range of

treatment options available for individuals with psychosis. AAPs, including medications such as clozapine (CLZ), risperidone (RIS), and olanzapine (OLZ), represented a significant improvement over first-generation antipsychotics. These medications offered several advantages, including enhanced efficacy in managing psychotic symptoms and a reduced risk of extrapyramidal side effects, such as involuntary movements and motor disturbances, which were commonly associated with typical antipsychotic drugs. The introduction of AAPs revolutionized psychiatric care by providing healthcare professionals with a broader array of treatment options for individuals with mental illness. These medications offered new possibilities for managing symptoms and improving the life quality for patients, empowering them to better cope with their condition and engage more fully in daily activities.

### 1.6 Caveats of AAP Therapies

Although AAPs have transformed the treatment of mental illnesses such as SZ and enhanced the well-being of numerous patients, their link to adverse metabolic effects underscores the need for vigilant monitoring and the development of better treatment strategies to enhance clinical outcomes. To progress towards our objectives, it is crucial to initially explore the peripheral molecular mechanisms driving these adverse metabolic effects, particularly how AAPs induce insulin resistance across various peripheral tissues. By conducting a thorough review of existing literature and experimental studies, we aim to elucidate the peripheral mechanisms responsible for insulin resistance by AAPs.

In recent decades, scientists have been engaged in unraveling the molecular mechanism behind the induction of metabolic dysfunctions by AAPs. Initially, the research primarily focused on examining AAP's effects on the central nervous system. The hypothalamus, the crucial organ for homeostasis, is involved in regulating food intake [17]. The effects of AAPs on

CNS histaminergic (H1), muscarinic (M1 and M3) receptors, and serotonergic (5HT1, 5HT2a, and 5HT2c) [18-20] lead to hyperphagia. In addition, AAPs have an impact on several pathways, such as the Endocannabinoid system (ECS) [21,22] and BDNF-mediated signaling [20,23,24], which contribute to hyperphagia [20], consequently leading to the development of metabolic dysfunctions.

### 1.7 Peripheral Mechanisms of AAPs-Induced Metabolic Adverse Effects

#### 1.7.1 The Overview of Peripheral Tissues and Insulin Resistance

Insulin, a hormone produced by the pancreatic β-cell, is important for glucose homeostasis [25]. Insulin resistance is a complex metabolic disorder featured with reduced sensitivity of peripheral tissues to the actions of insulin. Insulin resistance contributes significantly to the development of obesity, hyperinsulinemia, and type 2 diabetes [26]. Peripheral tissues, such as the liver, adipose tissues, and skeletal muscle, are crucial in the development of insulin resistance despite the involvement of multiple contributing factors.

Skeletal muscle, the largest organ in the body [27], is crucial for the clearance of glucose, and it accounts for more than 80% of the uptake of glucose in the postprandial state [28-31]. Due to the impaired insulin sensitivity and disrupted GLUT4 translocation [32-34], insulin's capacity is reduced to effectively stimulate glucose transport in skeletal muscle [34-38], ultimately leading to decreased glucose transportation and increased blood glucose levels. Moreover, studies indicate that in the condition of obesity, skeletal muscle experiences a heightened concentration of lipids that it struggles to efficiently oxidize. Consequently, muscle cell's lipid accumulations and intramuscular lipid (IMCL) buildup are believed to be associated with the onset of insulin resistance [39]. As a result, the activation of serine kinases can increase the serine phosphorylation of insulin receptor substrate 1 (IRS-1), thereby hindering insulin

signaling. [40]. Furthermore, the generation of reactive oxygen species (ROS) due to mitochondrial dysfunction is a mechanism by which oxidative stress in skeletal muscle contributes to insulin resistance [41].

The liver, a crucial organ in the body, is important in regulating the homeostasis of the metabolism, and it is central to insulin action [42]. All hormones released by the pancreas, including insulin and glucagon, undergo passage through the liver before entering the systemic circulation due to the direct drainage of pancreatic veins into the portal venous system [43]. Insulin boosts the utilization of glucose in the liver by expediting hepatic pathways responsible for glucose utilization, namely glycolysis and glycogenesis[42]; conversely, it inhibits the production of glucose by impeding pathways involved in glucose production, such as glycogenolysis and gluconeogenesis [42,44]. Approximately 70% of hepatic glucose output is derived from glycogenolysis, while the remaining 30% comes from gluconeogenesis [45]. However, in insulin resistance, the liver's responsiveness to insulin is impaired, leading to deceased glucose utilization and increased hepatic glucose production.

Adipose tissue also contributes to insulin resistance by releasing free fatty acids (FFAs) and adipokines. Increased concentrations of FFAs can hinder the responsiveness of insulin in specific body tissues like skeletal muscle, liver, and endothelial cells. [46]. The activation of different serine/threonine kinases by FFAs can interfere with insulin signaling by diminishing the tyrosine phosphorylation of insulin receptor substrate 1 or 2 (IRS 1/2) [46,47]. As a result, it could inhibit the IRS/PI3 kinase/Akt pathway, which is responsible for most of insulin's metabolic functions [46,48]. Furthermore, adipose tissue releases a range of bioactive substances called adipokines that exert a significant influence on insulin sensitivity [49].

Apart from peripheral tissues, insulin resistance is influenced by several factors,

including obesity, genetics, and medications. Obesity, one of the primary contributors of insulin resistance, triggers the release of inflammatory cytokines that impairs insulin signaling [50]. Genetics and family history can increase the likelihood of developing insulin resistance as well [51]. Furthermore, certain medications including steroids, HIV medications and blood pressure medications could further exacerbate the risk of insulin resistance; notably, atypical antipsychotics are known for inducing insulin resistance [52].

### 1.7.2 AAP Mediates IR in Skeletal Muscle

#### AAP Mediates IR in Skeletal Muscle Through Regulating Gene Expression

Previous studies have suggested that atypical antipsychotic medications may mediate insulin resistance (IR) in skeletal muscle through genetic alterations. AKT genes are known to play a crucial role in insulin signaling in skeletal muscle cells [53-55]. The scientists examined the methylation patterns specific to genes related to AKT in the promoters, using fasting skeletal muscle biopsies from individuals with bipolar disorder following treatment with AAPs [56]. The results revealed higher methylation levels in the AKT1 and AKT2 genes among AAP-treated patients [56]; thereby leading to impaired AKT functions since elevated methylation in gene promoters is associated with reduced gene expression and functionality [57,58]. The deficiency of AKT function has been proven to influence insulin functions as Akt knockout mice exhibit impaired insulin action in the adipose tissue, liver, and skeletal muscle [59]; plus, reduced Akt activities are observed in patients with type 2 diabetes [60]. Therefore, the increased methylation of AKT1 and AKT2 in patients treated with AAPs explains the observed reduction in insulin sensitivity in skeletal muscle linked to AAP treatment [61]. Furthermore, research in ICR mice has shown that quetiapine downregulates the expression of Pik3r1, which plays a key in regulating the insulin signaling pathway in the skeletal muscle of ICR mice [62]. These findings

collectively suggest that AAPs can interfere with insulin signaling in skeletal muscle, contributing to insulin resistance.

**AAP Mediates IR in Skeletal Muscle Through Altering Protein Actions**

Atypical antipsychotic medications (AAPs) have been implicated in mediating insulin resistance (IR) in skeletal muscle through insulin signaling alterations. Studies have demonstrated that treatment with AAPs, such as olanzapine and clozapine, can disrupt the insulin signaling cascade in skeletal muscle cells [63]. A study investigated rat L6 skeletal muscle cells treated with Olanzapine concluded the blocked phosphorylation of insulin-stimulated insulin receptor substrate-1 (IRS-1) [64], along with decreased phosphorylation of AKT and glycogen synthase kinase-3 (GSK-3α/β) [64], indicating impaired insulin signaling in L6 skeletal muscle cells [64]. Similarly, clozapine downregulated glucose uptake in L6 cells due to decreased IRS-1 tyrosine phosphorylation induced by insulin and lowered AKT $Ser^{473}$ phosphorylation [65], leading to the impairment of insulin signaling in L6 muscle cells [65].

Burghardt et al. conducted the first study exploring the abundance of skeletal muscle proteomics in patients undergoing AAP treatment [66]. Based on his findings, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation proteins, including YWHAQ, YWHAG, YWHAH, and YWHAZ, are upregulated due to AAPs, and they can exert effects on multiple insulin-related pathways in skeletal muscles [66]. First, the increasing of tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation proteins could lead to the activation of the P70S6K pathway [66]. P70S6K is a critical enzyme that plays an important role in the signaling cascade of insulin [67]; once activated, it leads to the downregulation of the insulin signaling pathway by causing serine phosphorylation of IRS-1[68-70]. Moreover, the elevation of tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation proteins influences the

insulin-like growth factor 1 (IGF-1) signaling[66], which results in the development of insulin resistance[71]. In addition, the heightened level of tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation proteins could alter the PI3K/AKT pathway [66]. Dysfunction of the PI3K/AKT pathway in different tissues of the body contributes to obesity and type 2 diabetes through insulin resistance. In a cyclical pattern, insulin resistance further aggravates the PI3K/AKT pathway, creating a detrimental loop [72].

The proteomics study also showed a decreased level of heat shock 70 kDa protein 6/7 (HSPA6/7) [66]. HSPA6/7 is a type of heat shock protein which has the potential to prevent insulin resistance in skeletal muscle [73,74]. As a result, the administration of AAP leads to a reduction in 70 kDa protein 6/7 (HSPA6/7), thereby diminishing the protective effect of skeletal muscle against insulin resistance.

**AAP Mediates IR in Skeletal Muscle Through Increasing Ceramides Level**

Prior to delving into the proteomic analysis, Burghardt et al. conducted a separate study in which they examined muscle biopsies from patients undergoing AAP treatment. This investigation aimed to assess changes in lipid content and their potential connections with insulin sensitivity. [56]. The study revealed elevated Ceramides (CERs) levels in the skeletal muscle of AAPs-treated patients, suggesting the rationale behind the development of insulin resistance. CERs, a type of sphingolipid that plays critical roles in cell signaling [75-78], are known for decreasing insulin sensitivity [79-82]. Due to the elevation of CERs after AAP treatment, insulin sensitivity could be impaired, thus leading to the development of insulin resistance in the skeletal muscle. Previous animal studies revealed that blocking ceramide synthesis could effectively improve insulin sensitivity [83].

The belief that elevated ceramides (CERs) lead to insulin resistance in skeletal muscle

due to their ability to activate multiple pathways associated with insulin actions as evidenced by multiple previous studies. First of all, ceramide acts as an agonist for MLK3, which is critical for the activation of c-Jun N-terminal kinase (JNK )triggered by TNF-alpha [84]. Therefore, increasing CERs could activate JNK [84,85], which is known for impairing the insulin signaling pathway [86-88]; ultimately, it develops insulin resistance in skeletal muscle. Protein kinase Cε, a member of the protein kinase C (PKC) family, exerts both transduction and modulatory effects on insulin action [89]. The heightened concentration of CERs could potentially restrict PKCε's capacity to create a signaling complex with Raf-1 and ERK [90]; as a result, the insulin signaling pathway is blunted in the skeletal muscle. The phosphoinositide 3-kinase (PI3K), critical in insulin signaling [91], could be antagonized by Ceramides [80]. Ceramides also inhibits the activation of protein kinase B (PKB) [92], an integral target of insulin signaling [93]. As for AAP treatment, these mechanisms exacerbate the insulin resistance in the skeletal muscle of patients.

**AAP Mediates IR in Skeletal Muscle Through Controlling Glucose Uptake**

The mechanism by which AAPs mediate insulin resistance in skeletal muscle appears to involve the regulation of glucose uptake, but the specific effects are complex and somewhat contradictory. For instance, in mouse C2C12 myoblasts, a study demonstrated that OLZ increased glucose uptake through the activating adenosine 5-monophosphate-activated protein kinase (AMPK) [94]. Conversely, some studies reported that OLZ exerts opposite inhibitory effects on glucose uptake in both C2C12 [95] and L6 muscle cells [65,96]. Interestingly, a similar study shows quetiapine treatment decreased insulin-stimulated glucose uptake in C2C12 myotubes as well [62]. In rat soleus skeletal muscle experiments, haloperidol, olanzapine, and clozapine did not exhibit any significant effects on glucose uptake [97], suggesting that the

impact of AAPs on glucose uptake might vary depending on the experimental model or cell type. Recent research suggests that AAPs might impede glucose uptake by disrupting serotonin signaling [98], which leads to GLUT4 translocation to the plasma membrane through serotonylation of Rab4, a small GTPase that becomes activated during the insulin-signaling pathway [99-101]. This disruption ultimately impacts the crucial process of GLUT4 translocation to the plasma membrane, which is essential for glucose uptake. OLZ, the most prescribed atypical antipsychotic, is presumed to be capable of directly interacting with GLUT4, thus disrupting glucose uptake [61]. This assumption arises from the finding that OLZ has the ability to bind to the same residue of Staphylococcus epidermidis glucose/H(+) symporter (GlcPSE) that is conserved in the glucose transporter as well [102].

Despite these diverse findings, the underlying mechanisms of how AAPs regulate glucose uptake in skeletal muscle are still debatable and require further investigation. Apart from the interference with serotonin signaling and direct binding to GLUT4, some researchers speculate that AMPK activation/expression via H1R blockade could directly influence GLUT4 translocation in skeletal muscle by AAPs [61]. However, AMPK activation is commonly believed to increase glucose uptake in skeletal muscle.

### 1.7.3 AAPs Mediate IR in Liver

It is crucial to elucidate the peripheral mechanisms through which AAPs induce insulin resistance in the liver. AAPs trigger metabolic dysfunction through hepatic glycogen disruption and impairment of the gluconeogenesis pathway.

**Evidence of Impaired Hepatic Insulin Resistance**

Numerous previous animal studies have been conducted to delve into the intricate effects of AAPs on insulin resistance in the liver. Notably, these investigations consistently reveal compelling evidence of disrupted glucose metabolism in rats following treatments with AAPs such as olanzapine or clozapine. These medications have been found to significantly reduce the glucose infusion rates in the treated animals [103-105], indicating the development of insulin resistance. Furthermore, elevated hepatic glucose output has been consistently observed in treated animals[103-105], further corroborating the impairment of insulin sensitivity and providing evidence of heightened gluconeogenesis and glycogenolysis in response to AAP administration. Interestingly, these effects persist across various experimental conditions, encompassing diverse administration routes, different dosages, and varying treatment durations. These collective findings not only present evidence of the insulin resistance induced by AAPs in the liver but also underscore the clinical significance of understanding the intricate molecular mechanisms driving these metabolic changes.

**AAPs and Hepatic Glycogen Disruption**

The liver plays a crucial role in maintaining glucose homeostasis by storing glucose in the form of glycogen, ensuring a steady supply of energy during periods of fasting or increased energy demands. Glycogen synthesis in hepatocytes, the primary liver cells, is tightly regulated by the enzymes glucokinase and glycogen phosphorylase, which govern the conversion of

glucose to glycogen and its subsequent breakdown into glucose. Additionally, serotonin, a neurotransmitter, influences hepatic glycogen synthesis with both stimulatory and inhibitory effects. However, the stimulatory actions appear to dominate, as shown in elegant studies by Tudhope, who demonstrated that the double 5-HT1/5-HT2 agonist α-methyl-5HT increased glycogen synthesis in rat hepatocytes [106]. Remarkably, this effect was diminished when hepatocytes were co-treated with α-methyl-5HT and OLZ [106]. Consequently, it appears that olanzapine hampers glycogen synthesis in hepatocytes, which might be a contributing factor to the hyperglycemia observed in individuals treated with atypical antipsychotic drugs. The underlying mechanism involves the inhibition of glycogen phosphorylase by α-methyl-5HT, a process pivotal for stimulating glycogen synthesis. AAPs such as CLZ and OLZ counteract this inactivation, impairing the normal glycogen synthesis cascade [107].

**Impairment of Gluconeogenesis Pathway**

Numerous studies have unveiled the impact of AAP treatments on the gluconeogenesis pathway in the liver, a critical process involved in glucose homeostasis. Studies conducted in both mice and rats have consistently revealed significant upregulations in key enzymes governing gluconeogenesis, namely glucose-6-phosphatase (G6pc) [108] and phosphoenolpyruvate carboxykinase (PEPCK) in the liver [95], upon administrations of common AAPs such as OLZ and CLZ, indicating that these medications may stimulate hepatic glucose production through enhanced gluconeogenesis. By promoting gluconeogenesis, AAPs disrupt the balance of glucose homeostasis in the liver, which leads to the development of hyperglycemia and insulin resistance. The increased hepatic glucose production exacerbates insulin resistance by overwhelming the body's ability to utilize glucose effectively, further contributing to dysregulated glucose metabolism. These findings collectively suggest that the intricate molecular

mechanisms by which AAPs meddle with glucose metabolism in the liver.

**Activation of AMPK and mTORC1 Signaling**

Long-term administration of OLZ induced a state of anaerobic glycolysis, causing an exhaustion of hepatic glycogen reserves, thus affecting glucose storage and availability. Meanwhile, this treatment also led to the activation of two key signaling pathways, AMPK, and subsequent mammalian target of rapamycin complex 1 (mTORC1), due to elevated levels of glutamate [109]. These findings shed light on the involvement of these pathways in contributing to insulin resistance caused by AAPs through peripheral mechanisms within the liver.

**Impaired Hepatic Insulin Signaling**

A growing number of studies have demonstrated the intricate impact of AAPs on hepatic insulin signaling, suggesting potential mechanisms underlying the metabolic dysregulation observed in patients undergoing AAP treatment. Notably, studies have consistently shown that AAPs induce impairments in the phosphorylation of critical downstream targets of insulin signaling, namely AKT (protein kinase B) and GSK3β (glycogen synthase kinase 3β) [110-112], within the liver. These reductions in phosphorylation levels are indicative of compromised insulin signaling pathways in liver, potentially contributing to the development of insulin resistance and glucose intolerance.

**Induced Oxidative Stress In the Liver**

AAPs can induce insulin resistance through peripheral mechanisms in the liver via oxidative stress, indicating a significant concern in their clinical use. Recent studies have shed light on the potential role of oxidative stress as a key contributor to insulin resistance induced by APDs, particularly in the liver.

An earlier investigation revealed that CLZ exhibits the most significant influence on

antioxidant enzyme function[113], aligning with its metabolic pathway predominantly mediated by cytochrome P450 (CYP) enzymes in the liver[114]. This metabolic process generates reactive oxygen species (ROS), including superoxide anion radical ($O^{2-}$) and hydrogen peroxide ($H_2O_2$), thereby contributing to oxidative stress [115]. Besides, CLZ treatment has been associated with an increase in superoxide dismutase (SOD) activity, indicating elevated $O_2^-$ production, along with elevated levels of glutathione peroxidase (GPx) activity and protein, likely in response to heightened $H_2O_2$ levels [113]. Besides CLZ, other AAPs, such as sertindole and ziprasidone, have been found to reduce catalase (CAT) activity, leading to $H_2O_2$ accumulation and exacerbation of oxidative stress in the liver [113]. The oxidative stress in the liver induced by AAPs contributes to insulin resistance by disrupting hepatic insulin signaling pathways [116], impairing insulin secretion [117], and promoting the development of metabolic disorders such as NAFLD [118].

### 1.7.4 AAPs Mediate IR in Adipose Tissue

#### Adipose Tissue Acts as a Target For AAP-Induced Insulin Resistance

In humans, adipose tissue is categorized into two main types: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT functions to store energy, whereas BAT converts WAT into heat [119]. Adipose tissue represents one of the crucial metabolic and endocrine organs in the human body. The development of insulin resistance induced by AAPs in adipose tissue is a complex and multifaceted process.

#### AAPs Directly Affect Insulin-Related Pathways in WAT

Classically, WAT has been acknowledged as playing a role in disposing of 5–15% of an oral glucose load [120], which could be associated with the development of insulin resistance induced by AAPs. AAPs can directly impact insulin action by affecting cellular functions within white adipocytes. For instance, Helliner et al. discovered that clozapine, risperidone, and olanzapine (at 5 mM concentration) could significantly reduce the maximal insulin-stimulated glucose transport rate by around 40-70% in adipocytes [121]. Additionally, Milica et al. found that aripiprazole (1–2 μM concentration) decreases glucose uptake in adipocytes [122]. Even at supra-therapeutic concentrations of aripiprazole (100 μM), basal and insulin-stimulated glucose uptake was reduced after pre-incubation in isolated abdominal subcutaneous adipocytes from humans [123]. However, in another study, Robinson et al. demonstrated that lower concentrations of olanzapine (0.07–0.35 μM) did not affect glucose transport in adipocytes [124]. The discrepancies between these findings may be attributed to differences in drug dosages. Importantly, conventional antipsychotic drugs (such as butyrophenone and trifluoperazine) did not impact glucose transport [121], which could help explain why TAPs are associated with a

lower risk of weight gain, obesity, insulin resistance, and diabetes in patients.

**AAPs Promote WAT Accumulation**

In addition to their direct effects, AAPs can indirectly contribute to insulin resistance through their influence on WAT. Multiple studies have consistently reported that AAP-induced insulin resistance is associated with increased weight gain, higher BMI, and the accumulation of intra-abdominal adiposity, especially in individuals undergoing prolonged AAP treatment [125]. At the molecular level, AAPs promote fat accumulation via stimulating preadipocyte differentiation, facilitating lipid storage, and reducing lipolysis.

Adipose tissue expansion can occur through an increase in the number of adipocytes (hyperplasia) and/or an increase in the size of individual adipocytes (hypertrophy) [126]. New adipocytes are generated through the recruitment and differentiation of precursor cells known as preadipocytes located within adipose tissue. This process is referred to as adipocyte differentiation or adipogenesis [127].

Previous research has shown that olanzapine enhances adipocyte differentiation and increases triglyceride accumulation during in vitro differentiation in both human and rodent precursor cells [128-131]. For example, human preadipocytes treated with clozapine (5 μM) exhibited a significantly higher percentage of differentiated cells than untreated controls [132]. Another study by Anitta et al. found that the expression levels of pro-adipogenic genes such as peroxisome proliferator-activated receptors (PPAR) -γ, PPAR-α, CCAAT/Enhancer Binding Protein (C/EBP-α) were increased after treatment with AAPs [133]. Additionally, there were increased expression levels of markers of adipogenic differentiation, such as fatty acid binding protein 4 (FABP4), as well as metabolic enzymes like lipoprotein lipase (LPL), which plays a role in transferring fatty acids from blood triacylglycerol to the triacylglycerol stores within

adipocytes, and acyl-CoA synthetase-1 (ACSL1) [133]. Sterol Regulatory Element-Binding Proteins (SREBPs) are known to regulate multiple genes involved in cholesterol synthesis and lipogenesis. Olanzapine has been shown to stimulate the expression of SREBP-1 in 3T3-L1 preadipocytes, subsequently upregulating its downstream genes involved in lipogenesis [131,133]. Clozapine has also been found to enhance the expression of ADD1/SREBP(1C) mRNA and increase triglyceride concentration in differentiated adipocytes [134]. These findings suggest that AAPs can influence adipocyte differentiation and metabolism, contributing to adipose tissue expansion and subsequent insulin resistance.

Moreover, adipocytes from rats chronically treated with olanzapine displayed decreased lipolytic activity, reduced hormone-sensitive lipase (HSL) expression, and increased fatty acid synthase (FAS) expression [135]. These changes were associated with increased fat deposition and larger adipocyte size. Rates of lipolysis in response to isoproterenol were reduced by several AAPs, including risperidone, clozapine, olanzapine, and quetiapine [121]. Acute treatment with therapeutic concentrations of olanzapine decreased basal lipolysis in isolated abdominal subcutaneous adipocytes from humans [123].

Additionally, research involving abdominal subcutaneous adipose tissue biopsies from healthy subjects revealed that isolated adipocytes or adipose tissue, when pre-incubated with AAPs, reduced gene expression related to mitochondrial function regulation, such as PPAR-γ Coactivator 1 Alpha (PPARGC1A), Pyruvate dehydrogenase kinase 4 (PDK4), and Carnitine Palmitoyl Transferase 1B (CPT1B), which are involved in mitochondrial function regulation [123]. The downregulation of these genes suggests that AAPs may negatively influence mitochondrial function in adipose tissue. Mitochondria are vital organelles responsible for energy production and metabolism within cells. Any disruption in mitochondrial function can

have profound effects on cellular energy balance and metabolism, potentially contributing to decreased β-oxidation and ATP production and increased ROS production, resulting in insulin resistance [136].

**AAPs Promote Adipose Secretion Thus Leading to Systemic Insulin Resistance**

AAPs can also trigger adipose tissue secretion, thereby inducing dyslipidemia and leading to elevated levels of proinflammatory cytokines within the circulatory system. Clozapine significantly inhibited the expression of both LPL mRNA and LPL protein in a dose-dependent and time-dependent manner [134]. The downregulation of LPL leads to the accumulation of triglycerides in the plasma, which subsequently contributes to insulin resistance [137]. Our previous longitudinal study suggested a positive correlation between alterations in plasma triglycerides and shifts in plasma macrophage migration inhibitory factor (MIF) levels following two months of olanzapine monotherapy [138]. Recent findings also indicated that MIF directly inhibits the expression and activity of LPL within murine adipose tissue, thereby impairing LPL's capacity to degrade plasma triglycerides, ultimately resulting in hypertriglyceridemia [139].

Notably, Anitta et al. observed an upregulation of the transcription factor Nuclear Factor Kappa B Subunit 1 (NF-KB1) and NF-KB1 target genes in adipocytes in response to these drugs, including the proinflammatory cytokines Tumor necrosis factor (TNF) -α, Interleukin (IL) -1β, IL-8 and Membrane cofactor protein (MCP) -1 [133]. Both olanzapine and aripiprazole, when administered at supra-therapeutic concentrations, exhibited downregulation of pro-inflammatory cytokines IL6 and IL1B genes after 72 hours of incubation in human abdominal subcutaneous adipocytes [123]. These findings provide additional evidence that AAP treatments alter the gene expression patterns in adipocytes, predisposing them to a state of low-level inflammation.

In our prior study, we established the involvement of MIF in the development of insulin resistance and hyperinsulinemia induced by olanzapine in individuals with schizophrenia [138]. Olanzapine was found to amplify *MIF* expression in the WAT, with the latter contributing to elevated plasma MIF levels in animal models. Elevated levels of circulating MIF were subsequently linked to the induction of insulin resistance within adipose tissue. This detrimental effect was mediated by suppressing the tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) [140] and disrupting the signaling pathway responsible for glucose metabolism [141]. Our findings concerning the intriguing relationship between MIF and olanzapine have given rise to a hypothesis proposing that plasma MIF levels may potentially govern the insulin resistance triggered by AAPs.

**AAPs regulate BAT metabolism**

While the dysfunction of WAT has been suggested as the primary contributor in the short term, reduced energy expenditure, particularly in thermogenesis and locomotor activity, has been implicated in insulin resistance during long-term AAP treatments. Promoting brown adipocyte differentiation has been shown to augment energy expenditure and reduce weight gain [142], whereas diminished brown adipocyte differentiation is associated with insulin resistance in humans [143]. Clozapine reversed BAT thermogenesis in rabbits [144]. In the mouse brown preadipocyte cell line, clozapine significantly down-regulated key brown adipogenesis markers, including PR domain containing 16 (PRDM16), C/EBPβ, PPARγ2, Uncoupling Protein (UCP) -1, Peroxisome proliferator-activated receptor-γ coactivator (PGC) -1α, and Cell Death Inducing DFFA Like Effector A (CIDEA) [145]. Clozapine also notably inhibited the mRNA expression of lipogenic genes Acetyl-CoA carboxylase (ACC), Stearoyl-CoA Desaturase 1 (SCD1), GLUT4, Apetala 2 (AP2), CD36, as well as adipokines such as resistin, leptin, and adiponectin

in BAT [145]. These results strongly indicate that AAP impedes brown adipocyte differentiation. The reduction of BAT exacerbates hypertriglyceridemia and obesity [146]. Serum hypertriglyceridemia, along with the subsequent deposition in WAT and ectopic storage in skeletal muscle and the liver, leads to decreased insulin sensitivity in these organs [147].

In established female rat models, Long-term olanzapine treatment led to a significant reduction in BAT temperature. This reduced BAT temperature was accompanied by decreased expressions of UCP1 and PGC-1α in BAT [148,149]. The decrease in BAT temperature induced by olanzapine also persisted along with reduced physical activity [149]. In pregnant rodents, olanzapine treatment induced alterations in WAT and BAT morphology in both poly I: C and saline-exposed offspring. This resulted in lipid accumulation, weight gain, and reduced expression of the BAT-specific marker molecule, UCP1 protein/gene [150]. Concurrently, olanzapine inhibited the directional differentiation and mitochondrial activity of C3H10T1/2 brown-like adipocytes [150]. These findings suggest that AAPs significantly impact the function of BAT, consequently contributing to the development of insulin resistance.

### 1.8 MIF and AAPs-induced metabolic adverse effects

Common metabolic abnormalities are intricately linked to adipose tissue dysfunction, leading to the secretion of adipokines, which serve as key contributors to the onset of insulin resistance, obesity, and hyperlipidemia. Therefore, elevated levels of circulating adipokines represent important indices of metabolic dysfunction and need to be fully elucidated.

The classical inflammatory cytokine, macrophage migration inhibitory factor (MIF) [151], is also recognized as an adipokine [152]. Obese individuals often display higher plasma MIF levels compared to their lean counterparts [153], suggesting a strong correlation between MIF and obesity. Plasma MIF levels positively correlate with insulin resistance in patients with type 2 diabetes (T2D) [154,155]. Additionally, they also play a contributory role in the development of non-inflammatory insulin resistance in obese animal models [156]. Furthermore, increased circulating MIF levels play a role in inducing hypertriglyceridemia by downregulating lipoprotein lipase (LPL) in adipose tissue [139]. Therefore, elevated plasma MIF levels serve as a significant indicator for monitoring the progression of metabolic dysfunction.

## Chapter II: Our project

### 2.1 Project Overview

Previous studies have established a potential relationship between MIF and severe mental disorders, such as schizophrenia (SZ) and bipolar disorders (BD). The *MIF* gene polymorphism has been linked to the onset and risk of SZ [157], as well as to attempted suicide in BD [158]. In Japanese patients with SZ, serum MIF level is higher compared to healthy controls, and it may be correlated with antipsychotic therapy [159,160]. We previously found that administering olanzapine (OLZ), a common AAP, as monotherapy over two months led to increased circulating MIF levels in first-episode SZ patients, and this rise was positively associated with elevated body weight gain, insulin resistance, and hyperlipidemia [161]. Our animal study revealed that the increase in circulating MIF levels primarily comes from MIF expression and release in adipocytes following OLZ administration [161]. Therefore, *Mif* knockout mice were able to mitigate OLZ-induced metabolic dysfunction [161]. This collective evidence suggests that MIF may serve as a crucial regulator for adverse metabolic effects induced by OLZ.

It should be noted that adverse metabolic effects are a common occurrence across various AAP therapies. Whether MIF plays a role in other AAP-induced metabolic abnormalities and whether it could serve as a unique marker for AAP-induced adverse metabolic effects remains to be elucidated. Thus, our current study enrolled healthy controls and patients with SZ who were receiving various antipsychotic monotherapies, including one typical and five atypical antipsychotics, respectively. We were investigating the correlation between MIF levels and metabolic side effects associated with antipsychotic treatments.

In summary, while the journey of antipsychotic treatment has come a long way, there is still much to be learned and accomplished in our quest to optimize patient outcomes. This thesis represents a step towards that goal, offering valuable insights into the peripheral mechanisms of atypical antipsychotics-induced insulin resistance and paving the way for future advancements in psychiatric pharmacotherapy based on our clinical pharmacological study.

## 2.2 MATERIALS AND METHODS

### 2.2.1 Study Design and Participants

The cross-sectional investigation comprised Han Chinese individuals for healthy controls and SZ patients. A total of 142 healthy subjects were recruited locally through advertisements. Chronic SZ patients were recruited from various healthcare centers, including the Shanghai Mental Health Center, Shanghai Civil Affairs First Mental Health Center, Cixi Mental Health Center, Quzhou Third People's Hospital, and Huzhou Third People's Hospital during the period spanning from January 2017 to January 2020. The Inclusion criteria for SZ patients included: (1) age eligibility was set between 18 and 65 years; (2) SZ diagnosis was made according to the Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM-5) by at least two separate psychiatrists*;* (3) SZ patients must have received at least six months of antipsychotic treatment. The exclusion criteria for SZ patients included (1) the presence of cerebral vascular disease, central nervous system diseases, severe physical diseases, and substance abuse/dependence; (2) genetic syndromes linked to obesity or other chronic conditions were grounds for exclusion; (3) individuals using medications for the treatment of insulin resistance or T2D were ineligible; or (4) pregnant or lactating women were also excluded. A total of 388 SZ patients met the aforementioned criteria and were ultimately included in the statistical analysis.

The authors affirm that all procedures in this study adhere to the ethical standards set by the relevant national and institutional committees on human experimentation and comply with the Helsinki Declaration of 1975, as revised in 2008. All procedures involving human subjects were approved by the Institutional Review Boards of the Shanghai Mental Health Center (2019-35R).

All potential participants were provided with comprehensive written information about the project.

### 2.2.2 Sample size calculation

Sample size calculation was performed using GPower v.3.1 9.7. The evaluation employed the analysis of variance (ANOVA) statistical method for 7 groups, expecting an effect size of 0.4 [162], with an alpha error of 0.05 and a power of 80%. Given equal sample sizes across the 7 groups, the minimum required sample size per group was determined to be 14 cases. Considering a dropout rate of 20%, each of the 7 groups was expanded to include 18 cases, resulting in a total sample size of 126 cases. This ensures the accuracy and scientific rigor of the study findings.

### 2.2.3 Collection of Data and Blood Samples

All measurements were performed by trained research nurses. All participants underwent waist circumference (WC) and hip circumference (HC) measurements. The Waist-hip ratio (WHR) was calculated by dividing WC by HC. Blood samples were collected between 7:00 and 9:00 AM following a 12–14 h fasting period. These collected blood serum samples were used to obtain the insulin, fasting glucose (Fpg), hemoglobin A1c (HbA1c), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG), Apolipoprotein A1 (ApoA1), Apolipoprotein B (ApoB), and the concentration of MIF. These metabolic parameters were detected by the Department of Clinical Laboratory, the Shanghai Mental Health Center. The levels of MIF were determined by ELISA using a commercially available kit (SEA698Hu, USCN Life, Wuhan, China), with a standard curve generated using recombinant human MIF. The mean intra- and inter-assay coefficient of variation values were 4.6% and 7.8%, respectively. The lower limit of detection was 0.122ng/ml. The HOMA-IR index was calculated using the formula: HOMA-IR = [Insulin μU/mL] x [Glucose mM]/22.5.

### 2.2.4 Clinical assessment

Clinical interviews with the participants were conducted in a structured format. Trained clinicians with experience in the protocol conducted these interviews. Socio-demographic information and clinical characteristics, including gender, age, education, age of onset, duration of illness, antipsychotic use, and other medications, were collected using a questionnaire. Clinical symptoms were assessed using the Positive and Negative Syndrome Scale (PANSS), and the inter-rater correlation coefficient of assessments exceeded 0.8.

### 2.2.5 Statistical analysis

Statistical analyses were conducted using GraphPad Prism version 8.0 (GraphPad Software, San Diego, USA) and SPSS 16.0 (SPSS, Chicago, USA). Data underwent normal distribution testing for continuous variables using the Kolmogorov-Smirnov one-sample test. Continuous variables were presented as means with standard deviations or medians with interquartile ranges, depending on their distribution. Parametrically distributed variables were analyzed using ANOVA, while non-parametric variables were assessed using the Kruskal-Wallis test. Gender variables were analyzed with the chi-square ($\chi 2$) test.

To compare the differences in plasma MIF levels and metabolic markers between groups, we conducted a multi-factor ANOVA. Post-hoc analyses (if applicable) were further conducted to explore pairwise differences between groups. Multiple linear regression was employed to determine correlations between MIF and other indices. In all these analyses, we incorporated relevant variables for adjustment, including age, gender, WHR, and PANSS scores. The hypothesis test was two-sided with a 5% level of statistical significance, and a 95% confidence interval was

applied.

## 2.3 RESULTS

### 2.3.1 Subject background characteristics

Among the SZ patients, 32 received monotherapy with chlorpromazine (CPZ), a typical antipsychotic (TAP), while the remaining patients obtained various AAP monotherapies over the past six months. Specifically, 114 patients were treated with OLZ, 77 patients received CLZ, and 41, 93 and 31 patients were treated with aripiprazole (APZ), risperidone (RIS), and quetiapine (QTP), respectively. A comprehensive overview of the baseline background characteristics for each group is shown in Table 1. The analysis revealed significant differences among the groups in terms of sex, age, education, and duration of illness ($p < 0.05$), while no significant difference was observed regarding the age of onset ($p = 0.838$). Although the total PANSS score varied among different patient groups, the negative score did not exhibit statistical significance ($p = 0.789$).

### 2.3.2 AAPs specifically upregulate plasma MIF levels in SZ patients

We examined plasma MIF levels in both healthy controls and SZ patients undergoing various antipsychotic monotherapies (Figure 1). CPZ did not change plasma MIF levels ($p=0.244$). However, all AAPs upregulate plasma MIF levels among SZ patients compared to healthy controls and patients receiving TAP treatment ($p < 0.05$). There were no significant differences in the degree of MIF elevation within the AAP groups.

### 2.3.3 MIF and waist-hip ratio (WHR) following AAP therapies

The waist-hip ratio (WHR) serves as a vital indicator for assessing abdominal obesity. WHR was significantly increased among all patients receiving AAPs compared to the healthy controls (Table S1). Furthermore, given the established association between MIF and the

development of abdominal adiposity [163], our multiple linear regression analysis revealed a positive correlation between plasma MIF levels and WHR ($\beta = 0.001$, $p = 0.011$).

### 2.3.4 MIF and AAP-induced insulin resistance

AAPs resulted in elevated HOMA-IR scores compared to healthy controls (Table S2). Our correlation analysis within the patient groups receiving AAPs (Table 2) unveiled a positive association between plasma MIF concentration and both HOMA-IR scores ($\beta = 0.024$, $p = 0.020$) and insulin levels ($\beta = 0.903$, $p = 0.001$). However, no statistically significant associations were found between MIF and Fpg ($p = 0.977$) or HbA1c ($p = 0.725$).

### 2.3.5 MIF is correlated with plasma triglyceride levels

Lipid metabolism indices, including TC, LDL, HDL, TG, ApoA1, and ApoB, were evaluated across seven groups (Table S3). The administration of AAPs did not exert an influence on TC levels but significantly decreased HDL levels in all groups. Specifically, OLZ and CLZ elevated LDL levels, while CLZ and APZ increased TG levels in comparison to healthy individuals (Table S3). Moreover, CLZ treatment led to increased levels of ApoB. These findings suggest that CLZ therapy induces more pronounced hyperlipidemia when compared to other AAPs. A positive correlation was identified solely between plasma MIF concentration with TC ($\beta = 0.012$, $p = 0.038$) and TG values ($\beta = 0.019$, $p = 0.001$) (Table 3).

### 2.3.6 MIF and TAP-induced metabolic alterations

Patients receiving the TAP treatment of CPZ did not exhibit alterations in their plasma MIF levels. However, they did experience elevated HOMA-IR, Fpg, and HbA1c levels when compared to healthy controls (Table S2). In contrast to the group receiving AAP treatment, these CPZ-treated patients did not manifest hyperinsulinemia, and correlation analysis also revealed no

significant relationship between MIF and glucose metabolism indices (all $p > 0.05$, Table S4). Furthermore, CPZ regulated lipid metabolism, characterized by decreased HDL levels ($p < 0.001$) and increased ApoA1 ($p < 0.001$) and ApoB levels ($p = 0.014$) (Table S3). Importantly, there was also no observed correlation between lipid metabolism indices and plasma MIF (all $p > 0.05$, Table S4).

## 2.4 DISCUSSION

While traditionally categorized as a cytokine, MIF has garnered increasing attention for its pivotal role in driving the progression of metabolic dysfunction [164]. We revealed an important association between the usage of AAPs and the upregulation of MIF levels in SZ patients. The presence of metabolic dysfunction is accompanied by increased circulating MIF levels in SZ patients undergoing AAP treatments. Notably, the heightened MIF levels are closely linked to insulin resistance, primarily relying on increased blood insulin levels rather than glucose. Furthermore, plasma MIF levels are associated with plasma TC and TG levels following AAP treatments. In contrast, despite the presence of adverse metabolic effects induced by TAP treatment, the plasma MIF level remains unchanged. Therefore, we propose that MIF could serve as a distinctive marker to monitor adverse metabolic effects induced by AAPs in clinical settings.

Adipokines are cell-signaling molecules derived from adipose tissue, responsible for regulating the body's metabolic state. Alterations in plasma adipokine levels frequently coincide with AAP-induced metabolic dysfunction, although the precise nature of this relationship remains unclear. SZ patients often exhibit higher levels of leptin compared to healthy controls, and these elevated levels can persist throughout antipsychotic therapy [165,166]. While OLZ, CLZ, and QTP are known to significantly increase leptin levels, it remains uncertain whether their influence on leptin levels is direct or mediated indirectly through effects on obesity and subsequent leptin resistance [165]. AAPs have also been observed to decrease adiponectin levels [167] and promote ghrelin secretion [168]. However, whether these alterations depend on adiposity and BMI remains uncertain. Unlike leptin, adiponectin, and ghrelin, MIF is directly upregulated in mouse adipose tissue and adipocytes by OLZ [161], and the upregulation of adipose MIF leads to elevated circulating MIF levels, which in turn contributes to the development of metabolic abnormalities

[161]. Indeed, OLZ monotherapy in SZ patients for two months also elevated plasma MIF levels, thereby contributing to insulin resistance, obesity, and hyperlipidemia [161]. Our findings regarding the interesting correlation between MIF and OLZ have prompted a hypothesis suggesting that plasma MIF levels could potentially influence the metabolic side effects induced by all major AAPs. However, this hypothesis remained unexplored until our current investigation. Our current study explores the involvement of MIF in the development of obesity, insulin resistance, and hyperlipidemia caused by other AAPs, including CLZ, APZ, RIS, and QTP, which are commonly prescribed in clinical settings.

The chronic therapy of AAPs is commonly associated with the development of obesity. In animal models, MIF plays a crucial role in regulating lipid storage in adipocytes, which leads to adipocyte hypertrophy and increased adiposity following high-fat diet feeding [169]. Our present study indicates a positive correlation between plasma MIF levels and AAP-induced enlargement of WHR, suggesting a potential role of MIF in accelerating the development of AAP-induced central obesity. Previously, our animal study also showed a novel molecular mechanism of MIF in modulating hyperphagia through an AMPK/neuropeptide, AgRP signaling pathway [161]. The extent to which elevated peripheral MIF can access the hypothalamus and regulate appetite in SZ patients undergoing AAP treatment remains largely unknown.

Plasma MIF levels are frequently associated with insulin resistance [154,155]. Our current study suggests a positive association between elevated plasma MIF levels and hyperinsulinemia following AAP treatment. OLZ amplified *Mif* expression in both the hypothalamus and adipose tissue, with the latter significantly contributing to the increase in plasma MIF levels in animal models [161]. Subsequently, the increased levels of circulating MIF were associated with the presence of insulin resistance within adipose tissue, facilitated by the inhibition of tyrosine

phosphorylation of insulin receptor substrate-1 (IRS-1) [140] and the disturbance of the signaling pathway responsible for glucose metabolism [140]. Interestingly, in our present study, we did not conclude any correlation between MIF and plasma glucose or HbA1c levels, despite a previous study suggesting a stepwise increase in serum MIF associated with the progression from impaired glucose tolerance (IGT) to type 2 diabetes (T2D) [170]. Two potential rationales may explain the phenomenon. First, the progression of insulin resistance stages has not reached a point where significant changes in glucose levels occur. Second, MIF has been reported to stimulate insulin release from beta cells [140,171]. It is currently unknown whether this effect contributes to the balance of dysfunctional glucose metabolism.

Moreover, our research revealed the presence of lipid abnormalities induced by AAPs, with high plasma MIF levels showing a specific correlation with plasma TG and TC values. Our previous longitudinal study suggested a positive correlation between changes in plasma TG and alterations in plasma MIF levels following two months of OLZ monotherapy [161]. Recent findings also indicated that MIF directly inhibits the expression and activity of lipoprotein lipase (LPL) in mouse adipose tissue [139], thereby impairing LPL's ability to break down plasma TG, resulting in hypertriglyceridemia. Further investigation is needed to determine whether LPL is involved in MIF-regulated AAP-induced hypertriglyceridemia in patients with SZ. This can be achieved by conducting a study where LPL is released using heparin, as described in previous studies [172].

Interestingly, elevated plasma MIF levels exhibit a positive correlation with TC rather than LDL. TC encompasses various cholesterol types carried by lipoprotein particles, such as LDL, HDL, intermediate-density lipoproteins (IDL), and very low-density lipoprotein (VLDL) [173]. While LDL accounts for 60% of total cholesterol in the body [174], other types of cholesterol also

play important roles in metabolism. Previous studies have suggested that diabetic patients often exhibit low LDL/high remnant cholesterol (RC) profiles rather than high LDL/low RC profiles [175], with this phenomenon being more pronounced in female patients [175]. Thus, our discovery regarding the association between MIF and TC may imply a potential role of MIF in regulating non-LDL cholesterols.

While the prevailing perspective suggests that TAPs are less likely to induce metabolic abnormalities than AAPs, our research and findings from other research groups [176,177] have demonstrated metabolic dysfunction induced by TAPs. In a cohort of SZ patients receiving CPZ monotherapy for over a decade, the prevalence of metabolic syndromes was found to be 31% [178]. In our current research, we did observe the occurrence of metabolic dysfunction in the CPZ group, characterized by insulin resistance and alterations in plasma levels of HDL, ApoA1, and ApoB levels. CPZ-induced insulin resistance appeared to be associated with increased plasma glucose levels rather than insulin levels, in contrast to the insulin resistance induced by MIF. In addition, CPZ did not elevate plasma MIF levels compared to those of healthy controls, although the sample size is small. The unchanged MIF levels in the CPZ group suggest that TAP-induced metabolic dysfunction is probably more predominantly associated with an MIF-independent mechanism. It should be noted that MIF has the capability to metabolize dopaminechrome into 3,4-dihydroxyphenylacetone [179], thereby alleviating extrapyramidal symptoms. Typically, the oxidation of dopamine leads to the formation of toxic dopaminechrome, a precursor of neuromelanin, which accumulates in neurons and astrocytes, resulting in extrapyramidal symptoms [180]. Thus, the MIF-independent mechanism in TAPs could partially explain their extrapyramidal side effects.

Although our findings present pioneering evidence supporting the link between plasma

MIF levels and AAPs-induced metabolic disturbances, our study design does come with several limitations. Firstly, our research design was cross-sectional, restricting our ability to establish the alterations between MIF levels and all the metabolic indexes before and after the monotherapy. The other limitation of our study was the medication dose was not factored into our statistical analysis, potentially influencing metabolic measures. Future studies employing longitudinal designs and incorporating baseline measurements would provide a more comprehensive understanding of the relationship between MIF and AAP-induced metabolic dysfunctions. Furthermore, all participants in our study were Han Chinese. There are 56 ethnic groups in China, with Han accounting for 91.51% and various ethnic minorities accounting for 8.49% [181]. Given that Han Chinese make up the majority, our sample adequately represents the Chinese and Eastern Asian populations. However, to validate and extend our findings, further exploration of diverse populations worldwide is necessary in the future.

In conclusion, we have identified MIF as a potential clinical marker for recognizing patients at risk of developing metabolic dysfunction induced by AAPs. Our study suggests that patients with elevated MIF levels may require more frequent monitoring to detect early signs of AAP-induced metabolic dysfunction. In addition, exploring interventions targeting MIF or its associated signaling pathways may be a possible strategy to prevent AAP-induced adverse metabolic effects and improve their therapeutic effects in clinical settings.

## Chapter III: Future Direction

### 3.1 Future Project Overview

Building on our findings that link MIF to metabolic dysfunction in the context of AAP therapy, our next phase of research will explore the metabolic impacts of CLZ and RIS both as monotherapies and in combination. Our works have established MIF as a crucial inflammatory cytokine and a vital metabolic indicator, especially in adverse metabolic effects of AAP-induced metabolic. This foundation supports our pursuit of a deeper understanding of the mechanisms by which AAPs influence metabolism.

### 3.2 Aims and Hypotheses of The Future Project

We aim to determine whether combined AAP therapies result in more pronounced metabolic disturbances than monotherapies and whether MIF levels mediate these effects. We hypothesize that Patients on combined AAP therapies will exhibit higher plasma MIF levels than those on monotherapies. There will be a strong association between elevated MIF levels and the severity of metabolic dysfunction in patients on combined therapies.

### 3.3 Methodological Approaches of The Future Project

We plan to recruit Chinese patients diagnosed with SZ, dividing them into three treatment groups: those on CLZ only, those on RIS only, and those on a combination of both drugs. We will measure plasma levels of MIF along with other metabolic indicators such as body mass index (BMI), lipid profiles, and glucose tolerance at baseline and intervals following treatment onset. Statistical analyses will be employed to assess differences in MIF levels and metabolic outcomes across the three groups and to explore correlations between these variables.

### 3.4 Potential Impact of The Future Project

This study aims to enhance our understanding of AAP-related metabolic pathways, especially under combined therapy conditions. Identifying a mechanistic link between MIF levels and metabolic side effects could lead to targeted interventions that mitigate these effects while optimizing psychiatric outcomes. Such advancements could significantly improve patient management and support the development of precision medicine approaches in psychiatric care.

### 3.5 Long-Term Goals

Beyond this study, we envision further research into MIF as a potential therapeutic target to reduce metabolic side effects in psychiatric treatments. We also plan to investigate other biomarkers that may interact with MIF under AAP treatment and to expand our study to include diverse populations to understand genetic variations in MIF response. These efforts are geared towards broadening the impact of our research, improving treatment strategies, and enhancing the quality of life for patients with severe mental disorders.

## Chapter V Tables and Supplementary Materials

Table 1. Demographic information and clinical characteristics of healthy controls and patients with SZ.

| Variables | HC (n=142) | CPZ (n=32) | OLZ (n=114) | CLZ (n=77) | APZ (n=41) | RIS (n=93) | QTP (n=31) | Test Statistic | p-Value |
|---|---|---|---|---|---|---|---|---|---|
| Age [a], yrs | 35.37 (12.64) | 55.13 (14.63) | 36.29 (13.91) | 47.73 (15.30) | 39.44 (17.45) | 36.79 (15.26) | 41.74 (14.63) | 13.990 | **0.000** |
| Sex [b] | 66 (46.5) / 76 (53.5) | 9 (28.1) /23 (71.9) | 41 (36.0) / 73 (64.0) | 20 (26.0) / 57 (74.0) | 20 (48.8) / 21 (51.2) | 37 (39.8) / 56 (60.2) | 11 (35.5) / 20 (64.5) | 12.700 | **0.048** |
| Education [a], yrs | 5.06 (1.37) | 3.66 (0.83) | 3.97 (0.98) | 3.43 (0.93) | 3.87 (0.86) | 4.04 (0.91) | 3.50 (0.95) | 26.066 | **0.000** |
| Age of onset [a], yrs | | 29.13 (12.39) | 27.81 (11.76) | 27.13 (9.92) | 27.10 (8.09) | 26.26 (10.67) | 29.17 (8.77) | 0.414 | 0.838 |
| Duration of illness [a], months | | 269.54 (193.92) | 121.85 (154.39) | 209.82 (194.85) | 216.07 (212.36) | 171.50 (162.57) | 163.87 (148.10) | 3.212 | **0.008** |
| PANSS score | | | | | | | | | |
| Positive [c] | | 14.00 (12.00) | 20.50 (15.00) | 15.00 (13.00) | 19.00 (13.00) | 19.00 (15.50) | 19.00 (9.00) | 19.774 | **0.001** |
| Negative [c] | | 14.00 (13.75) | 16.00 (11.50) | 16.00 (14.00) | 19.00 (10.5) | 16.00 (10.00) | 16.00 (12.00) | 2.416 | 0.789 |
| General [c] | | 28.50 (12.75) | 38.00 (21.5) | 33.00 (21.25) | 37.00 (32.75) | 34.00 (26.00) | 38.00 (14.00) | 20.344 | **0.001** |
| Total [c] | | 62.50 (27.25) | 79.00 (41.50) | 64.00 (38.75) | 77.50 (42.5) | 72.00 (42.00) | 79.00 (33.50) | 17.915 | **0.003** |

[a] Mean (SD); [b] Female n (%)/male n (%); [c] Median (IQR). Test Statistic: $\chi^2$ for the Chi-square test, H for the Kruskal-Wallis test, F for One-way ANOVA

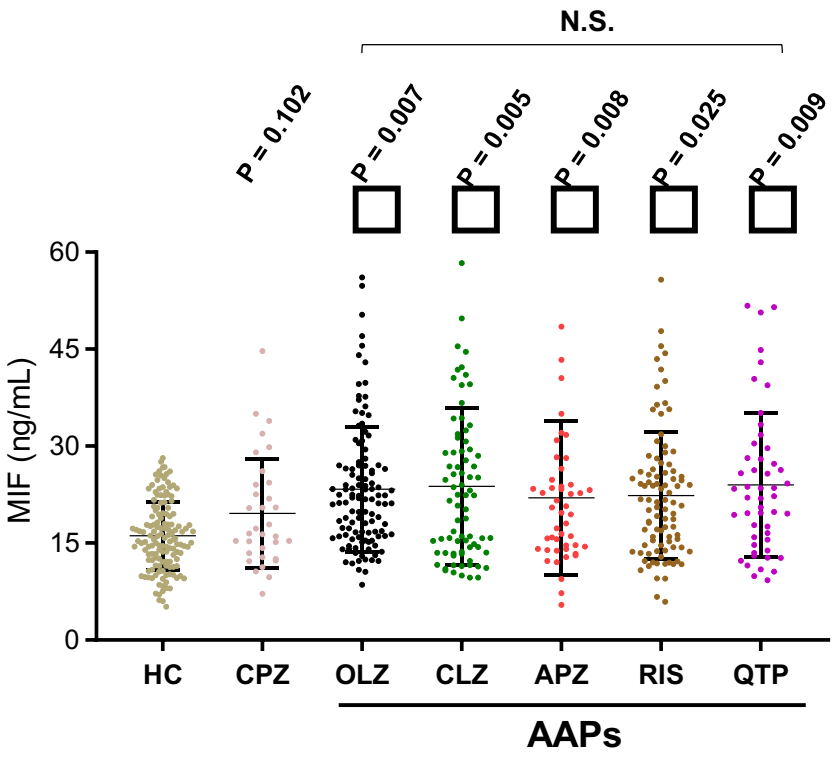


Figure 1. Plasma MIF levels in healthy controls and patients with SZ.
Dunnett's test was used for Post hoc multiple comparisons (to healthy controls, HC). All P values were adjusted for age, gender, and WHR in multi-factor ANOVA. * P value < 0.05.

Table 2. Association of plasma MIF concentration with glucose metabolism indexes in patients with SZ (OLZ, CLZ, APZ, RIS, QTP).

| Variables | β | p-value |
|---|---|---|
| HOMA-IR | 0.024 | **0.020** |
| Insulin | 0.903 | **0.001** |
| Fasting glucose (Fpg) | 0.001 | 0.977 |
| HbA1c | 0.003 | 0.752 |

All P values were adjusted for age, gender and PANSS.

Table 3. Association of plasma MIF concentration with lipid metabolism indexes in patients with SZ (OLZ, CLZ, APZ, RIS, QTP).

| Variables | β | p-value |
|---|---|---|
| TC | 0.012 | **0.038** |
| LDL | 0.008 | 0.081 |
| HDL | 0.001 | 0.694 |
| TG | 0.019 | **0.001** |
| ApoA1 | -0.001 | 0.663 |
| ApoB | 0.002 | 0.707 |

All P values were adjusted for age, gender and PANSS.

Table S1. The association of plasma MIF concentration with WHR in healthy controls and patients with SZ (OLZ, CLZ, APZ, RIS, QTP).

| WHR | mean ± SD | p-value |
|---|---|---|
| HC | 0.86 ± 0.09 | |
| OLZ | 0.90 ± 0.12 | **0.010** [a] |
| CLZ | 0.92 ± 0.07 | **0.000** [a] |
| APZ | 0.92 ± 0.07 | **0.004** [a] |
| RIS | 0.90 ± 0.10 | **0.006** [a] |
| QTP | 0.92 ± 0.07 | **0.011** [a] |
| F | 2.258 | **0.041** [b] |
| β | 0.001 | **0.011** [c] |

[a] P values of Dunnett's test which were adjusted for age and gender. [b] P values of multi-factor ANOVA which were adjusted for age and gender. [c] P values of multiple linear regression which were adjusted for age, gender and PANSS.

Table S2. Glucose metabolism indexes of healthy controls and patients with SZ.

| Variables | | HC (n=142) | CPZ (n=32) | OLZ (n=114) | CLZ (n=77) | APZ (n=41) | RIS (n=93) | QTP (n=31) | F | p-Value [b] |
|---|---|---|---|---|---|---|---|---|---|---|
| HOMA-IR | mean (SD) | 0.91 (0.47) | 1.72 (1.46) | 2.46 (2.06) | 2.06 (1.51) | 2.59 (1.97) | 2.15 (1.50) | 2.27 (2.10) | | |
| | p-value [a] | | **0.036** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | 7.744 | **0.000** |
| insulin | mean (SD), μU/ml | 26.43 (17.27) | 42.70 (51.73) | 70.40 (58.93) | 50.95 (44.07) | 72.18 (53.23) | 60.28 (39.98) | 62.54 (50.76) | | |
| | p-value [a] | | 0.289 | **0.000** | **0.001** | **0.000** | **0.000** | **0.000** | 6.812 | **0.000** |
| fpg | mean (SD), μU/ml | 4.65 (0.62) | 5.70 (1.45) | 5.25 (1.23) | 5.80 (1.76) | 5.69 (1.76) | 5.27 (1.10) | 5.16 (0.76) | | |
| | p-value [a] | | **0.000** | **0.000** | **0.000** | **0.000** | **0.001** | 0.154 | 5.168 | **0.000** |
| A1c | mean (SD), % | 5.35 (0.36) | 5.82 (0.97) | 5.53 (1.73) | 5.92 (1.33) | 6.00 (1.91) | 5.48 (1.02) | 5.37 (0.48) | | |
| | p-value [a] | | **0.043** | 0.440 | **0.000** | **0.000** | 0.858 | 1.000 | 2.032 | 0.063 |

[a] P values of Dunnett's test. [b] P values of multi-factor ANOVA
All P values were adjusted for age and gender.

Table S3. Lipid metabolism indexes of healthy controls and patients with SZ.

| Variables | | HC (n=142) | CPZ (n=32) | OLZ (n=114) | CLZ (n=77) | APZ (n=41) | RIS (n=93) | QTP (n=31) | F | p-Value [b] |
|---|---|---|---|---|---|---|---|---|---|---|
| TC | mean (SD), mmol/L | 4.22 (0.74) | 4.57 (1.05) | 4.43 (1.09) | 4.37 (1.08) | 4.29 (0.85) | 4.25 (0.91) | 4.69 (0.93) | | |
| | p-value [a] | | 0.257 | 0.334 | 0.788 | 0.998 | 1.000 | 0.064 | 1.928 | 0.078 |
| LDL | mean (SD), mmol/L | 2.43 (0.80) | 2.81 (0.95) | 2.69 (0.95) | 2.73 (0.78) | 2.65 (0.73) | 2.57 (0.77) | 2.75 (0.88) | | |
| | p-value [a] | | 0.058 | **0.036** | **0.026** | 0.448 | 0.636 | 0.163 | 2.409 | **0.029** |
| HDL | mean (SD), mmol/L | 1.67 (0.49) | 1.24 (0.39) | 1.29 (0.34) | 1.24 (0.40) | 1.30 (0.39) | 1.25 (0.35) | 1.43 (0.31) | | |
| | p-value [a] | | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.011** | 10.094 | **0.000** |
| TG | mean (SD), mmol/L | 1.14 (0.57) | 1.28 (0.66) | 1.22 (0.64) | 1.46 (0.96) | 1.56 (1.27) | 1.16 (0.69) | 1.33 (0.77) | | |
| | p-value [a] | | 0.777 | 0.831 | **0.002** | **0.001** | 1.000 | 0.498 | 3.731 | **0.002** |
| ApoA1 | mean (SD), g/L | 1.28 (0.19) | 1.54 (1.26) | 1.27 (0.27) | 1.29 (0.39) | 1.30 (0.27) | 1.24 (0.31) | 1.37 (0.32) | | |
| | p-value [a] | | **0.000** | 0.997 | 1.000 | 1.000 | 0.802 | 0.520 | 2.809 | **0.012** |
| ApoB | mean (SD), g/L | 0.78 (0.24) | 1.33 (1.60) | 0.91 (0.87) | 1.17 (1.65) | 0.81 (0.22) | 0.81 (0.30) | 0.87 (0.23) | | |
| | p-value [a] | | **0.014** | 0.650 | **0.005** | 1.000 | 1.000 | 0.990 | 1.211 | 0.302 |

[a] P values of Dunnett's test. [b] P values of multi-factor ANOVA
All P values were adjusted for age and gender.

Table S4. Association of plasma MIF concentration with metabolic index in patients with SZ (CPZ).

| Variables | β | p-value |
|---|---|---|
| HOMA-IR | 0.033 | 0.340 |
| Insulin | 0.860 | 0.468 |
| Fasting glucose | -0.002 | 0.953 |
| HbA1c | -0.014 | 0.533 |
| TC | 0.019 | 0.428 |
| LDL | 0.008 | 0.729 |
| HDL | 0.018 | 0.057 |
| TG | 0.005 | 0.727 |
| ApoA1 | -0.046 | 0.176 |
| ApoB | -0.023 | 0.655 |
| WHR | 0.001 | 0.862 |

All P values were adjusted for age, gender and PANSS.

## Reference


1. Watkins, T. (1992) Gods, Demons and Symbols of Ancient Mesopotamia - an Illustrated Dictionary - Black,J, Green,A. *Hist Today* 42, 52-52
2. Lam, C.S. *et al.* (2010) Chinese Lay Theory and Mental Illness Stigma: Implications for Research and Practices. *J Rehabil* 76, 35-40
3. Kleisiaris, C.F. *et al.* (2014) Health care practices in ancient Greece: The Hippocratic ideal. *J Med Ethics Hist Me* 7,
4. Brady, F.A. and Kelly, B.D. (2023) Asclepiades of Bithynia: Greek physician and medical reformer. *Irish J Med Sci* 192, 1775-1778. 10.1007/s11845-022-03216-2
5. Lamb, H.R. and Weinberger, L.E. (2016) Rediscovering the Concept of Asylum for Persons with Serious Mental Illness. *J Am Acad Psychiatry* 44, 106-110
6. George, P. *et al.* (2023) Cycles of reform in the history of psychosis treatment in the United States. *Ssm-Ment Health* 3. ARTN 100205

10.1016/j.ssmmh.2023.100205

7. Wellington, A. (2022) Dr Manfred J. Sakel: discoverer of insulin shock therapy - psychiatry in history. *Brit J Psychiat* 221, 682-682. PII S0007125022000733

10.1192/bjp.2022.73

8. West, F.H. *et al.* (1955) Insulin Coma Therapy in Schizophrenia - a 14-Year Follow-up Study. *Am J Psychiat* 111, 583-589. DOI 10.1176/ajp.111.8.583
9. Freudenthal, R. and Moncrieff, J. (2022) 'A landmark in psychiatric progress'? The role of evidence in the rise and fall of insulin coma therapy. *Hist Psychiatr* 33, 65-78. Artn 0957154x211062538

10.1177/0957154x211062538

10. Read, C.F. (1940) Consequences of metrazol shock therapy. *Am J Psychiat* 97, 667-676. DOI 10.1176/ajp.97.3.667
11. Trifu, S. *et al.* (2021) Efficacy of electroconvulsive therapy as a potential first-choice treatment in treatment-resistant depression (Review). *Exp Ther Med* 22. ARTN 1281

10.3892/etm.2021.10716

12. Schlich, T. (2015) Cutting the body to cure the mind. *Lancet Psychiat* 2, 390-392. Doi 10.1016/S2215-0366(15)00188-1
13. Tan, S.Y. and Yip, A. (2014) Antonio Egas Moniz (1874-1955): Lobotomy pioneer and Nobel laureate. *Singap Med J* 55, 175-176
14. Miller, A. (1967) The lobotomy patient--a decade later: a follow-up study of a research project started in 1948. *Can Med Assoc J* 96, 1095-1103
15. Majerus, B. (2016) Making Sense of the 'Chemical Revolution'. Patients' Voices on the Introduction of Neuroleptics in the 1950s. *Med Hist* 60, 54-66. 10.1017/mdh.2015.68
16. Stroup and Gray (2019) Management of common adverse effects of antipsychotic medications (vol 17, pg 341, 2018). *World Psychiatry* 18, 372-372
17. Ahima, R.S. and Antwi, D.A. (2008) Brain Regulation of Appetite and Satiety. *Endocrin Metab Clin* 37, 811-+. 10.1016/j.ecl.2008.08.005

18. Benarroch, L. *et al.* (2016) Atypical antipsychotics and effects on feeding: from mice to men. *Psychopharmacology* 233, 2629-2653. 10.1007/s00213-016-4324-8
19. Reynolds, G.P. and Kirk, S.L. (2010) Metabolic side effects of antipsychotic drug treatment - pharmacological mechanisms. *Pharmacol Therapeut* 125, 169-179. 10.1016/j.pharmthera.2009.10.010
20. Mukherjee, S. *et al.* (2022) Understanding the Effects of Antipsychotics on Appetite Control. *Front Nutr* 8. ARTN 815456

10.3389/fnut.2021.815456
21. Mastinu, A. *et al.* (2012) NESS038C6, a novel selective CB1 antagonist agent with anti-obesity activity and improved molecular profile. *Behav Brain Res* 234, 192-204. 10.1016/j.bbr.2012.06.033
22. Yang, D.D. *et al.* (2018) Orexin-A and endocannabinoid signaling regulate glucose-responsive arcuate nucleus neurons and feeding behavior in obese rats. *Neuropeptides* 69, 26-38. 10.1016/j.npep.2018.04.001
23. Zhang, C. *et al.* (2017) Metabolic adverse effects of olanzapine on cognitive dysfunction: A possible relationship between BDNF and TNF-alpha. *Psychoneuroendocrino* 81, 138-143. 10.1016/j.psyneuen.2017.04.014
24. Unger, T.J. *et al.* (2007) Selective deletion of Bdnf in the ventromedial and Dorsomedial hypothalamus of adult mice results in hyperphagic behavior and obesity. *J Neurosci* 27, 14265-14274. 10.1523/Jneurosci.3308-07.2007
25. Rorsman, P. and Ashcroft, F.M. (2018) PANCREATIC beta-CELL ELECTRICAL ACTIVITY AND INSULIN SECRETION: OF MICE AND MEN. *Physiol Rev* 98, 117-214. 10.1152/physrev.00008.2017
26. Wondmkun, Y.T. (2020) Obesity, Insulin Resistance, and Type 2 Diabetes: Associations and Therapeutic Implications. *Diabet Metab Synd Ob* 13, 3611-3616. 10.2147/Dmso.S275898
27. Pedersen, B.K. (2013) Muscle as a Secretory Organ. *Compr Physiol* 3, 1337-1362. 10.1002/cphy.c120033
28. DeFronzo, R.A. and Tripathy, D. (2009) Skeletal Muscle Insulin Resistance Is the Primary Defect in Type 2 Diabetes. *Diabetes Care* 32, S157-S163. 10.2337/dc09-S302
29. Ferrannini, E. *et al.* (1988) The Disposal of an Oral Glucose-Load in Patients with Non-Insulin-Dependent Diabetes. *Metabolism* 37, 79-85. Doi 10.1016/0026-0495(88)90033-9
30. Thiebaud, D. *et al.* (1982) The Effect of Graded Doses of Insulin on Total Glucose-Uptake, Glucose-Oxidation, and Glucose Storage in Man. *Diabetes* 31, 957-963. DOI 10.2337/diacare.31.11.957
31. Merz, K.E. and Thurmond, D.C. (2020) Role of Skeletal Muscle in Insulin Resistance and Glucose Uptake. *Compr Physiol* 10, 785-809. 10.1002/cphy.c190029
32. Klip, A. *et al.* (2019) Thirty sweet years of GLUT4. *J Biol Chem* 294, 11369-11381. 10.1074/jbc.REV119.008351

33. Shepherd, P.R. and Kahn, B.B. (1999) Mechanisms of disease - Glucose transporters and insulin action - Implications for insulin resistance and diabetes mellitus. *New Engl J Med* 341, 248-257. Doi 10.1056/Nejm199907223410406
34. Evans, P.L. *et al.* (2019) Regulation of Skeletal Muscle Glucose Transport and Glucose Metabolism by Exercise Training. *Nutrients* 11. ARTN 2432

10.3390/nu11102432
35. Lauritzen, H.P.M.M. (2009) In vivo imaging of GLUT4 translocation. *Appl Physiol Nutr Me* 34, 420-423. 10.1139/H09-043
36. Ryder, J.W. *et al.* (2000) Use of a novel impermeable biotinylated photolabeling reagent to assess insulin- and hypoxia-stimulated cell surface GLUT4 content in skeletal muscle from type 2 diabetic patients. *Diabetes* 49, 647-654. DOI 10.2337/diabetes.49.4.647
37. Zierath, J.R. *et al.* (1996) Insulin action on glucose transport and plasma membrane GLUT4 content in skeletal muscle from patients with NIDDM. *Diabetologia* 39, 1180-1189. Doi 10.1007/Bf02658504
38. Garvey, W.T. *et al.* (1998) Evidence for defects in the trafficking and translocation of GLUT4 glucose transporters in skeletal muscle as a cause of human insulin resistance. *Journal of Clinical Investigation* 101, 2377-2386. Doi 10.1172/Jci1557
39. Consitt, L.A. *et al.* (2009) Intramuscular Lipid Metabolism, Insulin Action, and Obesity. *Iubmb Life* 61, 47-55. 10.1002/iub.142
40. Gilbert, M. (2021) Role of skeletal muscle lipids in the pathogenesis of insulin resistance of obesity and type 2 diabetes. *J Diabetes Invest* 12, 1934-1941. 10.1111/jdi.13614
41. Bonnard, C. *et al.* (2008) Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice. *Journal of Clinical Investigation* 118, 789-800. 10.1172/Jci32601
42. Rahman, M.S. *et al.* (2021) Role of Insulin in Health and Disease: An Update. *Int J Mol Sci* 22. ARTN 6403

10.3390/ijms22126403
43. Carli, M. *et al.* (2021) Atypical Antipsychotics and Metabolic Syndrome: From Molecular Mechanisms to Clinical Differences. *Pharmaceuticals-Base* 14. ARTN 238

10.3390/ph14030238
44. Hatting, M. *et al.* (2018) Insulin regulation of gluconeogenesis. *Ann Ny Acad Sci* 1411, 21-35. 10.1111/nyas.13435
45. Han, H.S. *et al.* (2016) Regulation of glucose metabolism from a liver-centric perspective. *Exp Mol Med* 48. ARTN e218

10.1038/emm.2015.122
46. Boden, G. (2008) Obesity and Free Fatty Acids. *Endocrin Metab Clin* 37, 635-+. 10.1016/j.ecl.2008.06.007

47. Yu, C.L. *et al.* (2002) Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. *J Biol Chem* 277, 50230-50236. 10.1074/jbc.M200958200
48. Saltiel, A.R. and Kahn, C.R. (2001) Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 414, 799-806. DOI 10.1038/414799a
49. Rabe, K. *et al.* (2008) Adipokines and Insulin Resistance. *Mol Med* 14, 741-751. 10.2119/2008-00058.Rabe
50. Hardy, O.T. *et al.* (2012) What causes the insulin resistance underlying obesity? *Curr Opin Endocrinol* 19, 81-87. 10.1097/MED.0b013e3283514e13
51. Bener, A. *et al.* (2014) The potential impact of family history of metabolic syndrome and risk of type 2 diabetes mellitus: In a highly endogamous population. *Indian J Endocrinol Metab* 18, 202-209. 10.4103/2230-8210.129112
52. Greabu, M. *et al.* (2021) Drugs Interfering with Insulin Resistance and Their Influence on the Associated Hypermetabolic State in Severe Burns: A Narrative Review. *Int J Mol Sci* 22. ARTN 9782

10.3390/ijms22189782
53. Boucher, J. *et al.* (2014) Insulin Receptor Signaling in Normal and Insulin-Resistant States. *Csh Perspect Biol* 6. ARTN a009191

10.1101/cshperspect.a009191
54. Mackenzie, R.W.A. and Elliott, B.T. (2014) Akt/PKB activation and insulin signaling: a novel insulin signaling pathway in the treatment of type 2 diabetes. *Diabet Metab Synd Ob* 7, 55-64. Doi 10.2147/Dmso.S48260
55. Gabbouj, S. *et al.* (2019) Altered Insulin Signaling in Alzheimer's Disease Brain - Special Emphasis on PI3K-Akt Pathway. *Frontiers in Neuroscience* 13. ARTN 629

10.3389/fnins.2019.00629
56. Burghardt, K.J. *et al.* (2018) Association of Protein Kinase B (AKT) DNA Hypermethylation with Maintenance Atypical Antipsychotic Treatment in Patients with Bipolar Disorder. *Pharmacotherapy* 38, 428-435. 10.1002/phar.2097
57. Zemach, A. *et al.* (2010) Genome-Wide Evolutionary Analysis of Eukaryotic DNA Methylation. *Science* 328, 916-919. 10.1126/science.1186366
58. Feng, S.H. *et al.* (2010) Conservation and divergence of methylation patterning in plants and animals. *P Natl Acad Sci USA* 107, 8689-8694. 10.1073/pnas.1002720107
59. Cho, H. *et al.* (2001) Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). *Science* 292, 1728-1731. DOI 10.1126/science.292.5522.1728
60. Frojdo, S. *et al.* (2009) Alterations of insulin signaling in type 2 diabetes: A review of the current evidence from humans. *Bba-Mol Basis Dis* 1792, 83-92. 10.1016/j.bbadis.2008.10.019
61. Grajales, D. *et al.* (2019) Second-Generation Antipsychotics and Dysregulation of Glucose Metabolism: Beyond Weight Gain. *Cells-Basel* 8. ARTN 1336

10.3390/cells8111336

62. Nagashima, T. *et al.* (2016) Prevention of antipsychotic-induced hyperglycaemia by vitamin D: a data mining prediction followed by experimental exploration of the molecular mechanism. *Sci Rep-Uk* 6. ARTN 26375

10.1038/srep26375
63. Chen, J.Z. *et al.* (2017) Molecular Mechanisms of Antipsychotic Drug-Induced Diabetes. *Frontiers in Neuroscience* 11. ARTN 643

10.3389/fnins.2017.00643
64. Engl, J. *et al.* (2005) Olanzapine impairs glycogen synthesis and insulin signaling in L6 skeletal muscle cells. *Mol Psychiatry* 10, 1089-1096. 10.1038/sj.mp.4001729
65. Panariello, F. *et al.* (2012) Clozapine impairs insulin action by up-regulating akt phosphorylation and Ped/Pea-15 protein abundance. *J Cell Physiol* 227, 1485-1492. 10.1002/jcp.22864
66. Burghardt, K.J. *et al.* (2022) Profiling the Skeletal Muscle Proteome in Patients on Atypical Antipsychotics and Mood Stabilizers. *Brain Sci* 12. ARTN 259

10.3390/brainsci12020259
67. Gassaway, B.M. *et al.* (2018) PKC epsilon contributes to lipid-induced insulin resistance through cross talk with p70S6K and through previously unknown regulators of insulin signaling. *P Natl Acad Sci USA* 115, E8996-E9005. 10.1073/pnas.1804379115
68. Harrington, L.S. *et al.* (2004) The TSC1-2 tumor suppressor controls insulin-PI3K signaling via regulation of IRS proteins. *J Cell Biol* 166, 213-223. 10.1083/jcb.200403069
69. Um, S.H. *et al.* (2004) Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity (vol 431, pg 200, 2004). *Nature* 431, 485-485. 10.1038/nature02979
70. Ruffolo, S.C. *et al.* (2007) Basal activation of p70S6K results in adipose-specific insulin resistance in protein-tyrosine phosphatase 1B(-/-) mice. *J Biol Chem* 282, 30423-30433. 10.1074/jbc.M700697200
71. Friedrich, N. *et al.* (2012) The Association Between IGF-I and Insulin Resistance A general population study in Danish adults. *Diabetes Care* 35, 768-773. 10.2337/dc11-1833
72. Huang, X.J. *et al.* (2018) The PI3K/AKT pathway in obesity and type 2 diabetes. *Int J Biol Sci* 14, 1483-1496. 10.7150/ijbs.27173
73. Alemi, H. *et al.* (2019) Association of extracellular heat shock protein 70 and insulin resistance in type 2 diabetes; independent of obesity and C-reactive protein. *Cell Stress Chaperon* 24, 69-75. 10.1007/s12192-018-0942-x
74. Chichester, L. *et al.* (2015) Muscle Heat Shock Protein 70 Predicts Insulin Resistance With Aging. *J Gerontol a-Biol* 70, 155-162. 10.1093/gerona/glu015
75. Mencarelli, C. and Martinez-Martinez, P. (2013) Ceramide function in the brain: when a slight tilt is enough. *Cell Mol Life Sci* 70, 181-203. 10.1007/s00018-012-1038-x

76. Chalfant, C.E. and Spiegel, S. (2005) Sphingosine 1-phosphate and ceramide 1-phosphate: Expanding roles in cell signaling. *J Cell Sci* 118, 4605-4612. 10.1242/jcs.02637
77. Shalaby, Y.M. *et al.* (2022) Role of Ceramides in the Molecular Pathogenesis and Potential Therapeutic Strategies of Cardiometabolic Diseases: What we Know so Far. *Front Cell Dev Biol* 9. ARTN 816301

10.3389/fcell.2021.816301
78. Albeituni, S. and Stiban, J. (2019) Roles of Ceramides and Other Sphingolipids in Immune Cell Function and Inflammation. *Adv Exp Med Biol* 1161, 169-191. 10.1007/978-3-030-21735-8_15
79. Field, B.C. *et al.* (2020) The Role of Ceramides in Diabetes and Cardiovascular Disease Regulation of Ceramides by Adipokines. *Front Endocrinol* 11. ARTN 569250

10.3389/fendo.2020.569250
80. Sokolowska, E. and Blachnio-Zabielska, A. (2019) The Role of Ceramides in Insulin Resistance. *Front Endocrinol* 10. ARTN 577

10.3389/fendo.2019.00577
81. Summers, S.A. (2006) Ceramides in insulin resistance and lipotoxicity. *Prog Lipid Res* 45, 42-72. 10.1016/j.plipres.2005.11.002
82. Chathoth, S. *et al.* (2022) Insulin resistance induced by de novo pathway-generated C16-ceramide is associated with type 2 diabetes in an obese population. *Lipids Health Dis* 21. ARTN 24

10.1186/s12944-022-01634-w
83. Holland, W.L. *et al.* (2007) Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. *Cell Metab* 5, 167-179. 10.1016/j.cmet.2007.01.002
84. Sathyanarayana, P. *et al.* (2002) Activation of the Drosophila MLK by ceramide reveals TNF-alpha and ceramide as agonists of mammalian MLK3. *Mol Cell* 10, 1527-1533. Doi 10.1016/S1097-2765(02)00734-7
85. Chen, C.L. *et al.* (2008) Ceramide induces p38 MAPK and JNK activation through a mechanism involving a thioredoxin-interacting protein-mediated pathway. *Blood* 111, 4365-4374. 10.1182/blood-2007-08-106336
86. Feng, J. *et al.* (2020) The Role of JNk Signaling Pathway in Obesity-Driven Insulin Resistance. *Diabet Metab Synd Ob* 13, 1399-1406. 10.2147/Dmso.S236127
87. Yung, J.H.M. and Giacca, A. (2020) Role of c-Jun N-terminal Kinase (JNK) in Obesity and Type 2 Diabetes. *Cells-Basel* 9. ARTN 706

10.3390/cells9030706
88. Lanuza-Masdeu, J. *et al.* (2013) In vivo JNK activation in pancreatic beta-cells leads to glucose intolerance caused by insulin resistance in pancreas. *Diabetes* 62, 2308-2317. 10.2337/db12-1097

89. Sampson, S.R. and Cooper, D.R. (2006) Specific protein kinase C isoforms as transducers and modulators of insulin signaling. *Mol Genet Metab* 89, 32-47. 10.1016/j.ymgme.2006.04.017
90. Bourbon, N.A. *et al.* (2001) Inhibitory actions of ceramide upon PKC-epsilon/ERK interactions. *Am J Physiol-Cell Ph* 280, C1403-C1411. DOI 10.1152/ajpcell.2001.280.6.C1403
91. Hirsch, E. *et al.* (2007) Phosphoinositide 3-kinases as a common platform for multi-hormone signaling. *J Endocrinol* 194, 243-256. 10.1677/Joe-07-0097
92. Powell, D.J. *et al.* (2003) Ceramide disables 3-phosphoinositide binding to the pleckstrin homology domain of protein kinase B (PKB)/Akt by a PKC zeta-dependent mechanism. *Mol Cell Biol* 23, 7794-7808. 10.1128/Mcb.23.21.7794-7808.2003
93. Du, K.Y. *et al.* (2003) TRB3: A tribbles homolog that inhibits Akt/PKB activation by insulin in liver. *Science* 300, 1574-1577. DOI 10.1126/science.1079817
94. Kim, J.H. *et al.* (2010) Clozapine activates AMP-activated protein kinase (AMPK) in C2C12 myotube cells and stimulates glucose uptake. *Life Sci* 87, 42-48. 10.1016/j.lfs.2010.05.017
95. Tulipano, G. *et al.* (2007) Clozapine-induced alteration of glucose homeostasis in the rat: The contribution of hypothalamic-pituitary-adrenal axis activation. *Neuroendocrinology* 85, 61-70. 10.1159/000100981
96. Ardizzone, T.D. *et al.* (2001) Inhibition of glucose transport in PC12 cells by the atypical antipsychotic drugs risperidone and clozapine, and structural analogs of clozapine. *Brain Res* 923, 82-90. 10.1016/s0006-8993(01)03026-8
97. Smith, G.C. *et al.* (2008) Atypical antipsychotic drugs induce derangements in glucose homeostasis by acutely increasing glucagon secretion and hepatic glucose output in the rat. *Diabetologia* 51, 2309-2317. 10.1007/s00125-008-1152-3
98. Al-Zoairy, R. *et al.* (2013) The effects of psychotropic drugs on the regulation of glucose metabolism. *Curr Diabetes Rev* 9, 362-370. 10.2174/15733998113099990067
99. Al-Zoairy, R. *et al.* (2017) Serotonin improves glucose metabolism by Serotonylation of the small GTPase Rab4 in L6 skeletal muscle cells. *Diabetol Metab Syndr* 9, 1. 10.1186/s13098-016-0201-1
100. Redpath, G. and Deo, N. (2022) Serotonin: an overlooked regulator of endocytosis and endosomal sorting? *Biol Open* 11. 10.1242/bio.059057
101. Zhong, X. *et al.* (2022) Dynamic transcriptome analysis of the muscles in high-fat diet-induced obese zebrafish (Danio rerio) under 5-HT treatment. *Gene* 819, 146265. 10.1016/j.gene.2022.146265
102. Babkin, P. *et al.* (2015) Antipsychotics inhibit glucose transport: Determination of olanzapine binding site in Staphylococcus epidermidis glucose/H(+) symporter. *FEBS Open Bio* 5, 335-340. 10.1016/j.fob.2015.04.006
103. Houseknecht, K.L. *et al.* (2007) Acute effects of atypical antipsychotics on whole-body insulin resistance in rats: Implications for adverse metabolic effects. *Neuropsychopharmacol* 32, 289-297. 10.1038/sj.npp.1301209

104. Chintoh, A.F. *et al.* (2008) Insulin resistance and decreased glucose-stimulated insulin secretion after acute olanzapine administration. *J Clin Psychopharm* 28, 494-499. 10.1097/JCP.0b013e318184b4c5
105. Girault, E.M. *et al.* (2012) Acute peripheral but not central administration of olanzapine induces hyperglycemia associated with hepatic and extra-hepatic insulin resistance. *PLoS One* 7, e43244. 10.1371/journal.pone.0043244
106. Tudhope, S.J. *et al.* (2012) A Novel Mechanism for Regulating Hepatic Glycogen Synthesis Involving Serotonin and Cyclin-Dependent Kinase-5. *Diabetes* 61, 49-60. 10.2337/db11-0870
107. Hampson, L.J. *et al.* (2007) Stimulation of glycogen synthesis and inactivation of phosphorylase in hepatocytes by serotonergic mechanisms, and counter-regulation by atypical antipsychotic drugs. *Diabetologia* 50, 1743-1751. 10.1007/s00125-007-0696-y
108. Ikegami, M. *et al.* (2013) Olanzapine increases hepatic glucose production through the activation of hypothalamic adenosine 5′ -monophosphate-activated protein kinase. *Diabetes Obes Metab* 15, 1128-1135. 10.1111/dom.12148
109. Schmidt, R.H. *et al.* (2013) Olanzapine Activates Hepatic Mammalian Target of Rapamycin: New Mechanistic Insight into Metabolic Dysregulation with Atypical Antipsychotic Drugs. *J Pharmacol Exp Ther* 347, 126-135. 10.1124/jpet.113.207621
110. Liu, X.M. *et al.* (2017) Time-dependent changes and potential mechanisms of glucose-lipid metabolic disorders associated with chronic clozapine or olanzapine treatment in rats. *Sci Rep* 7. ARTN 2762

10.1038/s41598-017-02884-w
111. Townsend, L.K. *et al.* (2018) Obesity exacerbates the acute metabolic side effects of olanzapine. *Psychoneuroendocrino* 88, 121-128. 10.1016/j.psyneuen.2017.12.004
112. Park, S. *et al.* (2007) Chlorpromazine exacerbates hepatic insulin sensitivity via attenuating insulin and leptin signaling pathway, while exercise partially reverses the adverse effects. *Life Sciences* 80, 2428-2435. 10.1016/j.lfs.2007.04.001
113. Arizanovic, L.P. *et al.* (2021) Effects of several atypical antipsychotics closapine, sertindole or ziprasidone on hepatic antioxidant enzymes: Possible role in drug-induced liver dysfunction. *J Toxicol Env Heal A* 84, 173-182. 10.1080/15287394.2020.1844827
114. Urichuk, L. *et al.* (2008) Metabolism of atypical antipsychotics: Involvement of cytochrome P450 enzymes and relevance for drug-drug interactions. *Curr Drug Metab* 9, 410-418. Doi 10.2174/138920008784746373
115. Gonzalez, F.J. (2005) Role of cytochromes P450 in chemical toxicity and oxidative stress: studies with CYP2E1. *Mutat Res-Fund Mol M* 569, 101-110. 10.1016/j.mrfmmm.2004.04.021
116. Hurrle, S. and Hsu, W.H. (2017) The etiology of oxidative stress in insulin resistance. *Biomed J* 40, 257-262. 10.1016/j.bj.2017.06.007
117. Tangvarasittichai, S. (2015) Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. *World J Diabetes* 6, 456-480. 10.4239/wjd.v6.i3.456

118. Bovi, A.P.D. *et al.* (2021) Oxidative Stress in Non-alcoholic Fatty Liver Disease. An Updated Mini Review. *Front Med-Lausanne* 8. ARTN 595371

10.3389/fmed.2021.595371
119. Poher, A.L. *et al.* (2015) Brown adipose tissue activity as a target for the treatment of obesity/insulin resistance. *Frontiers in physiology* 6, 4. 10.3389/fphys.2015.00004
120. Engl, J. *et al.* (2007) Atypical antipsychotic drugs directly impair insulin action in adipocytes: effects on glucose transport, lipogenesis, and antilipolysis. *Neuropsychopharmacology* 32, 2431-2432; author reply 2433-2434. 10.1038/sj.npp.1301366
121. Vestri, H.S. *et al.* (2007) Atypical antipsychotic drugs directly impair insulin action in adipocytes: effects on glucose transport, lipogenesis, and antilipolysis. *Neuropsychopharmacology* 32, 765-772. 10.1038/sj.npp.1301142
122. Vranic, M. *et al.* (2023) Effects of the second-generation antipsychotic drugs aripiprazole and olanzapine on human adipocyte differentiation. *Molecular and Cellular Endocrinology* 561, 111828. https://doi.org/10.1016/j.mce.2022.111828
123. Sarsenbayeva, A. *et al.* (2019) Effects of second-generation antipsychotics on human subcutaneous adipose tissue metabolism. *Psychoneuroendocrinology* 110, 104445. 10.1016/j.psyneuen.2019.104445
124. Robinson, K.A. *et al.* (2006) At therapeutic concentrations, olanzapine does not affect basal or insulin-stimulated glucose transport in 3T3-L1 adipocytes. *Prog Neuropsychopharmacol Biol Psychiatry* 30, 93-98. 10.1016/j.pnpbp.2005.06.008
125. Bou Khalil, R. (2012) Atypical antipsychotic drugs, schizophrenia, and metabolic syndrome in non-Euro-American societies. *Clinical neuropharmacology* 35, 141-147. 10.1097/WNF.0b013e31824d5288
126. Fuster, J.J. *et al.* (2016) Obesity-Induced Changes in Adipose Tissue Microenvironment and Their Impact on Cardiovascular Disease. *Circulation research* 118, 1786-1807. 10.1161/circresaha.115.306885
127. Tang, Q.Q. and Lane, M.D. (2012) Adipogenesis: from stem cell to adipocyte. *Annual review of biochemistry* 81, 715-736. 10.1146/annurev-biochem-052110-115718
128. Bába, L.I. *et al.* (2019) Effects of Cariprazine, Aripiprazole, and Olanzapine on Mouse Fibroblast Culture: Changes in Adiponectin Contents in Supernatants, Triglyceride Accumulation, and Peroxisome Proliferator-Activated Receptor-γ Expression. *Medicina (Kaunas, Lithuania)* 55. 10.3390/medicina55050160
129. Nimura, S. *et al.* (2015) Olanzapine promotes the accumulation of lipid droplets and the expression of multiple perilipins in human adipocytes. *Biochemical and biophysical research communications* 467, 906-912. 10.1016/j.bbrc.2015.10.045
130. Sertié, A.L. *et al.* (2011) Effects of antipsychotics with different weight gain liabilities on human in vitro models of adipose tissue differentiation and metabolism. *Prog Neuropsychopharmacol Biol Psychiatry* 35, 1884-1890. 10.1016/j.pnpbp.2011.07.017
131. Yang, L.H. *et al.* (2007) Olanzapine induces SREBP-1-related adipogenesis in 3T3-L1 cells. *Pharmacological research* 56, 202-208. 10.1016/j.phrs.2007.05.007

132. Hemmrich, K. *et al.* (2006) Clozapine enhances differentiation of adipocyte progenitor cells. *Mol Psychiatry* 11, 980-981. 10.1038/sj.mp.4001892
133. Sárvári, A.K. *et al.* (2014) Atypical antipsychotics induce both proinflammatory and adipogenic gene expression in human adipocytes in vitro. *Biochemical and biophysical research communications* 450, 1383-1389. 10.1016/j.bbrc.2014.07.005
134. Yang, Z. *et al.* (2009) Evidence for an effect of clozapine on the regulation of fat-cell derived factors. *Clinica chimica acta; international journal of clinical chemistry* 408, 98-104. 10.1016/j.cca.2009.07.021
135. Minet-Ringuet, J. *et al.* (2007) Alterations of lipid metabolism and gene expression in rat adipocytes during chronic olanzapine treatment. *Mol Psychiatry* 12, 562-571. 10.1038/sj.mp.4001948
136. Chung, J.Y. *et al.* (2022) Role of ketone bodies in diabetes-induced dementia: sirtuins, insulin resistance, synaptic plasticity, mitochondrial dysfunction, and neurotransmitter. *Nutrition reviews* 80, 774-785. 10.1093/nutrit/nuab118
137. Huang, Y. *et al.* (2013) Lipoprotein lipase links vitamin D, insulin resistance, and type 2 diabetes: a cross-sectional epidemiological study. *Cardiovascular Diabetology* 12, 17. 10.1186/1475-2840-12-17
138. Cui, D. *et al.* (2018) Macrophage migration inhibitory factor mediates metabolic dysfunction induced by atypical antipsychotic therapy. *J Clin Invest* 128, 4997-5007. 10.1172/JCI93090
139. HUANG, Y. *et al.* (2023) 6-OR: PAR2 Downregulates Adipose LPL through MIF—A Novel Adipose Mechanism Contributing to the Development of Hypertriglyceridemia. *Diabetes* 72. 10.2337/db23-6-OR
140. Atsumi, T. *et al.* (2007) The proinflammatory cytokine macrophage migration inhibitory factor regulates glucose metabolism during systemic inflammation. *J Immunol* 179, 5399-5406. DOI 10.4049/jimmunol.179.8.5399
141. Kleemann, R. and Bucala, R. (2010) Macrophage Migration Inhibitory Factor: Critical Role in Obesity, Insulin Resistance, and Associated Comorbidities. *Mediat Inflamm* 2010. Artn 610479

10.1155/2010/610479
142. Tseng, Y.H. *et al.* (2008) New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 454, 1000-1004. 10.1038/nature07221
143. Yang, X. *et al.* (2003) Reduced expression of FOXC2 and brown adipogenic genes in human subjects with insulin resistance. *Obesity research* 11, 1182-1191. 10.1038/oby.2003.163
144. Blessing, W.W. *et al.* (2006) Clozapine reverses increased brown adipose tissue thermogenesis induced by 3,4-methylenedioxymethamphetamine and by cold exposure in conscious rats. *Neuroscience* 141, 2067-2073. 10.1016/j.neuroscience.2006.05.050
145. Oh, J.E. *et al.* (2012) Inhibition of mouse brown adipocyte differentiation by second-generation antipsychotics. *Experimental & molecular medicine* 44, 545-553. 10.3858/emm.2012.44.9.062

146. Dulloo, A.G. and Miller, D.S. (1984) Energy balance following sympathetic denervation of brown adipose tissue. *Canadian journal of physiology and pharmacology* 62, 235-240. 10.1139/y84-035
147. Maliszewska, K. *et al.* (2019) The interplay between muscle mass decline, obesity, and type 2 diabetes. *Polish archives of internal medicine* 129, 809-816. 10.20452/pamw.15025
148. Zhang, Q. *et al.* (2014) Olanzapine reduced brown adipose tissue thermogenesis and locomotor activity in female rats. *Prog Neuropsychopharmacol Biol Psychiatry* 51, 172-180. 10.1016/j.pnpbp.2014.02.003
149. Stefanidis, A. *et al.* (2009) The role of thermogenesis in antipsychotic drug-induced weight gain. *Obesity (Silver Spring, Md.)* 17, 16-24. 10.1038/oby.2008.468
150. Chen, X. *et al.* (2022) Olanzapine induces weight gain in offspring of prenatally exposed poly I:C rats by reducing brown fat thermogenic activity. *Frontiers in pharmacology* 13, 1001919. 10.3389/fphar.2022.1001919
151. Calandra, T. and Roger, T. (2003) Macrophage migration inhibitory factor: a regulator of innate immunity. *Nature reviews. Immunology* 3, 791-800. 10.1038/nri1200
152. Kim, B.S. *et al.* (2015) The macrophage migration inhibitory factor protein superfamily in obesity and wound repair. *Experimental & molecular medicine* 47, e161. 10.1038/emm.2015.26
153. Morrison, M.C. and Kleemann, R. (2015) Role of Macrophage Migration Inhibitory Factor in Obesity, Insulin Resistance, Type 2 Diabetes, and Associated Hepatic Co-Morbidities: A Comprehensive Review of Human and Rodent Studies. *Frontiers in immunology* 6, 308. 10.3389/fimmu.2015.00308
154. Yabunaka, N. *et al.* (2000) Elevated serum content of macrophage migration inhibitory factor in patients with type 2 diabetes. *Diabetes care* 23, 256-258. 10.2337/diacare.23.2.256
155. Herder, C. *et al.* (2006) Association of systemic concentrations of macrophage migration inhibitory factor with impaired glucose tolerance and type 2 diabetes: results from the Cooperative Health Research in the Region of Augsburg, Survey 4 (KORA S4). *Diabetes care* 29, 368-371. 10.2337/diacare.29.02.06.dc05-1474
156. Huang, Y. *et al.* (2023) A pref-1-controlled non-inflammatory mechanism of insulin resistance. *iScience* 26, 106923. 10.1016/j.isci.2023.106923
157. Aytac, H.M. *et al.* (2021) Macrophage Migration Inhibitory Factor - 173 G/C Polymorphism is Associated With The Age of Onset and Insight in Schizophrenia in the Turkish Population. *Neurological research* 43, 977-984. 10.1080/01616412.2021.1948748
158. Aytac, H.M. *et al.* (2020) Association of MIF and MBL2 gene polymorphisms with attempted suicide in patients diagnosed with schizophrenia or bipolar disorder. *Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia* 78, 264-268. 10.1016/j.jocn.2020.04.001
159. Okazaki, S. *et al.* (2018) Increased serum levels and promoter polymorphisms of macrophage migration inhibitory factor in schizophrenia. *Progress in neuro-psychopharmacology & biological psychiatry* 83, 33-41. 10.1016/j.pnpbp.2018.01.001

160. Okazaki, S. *et al.* (2021) Clozapine increases macrophage migration inhibitory factor (MIF) expression via increasing histone acetylation of MIF promoter in astrocytes. *Journal of psychiatric research* 135, 237-242. 10.1016/j.jpsychires.2021.01.033
161. Cui, D.H. *et al.* (2018) Macrophage migration inhibitory factor mediates metabolic dysfunction induced by atypical antipsychotic therapy. *Journal of Clinical Investigation* 128, 4997-5007. 10.1172/Jci93090
162. Cohen, J. (1988) *Statistical Power Analysis for the Behavioral Sciences* L. Erlbaum Associates
163. Koska, J. *et al.* (2009) mRNA concentrations of MIF in subcutaneous abdominal adipose cells are associated with adipocyte size and insulin action. *International journal of obesity (2005)* 33, 842-850. 10.1038/ijo.2009.106
164. Bilgir, O. *et al.* (2018) Relationship Between Serum Macrophage Migration Inhibitory Factor Level and Insulin Resistance, High-Sensitivity C-Reactive Protein and Visceral Fat Mass in Prediabetes. *Am J Med Sci* 355, 37-43. DOI 10.1016/j.amjms.2017.10.006
165. Potvin, S. *et al.* (2015) Antipsychotic-induced changes in blood levels of leptin in schizophrenia: a meta-analysis. *Canadian journal of psychiatry. Revue canadienne de psychiatrie* 60, S26-34
166. Peña, R. *et al.* (2008) Frequency of abnormal correlation between leptin and the body mass index during first and second generation antipsychotic drug treatment. *Schizophrenia research* 106, 315-319. 10.1016/j.schres.2008.08.031
167. Chen, C.Y. *et al.* (2020) The Role of Adiponectin in the Pathogenesis of Metabolic Disturbances in Patients With Schizophrenia. *Frontiers in psychiatry* 11, 605124. 10.3389/fpsyt.2020.605124
168. Jin, H. *et al.* (2008) Impact of atypical antipsychotic therapy on leptin, ghrelin, and adiponectin. *Schizophrenia research* 100, 70-85. 10.1016/j.schres.2007.11.026
169. Chen, L. *et al.* (2024) Extracellular macrophage migration inhibitory factor (MIF) downregulates adipose hormone-sensitive lipase (HSL) and contributes to obesity. *Molecular metabolism* 79, 101834. 10.1016/j.molmet.2023.101834
170. Herder, C. *et al.* (2006) Association of systemic concentrations of macrophage migration inhibitory factor with impaired glucose tolerance and type 2 diabetes - Results from the Cooperative Health Research in the Region of Augsburg, Survey 4 (KORA S4). *Diabetes Care* 29, 368-371. DOI 10.2337/diacare.29.02.06.dc05-1474
171. Waeber, G. *et al.* (1997) Insulin secretion is regulated by the glucose-dependent production of islet beta cell macrophage migration inhibitory factor. *Proceedings of the National Academy of Sciences of the United States of America* 94, 4782-4787. 10.1073/pnas.94.9.4782
172. Kobayashi, J. *et al.* (2001) Pre-heparin plasma lipoprotein lipase mass: correlation with intra-abdominal visceral fat accumulation. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme* 33, 412-416. 10.1055/s-2001-16230
173. van Wijk, D.F. *et al.* (2009) Lipid measures and cardiovascular disease prediction. *Disease markers* 26, 209-216. 10.3233/dma-2009-0636

174. Yang, W. *et al.* (2020) Effects of different fatty acids on BRL3A rat liver cell damage. *Journal of cellular physiology* 235, 6246-6256. 10.1002/jcp.29553
175. Hu, X. *et al.* (2022) The role of remnant cholesterol beyond low-density lipoprotein cholesterol in diabetes mellitus. *Cardiovascular diabetology* 21, 117. 10.1186/s12933-022-01554-0
176. Chikowe, I. *et al.* (2019) Adverse drug reactions experienced by out-patients taking chlorpromazine or haloperidol at Zomba Mental Hospital, Malawi. *BMC research notes* 12, 376. 10.1186/s13104-019-4398-6
177. Allison, D.B. *et al.* (1999) Antipsychotic-induced weight gain: a comprehensive research synthesis. *The American journal of psychiatry* 156, 1686-1696. 10.1176/ajp.156.11.1686
178. Xing, M. *et al.* (2022) Differing Prevalence and Correlates of Metabolic Syndromes Between Chlorpromazine and Clozapine: A 10-year Retrospective Study of a Male Chinese Cohort. *Current neuropharmacology* 20, 1969-1977. 10.2174/1570159x20666220302153123
179. Matsunaga, J. *et al.* (1999) Enzyme activity of macrophage migration inhibitory factor toward oxidized catecholamines. *The Journal of biological chemistry* 274, 3268-3271. 10.1074/jbc.274.6.3268
180. Smythies, J. (1996) On the functional of neuromelanin. *Proceedings. Biological sciences* 263, 487-489. 10.1098/rspb.1996.0073
181. Wu, X. (2014) From Assimilation to Autonomy: Realizing Ethnic Minority Rights in China's National Autonomous Regions. *Chinese Journal of International Law* 13, 55-90. 10.1093/chinesejil/jmu006 %J Chinese Journal of International Law