Statins in depression:

a meta-analytic, epidemiological, and experimental medicine approach

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Preface

Depression has a substantial clinical, economic, and societal burden worldwide[1,2]. This disorder is characterised by several disabling symptoms including: low mood and apathy, anhedonia, fatigue and anergia; feelings of worthlessness, helplessness, and hopelessness; disturbances of sleep, appetite, libido, and psychomotor activity; impaired concentration, attention, and memory; self-harm and suicidality[3]. Both psychotherapy and pharmacotherapy are valuable treatment strategies for patients suffering from depressive disorders[4,5], but international clinical guidelines endorse the use of antidepressant medications to treat moderate to severe depression in adults[6-10] as the magnitude of their effect is proportional to disease severity[11].

Nevertheless, only half of such patients respond to first-line antidepressants, and one-third still suffer from impairing depressive symptoms after four treatment steps over one year[12]. Concordance with treatment is a further significant issue[13], with one-quarter of patients discontinuing antidepressants due to any cause, and one-tenth suspending treatment because of intolerable side-effects after two months[14]. Also, most licensed antidepressants, which work in the main by modulating monoaminergic neurotransmission, are characterised by a delayed therapeutic onset[15], further compounding the effective management of this condition[16].

Overall, there is a compelling need to identify novel molecular targets in depression and thus new antidepressants that can act on them[17], particularly with a view to tailoring treatment to the individual patient[18]. Regrettably, clinical research and drug development in this area is challenging[19], and indeed several pharmaceutical companies have disinvested from it[20]. An innovative and promising approach proposes instead to repurpose existing medications with a well-defined safety profile and capable of targeting emerging physiopathological pathways implicated in depression[21-23].

Nowadays, the conceptual framework surrounding depressive disorders encompasses a variety of psychological and somatic mechanisms including deeply interconnected neurobiological, endocrine-metabolic, cardiovascular, and immunological systems[24-29]. In this context, pre-clinical and clinical studies consistently show that the 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase inhibitors, or statins, are intriguing candidates for repurposing in neuropsychiatric disorders[30-34], and especially in depression[23,35-39].

In this dissertation, I will add to the literature on the topic of statins for depressive disorders. In the introduction, I provide an overview of the pharmacology of statins and the pre-clinical and clinical evidence on the effects of these drugs in depression (Chapter 1). Six experimental chapters follow, including two studies per each methodology employed: meta-analytic (Chapters 2-3), epidemiological (Chapters 4-5), and experimental medicine (Chapters 6-7). Finally, I discuss a follow-up, ongoing experimental medicine study that I am currently conducting, as well as avenues for further research in this field (Chapter 7).

Chapter 1.

Introduction

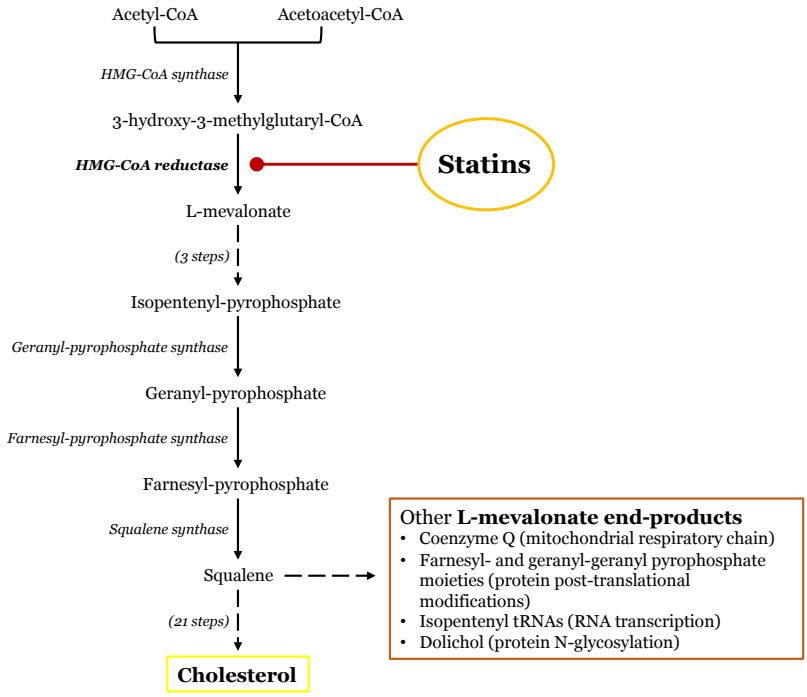
Statins are a class of lipid-lowering drugs used for the prevention and treatment of cardiovascular, cerebrovascular, and metabolic disorders[40]. Thanks to their established effectiveness and safety in these conditions, they are among the most widely prescribed medications worldwide[41,42] and can be found in the World Health Organization model list of essential medicines[43].

1.1 Principles of general pharmacology of statins

Historically, statins' primary mechanism of action was recognised as the competitive, reversible antagonism of liver 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase (Figure 1.1), the rate-limiting enzyme in cholesterol biosynthesis[44]. All statins share a common pharmacophore, a dihydroheptanoic acid, that resembles endogenous HMG-CoA[45]. As such, they can bind the catalytic domain of HMG-CoA reductase, causing steric hindrance thus preventing endogenous HMG-CoA from reaching the enzyme active site[46]. By inhibiting HMG-CoA reductase, statins thwart the physiological production of cholesterol with subsequent decline of its blood levels[47]. The ensuing reduction in cholesterol concentration within hepatocytes triggers the upregulation of low-density lipoprotein (LDL)-receptor via sterol regulatory element binding proteins[48], leading to increased uptake of LDL cholesterol from systemic circulation[49]. In other words, statins' cholesterol-lowering properties depend not only on mere reduction of cholesterol biosynthesis from the liver, but also on the consequent substantial upsurge in LDL clearance from plasma. Ancillary mechanisms of cholesterol reduction comprise inhibition of hepatic synthesis of apolipoprotein B100[50] and decreased production and secretion of triglyceride-rich lipoproteins[51]. Overall, the effects on lipid profile include

substantial contractions in total cholesterol, LDL, and triglycerides, as well as an accrue in high-density lipoproteins (HDLs)[52]. Additionally, statins differ from other lipid-lowering agents because their upstream inhibition of the mevalonate pathway affects several end-products other than cholesterol, which are responsible for numerous homeostatic processes, including Coenzyme Q (mitochondrial respiratory chain), farnesyl- and geranyl-geranyl pyrophosphate moieties (protein post-translational modifications), isopentenyl tRNAs (RNA transcription), and dolichol (protein N-glycosylation)[53]. On this basis, statins are described as possessing "pleiotropic effects"[54], whether positive or negative[55], of which the immunomodulatory ones are best characterised today[56].

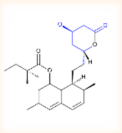
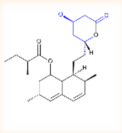
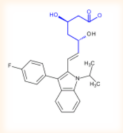
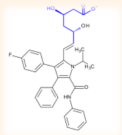
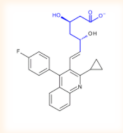
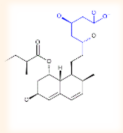
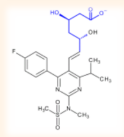
Figure 1.1 *Pharmacodynamics of statins*



The pharmacokinetics of individual statins (Table 1.1) is related instead to their diverse hydrophobic ring systems covalently bound to the shared pharmacophore[52]. From this

perspective, statins are divided into two categories: type I, natural or semi-synthetic/fungal-derived statins (i.e., simvastatin, lovastatin, pravastatin) and type II, fully-synthetic statins (i.e., fluvastatin, atorvastatin, pitavastatin, rosuvastatin)[57]. Simvastatin and lovastatin are pro-drugs, requiring hepatic metabolism to achieve their active state, whereas other statins are administered as active molecules[58]. As sterol synthesis occurs mainly at night-time, statins are more effective when taken in the evening[59]. Atorvastatin and rosuvastatin are known to interact more strongly with HMG-CoA reductase thanks to their greater hydrogen-binding capacity and are therefore considered to have higher potency[60]. Simvastatin is highly lipophilic, lovastatin, fluvastatin, atorvastatin, and pitavastatin show moderate lipophilicity, while pravastatin and rosuvastatin are hydrophilic[52,58]. Lipophilic statins can passively enter bodily tissues, thus generally being associated with worse side effects, but they can also cross the blood–brain barrier (BBB) and penetrate the central nervous system (CNS); on the other hand, hydrophilic statins are more hepato-selective, so that other effects, aside from their lipid-lowering one, tend to be less intense[61]. Importantly, recent studies confirm that statins express their pleiotropic activities differentially, based on both their potency and lipophilicity/hydrophilicity[62].

Table 1.1 *Pharmacokinetics of statins*[*]

	Simvastatin	Lovastatin	Fluvastatin	Atorvastatin	Pitavastatin	Pravastatin	Rosuvastatin
Molecular structure							
Type	I	I	II	II	II	I	II
Pro-drug	Yes	Yes	No	No	No	No	No
Dosing time	Evening	Any, with food	Bedtime	Any	Any	Bedtime	Any
Half-life	3h	3h	2.3h	14h	12h	1.3-2.7h	19h
Bioavailability	<5%	5%	9-50%	12%	51%	18%	20%
Protein binding	95%	95%	98%	98%	96%	50%	90%
CYP substrate	3A4	3A4	2C9	3A4	2C8, limited 2C9	Limited 3A4	Limited 2C9
Lipophilicity (log octanol–water distribution coefficient)	Lipophilic (4.40)	Lipophilic (3.91)	Lipophilic (1.73)	Lipophilic (1.53)	Lipophilic (1.50)	Hydrophilic (-0.47)	Hydrophilic (-0.25 to -0.50)
Excretion	13% renal 60% faecal	10% renal 83% faecal	6% renal 93% faecal	<2% renal >98% faecal	2% renal 79% faecal	20% renal 70% faecal	10% renal 90% faecal

1.2 Pharmacological activity of statins in the central nervous system

We have clear evidence today that all statins can reach the CNS. These molecules are detected in rodent brain after a few hours following a single dose administration[63]. Both lipophilic and hydrophilic statins can be found in the neuroparenchyma of animals[64] and humans[65], with functional magnetic resonance imaging (fMRI) studies displaying their effect on neural activity[66]. Hydrophilic statins (i.e., pravastatin, rosuvastatin) require carrier-mediated passage through the BBB[67,68], while lipophilic ones (i.e., simvastatin, lovastatin, fluvastatin, atorvastatin, pitavastatin) cross it via both simple diffusion and active transporters[69]. Nevertheless, statins seem to accumulate within the CNS at rates and concentrations that reflect their lipid solubility; therefore, the greater the lipophilicity, the

[*]Adapted from McFarland et al., 2014; DOI:10.3390/ijms151120607

higher their levels in the brain[32]. Statins' variability in neural entry/efflux and indeed potency emphasises that these molecules should be considered individually with respect to their pharmacological activity in the CNS[52].

Consistent data advise that statins can affect brain function both directly, via local neurobiological effects, and indirectly, by influencing deeply interconnected systemic processes (i.e., endocrine-metabolic, cardiovascular, immunological) that ultimately have an impact on the CNS[32,70].

The local CNS effects of statins involve brain lipids, neurotransmission, neurogenesis, and neuroprotection from trauma, inflammation, and oxidative stress[32]. Firstly, it should be noted that cholesterol and other end-products of the mevalonate pathway are especially abundant in the CNS, where they serve many essential physiological functions[71]. These molecules are rather metabolically inert in the adult brain: their half-life spans from months to years[72], and only some 0.02% undergo daily turnover through *de novo* synthesis mainly by astrocytes[73], meaning that there is no need to rely on uptake from systemic circulation[74]. Nevertheless, even short-term statins administration seems to cause acute disruption in the homeostasis of these metabolites in the CNS[64], whereas chronic statin use determines further reductions in brain cholesterol[75] and other mevalonate end-products[76], either directly through HMG-CoA reductase inhibition, or secondarily via a "sink effect"[77]. Modulation of these lipids within the CNS leads to changes in brain function and behaviour, and is therefore associated with neuropsychological diseases[71] and their treatment[78].

Secondly, statins have widespread effects on neurotransmission, involving monoaminergic, cholinergic, and glutamatergic systems implicated in a variety of neuropsychiatric disorders: both cholesterol-dependent and unrelated (e.g., anti-

inflammatory, antioxidant) mechanisms can explain such alterations in neurotransmitters levels[79].

Thirdly, statins are ligands of peroxisome proliferator-activated receptor (PPAR)α, which drives the expression of neurotrophins such as brain-derived neurotrophic factor (BDNF)[80]. Furthermore, statin-dependent inhibition of the mevalonate pathway stimulates hippocampal neurogenesis via Wnt signalling[81] and promotes neurite outgrowth[82], though also appearing to inhibit synaptic spurring[83].

Finally, statins can be neuroprotective against a variety of stressors. Following traumatic injury, statin use is associated with reduced neuronal loss[84] and increased tissue recovery via vascular endothelial growth factor (VEGF) and activation of the PI3K/Akt-BDNF pathway[85]. Likewise, the suppression of certain mevalonate metabolites mediated by statins dampens the production of pro-inflammatory cytokines[86] and free radicals[87] such as reactive oxygen species (ROS) and nitric oxide (NO), thus protecting neurons from leaky BBB [88] and overly activated microglia[89] (i.e., neuroinflammation), as well as oxidative stress[90].

The systemic, peripheral effects of statins include a wealth of endocrine-metabolic, cardiovascular, immunological, and other (e.g., musculoskeletal) changes, part of their dubbed "pleiotropy"[70], whose detailed description would go beyond the scope of this chapter. In addition to, and independently from their established activity on the metabolism of bodily lipids[57,91], statins can regulate critical functions of endocrine (e.g., cortisol[92,93] and insulin secretion[94,95]), cardiovascular (e.g., endothelial function, platelet activation, and atherogenesis[96]), and immune (e.g., regulation of innate immunity via pro- and anti-inflammatory cytokines[97,98] and of adaptive immunity via inhibition of antigen-1 leukocytes (LFA-1)[99], T-cell activation[100], and regulatory T-cells induction[101]) systems

and their related clinical outcomes. All these processes share profound interactions with each other[102-105], not to mention their substantial crosstalk with several of the neurobiological mechanisms described above [32,70].

Despite the growing amount of literature available on this topic, the resulting effects of statins in neuropsychiatric disorders[31] and related psychopathological domains[106] remain controversial. Early studies had raised concerns about possible associations between statins use and adverse neuropsychiatric outcomes, especially affecting mood and cognition[107,108]. However, more recent evidence from pre-clinical[109] and clinical[110,111] research indicates that statins may play a role in the treatment of neuropsychiatric syndromes, particularly in the context of affective disorders such as depression[108,112]. In the next two sections, I outline such pre-clinical and clinical evidence on the effects of statins in depression.

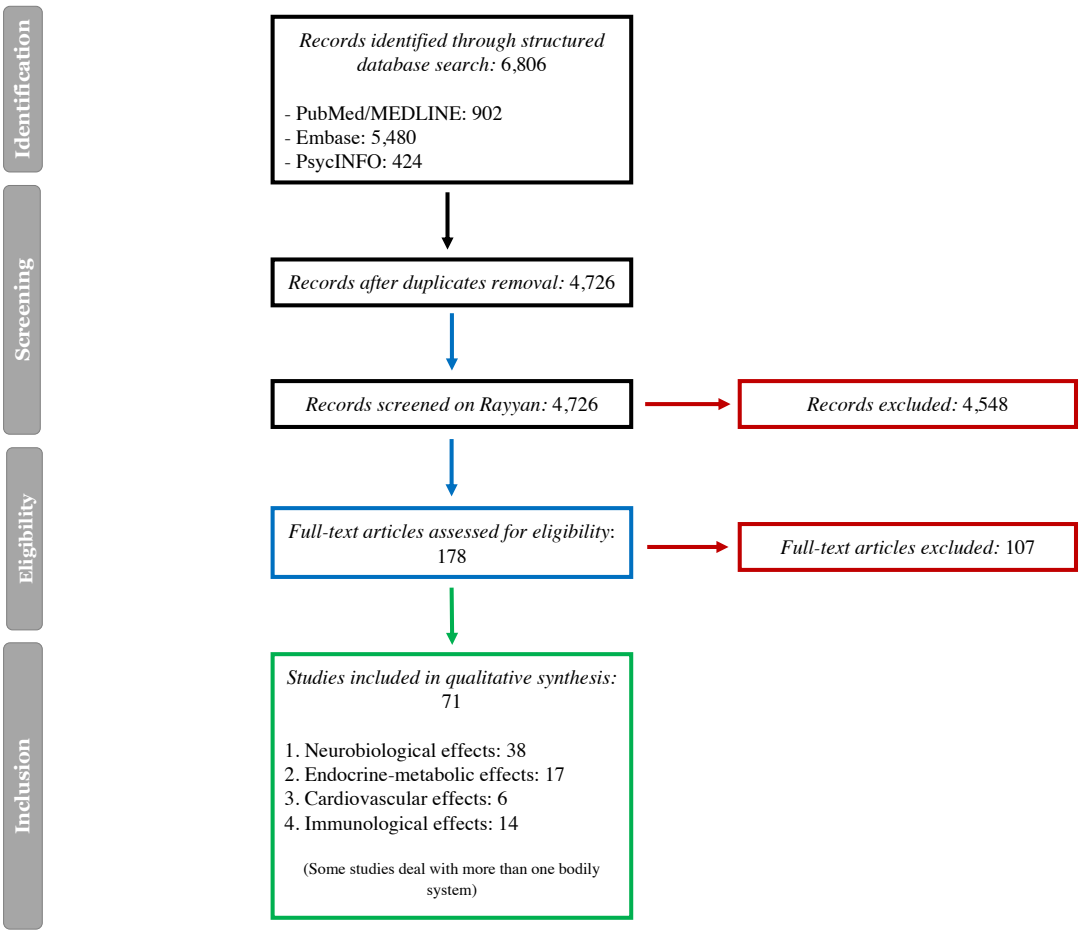
1.3 Statins in depression: pre-clinical evidence

Traditionally, evidence from *in vitro*, animal, and translational research used to be gathered and presented by means of narrative reviews. As these studies tend to be abundant yet less methodically organised on search engines and databases than their clinical counterparts, systematically searching for relevant pre-clinical evidence can be a daunting task, though a profitable one [113], so that machine learning approaches have been developed to support with such enterprise[114]. Meanwhile, scoping reviews have emerged as a valid alternative when the aim is to ascertain knowledge gaps, inspect a body of literature, and elucidate concepts[115]. In this section, I employ a mixed model involving the use of a broad search strategy (Appendix A1.1) on three major databases (i.e., Pubmed/MEDLINE, Embase, PsychINFO) via OvidSP on 8th April 2022, a web-based

software (i.e., Rayyan)[116] for semi-automated text mining, and extensive forward/backward searching techniques. Rayyan is the most popular, well-validated tool to support articles screening for systematic reviews[420]. This software processes the input citation files by extracting different metadata (e.g., title, authors) and computing others (e.g., MeSH terms). These variables then populate the features in the review workbench to further explore and filter the studies. Users can filter studies based on customisable lists of keywords that most likely hint to either include or exclude a study. Citations can also be labelled and reasons for exclusion can be further defined to facilitate sharing and tracking of these decisions. Importantly, Rayyan iteratively learns from users' decisions to include or exclude studies, which is then used to build progressively improved algorithms offering suggestions on studies awaiting screening. Validation models for Rayyan show an accuracy of 0.87 ± 0.09 and time saving of 0.49% ± 0.18% compared to manual screening[116].

The search flow chart is in Figure 1.2, for a total of 71 studies.

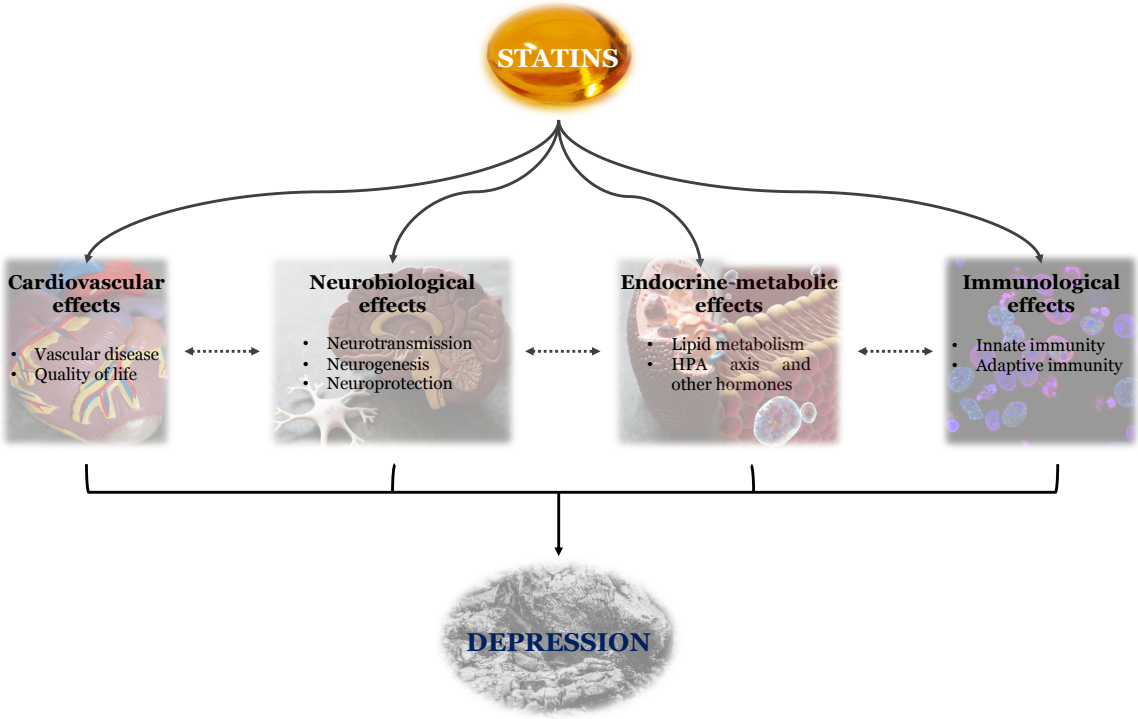
Figure 1.2 *Flow chart of the search for pre-clinical studies*



Four studies assess the putative antidepressant activity of statins in animal models of depression without further investigating their underlying mechanism of action. These show that the highly-lipophilic simvastatin and lovastatin have antidepressant-like effects in rats or mice[117], while the less lipophilic atorvastatin[118] and pitavastatin[119] failed to do so. However, atorvastatin, pitavastatin, and the hydrophilic rosuvastatin display antianxiety properties[120].

In keeping with the data presented above (section 1.2), mechanistic evidence shows that several intertwined neurobiological, endocrine-metabolic, cardiovascular, and immunological processes may contribute to the effects of statins in depression (Figure 1.3).

Figure 1.3 *Mechanisms of statins effects in depression*



I now describe the remaining studies following these categories, though it must be reiterated that these mechanisms are entangled and bound to strongly influence each other.

1.3.1 Neurobiological effects of statins in depression

These include effects on neurotransmission, neurogenesis, and neuroprotection.

1.3.1.1 Neurotransmission

The pathophysiology of depression is classically associated to anomalies in monoaminergic (i.e., serotonin or 5-hydroxytryptamine, 5HT; noradrenaline, NA; dopamine, DA) neurotransmission[121], though more recently glutamatergic, γ-aminobutyric acid (GABA)ergic, and cholinergic receptors have been implicated[122].

Numerous studies indicate that statins can alter synaptic transmission by modulating the function of several of these neurotransmitter receptors and their ligands[79].

1.3.1.1.1 Serotonin

In vitro, statin-induced cholesterol depletion impairs $5HT_{1A}$[123] and $5HT_3$[124] receptor function. Simvastatin however also increases serotonin reuptake by augmenting serotonin transporter (SERT) activity via both cholesterol-mediated[125] and independent[126] pathways. In animal models, an antidepressant-like effect of simvastatin appears linked to an increase in tryptophan, a serotonin precursor, through the inhibition of indoleamine 2,3-dioxygenase (IDO)[127], and in hippocampal serotonin[128]. Conversely, serotonin depletion or $5HT_{1A}$ and $5HT_{2A/C}$ receptor antagonism abolish the antidepressant effect of atorvastatin[129]. These findings have not been replicated in human studies assessing 5HT neuroendocrine function and plasma tryptophan in hypercholesterolaemic patients receiving statins[130].

Statins can also modulate the serotonergic effects of some antidepressants *in vitro*, via the tyrosine kinase receptor 2 (TRKB) domain of BDNF receptor[131]. In animals, the antidepressant effect of selective serotonin reuptake inhibitors (SSRIs) seems potentiated by several statins[127,129,132,133], possibly involving pharmacokinetics interactions [134-136], but the same does not apply to tricyclic antidepressants (TCAs)[127].

1.3.1.1.2 Dopamine

The dopaminergic system is affected by statins administration, but while certain studies demonstrate the occurrence of concomitant dopaminergic and antidepressant- or anxiolytic-like effects[127], perhaps mediated by interaction with BDNF function[137,138] or potentiation of dopaminergic drugs[139] for simvastatin, others fail to show any concurrent changes in animal depressive or anxiety behaviour for atorvastatin[140].

1.3.1.1.3 Glutamate and GABA

Simvastatin[127,141-143] and atorvastatin[144] antidepressant and anxiolytic properties in rats seem associated to the glutamate N-methyl-D-aspartate (NMDA) receptor expression and blockade, especially in the hippocampus and amygdala. However, another study shows that while atorvastatin seems to affect hippocampal glutamate and GABA, no concurrent effect on depression or anxiety can be observed[140].

1.3.1.1.4 Other neurotransmitters

Less conventional pathways have also been explored in animal models of depression, showing that simvastatin may elicit antidepressant action via opioid-[145] and endocannabinoid-mediated[146] neurotransmission, though it might also increase the concentration of the anxiogenic cholecystokinin-tetrapeptide in the cerebrospinal fluid (CSF) of healthy human subjects[147].

1.3.1.2 Neurogenesis

Processes of hippocampal neurogenesis and neuroplasticity, largely controlled by neurotrophins such as BDNF[148], are considered today a hallmark of depressive disorder and antidepressant action[149]. Emerging evidence from animal studies suggests that lovastatin and atorvastatin may enhance the proteolytic cleavage of pro-BDNF[150-152], BDNF hippocampal concentrations[144,153], and α7nAChR-mediated activation of the PI3K/Akt-BDNF pathway[154], with consequent positive influence on depressive behaviour. Agmatine and imidazoline receptors, whose function broadly relates to BDNF neurogenesis, NMDA neuroprotection, and monoamine regulation, have also been involved in the antidepressant-like effect of simvastatin and atorvastatin[155]. On the other

hand, simvastatin or rosuvastatin administration seems associated with lower hippocampal BDNF and anxiogenic response in rats[156].

1.3.1.3 Neuroprotection

Excitotoxicity[157] and oxidative stress due to reactive oxygen and nitrogen species in the brain[158] are strictly related, highly depressogenic triggers. Numerous animal studies show that the antidepressant effect of statins may occur because of decreasing glutamate-NMDA excitotoxicity[159], PPARg-mediated[160,161] or inflammation-related[162,163] nitrosative and oxidative stress, or all the above[144,164], while also inducing neuroprotective pathways such as protein kinase B (PKB)/Akt[159] and PI3K/Akt-GSK3b/mTOR[165] signalling.

1.3.2 Endocrine-metabolic effects of statins in depression

These include effects on lipid metabolism and on the hypothalamic-pituitary-adrenal (HPA) axis and other hormones.

1.3.2.1 Lipid metabolism

Lipids in the CNS and peripheral circulation interact with biological pathways implicated in depression[166] and antidepressant action[167]. Intriguingly, lipid homeostasis is critical to several interconnected mechanisms involved in mood regulation, anxiety, and suicidal behaviour, including serotonin neurotransmission[168-170], neurogenesis[171], neuroprotection from excitotoxicity[142], and systemic inflammation[170]. From a clinical standpoint, dyslipidaemia and depression, its severity and prospective course appear associated[172], while SSRI-induced increase in cholesterol has been argued to be protective against depression[173]. Correlations between depressive symptomatology and both raised[174,175] and

diminished[176-179] concentrations of circulating lipids, including total cholesterol, LDL, HDL, triglycerides, w-3 polyunsaturated fatty acids (PUFA), can be observed. These associations may differ between men and women[180]. Some studies highlight a link between cholesterol and anxiety, rather than an effect on mood[181]. Nevertheless, changes in lipid metabolism have been proposed as potential biomarkers for depressive disorders [182].

In keeping with the variable findings above, the effects of statins on lipid metabolism and thus depression seem conflicting. An *in vitro* study shows that statin-mediated cholesterol depletion inhibits $5HT_{1A}$ receptor dynamics[123]. High-fat diet induces depressive and anxiety behaviours in rats, but these effects are counteracted by simvastatin[183]. Also, simvastatin administration affects mevalonate metabolites within the hippocampus and prefrontal cortex of rodents, with consequent modulation of emotional cognition[53], though another study highlights a detrimental association between cholesterol-lowering and altered behaviour, weight loss, and circadian disruption[184]. On the other hand, an antidepressant and anxiolytic effect of several statins is observed in rats in the absence of concurrent changes in plasma cholesterol[185]. An intriguing study in humans reports that, despite low LDL cholesterol levels correlating with depression, as also described above, such association is not observed when the cholesterol lowering is achieved via statins[186]. Furthermore, failure to improve the lipid profile, following statin therapy, in patients who suffered a myocardial infarction, seems associated with a higher incidence of depression at six months[187].

1.3.2.2 Hypothalamic-pituitary-adrenal axis and other hormones

Disturbances of glucocorticoids and the HPA or "stress" axis, closely related to abnormal inflammatory response, are known to play a major role in the pathophysiology of

depression[188], with elevated plasmatic cortisol potentially predicting the development of depressive disorders[189].

Some reviews have hypothesised that statins may mediate the relationship between lipid metabolism, stress, inflammation, and depression in animals[190] and humans[191,192]. Simvastatin[128] and atorvastatin[193] reduce glucocorticoid levels while expressing antidepressant-like effects in rats. Equally, the depressive[194] and anxiety[195] behaviours caused by chronic mild stress are neutralised with statins use. In humans however statins have been observed to have no effect on glucocorticoid receptors[196] or to even increase serum cortisol[92], although neither study specifically addresses whether these events eventually lead to the development of depression. A case report instead describes the onset of depressive symptomatology in a male whose simvastatin initiation was associated with a reduction of testosterone levels[197].

1.3.3 Cardiovascular effects of statins in depression

These include effects on vascular disease and overall quality of life.

1.3.3.1 Vascular disease

Atherosclerosis and endothelial dysfunction, which statins lessen via both cholesterol-mediated and other mevalonate-dependant pathways, appear involved in depression[198,199], especially in late-life according to the "vascular depression hypothesis"[200].

Statins are considered excellent candidates for reducing vascular dysfunction of the small white matter vessels in the neuroparenchyma, with consequent positive effects on depression[201]. In obese rats, atorvastatin administration reduces thromboxane and improves vascular reactivity while decreasing depressive-like behaviour[193]. One recent

human study shows that low doses of statins in depressed participants determine blood flow changes in key brain areas of mood and cognitive control as well as an improvement in depressive symptoms and markers of endothelial function[202,203].

1.3.3.2 Quality of life

On the back of strong bidirectional links between depression, cardiovascular disorders, and quality of life[204], some authors argue the ability of statins to prevent cardiovascular and cerebrovascular accidents can lead to improved quality of life and thus lower onset of depressive disorders[205,206]. However, no studies that explicitly investigate this issue could be retrieved.

1.3.4 Immunological effects of statins in depression

These include effects on innate immunity or inflammation and adaptive immunity.

1.3.4.1 Innate immunity (inflammation)

Extensive evidence suggests that immune processes, especially inflammatory ones, are prominent in depression pathophysiology[207]. Both peripheral and CNS inflammation appear causally involved[208].

Simvastatin[209-213] and atorvastatin[214] reduce depressive-like symptoms in animals by decreasing neuroinflammation thanks to the suppression of pro-inflammatory cytokines, P2X7-inflammasome complex, and microglia activation. In addition, reduction of circulating tumor necrosis factor (TNF)a by simvastatin is likewise associated with improved depressive behaviour[193].

Some translational human studies indicate that statins might positively affect mood by offsetting the peripheral pro-inflammatory effects of interleukin (IL)1b[215], IL6 and IL18[216,217], and C-reactive protein (CRP)[218]. Nonetheless, a study on a functional genetic polymorphism of superoxide dismutase (SOD)2, an enzyme responsible for the anti-inflammatory activity of rosuvastatin, could not observe any association with antidepressant response or CRP[219].

1.3.4.2 Adaptive immunity

Though with less consistency, disruptions in adaptive immunity (i.e., acquired humoral and cell-mediated immune system) have been implicated in depression [220].

No studies could be identified that directly assessed the effect of statins on these mechanisms. One study shows that atorvastatin can inhibit interferon (IFN)g-dependant cellular immunity, which is related to increased tryptophan availability[221]. Since tryptophan is the precursor of serotonin, it is suggested that statins might reduce the risk of depression by decreasing immune-mediated tryptophan degradation[222,223].

1.3.5 Summary

Overall, most pre-clinical evidence points toward an antidepressant and anxiolytic effect of statins by means of neurobiological, endocrine-metabolic, cardiovascular, and immunological mechanisms largely communicating with each other. A minority of studies report no effect, or even depressogenic and anxiogenic ones. Negative pre-clinical findings though are particularly susceptible to publication bias[224], therefore the paucity of the latter associations should be taken with caution. It is also possible that some studies may have escaped the articles' screening performed via Rayyan.

In any case, translation of findings from *in vitro*, animal, and indeed human studies to medical practice remains a particular challenge for mental illness[225]. Current animal models of neuropsychiatric disorders show significant limitations, which range from weak validation to poor predictive power for drug efficacy in human disease. Many of the symptoms observed in psychiatric disorders in humans (e.g., dysthymia, delusions, hallucinations) cannot be convincingly established in animals, and even when there are reasonable correlates (e.g., anxious or abnormal behavior, lack of motivation), the correspondence only seems approximate. Furthermore, psychiatric diagnoses are currently based on groups of symptoms which, in the absence of widely accepted abnormalities of molecules, synapses, cells, or neural circuits, are unlikely to be reflected in their full spectrum in animals. In the case of depression, along with low mood, several other symptoms can be seen in variable clusters among patients, such as anhedonia, guilt, neurovegetative symptoms including poor sleep, appetite, libido, and energy levels, and suicidality; of these, only a subset (e.g., neurovegetative symptoms) can be objectively measured in animals. Moreover, physiopathological anomalies involving the immune (e.g., excess inflammation) and endocrine (HPA axis hyperreactivity) systems are inconsistently observed in people with depression, therefore their validity in animal models remains scant.

Therefore, we now focus on the numerous studies that have assessed the clinical effects of statins in the context of depression.

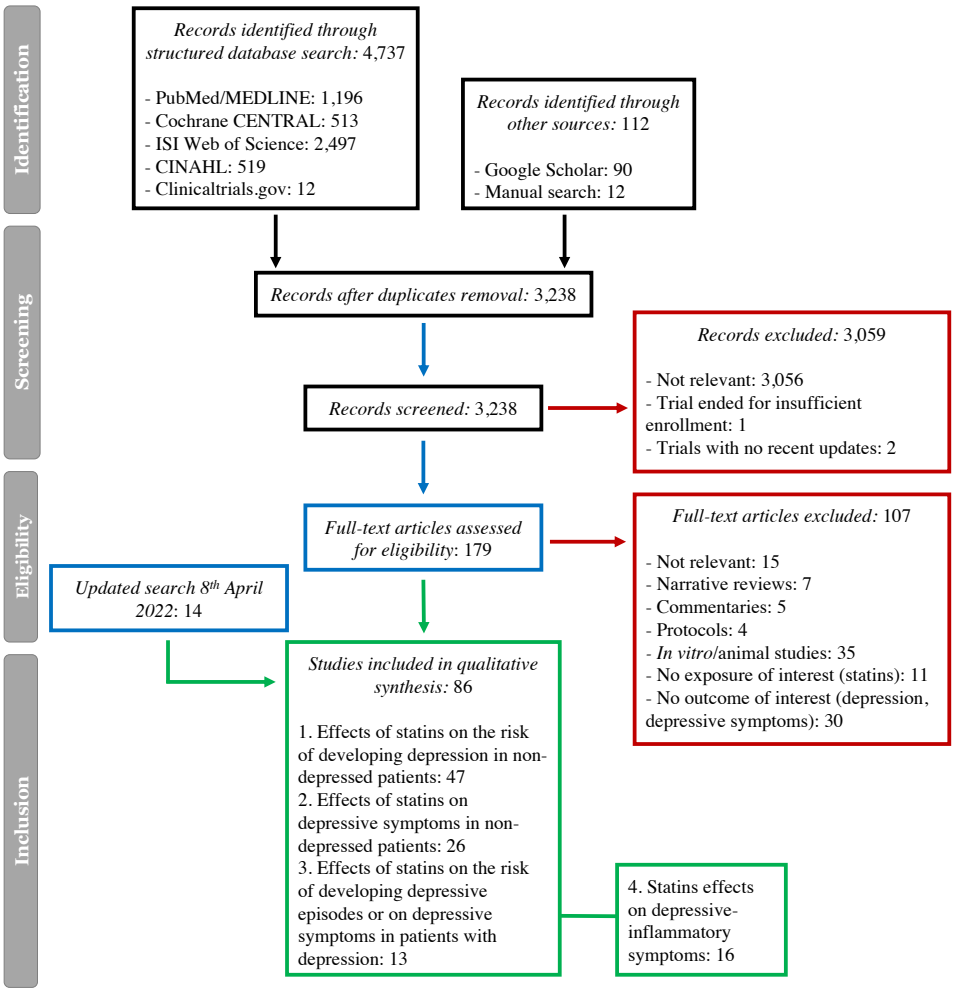
1.4 Statins in depression: clinical studies*

This section includes a systematic narrative review of clinical studies that report on any statin and their effect on depression, mood scores, or depressive-inflammatory symptoms (i.e., symptoms that appear particularly associated to an inflammatory phenotype of depression, such as anhedonia, psychomotor retardation, anxiety, and sleep disturbance, and that can be experimentally induced in animals or humans via exposure to an inflammatory trigger)[207] in people with depressive disorders.

The original literature search employs a previously validated search strategy (Appendix A1.2) run on PubMed/MEDLINE, Cochrane CENTRAL, ISI Web of Science, CINAHL, and ClinicalTrials.gov databases, as well as an unstructured search of Google Scholar for grey literature, from date of inception until 9th April 2021, leading to the inclusion of 72 studies. Exclusion criteria were: *in vitro* and animal studies; narrative reviews, letters, and commentaries not reporting new data; protocols; articles not involving the intervention/exposure (i.e., any statin) or outcomes (i.e., depression, mood scores, depressive-inflammatory symptoms in depressed patients) of interest. I have regularly updated this search until 8th April 2022, using the search algorithm described in section 1.3 and Appendix A1.1, ultimately adding 14 records, for a total of 86 studies. The search flow chart is in Figure 1.4.

*This section is based on the paper: "Statins in Depression: An Evidence-Based Overview of Mechanisms and Clinical Studies", De Giorgi et al., 2021; DOI:10.3389/fpsyt.2021.702617. Co-authors permission to reuse material is in Appendix, miscellanea

Figure 1.4 *Flow chart of the search for clinical studies*



Four areas of interest can be identified: effects of statins on the risk of developing depression in non-depressed patients (47 studies); effects of statins on depressive symptoms in non-depressed patients (26 studies); effects of statins on the risk of developing depressive episodes or on depressive symptoms in patients with depression (13 studies); effects of statins on depressive-inflammatory symptoms in patients with depression (16 studies). Point estimates with confidence intervals are reported for all studies, either extracted from the paper or computed from the data available; however, where this was not possible, the raw data or statistical test results provided in the individual

record are described. Studies are summarised in their respective category in Appendix A1.3, and an overview is provided in Table 1.2.

Table 1.2 *Overview of clinical studies*

	Positive effect	**No effect**	**Negative effect**
Meta-analyses of randomised controlled trials	Bai 2020 De Giorgi 2021 Köhler-Forsberg 2019 Lee 2021 Salagre 2016	De Giorgi 2022 Hang 2021 O'Neil 2012	
Clinical trials, randomised	Gengo 1995 Ghanizadeh 2013 Gougol 2015 Haghighi 2014 Sparks 2005	Berk 2020 Carlsson 2002 Chan 2017 Harrison 1994 Massardo 2022 Morales 2006	Hyyppä 2003

		Muldoon 2000	
		Robertson 2017	
		Santanello 1997	
		Sheridan 2014	
		Soh 2020	
		Stewart 2000	
		Visseren 2001	
		Wardle 1996	
Clinical trials, non-randomised	Krysiak 2018		
	Ormiston 2003		
Meta-analyses of observational studies	Parsaik 2014	Lee 2020	
		Zhang 2022	
	Chuang 2014	Al Badarin 2013	Kang 2015

Observational studies, cohort	Kang 2016	Asplund 2011	Köhler-Forsberg 2019
	Katzan 2017	Feng 2010	Li 2021
	Kessing 2019	Glaus 2015	
	Khokhar 2018	Hoogwegt 2013	
	Kim 2014	Huang 2017	
	Kim 2015a	Kim 2015b	
	Kim 2018	Mansi 2013	
	Köhler 2016	Medici 2017	
	Molero 2020	Smeeth 2009	
	Otte 2012	Wee 2016	
	Pasco 2010	Williams 2016	
	Redlich 2014		
	Stafford 2011		

	Wang 2017		
	Wium-Andersen 2017a		
	Wium-Andersen 2017b		
	Yeh 2019		
	Young-Xu 2003		
Observational studies, case-control	Williams 2016	Pasco 2010	
	Yang 2003		
Observational studies, cross-sectional	Otte 2012	Agustini 2019	Alghamdi 2018
	Feng 2008	Lindberg 1998	Asuzu 2015
	Mandas 2014	Williams 2015	Boumendil 1995
		Agostini 2007	Leutner 2021
		Olson 2008	
Case series			Cham 2016

	Davidson 1996
	Duits 1993
	Lechleitner 1992
	Rosenson 1993
	Tatley 2007

1.4.1 Effects of statins on the risk of developing depression in non-depressed patients

Forty-seven records (3 pair-wise meta-analyses, 26 cohort studies, 2 cohort and case-control studies, 1 cohort and cross-sectional study, 1 case-control study, 8 cross-sectional studies, and 6 case series) investigating the effects of statins on the risk of developing depression in non-depressed patients are included.

1.4.1.1 Meta-analyses

The earliest meta-analysis comprises 4 cohort, 2 nested case-control, and 1 cross-sectional studies over 9,187 non-depressed participants with median follow-up of 5 years and shows that statin users are 32% less likely to develop depression (95%CI 0.52 to 0.89)[226]. One of the included studies reporting no association[227] accounts for most of the meta-analysis' high heterogeneity (I^2 55%); when removed, the association with a possible antidepressant effect becomes stronger (OR 0.63, 95%CI 0.43 to 0.93) while heterogeneity decreases sensibly (I^2 2%). A larger meta-analysis of 13 observational studies over 5,035,070 participants initially reports comparable results (OR 0.85; 95%CI 0.72 to 0.99), but no association between statin use and depression risk could be confirmed when a trim-and-fill analysis (i.e., a method to correct for publication bias) is used (OR 0.87, 95%CI 0.74 to 1.02), suggesting that some smaller observational studies with negative results may have not been published[228]. The most recent meta-analysis investigates the association between several cardiovascular medications and affective symptoms and reports on 19 observational studies over 178,278 participants on statins, again not finding any association between these drugs and depression (OR 0.78, 95%CI 0.57 to 1.06)[229].

1.4.1.2 Cohort studies

Of the 29 cohort studies, 16 have a prospective cohort while 13 a historical cohort design.

Ten studies involve non-psychiatric populations with no specific physical comorbidities. A small study on 1,631 adults followed for 5.2 years finds no association (HR 1.25, 95%CI 0.73 to 2.14) between self-reported statin use and a formal diagnosis of depression[230]. A strong protective effect (HR 0.20, 95%CI 0.04 to 0.85) of statins and aspirin on the risk of developing depression is instead seen in 345 women[231], while another study does not replicate such a finding (HR 0.55, 95%CI 0.23 to 1.32) in 836 men[232]. No association (OR 1.02, 95%CI 0.94 to 1.11) between statin use and mood disorders is likewise reported in 13,944 adults[233]. A larger study on 129,288 statin users and 600,241 matched non-users followed for 4.3 years does identify any association (HR 1.01, 99%CI 0.96 to 1.06) between statin use and initiation of antidepressant treatment[234], while a similar study on 193,977 statin users and 193,977 matched non-users, highlights an increased risk of depression (HR 1.33, 95%CI 1.31 to 1.35) in the exposed group over 6.8 years, but such association becomes non-significant when adjusting for antidepressant use[235].

Comparable results are found in a recent, within-subject epidemiological investigation of 1,149,384 statin users followed for 8 years, which shows that presentations to psychiatric services for depressive disorders are less frequent during periods on statins, compared to periods off them (HR 0.91, 95%CI 0.88 to 0.94)[236]. Similarly, a study over 21 years of prescription data from 497,080 statin users finds that these medications seem to decrease the rate of incident depression (trend test for statin prescription 0.92, 95%CI 0.92 to 0.95)[237], in line with the results of a previous nationwide cohort study (N 4,607,990, OR 0.92, 95%CI 0.89 to 0.96) additionally suggesting a protective effect for the highly-lipophilic simvastatin (OR 0.93, 95%CI 0.89 to 0.97) and a harmful one for the more

potent but less lipophilic atorvastatin (OR 1.11, 95%CI 1.01 to 1.22) on the risk of developing depression[238]. In contrast with the latter, another study on 299,298 participants comparing lipophilic versus hydrophilic statins over a 3-year follow-up highlights a worsened risk of depression for simvastatin only (HR 1.09, 95%CI 1.02 to 1.16), but not for all lipophilic statins compared to hydrophilic ones (HR 1.05 95%CI 1.00 to 1.10)[239].

The remaining cohort studies focus on patients with comorbid somatic illness. One explores the relationship between hyperlipidaemia, statins use, and depression: 26,852 hyperlipidaemic and 107,408 non-hyperlipidaemic patients are compared over 4 years, showing a lower risk of depression among hyperlipidaemic patients receiving statins (HR 0.81, 95%CI 0.69 to 0.96), while no effect is observed in non-hyperlipidaemic participants[240]. Five studies include people with cardiovascular conditions. One historical cohort study on 91,842 patients with acute coronary syndrome (ACS) and 91,860 non-ACS matched controls shows that statin use is associated with decreased risk of both early (<1 year) (HR 0.94, 95%CI 0.86 to 0.94) and late (>12 years) (HR 0.96, 95%CI 0.82 to 0.90) depression, but only in the ACS group[241]. The other studies on cardiological patients are prospective and include smaller samples between 193 and 711 participants: all report a lower risk of depression at 9 months (OR 0.21, 95%CI0.052 to 0.876)[242], 1 year (23.3% *versus* 32.8)[217], 4 years (OR 0.63, 95%CI 0.43 to 0.93)[243], and 6 years (OR 0.62, 95%CI 0.41 to 0.95)[244] in statin users with ACS or coronary artery disease (CAD). Eight studies involve patients with cerebrovascular disease. Two show increased risk of post-stroke depression among statin users compared to non-users (N 11,218, HR 1.59, 95%CI 1.30 to 1.95)[245] and in pre- and post-stroke statin users, compared to pre-stroke users only (N 1,371, HR 1.99, p 0.037)[246] over 1 year. A larger study on 70,706 stroke patients shows however no effect (OR 0.99, 95%CI 0.95 to 1.03) of statin prescription on self-reported low mood or antidepressant use at a 3-month follow-up[247]. All remaining studies indicate

a beneficial effect of statins on post-stroke depression at several timepoints: four are on smaller samples of 288 (OR 0.54, 95%CI 0.49 to 0.87)[248], 286 (Wald 8.477, p 0.004)[216], 803 (MD -1.91, 95%CI -3.34 to -0.47)[249], and 1,023 participants (3-month OR 0.69, 95%CI 0.49 to 0.96; 3-year OR 0.40, 95%CI 0.20 to 0.90)[250], whereas a fifth on a larger historical cohort of 147,487 stroke patients and 160,235 matched individuals followed for 12 years shows a risk reduction among both stroke patients (HR 0.90, 95%CI 0.87 to 0.93) and non-stroke patients (HR 0.90, 95%CI 0.86 to 0.94) using statins[251]. Two studies assess survivors of traumatic brain injury. One shows that statin use is associated with fewer depression diagnoses at 6 months (N 100,515, RR 0.85, 95%CI 0.79 to 0.90)[252], while the other reports a higher risk of depression in patients that are also hyperlipidaemic (N 3,792 HR 1.61, 95% CI 1.03 to 2.53), but no significant difference between patients treated with statins or not, regardless of their hyperlipidaemic (HR 0.63, 95%CI 0.34 to 1.17) or normolipidaemic (HR 1.02, 95%CI 0.55 to 1.89) status at 3 years[253]. One study of 9,139 patients with chronic respiratory syndrome sees statin users at lower risk of depression for up to 11 years compared to non-users (HR 0.36, 95%CI 0.25 to 0.53)[254]. Finally, two studies on 408 hyperlipidaemic head and neck cancer patients at 1 year (HR 0.85, 95%CI 0.46 to 1.57)[255] and 12,176 patients under intensive care at 3 years (RR 1.04, 95%CI 0.96 to 1.13)[256] finds no effect of statins on depression risk.

1.4.1.3 Case-control studies

Two cohort studies on non-depressed patients also include a case-control analysis: one reports non-significant results (N 345 females, OR 0.13, 95%CI 0.02 to 1.02)[231], whilst the other shows a strong protective effect of statins (N 937 males, OR 0.10, 95%CI 0.10 to 0.40)[232]. Another case-control study on 366 hyperlipidaemic participants shows a

decreased risk of new-onset depression in statin users versus non-users (OR 0.40, 95%CI= 0.20 to 0.90)[205].

1.4.1.4 Cross-sectional studies

Nine cross-sectional studies are here presented. A recent study on a nationwide database (N 7,481,168) finds an overrepresentation of depression in statin users compared to non-users (OR 1.22, 95%CI 1.20 to 1.25), but this association is reportedly reversed when considering low dosages of statins, and worsened in high-dose statin users[257]. Finally, a cross-sectional study observes increased absenteeism from work due to depression among employees reporting statin use (N 17,244, prevalence ratio 2.18, 95%CI 1.18 to 4.03)[258]. Another investigation using a Mendelian randomisation methodology similarly found 12% increased odds of depression in people using statins (N 188,000, 95%CI 1.04 to 1.18)[259]. A small (N 166) within-subject study finds no change (RR 1.06, 95%CI 0.79 to 1.45) in the redemption of antidepressants prescription before *versus* after the initiation of statins[227]. One article on patients with heart conditions shows a reduction in the risk of developing depression in statin users (N 965, OR 0.66, 95%CI 0.45 to 0.98)[244]. All other studies specifically comprise elderly participants. Outcomes based on standardised scales for depression score above threshold are reported in three studies with conflicting results: one shows a decreased prevalence of depression in statin users (N 2,804, OR 0.71, 95%CI 0.52 to 0.97)[260], another no difference among South Asian and African-Caribbean (N 695, OR 1.67, 95%CI 0.97 to 2.88) nor White Europeans (N 638, OR 0.54, 95%CI 0.26 to 1.13) statin users[261], in spite of a significant ethnicity-statin interaction (p 0.041), as well as a further study in 19,114 community-dwelling participants showing no association (OR 1.09, 95%CI 0.98 to 1.20)[262]. Finally, a noteworthy study in 208 healthy octogenarians shows that the odds of depression are higher among statin users (OR 2.50, 95%CI 1.10 to

5.60), but this seems particularly true in patients who have low cholesterol, whereas such association wanes in those who have high baseline cholesterol, suggesting that cholesterol homeostasis could play an important mediating role[263].

1.4.1.5 Case series

Six case series are included, all expectedly reporting negative effects of statins on affective and other psychiatric symptoms. The largest one investigates accounts from the New Zealand Centre for Adverse Reaction Monitoring and shows that, of the 203 reports of psychiatric adverse events associated with statins, 67 concern mood disorders[264]. Another reports 12 cases of onset of violent ideation, irritability, depression, and suicide that resolved upon statins discontinuation and recurred on re-challenging[265]. One case series of 6 males with hypercholesterolaemia treated with statins shows that depression scores worsened in 4 of them by 6 weeks, with 2 reaching the threshold for a mild depressive episode[266]. Two further case series include 8 reports of female patients suffering depressive and psychotic symptoms after initiation of pravastatin and simvastatin that resolved on discontinuation[267,268], and a similar clinical course is described in another article concerning 2 males treated with lovastatin[269].

1.4.2 Effects of statins on depressive symptoms in non-depressed patients

Twenty-six records (1 network meta-analysis and 3 pair-wise meta-analyses, 16 clinical trials, 3 cohort studies, 3 cross-sectional studies) investigating the effects of statins on depressive symptoms in non-depressed patients are included.

1.4.2.1 Meta-analyses

Most retrieved meta-analyses show similar results. An earlier pair-wise meta-analysis of 7 RCTs (N 2105) with follow-up between 4 weeks to 4 years shows no overall difference in depression scores between participants receiving statins or placebo (SMD -0.08, 95%CI -0.29 to 0.12)[270]. A more recent pair-wise meta-analysis assesses all anti-inflammatory drugs in a mixed sample of non-depressed patients with baseline depressive symptoms and patients with a diagnosis of depression: with regards to the statin trials (both in add-on to antidepressants and in monotherapy), 7 different RCTs (N 1,576) are retrieved and support a small antidepressant effect size for statins (SMD -0.26, 95%CI -0.48 to -0.04)[271]. A larger pair-wise meta-analysis on statins only, though again on a diverse sample of participants, includes 10 RCTs (N 2,517) and confirms the above findings (SMD -0.31, 95%CI -0.52 to -0.09), though with high heterogeneity and unclear risk of bias[272]. Despite the overlapping inclusion/exclusion criteria of these meta-analyses, only 1 article is included in all of them[273], whereas 2 studies[274,275] are only in the older one by O'Neil and colleagues, 2 studies[276,277] only in Köhler-Forsberg and colleagues, 1 study[278] only in Yatham and colleagues, and 4 studies[279-282] in those by Yatham and colleagues and O'Neil and colleagues, but not by Köhler-Forsberg and colleagues. Finally, a recent network meta-analysis of 13 RCTs (N 2,479), also containing a pair-wise component, shows that statins are overall better than placebo in reducing depressive symptoms (SMD -0.35, 95%CI -0.59 to -0.11), with atorvastatin seemingly the most efficacious one[283].

1.4.2.2 Clinical trials

Sixteen trials assess the effect of statins on mood scores in people with or without subclinical depression. Three RCTs demonstrate opposing effects of statin administration on depression scores. A crossover RCT including 36 hypercholesteraemic participants

followed-up for 4 weeks shows a reduction in depressive symptoms due to lovastatin and pravastatin (SMD -0.63, 95%CI -1.21 to -0.05)[279]. Likewise, a RCT of 63 elderly patients with mild-to-moderate Alzheimer disease finds a large reduction in depressive symptoms (MD= -2.52, 95%CI= -3.18 to -1.87) associated with high-dose atorvastatin over 52 weeks[284]. In contrast, another crossover RCT assessing the separate and combined effects of simvastatin and Mediterranean-type diet in 120 hyperlipidaemic men followed-up for 24 weeks highlights an increase in depressive scores in the statin group (MD 0.06, 95%CI 0.01 to 0.12)[274]. None of the remaining trials report significant changes in depression scores associated with statins. One study on 25 healthy volunteers shows no effect of simvastatin or pravastatin compared to placebo on depressive symptoms at 4 weeks (MD 0.05, 95%CI -0.51 to 0.60)[280]. Similar results are reported in a sample of 209 hyperlipidaemic adults treated with lovastatin *versus* placebo for 6 months (SMD 0.21, 95%CI -0.07 to 0.49)[281]. Two further RCTs on larger samples (N 1,130 and N 621) of individuals with heart conditions and longer follow-ups (4 years and 152 weeks) respectively find no differences in depression scores between study arms (MD 0.49, 95%CI -0.30 to 1.28[273] and $\chi 2$ 1.66, linear trend 0.08[282]). Three trials on 431[277], 80[275], and 41[276] older adults separately show no differences (SMD -0.08, 95%CI-0.29 to 0.14; SMD 0.00, 95%CI-0.46 to 0.46; SMD 0.09, 95%CI -0.57 to 0.76) at 15 weeks, 6 months, and 1 year in depression scores. A trial on 140 patients with multiple sclerosis finds no difference in depressive symptoms (SMD -1.00, 95%CI -3.20 to 1.20) between simvastatin treatment and placebo at 2 years[285]. No difference (SMD 0.05, 95%CI -0.49 to 0.59) is again reported in 52 mild traumatic brain injury patients using atorvastatin for 3 months[278]. Two RCTs employ the less commonly prescribed fluvastatin: one in 60 patients with hepatitis C[286]., and one in 87 patients with comorbid type 2 diabetes mellitus

and dyslipidaemia[287], yet neither highlight any difference between study arms (respectively, MD -0.11, SE 0.76 and SMD -0.28 95%CI -0.70 to 0.14).

Two further trials are non-controlled and non-randomised. One pilot (N 12) double-blind study does not include a placebo arm and reports an improvement in depressive scores (t 2.27, df 11) following treatment with statins[288]. Another on 14 hypercholesteraemic women receiving 24 weeks treatment with atorvastatin and 14 normolipidaemic patients left untreated shows that depression scores at baseline are significantly higher among the hypercholesteraemic ones ($p<0.05$) and treatment with statins normalises such scores[289].

1.4.2.3 Cohort studies

All 3 cohort studies for this category show no effect of statins on depression. One on 1,691 patients with ACS observes that statin prescription does not seem to affect the otherwise progressive decrease in depression scores (MD -0.05, 95%CI -0.67 to 0.58) at 1 year follow-up[290]. Similar results (MD -0.97, 95%CI -1.99 to 0.05) are reported in 409 patients with an implanted cardiac device[291]. Another study on 1,803 elderly participants concludes that statin use does not correlate with increased depressive symptoms (p 0.23) at 1.5 years follow-up, though *post-hoc* analyses reportedly suggest a protective effect of statins in female participants and an opposite effect in men[260].

1.4.2.4 Cross-sectional studies

Of the 3 cross-sectional studies retrieved, 2 do not show any association between statins use and depression scores: one conducted on 756 elderly veterans (beta -0.18, 95%CI -0.69 to 0.33) [292] and the other on 525 female patients with heart conditions (p 0.94)[293]. The

remaining study involves again elderly dyslipidaemic patients but instead reports an increase in depressive symptoms in statin users (N 329, p 0.018)[294].

1.4.3 Effects of statins on the risk of developing depressive episodes or on depressive symptoms in patients with depression

Thirteen records (1 network meta-analysis and 3 pair-wise meta-analyses, 7 clinical trials, 2 cohort studies) investigating the effects of statins on the risk of developing depressive episodes or on depressive symptoms in patients with depression are included.

1.4.3.1 Meta-analyses

Two pair-wise meta-analyses report results on the effect of statins as add-on treatment in depressed patients, with similar results. They both include 3 RCTs[295-297] and show an improvement in depressive symptoms (SMD -0.73, 95%CI -1.04 to -0.42[298]; SMD -0.65, 95%CI -0.96 to -0.33[299]) due to statin add-on therapy. One meta-analysis that uses a network model, also comparing other anti-inflammatory medications, includes only the 3 abovementioned trials with regards to statins, yet does not observe any effect on depression (RR 0.68, 95%CI 0.38 to 1.18)[300].

1.4.3.2 Clinical trials

Seven clinical trials assess the effect of statins in depressed patients, 4 of which are included in some of the meta-analyses described above. In the earliest of these, 68 depressed patients are randomised to 6 weeks of either fluoxetine plus lovastatin or fluoxetine plus placebo: depression scores decrease considerably in both groups, but more noticeably in the combination treatment group (t 3.4, df 60)[295]. A similar 6-week trial on

simvastatin plus fluoxetine randomises 48 depressed patients and shows comparable results (MD 4.81, p 0.02), though overall remission rates are not significantly different between study arms (59% versus 45%, p 0.36)[297]. In a 12-week trial, 60 depressed patients receive citalopram for 1 week and are then randomised to either atorvastatin or placebo adjunction: results show significantly lower depressive scores ($F_{3,174}$ 8.93) and increased partial remission (OR 8.83, 95%CI 1.02 to 76.96) for the statin group[296]. A larger RCT includes 130 depressed patients aged 15 to 25 years old, randomised to receive either treatment as usual (TAU) plus placebo, or TAU plus aspirin, or TAU plus rosuvastatin, and finds that changes in depression scores are not significantly different when comparing rosuvastatin and placebo group (MD -4.20, 95%CI -9.10 to 0.60) nor when comparing rosuvastatin and aspirin groups (MD -6.40, 95%CI -11.70 to 1.20)[301]. Another RCT compares the relapse risk in a sample of 60 bipolar or depressed patients randomised to either lithium plus atorvastatin or lithium plus placebo, and reports no effect $\chi 2$ 0.148, p 0.70)[302]. A small (N 10), recent RCT randomises participants to either rosuvastatin or placebo plus SSRI: improvements on depression scales are reported for both groups, and these seem larger in those treated with a statin on one of the scales (BDI change from baseline, placebo p 0.038 *versus* rosuvastatin p< 0.001), but no test for directly comparing the two groups is performed[203]. Finally, a RCT comparing the antidepressant effects of simvastatin *versus* atorvastatin, without a placebo arm, in 46 post-coronary artery bypass patients with comorbid mild-to-moderate depression shows that depression scores at 6 weeks decrease more prominently in the simvastatin group (MD 3.63, 95%CI 0.44 to 6.51)[303].

1.4.3.3 Cohort studies

Two cohort studies assess the effect of statins in depressed patients. Kim and colleagues analyse 1-year follow-up data of a previous 24-week RCT of escitalopram in 300 patients with comorbid ACS and depression: statin users show higher response rates on two different scales, namely the Beck Depression Inventory and the Hamilton Depression Rating Scale (OR 2.23 to 2.82, 95%CI range 1.11 to 5.90) but notably no differences in response (OR 0.89 to 1.19, 95%CI range 0.36 to 3.18) when considering the effect of statin monotherapy in patients not on antidepressants (N 146); moreover, a higher response rate is observed in users of lipophilic *versus* hydrophilic statins users (OR 2.91, 95%CI 1.21 to 6.99[304]. Then, a large (N 872,216) historical cohort study compares several outcomes associated with depression in a group of SSRI plus statin and a group of SSRI-only users, showing a lower risk for psychiatric hospital contacts (HR 0.75, 95%CI 0.69 to 0.82) and psychiatric hospital contacts specifically due to depression (HR 0.64, 95%CI 0.55 to 0.75) among statin users, with no differences in all-cause mortality (HR 1.04, 95%CI 0.96 to 1.12) and suicidality (HR 0.85, 95%CI 0.61 to 1.18) at 3 years[305].

1.4.4 Effects of statins on depressive-inflammatory symptoms in patients with depression

Sixteen of the studies described above also investigate the effects of statins on depressive-inflammatory symptoms in patients with depression – symptoms so defined because inflammatory stimuli seem to particularly induce them[207]

1.4.4.1 Anhedonia

No paper specifically addressing the effect of statins on anhedonia can be identified.

1.4.4.2 Psychomotor retardation

Three of the abovementioned RCTs also examine the effect of statins on psychomotor retardation. A study on 209 hyperlipidaemic adults reports an improved psychomotor activity in the placebo group *versus* lovastatin-treated subjects (Z score 0.17, 95%CI 0.05 to 0.28)[281]. The two remaining studies describe however no difference between statin and placebo groups on a test assessing psychomotor retardation, though such data are not reported[277,280].

1.4.4.3 Anxiety

Nine studies measure anxiety as well as depression scores. A meta-analysis of two cross-sectional studies in 8,692 people with background cardiovascular disorders observes that statin use seems associated with lower anxiety scores or diagnosis of anxiety disorders (OR 0.79, 95%CI 0.71 to 0.88)[229]. A recent RCT finds no difference in an anxiety scale scores between rosuvastatin and placebo groups (MD -0.60, 95%CI -1.70 to 3.00)[301], and the same in a crossover RCT on 25 healthy volunteers using pravastatin (mean 3.2, 95%CI 2.0 to 4.4), simvastatin (mean 2.5, 95%CI 1.7 to 3.3), and placebo (mean 3.1, 95%CI 2.2 to 4.0)[280]. Another RCT on 621 adults with CAD shows no difference in anxiety scores ($\chi 2$ 3.57, linear trend 0.07) between simvastatin and placebo after a 152-week follow-up[282]. Likewise, two further RCTs on patients with previous ACS (N 1,130)[273] and hyperlipidaemia (N 120)[274] do not show any effect of statins on anxiety (MD 0.49, 95%CI -0.30 to 1.28; MD 0.00, 95%CI -0.05 to 0.05, respectively). Conversely, a cohort study on 371 CAD patients highlights an improvement in anxiety among statin users (OR 0.69, 95%CI0.47 to 0.99)[243]. Another cohort study on patients with implantable cardiac device (N 409) reports non-significant differences in anxiety scores when statins are used (MD -0.81, 95%CI -1.80 to 0.18)[291]. Lastly, a recent cohort study on a nationwide register of

statin users (N 1,149,384) shows no difference in the risk of anxiety disorders presentation when people are on statins compared to periods off statins (HR 0.99, 95%CI 0.95 to 1.02)[236].

1.4.4.4 Sleep

Four papers also investigate the effect of statins on sleep symptoms in depression, and none can find any association. Harrison and colleagues do not find differences on sleep scale scores between simvastatin (mean 47.0, 95%CI 44.9 to 49.1), pravastatin (mean 51.4, 95%CI 48.4 to 54.6), and placebo (mean 50.1, 95%CI 46.4 to 53.8)[280]. A positive effect of simvastatin, though of unclear clinical and statistical value, is seen in a sample of CAD patients, with sleep disturbances reported by 48.8% of patients taking simvastatin and 54% of those taking placebo[282]. Two further RCTs on respectively 431[277] and 41[276] older adults randomised to statins or placebo do not identify any changes on a sleep dysfunction scale (p 0.93; p 0.76, respectively).

1.4.5 Summary

In this section, I have provided a descriptive overview of 86 clinical studies on the effects of statins in depression. The high number of articles retrieved, as well as the presence of a trend for larger and more robust studies, emphasise the interest of the scientific community in this research area with potential implications for clinical practice. Such awareness likely stems from two factors: firstly, statins are among the most commonly prescribed medications worldwide[41], therefore the discovery that their use is associated with either depressogenic or antidepressant effects would have a substantial impact on public health or psychiatric care respectively; secondly, the relative lack of any breakthrough development of novel antidepressants capable of targeting alternative

biological pathways and relatively free from concerns about adverse events[19,306] makes the potential use of statins in depression especially appealing.

Whether statins are a public health concern because of depressogenic activity in non-depressed people seems less likely, from a purely numerical perspective, when looking at the majority of studies reporting either no effect (37) or indeed a positive one (23) on depression, as compared to those showing a negative effect (14). Epidemiological data is particularly informative here: the largest meta-analysis of observational studies includes over 5 million non-depressed participants and does not observe any association between statin use and the risk of developing depression[228]. Data on the effect of statins on depression scores, which are more sensitive to smaller changes in mental state, in non-depressed or mixed populations are only available for small samples mainly from RCTs[270-272,283], likely due to national registers not routinely recording measures on depression scales – an important avenue for further research. Still, taken together, these results might provide some reassurance that statins are unlikely to provoke the onset of depressive episodes in otherwise healthy populations.

For studies directed at groups of depressed patients, numbers appear even more favourable (positive effect, 8 studies; no effect, 5 studies; negative effect, no studies). This crude dichotomy suggests that statins might indeed have antidepressant action in people suffering from depression, while they do not improve mood in non-depressed subjects, as it is the case for conventional antidepressants. In line with this, a pair-wise meta-analysis of RCTs on a mixed population hints, via a subgroup analysis, that only the depressed sample shows improved mood while on statins, whereas such effect is not apparent in the non-depressed subgroup[272].

Matching this concept to the previously described pre-clinical findings, statins might express an antidepressant activity only in those people with underlying abnormal neurobiological, endocrine-metabolic, cardiovascular, or immunological processes that directly contribute to a depressive symptomatology – if indeed these can be identified; on the other hand, statins would have no effect or even a negative one if they perturbed physiological processes that are necessary for neuronal integrity and the upkeep of a normal mental state, especially in the absence of any baseline depressive disorder.

Several demographic and clinical variables linked to these biological systems could likewise play a significant role. For example, elderly people are more likely to present with immunological and metabolic anomalies[307] and in line with this, the older the patient, the higher the apparent benefit of statins on depression[238], and perhaps vice versa in younger populations[301]. Sex differences in immune function might explain why women, generally more prone to over reactivity of the immune system[308], seem to respond more than men to the antidepressant effect of statins[231,232,260]. Physical comorbidities such as dyslipidaemia[253], cardiovascular[241] or cerebrovascular[251] diseases, and inflammation[216,217] can likewise mediate the antidepressant activity of statins. Finally, the use of a statin in addition to an antidepressant, as compared to a statin alone, could differentially explain the observed improvement in depressive symptoms[272,304].

Another factor potentially contributing to the heterogeneity of these findings relates to the notion that all statins are equally capable of expressing an antidepressant effect or indeed any neurobiological effect at all. As seen before, despite most statins sharing similar pharmacodynamic properties, their pharmacokinetics and especially lipophilicity, thus ability to cross the BBB, vary dramatically[52]. Accordingly, all retrieved RCTs that employ a lipophilic statin (i.e., lovastatin, atorvastatin, simvastatin) in depressed patients observe

an improvement in depressive symptoms[295-297], while the only trial that could not replicate these results uses the hydrophilic rosuvastatin[301]. Simvastatin, the most lipophilic molecule, also displays a more pronounced antidepressant activity compared to the less lipophilic atorvastatin[303,309], with certain observational studies replicating this finding in non-depressed populations[236,238], though other studies appear in contrast[239,283].

Finally, despite some evidence connecting inflammation with the beneficial effects of statins in depression, few to no studies explicitly addressing whether statins affect cardinal depressive-inflammatory symptoms of psychomotor retardation and anhedonia[207] could be retrieved. Conversely, more data are available for anxiety and sleep disturbance. Pre-clinical evidence, reported above, supports both an anxiogenic[147,156] and anti-anxiety[120,127,183,195] effect of statins, yet most clinical studies show no effect[236,301] or indeed an improvement[243] of anxiety scores in statin users. Moreover, in contrast with extensive literature describing an association between statin use and sleep problems in the general population[310], none of the retrieved studies report similar adverse events in the context of depression.

1.5 Conclusion

Following an outline of statins' pharmacology, this introductory chapter offers a comprehensive review of the literature on the effects of statins in depression. In spite of the existing amount of data, several knowledge gaps and inconsistencies remain. This doctoral thesis aims to fill some of these gaps and resolve certain discrepancies of the available scholarship.

Firstly, clinical trials and pooled analyses of them are unclear on whether statins can express any activity in depression regardless of concurrent use of antidepressant

medications. Therefore, I will conduct two new meta-analyses of randomised controlled trials: one comparing statin against placebo (Chapter 2), another statin plus antidepressant *versus* antidepressant alone (Chapter 3), investigating the antidepressant efficacy, treatment discontinuations, and adverse events of such interventions. I will then address the same issue, but from an epidemiological perspective, respectively in Chapter 4 and Chapter 5. These studies, which involve the electronic health records from an innovative UK-based primary care database, can benefit from real-world information from a large, unselected population with depressive disorders, including their mood scores over longer follow-ups, thus providing a precious complement to data from the clinical trials above. Finally, no study to date has assessed the behavioural effects of statins on a cognitive neuropsychological model of depression under laboratory conditions. Therefore, using an experimental medicine framework, I will explore the neuropsychological effects of the more potent but less lipophilic atorvastatin (Chapter 6) and the highly lipophilic simvastatin (Chapter 7) in two samples of healthy volunteers.

Chapter 2. Effects of statin monotherapy in depression – a meta-analysis of randomised controlled trials*

*This chapter is based on the paper: "The effects of statin monotherapy on depressive symptoms: a systematic review and meta-analysis", De Giorgi et al., 2022; DOI:10.1016/j.jad.2022.05.113. Co-authors permission to reuse material is in Appendix, miscellanea

2.1 Introduction

In Chapter 1, I have discussed the pre-clinical and clinical evidence for the effects of statins in depression. Whether statins can reduce depressive symptoms regardless of concurrent antidepressant medications, and indeed on the background of a diagnosis of subclinical rather than depression, remains an equivocal matter[311]. A meta-analysis of randomised controlled trials considering statins both with and without antidepressants in mixed populations with depressive symptoms described an overall benefit of such interventions on depression rating scores [N 2,517, standardised mean difference (SMD) -0.31, 95% confidence interval (CI) -0.52 to -0.09], but this outcome seemed mostly driven by those few studies that employed statins along with antidepressants in patients diagnosed with depression[272]. A similar issue likely affected a network meta-analysis aiming to assess the comparative efficacy of different statins[283]. Findings from observational studies appear more conflicting, since no effect [N 5,035,070, odds ratio (OR) 0.87, 95%CI 0.74 to 1.02][228], a reduction [N 1,149,384, hazard ratio (HR) 0.91, 95%CI 0.88 to 0.94][236], and an increase (N 7,481,000, OR 1.22, 95%CI 1.20 to 1.25)[257] in incident depression were found for statin users.

In view of this uncertainty, I present a systematic review and meta-analysis of randomised controlled trials to investigate the effects of any statin monotherapy in adult participants with depressive symptoms.

2.2 Methods

The study protocol was registered on PROSPERO international prospective register of systematic reviews with reference CRD42022306653 and is available at https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=306653.

2.2.1 Search strategy

I run an extensive search of the PubMed/MEDLINE, Embase, and PsycINFO databases via Ovid SP, as well as Cochrane CENTRAL, ClinicalTrials.gov, and WHO portal from date of inception until 22nd of January 2022, with no language restrictions. References of the included papers were manually screened for further relevant material. The search algorithms (Appendix A2.1) combined index terms and free-text words for statins, depression/depressive symptoms, and clinical trials.

2.2.2 Inclusion criteria

Randomised controlled trials that compared the effects of any statin monotherapy (i.e., not in add-on to any antidepressant strategy) *versus* any control condition on depressive symptoms were included. Quasi-randomised trials, such as those allocating treatment by using alternate days of the week, were excluded. For trials with a cross-over design, data from the first randomisation period was extracted and analysed whenever possible.

2.2.3 Outcomes

For efficacy, results were computed at studies' endpoints as well as at the following timepoints (or their closest in a contiguous range): early (<6 months), medium (6-12

months), long (>12 months). For acceptability, tolerability, and safety, results were calculated at the studies' endpoints.

2.2.3.1 Primary outcome

The primary efficacy outcome was the mean value on any standardised scale for depressive symptoms (e.g., Center for Epidemiologic Studies Depression Scale, Geriatric Depression Scale, Hamilton Depression Rating Scale) at studies' endpoint.

2.2.3.2 Secondary outcomes

Secondary outcomes included: efficacy, measured as the mean value on any standardised scale for depressive symptoms at three timepoints: early (<6 months), medium (6-12 months), long (>12 months); acceptability, measured as the number of participants discontinuing treatment (dropouts) due to any cause at studies' endpoints; tolerability, measured as the number of participants discontinuing treatment (dropouts) due to adverse events at studies' endpoints; safety, measured as the number of participants with any adverse events, self-harm episodes, and suicides at studies' endpoints.

2.2.4 Study selection, data extraction, quality assessment

Two researchers (NRP, SW) independently screened the retrieved records' title/abstract and assessed the relevant full-text articles for eligibility. Any disagreement was discussed with a third researcher (RDG) and resolved by *consensus*.

Three researchers (NRP, RDG, SW) used a structured data extraction spreadsheet to ensure consistency of appraisal for each study. For the included studies, data about first authors' name, year of publication, study design, sample size and characteristics,

intervention and comparison details, length of follow-up, primary and secondary outcome measures of interest with point estimates were extracted. Graph data was extracted using WebPlotDigitizer v4.2 software[312]. For means and standard deviations not readily available, I estimated values from medians and interquartile ranges or employed the RevMan v5.4 software calculator according to guidelines[313,314].

Risk of bias of the included trials was assessed using the "RoB 2: A revised Cochrane risk-of-bias tool for randomised trials"[315], and the certainty of evidence for all outcomes via GRADEpro GDT[316]. The quality assessment was performed by two independent raters (NRP, RDG) and disagreements were discussed with another member of the review group (SW) and resolved by *consensus*.

2.2.5 Statistical analysis

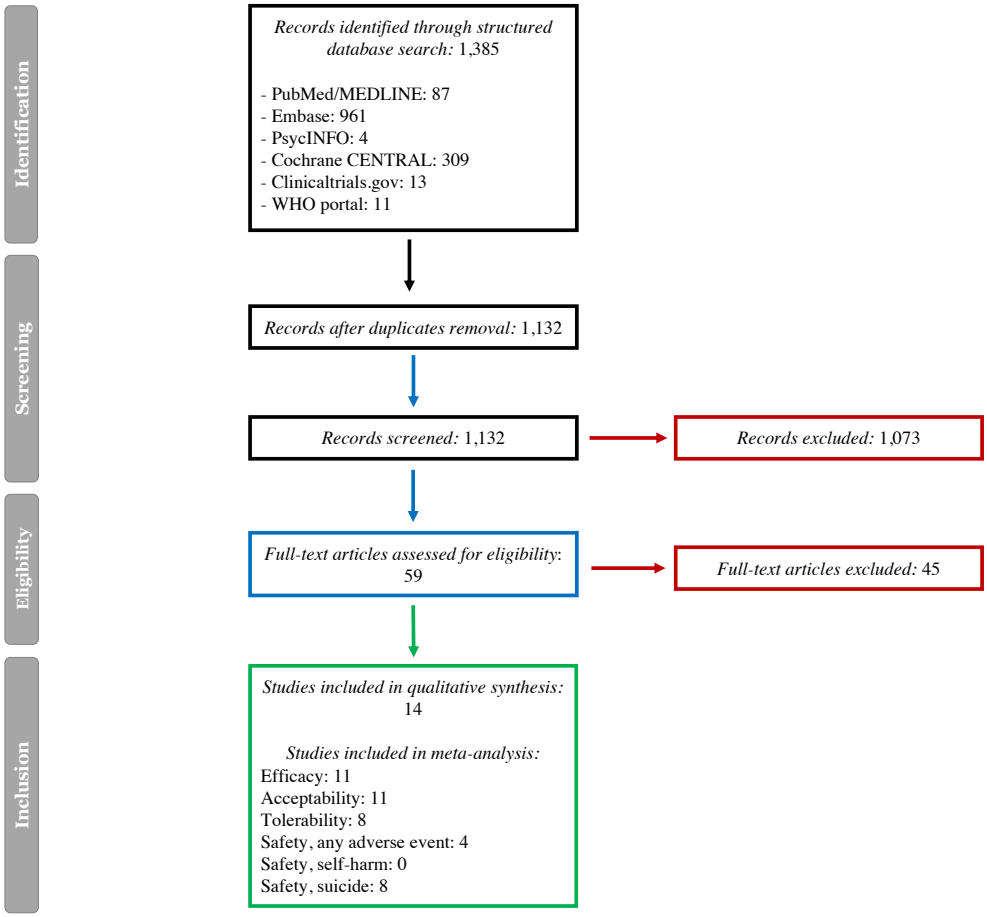
All the extracted data was analysed using STATA v17 software[317]. Data from rating scales for depression were analysed as a continuous variable using standardised mean difference (SMD, as different depression rating scales were used) with 95% confidence intervals (CI), employing random-effect models, more conservative than a fixed-effect model, in view of the heterogeneous study populations included. For evaluating the clinical significance of SMD values, the effect size was considered 'small' if SMD <0.40, 'moderate' if SMD 0.40 to 0.70, and 'large' if SMD >0.70[313]. All the other quantitative data (e.g., number of participants discontinuing treatment) were analysed as dichotomous variables using relative risk (RR) with 95%CI, employing random-effect models. Heterogeneity between studies was investigated through the I^2, t^2, and *p-value* statistic and by visual inspection of forest plots. If at least ten studies were available, funnel plots and

Egger's test to detect publication bias were used[313]. Sensitivity and subgroup analyses were conducted, as appropriate, to verify the robustness of the primary outcome findings.

2.3 Results

The literature search is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline[318] in Figure 2.1. In summary, it retrieved 1,385 records from electronic databases (Cochrane CENTRAL: 309, Embase: 961, PubMed/MEDLINE: 87, PsycINFO: 4, ClinicalTrials.gov: 13; WHO portal: 11), and no further papers from the manual search. After duplicates removal, 1,132 records' title and abstract were screened by applying the inclusion/exclusion criteria previously described. Fifty-nine potentially eligible articles were assessed full text, of which fourteen were ultimately included in this systematic review.

Figure 2.1 *PRISMA flow diagram*



2.3.1 Trials characteristics and risk of bias assessment

The characteristics of the fourteen retrieved trials[273-282,284-287] are in Table 2.1. The study samples included: nine trials in people with hyperlipidaemia[274-277,279,281] of which two involving patients with comorbid coronary artery disease[273,282] while one included comorbid type-2 diabetes mellitus[287]; one trial in healthy volunteers[280]; one trial in patients with secondary progressive multiple sclerosis[285]; one trial in adults with traumatic brain injury[278]; one trial in patients diagnosed with mild-to-moderate Alzheimer disease[284]; one trial in individuals affected by chronic hepatitis C[286]. All trials had been conducted in an adult population, though three focussed on older adults groups[275-277] and one was in males

only[274]. Baseline diagnosis of depression and antidepressant use were not always reported (Appendix A2.2), but overall rates of both seemed low or were explicitly excluded. The majority of trials employed a lipophilic statin (i.e., simvastatin, lovastatin, fluvastatin, atorvastatin)[274,275,277-282,284-287], though some used a hydrophilic statin (i.e., pravastatin)[273,276,279,280]. The range of follow-up was very wide between 4 to 152 weeks.

The risk of bias tables, including explanatory notes and overall bias across trials, are in Appendix A2.3. In summary, nine trials were judged at 'low' risk of bias[273,274,277,279-281,284,285] and five with 'some concerns'[275,276,278,282,287]. All trials described appropriate randomisation procedures, so they scored 'low' for this crucial domain. A common reason for downgrading several trials was the lack of an intention-to-treat analysis[274,275,278,280,282,287] despite the presence of missing data. Many trials lacked a pre-specified statistical analysis or pre-registered protocol[273-275,277,278,280-282,284,287], but it should be noted that the latter was not usual practice at the time that the majority of these studies had been conducted.

Table 2.1 *Trials characteristics*

Study ID	Study design	Population	Intervention	Comparator	Follow-up	Primary outcome measure scale
Carlsson 2002	RCT crossover	41 hyperlipidaemic older adults aged ≥70 years	Pravastatin 20mg → pravastatin 20mg + tocopherol 400IU	Placebo + tocopherol → pravastatin + tocopherol	1 year	GDS
Chan 2017	RCT	140 secondary progressive multiple sclerosis adults	Simvastatin 80mg	Placebo	2 years	HDRS
Gengo 1995	RCT crossover	36 hyperlipidaemic adults	Lovastatin 40mg, pravastatin 40mg	Placebo	4 weeks	POMS
Harrison 1994	RCT crossover	25 healthy adults	Simvastatin 40mg, pravastatin 40mg	Placebo	4 weeks	HADS
Hyyppä 2003	RCT crossover	120 hyperlipidaemic men aged 35-64 years	Simvastatin 20mg	Placebo	24 weeks	BDI
Morales 2006	RCT	80 hyperlipidaemic older adults aged ≥65 years	Simvastatin (up to) 20mg	Placebo	15 weeks	CES-D
Muldoon 2000	RCT	209 hyperlipidaemic adults	Lovastatin 20mg	Placebo	6 months	HDRS
Robertson 2017	RCT	52 mild TBI adults aged 18-50 years	Atorvastatin 1mg/kg/die (up to 80mg/die)	Placebo	3 months	CES-D

Santanello 1997	RCT	431 hyperlipidaemic older adults aged ≥65 years	Lovastatin 20-40mg	Placebo	6 months	CES-D
Sheridan 2014	RCT	60 chronic hepatitis C adults	Fluvastatin 40-80mg, Fluvastatin 40-80mg + w-3 PUFA 1g, Fluvastatin 40-80mg + w-3 PUFA 2-4g	Placebo, w-3 PUFA 1g, w-3 PUFA 2-4g	12 weeks	HADS
Sparks 2005	RCT	63 mild-to-moderate Alzheimer disease adults	Atorvastatin 80mg	Placebo	12 months	GDS
Stewart 2000	RCT	1,130 hyperlipidaemic adults with CAD	Pravastatin 40mg	Placebo	4 years	GHQ
Visseren 2001	RCT	87 hyperlipidaemic adults with type 2 diabetes mellitus	Fluvastatin 40mg	Placebo	12 weeks	Zung SDS
Wardle 1996	RCT	621 hyperlipidaemic adults aged 40-75 years with CAD	Simvastatin 20-40mg	Placebo	3 years	POMS

BDI: Beck Depression Inventory; CAD: Coronary Artery Disease; CES-D: Centre for Epidemiological Studies Depression; GDS: Geriatric Depression Scale; GHQ: General Health Questionnaire; HADS: Hospital Anxiety and Depression Scale; HDRS: Hamilton Depression Rating Scale; w-3 PUFA: omega-3 poly-unsaturated fatty acids; POMS: Profile of Mood States; RCT: Randomised Controlled Trial; SDS: Self-Rating Depression Scale; TBI: Traumatic Brain Injury

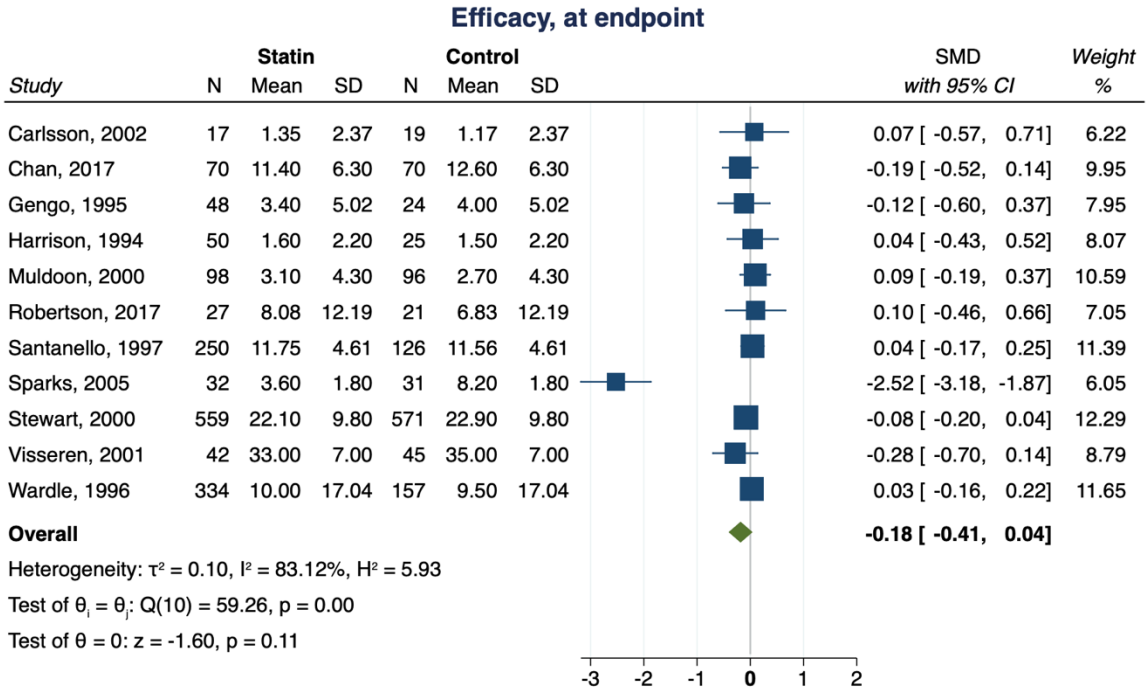
2.3.2 Data analysis

For all outcomes, forest plots (overleaf and in the Appendix) display effect sizes (SMD for continuous outcomes, RR for dichotomous outcomes) with 95%CI for each individual trial, as well as pooled results and heterogeneity. Certainty of the evidence is reported in the GRADE table (Appendix A2.4).

2.3.2.1 Efficacy

Eleven trials[273,276-282,284,285,287] comprising a total of 2,712 participants were included in the meta-analysis for the primary outcome of efficacy as mean value on any standardised scale for depressive symptoms at studies' endpoint (Figure 2.2). This showed a small effect of statins in improving depressive scores compared to placebo that did not reach statistical significance (SMD -0.18, 95%CI -0.41 to 0.04). The degree of certainty for this outcome was 'very low' due to serious concerns about inconsistency, indirectness, and imprecision. No publication bias was identified (Appendix A2.5). Heterogeneity was substantial (I^2 83.12%) and driven only by one study[284], which had found a large positive effect of statins on depression scores in a sample of patients with mild-to-moderate Alzheimer's disease (N 63, SMD -2.52, 95%CI -3.18 to -1.87).

Figure 2.2 *Forest plot for efficacy (mean value for depressive symptoms) at endpoint*



I therefore conducted a sensitivity analysis (Appendix A2.6) by excluding this latter trial: heterogeneity decreased (I^2 0.00%), but the overall results did not appreciably change (N 2,649, SMD -0.04, 95%CI -0.12 to 0.04), although this allowed to increase the certainty of such evidence to 'moderate', with the only serious concerns remaining for indirectness. To further check the robustness of this finding, a subgroup analysis was performed (Appendix A2.7) only on those seven trials with a similar population of patients with baseline dyslipidaemia[273,276,277,279,281,282,287]: again, outcome data consistently showed a non-significant difference between study arms (N 2,386, SMD -0.03, 95%CI -0.12 to 0.05), but the certainty of evidence could be upgraded to 'high'. Finally, to explore for potentially different effects between lipophilic and hydrophilic statins, I conducted another sensitivity analysis by excluding data measured for hydrophilic statins (Appendix A2.8), yet results remained consistently non-significant (N 1497, SMD -0.29; 95%CI -0.62 to 0.03, 'very low' certainty).

Comparable results were observed at all timepoints considered (Appendix A2.9): early (<6 months, N 282, SMD -0.09, 95%CI -0.33 to 0.15, 'low' certainty), medium (6-12 months, N 1,799, SMD -0.38, 95%CI -0.84 to 0.08, 'very low' certainty), and late (>12 months, N 1,761, SMD 0.06, 95%CI -0.16 to 0.03, 'high' certainty).

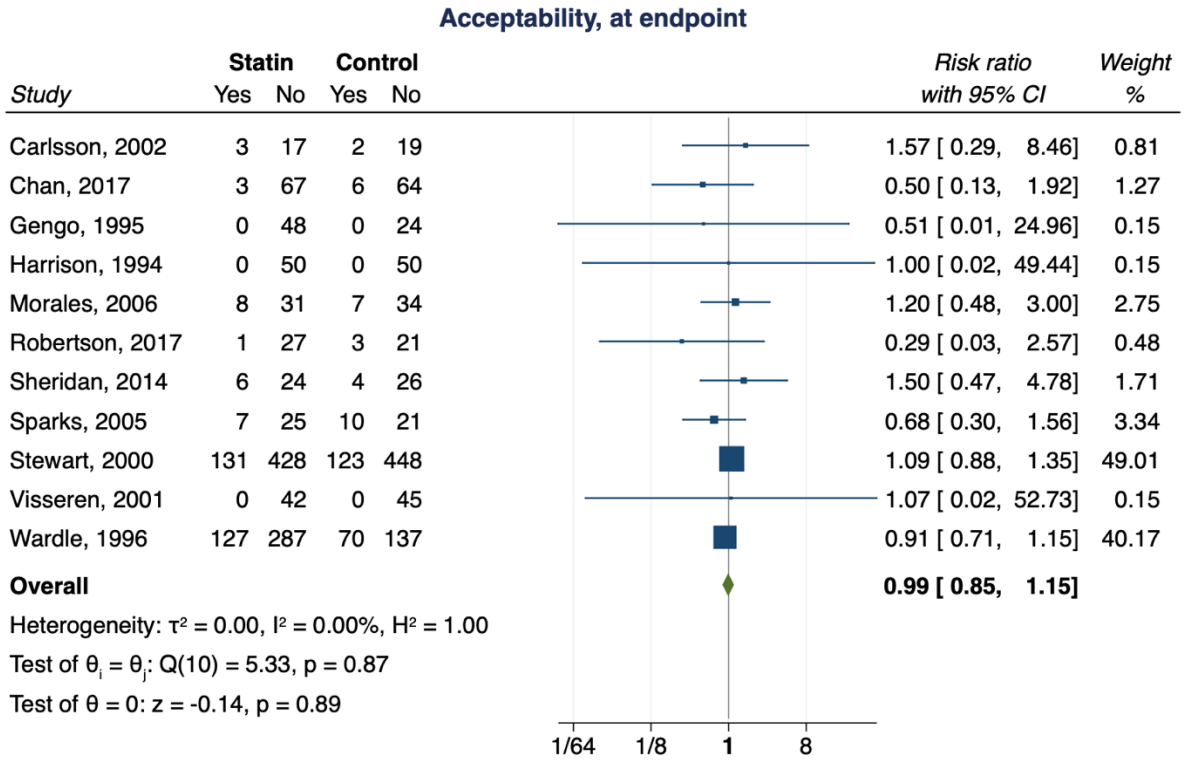
Three trials[274,275] reported data that could not be pooled for this outcome measure because no adequate summary statistics was provided for each study arm. Hyyppä et al., found a negative effect of simvastatin on depressive symptoms [N 120, mean difference (MD) 0.06, standard error (SE) 0.03, p 0.016][274]. Also, Morales et al., observed a higher number of people taking statins versus placebo (4/31 *versus* 1/34) with a score in keeping with a diagnosis of depression (i.e., >16 on the Center for Epidemiological Studies Depression scale)[275]. Conversely, Sheridan et al., did not identify any statistically significant effect of fluvastatin on depression scores (N 50; MD -0.11, SE 0.76; p 0.886)[286].

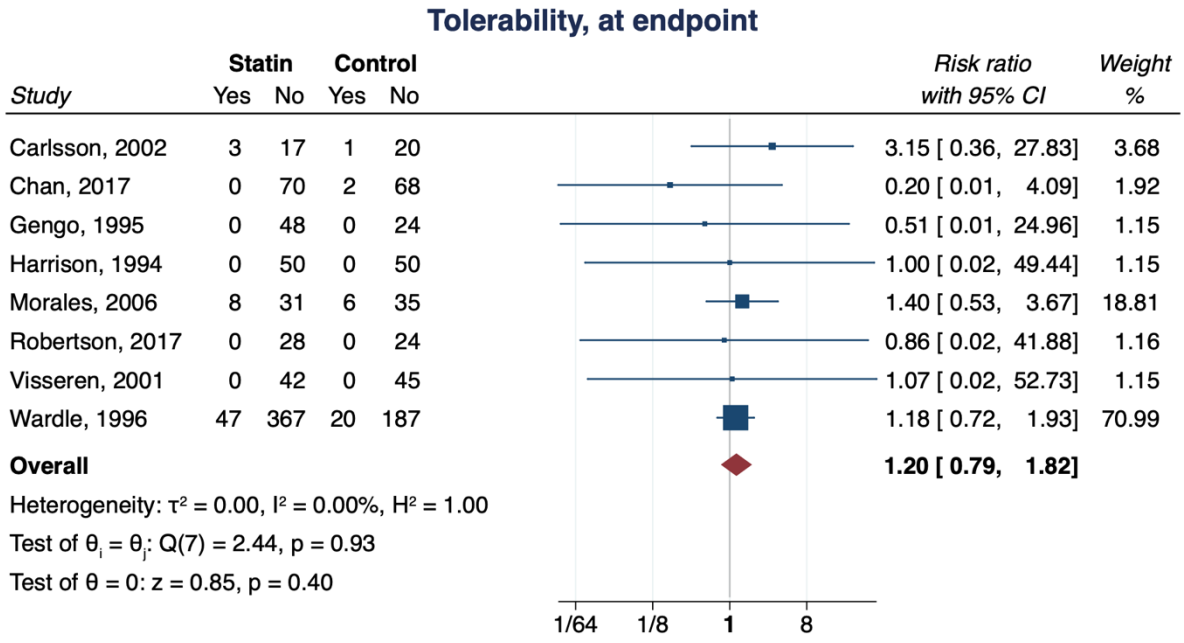
2.3.2.2 Acceptability, tolerability

Treatment acceptability (Figure 2.3) did not differ between the statin and control groups (N 2,446, RR 0.99, 95%CI 0.85 to 1.15, 'moderate' certainty), with no heterogeneity.

Treatment tolerability (Figure 2.3) was slightly worse for the statin group, but this difference was not statistically significant (N 1,193, RR 1.20, 95%CI 0.79 to 1.82, 'moderate' certainty), and no heterogeneity was identified.

Figure 2.3 *Forest plot for acceptability (number of participants discontinuing treatment due to any cause) and tolerability (number of participants discontinuing treatment due to adverse events)*





Two trials[277,281] reported data that could not be pooled for these outcome measures as the number of participants discontinuing treatment was not divided per study arm. Muldoon et al., said that 15 participants withdrew, of which one due to an adverse drug reaction (rash), out of the 209 randomised[281]. Similarly, Santanello et al., described that 55 participants withdrew, of which 16 due to adverse events, out of the 431 randomised[277].

2.3.2.3 Safety

Adverse events were inconsistently reported among trials (Appendix A2.10). For participants experiencing any adverse events (N 289, RR 1.07, 95%CI 0.66 to 1.73, 'moderate' certainty) and suicides (N 1,398, RR 0.64, 95%CI 0.17 to 2.45, 'very low' certainty), pooled estimates were not statistically significant, with large confidence intervals due to the very low number of events reported, and no evidence of heterogeneity. For self-harm, no events were observed in any of the included trials for either arm, so these data were not pooled according to guidelines[313].

Five trials[276-278,282] reported the total number of adverse events per study arm: overall, 4,750 adverse events were reported over 742 participants on statins versus 2,389 adverse events over 408 participants in the control groups.

2.4 Discussion

In this systematic review and meta-analysis, I have investigated the effects of statin monotherapy in adults with depressive symptoms over the available randomised controlled trials. Overall, there is no evidence that statins, without concurrent antidepressant treatment, have a significant advantage over placebo in reducing depressive symptoms in the general population. This result remains consistent at all timepoints

considered and after sensitivity and subgroup analyses, thus increasing confidence in its validity. Likewise, none of the secondary outcomes, namely acceptability, tolerability, and safety show any difference between treatment with statins or placebo.

On the surface, the negative findings for efficacy appear in contrast with the positive results of several previous meta-analyses of randomised controlled trials[271,272,283]. These earlier studies had all included, amongst others, three positive trials where statins had been given in add-on to antidepressants in patients explicitly diagnosed with depression[295-297]. Indeed, other meta-analyses that focussed on these specific intervention and population had highlighted the benefit of treating depressed participants by combining statins and antidepressants[298,299]. My present work excluded instead trials investigating add-on antidepressant strategies; nevertheless, the more homogeneous study sample was larger than earlier ones as the comprehensive search strategy retrieved several trials that had escaped previous meta-analyses. Some epidemiological evidence has suggested that statin users may have a lower risk of incident depression compared to non-users irrespective of concurrent antidepressant use[236,237]. However, this benefit was not observed in meta-analyses of observational studies[228,229], and indeed a negative effect was seen in some recent epidemiological investigations[257,259].

Findings for acceptability, tolerability, and safety are more difficult to contextualise as no equally designed study that had specifically assessed these outcomes could be identified. On the whole, the established safety profile of statins in the general population seems confirmed over the wide follow-up range (i.e., 4 to 152 weeks) included in the current meta-analysis.

2.4.1 Limitations

This study has several limitations that must be considered when interpreting its findings.

Although its search was extensive and queried several databases, there may still be trials that could have been missed. This issue might however be marginal as demonstrated by the lack of evidence for publication bias as well as the inclusion of many studies that were not retrieved in other recent systematic reviews.

Compared to the latter, I strived for a more uniform study population by excluding previously included trials that had investigated the use of statins plus antidepressants *versus* antidepressants alone in patients with a diagnosis of depression, as these studies answer a different clinical and research question about the value of statin as add-on therapy in depressed patients. However, I still pooled data from a variety of participants with very different background diagnoses and comorbidities. Baseline presence of depressive disorders and antidepressant use, though likely low or even specifically excluded, were inconsistently described – which may have introduced bias. On the other hand, the inclusion of a more varied population increases results' generalisability. The analysis within the larger subgroup of patients with dyslipidaemia, whose findings were consistent with the primary outcome ones, appears reassuring in this respect. However, it remains possible that other subgroups of patients may be more liable to show a better (or worse) effect on depressive symptoms of statins given in monotherapy – something that could have gone unnoticed in certain studies because of low sample sizes and thus lack of sufficient power. In this regard, the trial by Sparks et al.,[284] was a noteworthy outlier as it found a strong antidepressant effect of atorvastatin monotherapy in people with mild-to-moderate Alzheimer disease. Indeed, a precision approach to future clinical trials would see the *a priori* selection of target populations expected to respond to a specific

intervention: for example, in the case of medications with an anti-inflammatory potential such as statins, populations with raised inflammatory markers (e.g., C-reactive protein), background inflammatory conditions (e.g., obesity), or an inflammatory phenotype of depression (e.g., prominent anxiety and anhedonia) should be considered[207]. Equally, the underlying metabolic profile of the study participants is likely to play a major role[25]. Changes in these individual variables are not usually tested in randomised controlled trials, though there is evidence that they may have clinical meaning. For example, previous trials showed that stratifying patients for high *versus* low levels of C-reactive protein[418], or specifically assessing the depressive-inflammatory symptom of anhedonia[419] may highlight beneficial effects of anti-inflammatory medications that would have otherwise gone unnoticed.

On a similar note of precision, it is also plausible that individual statins might differentially elicit an antidepressant response according to their lipophilicity and thus brain penetrance[238] – something that I did not fully investigate in this pair-wise meta-analysis. The sensitivity analysis excluding those data that had measured the effects of hydrophilic statins did not steer efficacy findings; however, only a network meta-analysis could rank among different statins[319] and potentially identify a stepwise antidepressant effect according to lipophilicity. Some available network meta-analyses have shown conflicting results [229,283], but this might be due again to the mixing of study populations and interventions under examination[320]. A network meta-analysis of the more homogeneous trials hereby included could shed some light on this matter.

Finally, the evaluation of the trials' risk of bias and the grading of pooled evidence may have been more lenient than commonly advised[313]. For instance, I chose not to consider the lack of a pre-specified protocol as something that would put the trial at overall high

risk of bias because many studies were old, protocol registration was not in practice at the time, and the lack of it is unlikely to have led the study authors to report non-significant results. The reasoning behind the quality assessment was discussed in depth and eventually agreed among study authors, then described in full in the relevant tables (Appendix A2.3 and A3.4). Aside that, the study methodology and statistical analysis, as per pre-registered protocol, were robust and followed the recommended guidelines for systematic reviews which, along with low heterogeneity for most outcomes, support the validity of its results.

2.4.2 Conclusion

Evidence from this systematic review and meta-analysis does not support the antidepressant efficacy of statin monotherapy in the general population. A prospective clinical trial of three study arms comparing a statin alone, an antidepressant alone, and placebo could ultimately corroborate this finding. Meanwhile, existing data from pre-clinical (section 1.3.1.1.1) and clinical (sections 1.4.2.1 and 1.4.3.1) evidence suggests that statins, rather than being antidepressant molecules *per se*, might be seen as an augmentation strategy with conventional antidepressants for the treatment of people with depression. I therefore explore this possibility in the next Chapter 3.

Chapter 3. Effects of statin in add-on to antidepressant in depression – a meta-analysis of randomised controlled trials*

*This chapter is based on the paper: "Statins for major depressive disorder: A systematic review and meta-analysis of randomized controlled trials", De Giorgi et al., 2021; DOI:10.1371/journal.pone.0249409. Co-authors permission to reuse material is in Appendix, miscellanea

3.1 Introduction

In Chapter 2, the meta-analysis of randomised controlled trials investigating statin monotherapy in the general population with depressive symptoms has not shown any antidepressant effect compared to placebo. This was in keeping with a subgroup analysis of a previously published work[272]. Two further meta-analyses[298,299] which focussed instead only on those trials employing a statin in addition to an antidepressant in patients formally diagnosed with depressive disorder[295-297] had suggested the efficacy of this intervention [respectively, N 165, standardised mean difference (SMD) -0.65, 95% confidence interval (CI) -0.96 to -0.33; N 166, SMD -0.73, 95%CI -1.04 to -0.42]. A historical cohort study (N 872,216) comparing people using selective serotonin reuptake inhibitors (SSRIs) plus statin *versus* SSRI-only users had similarly shown a lower risk for psychiatric hospital contacts due to depression [hazard ratio (HR) 0.64, 95%CI 0.55 to 0.75] among statin users[305]. In section 1.3.1.1.1, I have also reported several pre-clinical studies highlighting favourable pharmacodynamics and pharmacokinetics interactions between statins and commonly-used antidepressants[127,129,131-136]. Overall, there is suggestive evidence for the combined use of statins and antidepressants in depression. Nevertheless, the earlier meta-analyses[298,299] of randomised controlled trials simply assessed the change in depression scores as their outcome, whereas other important effects such as antidepressant response and remission, treatment discontinuation, and adverse events had been overlooked. Furthermore, new trials may have been published since.

In view of these issues, I present an updated systematic review and meta-analysis of randomised controlled trials to investigate the efficacy, acceptability, tolerability, and safety of statins in add-on to antidepressant strategies in patients with depressive disorders.

3.2 Methods

The study protocol was registered on PROSPERO international prospective register of systematic reviews with reference CRD42020170938 and is available at https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=170938.

3.2.1 Search strategy

I run an extensive search of the PubMed/MEDLINE, Cochrane CENTRAL, ISI Web of Science, CINAHL, and ClinicalTrials.gov databases from date of inception until 1st September 2020, with no language restrictions. References of the included papers were manually screened for further relevant material. The search algorithm (Appendix A3.1) combined index terms and free-text words for statins, depression, and antidepressants.

3.2.2 Inclusion criteria

Randomised controlled trials that compared the effects of any statin in add-on to any antidepressant strategy *versus* any control condition on depressive symptoms were included. Quasi-randomised trials, such as those allocating treatment by using alternate days of the week, were excluded. For trials with a cross-over design, data from the first randomisation period was extracted and analysed whenever possible.

Compared to the meta-analysis in Chapter 2, the study population has to be further defined here. Studies involving patients aged 12 years or older, of both sexes, with a primary diagnosis of major depression according to the diagnostic and statistical manual of mental disorders (DSM)-III to DSM-5, international classification of diseases (ICD)-10, Feighner criteria, or Research Diagnostic criteria were included. Studies using ICD-9 were excluded as this system does not use operationalised criteria. A concurrent secondary diagnosis of

another psychiatric or medical disorder, as assessed by the trial's authors according to the diagnostic manuals described above, was not considered as an exclusion criterion; however, a concurrent primary diagnosis of another psychiatric disorders was an exclusion criterion.

3.2.3 Outcomes

For efficacy, results were computed at the following endpoints (or their closest in a contiguous range): 2 weeks (1-2 weeks), 4 weeks (3-5 weeks), 8 weeks (6-10 weeks), 12 weeks (11-14 weeks). For acceptability, tolerability, and safety, results were calculated at studies' endpoints.

3.2.3.1 Primary outcome

The primary efficacy outcome was the mean value on any standardised scale for depressive symptoms (e.g., Center for Epidemiologic Studies Depression Scale, Geriatric Depression Scale, Hamilton Depression Rating Scale) at 8 weeks.

3.2.3.2 Secondary outcomes

Secondary outcomes included: efficacy, measured as the mean value on any standardised scale for depressive symptoms at 2 weeks, 4 weeks, and 12 weeks; efficacy as response, measured as number of participants with a 50% reduction on any standardised scale for depressive symptoms at 2 weeks, 4 weeks, 8 weeks, and 12 weeks; efficacy as remission, measured as number of participants with a depression score below a pre-specified threshold (i.e., no clinical depression) on any standardised scale for depressive symptoms (e.g., <7 for HDRS) at 2 weeks, 4 weeks, 8 weeks, and 12 weeks; acceptability, measured

as the number of participants discontinuing treatment (dropouts) due to any cause at studies' endpoints; tolerability, measured as the number of participants discontinuing treatment (dropouts) due to adverse events at studies' endpoints; safety, measured as the number and type of adverse events at studies' endpoints.

3.2.4 Study selection, data extraction, quality assessment

Two researchers (RDG, NRP) independently screened the retrieved records' title/abstract and assessed the relevant full-text articles for eligibility. Any disagreement was discussed with a third researcher (FDC) and resolved by *consensus*.

Three researchers (FDC, NRP, RDG) used a structured data extraction spreadsheet to ensure consistency of appraisal for each study. For the included studies, data about first authors' name, year of publication, study design, sample size and characteristics, intervention and comparison details, length of follow-up, primary and secondary outcome measures of interest with point estimates were extracted. Graph data was extracted using WebPlotDigitizer v4.2 software[312]. For means and standard deviations not readily available, I estimated values from medians and interquartile ranges or employed the RevMan v5.4 software calculator according to guidelines[313,314].

Risk of bias of the included trials was assessed using the "RoB 2: A revised Cochrane risk-of-bias tool for randomised trials"[315], and the certainty of evidence for all outcomes via GRADEpro GDT[316]. The quality assessment was performed by two independent raters (FDC, RDG) and disagreements were discussed with another member of the review group (NRP) and resolved by *consensus*.

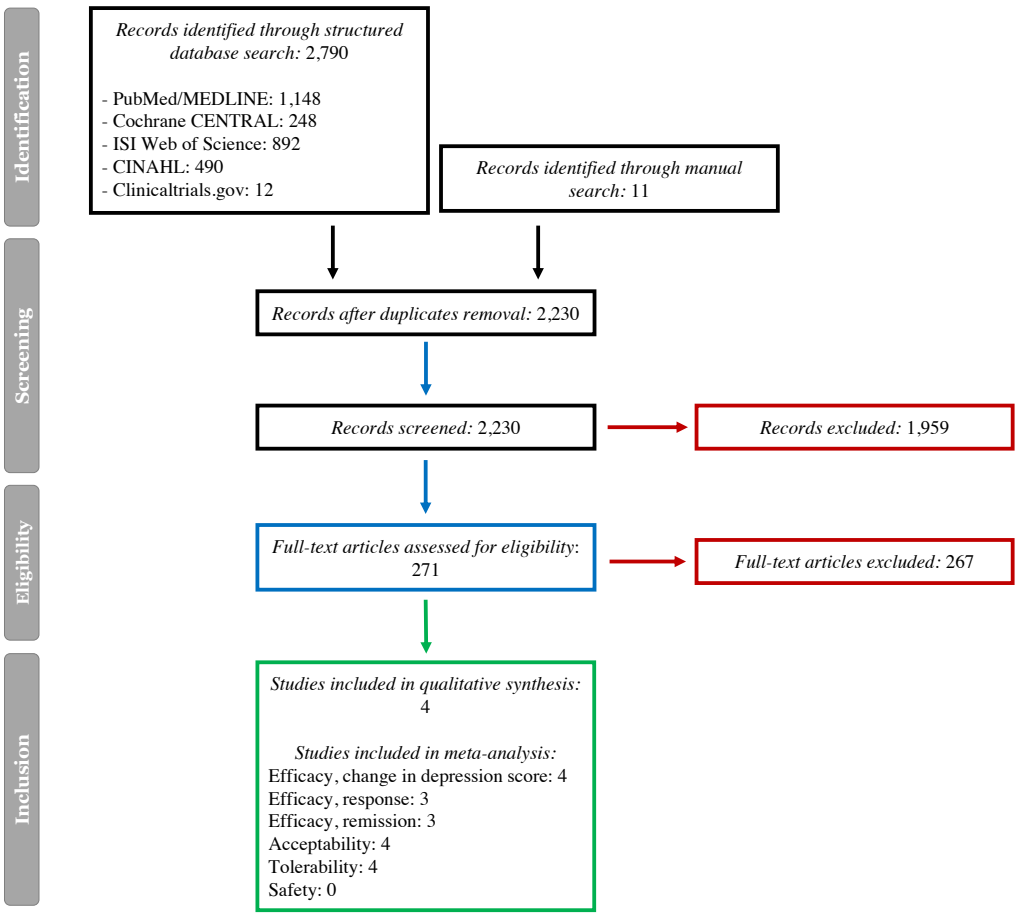
3.2.5 Statistical analysis

All the extracted data was analysed using STATA v17 software[317]. Data from rating scales for depression were analysed as a continuous variable using standardised mean difference (SMD, as different depression rating scales were used) with 95% confidence intervals (CI), employing random-effect models, more conservative than a fixed-effect model. For evaluating the clinical significance of SMD values, the effect size was considered 'small' if SMD <0.40, 'moderate' if SMD 0.40 to 0.70, and 'large' if SMD >0.70[313]. All the other quantitative data (e.g., number of participants discontinuing treatment) were analysed as dichotomous variables using relative risk (RR) with 95%CI, employing random-effect models. Non-quantitative data (e.g., type of adverse events) were presented descriptively. Heterogeneity between studies was investigated through the I^2, t^2, and *p-value* statistic and by visual inspection of the forest plots. If at least ten studies were available, funnel plots and Egger's test to detect publication bias were used[313].

3.3 Results

The literature search is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline[318] in Figure 3.1. In summary, it retrieved 2,790 records from electronic databases (PubMed/MEDLINE: 1,148, Cochrane CENTRAL: 248, ISI Web of Science: 892, CINAHL: 490, ClinicalTrials.gov: 12), and further 11 papers from the manual search. After duplicates removal, 2,230 records' titles and abstracts were screened by applying the inclusion/exclusion criteria previously described. The remaining 271 articles were assessed full text, of which four were ultimately included in this systematic review.

Figure 3.1 *PRISMA flow diagram*



3.3.1 Trials characteristics and risk of bias assessment

The characteristics of the four retrieved trials[295-297,301] are in Table 3.1. All were published in English, but three[295-297] were conducted in Iran and one[301] in Australia. They all involved patients with diagnosed major depressive disorder, though one[301] included a younger population (i.e., 15-25 years old). Though previous episodes of depression were reported, it is unclear for all trials whether the study population included patients with treatment-resistant depression. All trials were placebo-controlled and used different statins (i.e., rosuvastatin, lovastatin, simvastatin, atorvastatin) in addition to an antidepressant strategy. Follow-ups ranged from 6 to 12 weeks.

The risk of bias tables, including explanatory notes and overall bias across trials, are in Appendix A3.2. In summary, the higher-quality studies were the more recent ones[296,297,301], which were reported in more detail and had 'low' risk of bias for most items. The older study by Ghanizadeh et al.,[295] did not describe any procedure for allocation concealment, therefore it was scored as 'some concerns' for the item of deviations from intended interventions as well as its linked one of measurement of the outcome. The study by Gougol et al,[297] had 'some concerns' for the item of missing outcome data since no reason was given for dropouts and it did not describe whether an intention-to-treat analysis was employed. A study protocol had been previously registered for all trials; however, one trial had been registered only after recruitment had started, hence it was scored as 'some concerns' for selection of the reported result[295].

Table 3.1 Trials characteristics

Study ID	Study design	Population	Intervention	Comparator	Follow-up	Primary outcome measure
Berk 2020	RCT	90 MDD patients, 15-25yo, baseline moderate to severe depression (MADRS ≥20)	TAU + rosuvastatin 10mg	TAU + placebo	12 weeks	MADRS
Ghanizadeh 2013	RCT	68 MDD patients, 17-70yo, baseline moderate to severe depression (HDRS-17 ≥17)	Fluoxetine (up to) 40mg + lovastatin 30mg	Fluoxetine (up to) 40mg + placebo	6 weeks	HDRS-17
Gougol 2015	RCT	48 MDD patients, 20-70yo, baseline moderate to severe depression (HDRS-17 ≥22)	Fluoxetine (up to) 40mg + simvastatin 20mg	Fluoxetine (up to) 40mg + placebo	6 weeks	HDRS-17
Haghighi 2014	RCT	60 MDD patients, 18-50yo, baseline severe depression (HDRS-21) ≥25	Citalopram 40mg + atorvastatin 20mg	Citalopram 40mg + placebo	12 weeks	HDRS-21

CABG: Coronary Artery Bypass Graft; HDRS-17: Hamilton Depression Rating Scale, 17 items; HDRS-21: Hamilton Depression Rating Scale, 21 items; MADRS: Montgomery-Åsberg Depression Rating Scale; MDD: Major Depressive Disorder; RCT: Randomised Controlled Trial; TAU: Treatment As Usual (included case management, cognitive behavioural therapy, or pharmacotherapy with antidepressant).

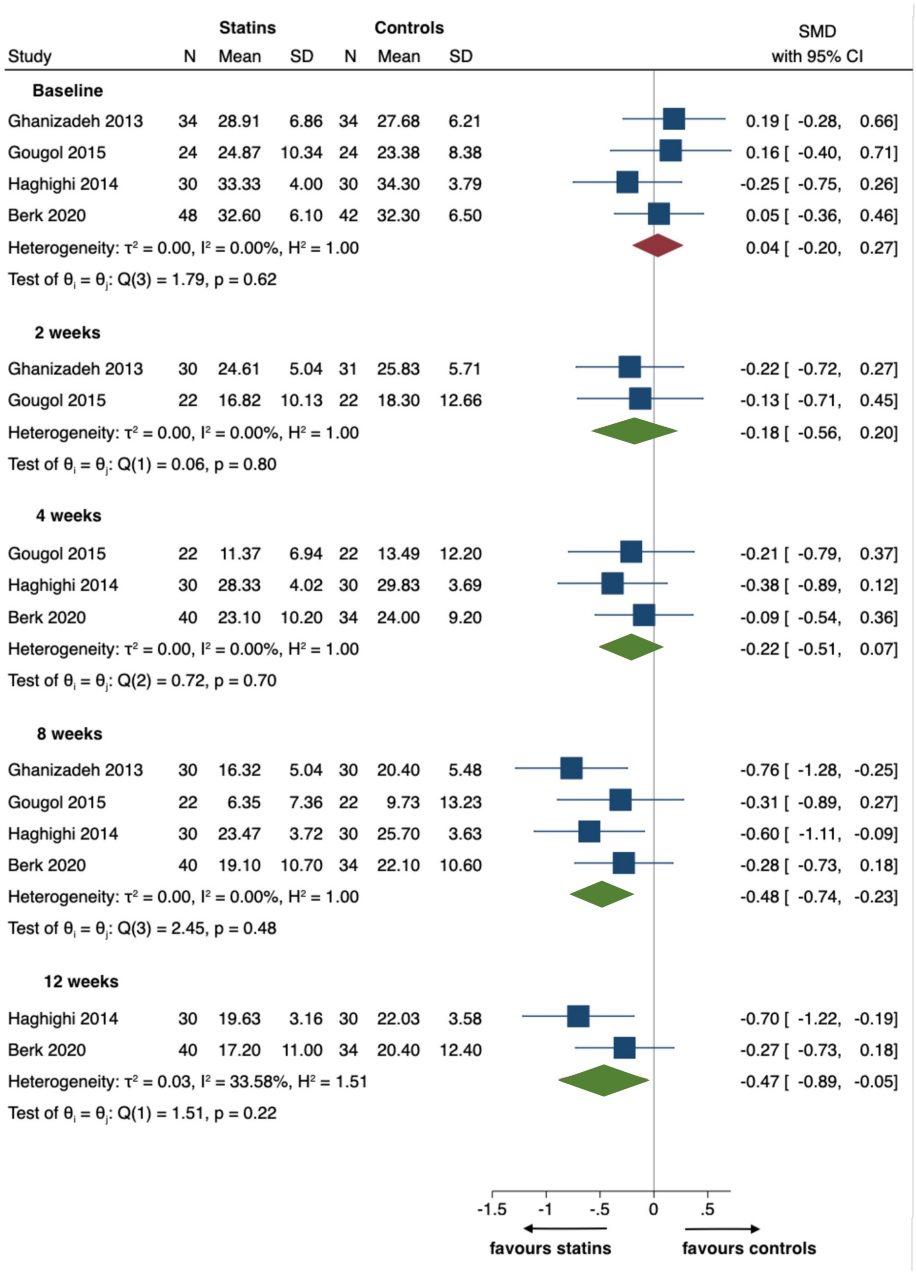
3.3.2 Data analysis

For all outcomes, forest plots (below and in the Appendix) display effect sizes (SMD for continuous outcomes, RR for dichotomous outcomes) with 95%CI from each individual trial, as well as pooled results and heterogeneity. Certainty of the evidence is reported in the GRADE table (Appendix A2.3). Publication bias was not assessed because the number of studies retrieved was below the minimum of ten trials generally recommended for this analysis[313].

3.3.2.1 Efficacy

Four trials [295-297,301] comprising a total of 238 participants were included in the meta-analysis for the primary outcome of efficacy as mean value for depressive symptoms at 8 weeks (Figure 3.2). This showed a moderate effect of any statin plus any antidepressant in improving depression scores compared to control (SMD -0.48, 95%CI -0.74 to -0.22), which was statistically significant, but was downgraded for imprecision due to low sample size, therefore the degree of certainty was 'moderate'. Efficacy at 2 weeks (two trials[295,297], N 105, SMD -0.18, 95%CI-0.57 to 0.20) and 4 weeks (three trials[296,297,301], N 178, SMD -0.22, 95%CI -0.51 to 0.08) showed a progressive trend towards improvement for the statins' arm, which was however not statistically significant. Efficacy at 12 weeks (two trials[296,301], N 134, SMD-0.47, 95%CI -0.89 to -0.05) showed a moderate effect for the intervention arm in improving depression scores, which was statistically significant. Calculation of I^2 did not show any significant heterogeneity for these efficacy outcomes (I^2 0% to 33.58%).

Figure 3.2 *Forest plot for efficacy (mean value for depressive symptoms) at all timepoints*



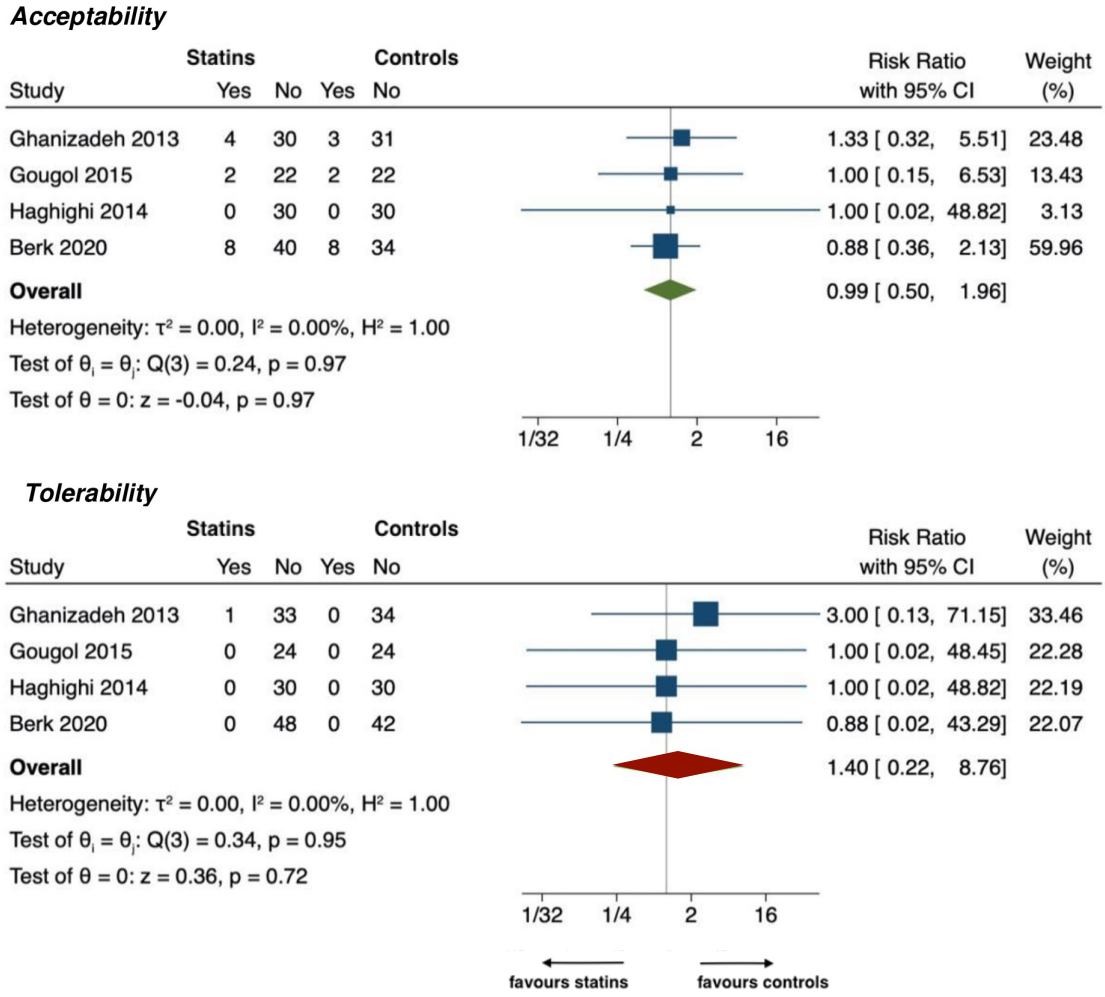
Data for response and remission was available over three trials: one[297] for response and remission at 8 weeks, and two[296,301] for response and remission at 12 weeks (Appendix A3.4), while no studies had measured response and remission at 2 or 4 weeks. Statin plus antidepressant treatment was associated with increased response at 8 weeks in only one trial[297] (N 48, RR 1.57, 95%CI 1.10 to 2.25), but remission at 8 weeks and response and

remission at 12 weeks did not show any statistically significant difference with control[296,301]. Calculation of I^2 did not show any heterogeneity for response and remission.

3.3.2.2 Acceptability, tolerability

Neither treatment acceptability (N 238, RR 0.99, 95%CI 0.50 to 1.96) nor tolerability (N 238, RR 1.40, 95%CI 0.22 to 8.76) proved significantly different between statin plus antidepressant *versus* antidepressant alone, with no evidence of heterogeneity (Figure 3.3). Acceptability and tolerability were both downgraded by two levels for imprecision due to low sample size and wide confidence intervals, scoring a 'low' degree of certainty.

Figure 3.3 *Forest plot for acceptability (number of participants discontinuing treatment due to any cause) and tolerability (number of participants discontinuing treatment due to adverse events)*



3.3.2.3 Safety

Combining a statin with antidepressant treatment appeared a safe strategy in this sample of depressed participants, with no report of serious adverse events across all trials[295-297,301]. Commonly reported side-effects were gastrointestinal (e.g., nausea, vomiting, and decreased appetite), pain (e.g., generalised pain, headache, abdominal pain, backpain), psychiatric (e.g., nervousness and insomnia), itches, and common cold. Two trials

reported the number and type of side-effects separately for statins (specifically, lovastatin and rosuvastatin) and control[295,301], with no evidence of difference between study arms. Gougol et al., did not report detailed data about side-effects but claimed that no significant difference was found between simvastatin and control[297]. Haghighi et al., reported the pooled number of individual side-effects for patients on atorvastatin and control, but no information was available to compare between the two[296].

3.4 Discussion

In this systematic review and meta-analysis, I have investigated the effects of any statin in add-on to any antidepressant strategy in patients with depressive disorders over the available randomised controlled trials. Overall, statins appear efficacious in addition to antidepressants at 8 weeks and 12 weeks, with a trend towards improvement seen as early as the second week of treatment.

Compared to similar previous meta-analyses[298,299], a recent trial[301] could be added, which led to a slightly lower effect size for the primary outcome of efficacy at 8 weeks. This study[301] had not shown a statistically significant difference in efficacy between study arms, which appeared mainly driven by large standard deviations, though there was a trend towards improvement for people on statin. Interestingly, the sample size calculation of this trial had been estimated at 270 participants for a 80% power, but only 130 participants were eventually randomised, therefore such underpower might explain why the positive result for statins did not reach statistical significance. From a methodological perspective, this trial had higher quality according to the risk of bias assessment; however, unlike the other three studies[295-297] here reported, it had employed a hydrophilic molecule, rosuvastatin. The other statins tested, namely atorvastatin, lovastatin, and simvastatin are

lipophilic and therefore, as previously discussed, more capable to cross the blood-brain barrier (BBB)[52]. Thus, the lower effect size seen for rosuvastatin may also be explained by its lesser capability to enter the central nervous system (CNS). Another important observation is that the rosuvastatin trial[301] also involved a younger (15 to 25 years old) sample. Older populations are more likely to have contextual inflammation related to several factors (e.g., higher body mass index, stress, and chronic ailments), a concept known as "inflammaging"[307]. As previously mentioned, some researchers emphasise the importance of targeting a specific subset of depressed patients with concomitant increased inflammatory markers when using anti-inflammatories for antidepressant purposes[207]: in other words, applying a precision medicine approach to a psychopharmacological treatment. In view of this, a sample that is less prone to baseline inflammation because of young age and that had not been pre-selected for elevated inflammatory markers might see less benefit from using a statin as adjunct antidepressant treatment. Accordingly, an earlier cohort study had shown a stepwise reduction in the odds of developing depression with increasing age for statin users[238]. Considering these two concepts (i.e., statin's lipophilicity, need to target specific patients groups) together, two ongoing randomised controlled trials employing the highly lipophilic simvastatin in add-on to antidepressants, respectively in patients with treatment-resistant depression[321] and in people with comorbid depression and obesity[322] might prove illuminating.

Several other outcomes were assessed in this meta-analysis. However, apart from an increased response rate at 8 weeks of treatment, combined statins and antidepressants did not show any statistically significant difference for remission at 8 weeks, response and remission at 12 weeks, and acceptability and tolerability at studies' endpoints. Commonly reported side-effects were similar between study arms. The latter findings are of potential interest in further confirming the known safety of statins in a psychiatric population

affected by depressive disorders; however, it should be noted that adverse events manifesting with long-term statin use may have not been captured by the relatively short follow-ups (i.e., up to 12 weeks) of the present trials.

3.4.1 Limitations

This study has several limitations that must be considered when interpreting its findings.

There may be trials that have not been included, though the comprehensive search strategy should have minimised such risk. Publication bias could not be formally assessed, so it is possible that studies showing no effect or negative ones of statins on mood may have not been published.

The overall sample size for all measured outcomes was small, as only four articles[295-297,301] could be retrieved: such limited number of participants was the main reason why the certainty of the findings was downgraded. The length of follow-up did not go further than 12 weeks, hence late effects of statins could have been missed. Three[295-297] out of four of the included trials had been conducted in a single country, which may limit the generalisability of the pooled estimates to other countries[421].

Aside that, the study methodology and statistical analysis, as per pre-registered protocol, were robust and followed the recommended guidelines for systematic reviews which, along with low heterogeneity for most outcomes, support the validity of its results.

3.4.2 Conclusion

Evidence from this systematic review and meta-analysis suggests the efficacy, acceptability, tolerability, and safety of statins in add-on to antidepressant treatments for patients with depressive disorders. Larger and longer clinical trials in a variety of settings

are needed to confirm these findings. However, trials sometimes struggle to overcome these issues because of practical barriers related to patients' recruitment, retention, and safety[323]. I therefore complement the findings from Chapter 2 and Chapter 3 using an alternative, epidemiological approach, respectively in Chapter 4 and Chapter 5.

Chapter 4. Effects of statin monotherapy in depression – a real-world epidemiological study

4.1 Introduction

As seen in Chapter 1, and reiterated in other reviews[31,107], the pre-clinical and clinical evidence for the effects of statins in neuropsychiatric disorders, including depression, appear variable, with past reports raising concerns about statins' association with several neuropsychiatric adverse events, including depressive and anxiety symptoms, self-harm and suicidality, sleep disturbance, and cognitive dysfunction. More recent works have however advised that these previously observed associations may have been driven by confounds[235] or other bias [106]. Some pre-clinical [109] and clinical evidence [110,111] in fact suggest that statins may have a positive impact on neuropsychiatric symptoms, especially depression.

The potential antidepressant activity of statins and their neuropsychiatric adverse events profile in people with depressive disorders, regardless of concurrent antidepressant use, are relatively unexplored or anyway ambiguous[311,324]. In Chapter 2, I have presented a meta-analysis of fourteen randomised controlled trials showing no difference between statin monotherapy and placebo on depression scores [N 2,712, standardised mean difference (SMD) -0.18, 95% confidence interval (CI) -0.41 to 0.04] and adverse outcomes in the general population with depressive symptoms. Efficacy findings are in line with a previous similar work[272], whereas those for the latter are less certain since few to no studies had specifically assessed them before. In this respect, observational data are an invaluable complement: compared to trials, causal inference between the exposure and outcome of interest cannot be automatically assumed, but other aspects of internal and external validity can benefit from their larger unselected study populations and longer follow-ups[323]. However, epidemiological studies have thus far provided conflicting results, including higher [N 7,481,000, odds ratio (OR) 1.22, 95%CI 1.20 to 1.25][257], lower

[N 1,149,384, hazard ratio (HR) 0.91, 95%CI 0.88 to 0.94][236], and unchanged (N 5,035,070, OR 0.87, 95%CI 0.74 to 1.02)[228] depression risk in individuals taking statins. Moreover, none of these investigations had been specifically conducted on people with a background diagnosis of depression, and other adverse neuropsychiatric outcomes had not been systematically assessed. Aside from differences in their design, inconsistencies among clinical trials and observational studies might also be explained by small sample sizes, selection of different study populations and endpoints, inadequate control for possible confounders, and short follow-ups.

In view of these discrepancies, I present a real-world epidemiological study of a primary care database to investigate the putative antidepressant efficacy and neuropsychiatric adverse events associated with statin use *versus* non-use in patients with depression.

4.2 Methods

This study was part of a larger project[325] registered on QResearch (OX3 "Choosing the right antidepressant for depressive disorder"), independently reviewed and approved by the QResearch scientific committee (18/EM/0400). The study-specific protocol is available at https://osf.io/96zcn/.

4.2.1 Database

QResearch primary care research registry (www.QResearch.org) was investigated. This database includes the anonymised electronic healthcare records of over 35 million patients registered with 1,574 practices in England, recording demographic data (e.g., age, gender), characteristics (e.g., height, weight, smoking status), symptoms and adverse events, clinical diagnoses, and prescribed medications[326]. The Egton Medical Information

Systems (EMIS), managed centrally by QResearch, was used to obtain consent to provide data.

4.2.2 Cohort construction

Patients aged 18-100 years, newly diagnosed with a depressive disorder (i.e., study entry date) according to validated "Read codes"[327,328] (Appendix A4.1), registered with English practices between 1st January 1998 and 15th August 2020 for at least 12 months were included. Exclusion criteria encompassed: any prescription of antidepressants or any depression diagnosis before the study entry date; any depression diagnosis made 2 months before or 2 months after starting an antidepressant; a lifetime diagnosis of schizophrenia spectrum disorder or bipolar affective disorder; any diagnosis of post-partum depression within 180 days before or up to 180 days after the first diagnosis of depression; any prescription of antipsychotics, mood stabilisers, or more than one antidepressant at baseline. Participants in the study cohort were followed up for 12 months.

4.2.3 Exposure and comparison

The exposure under investigation was the use at baseline of any UK-licensed statin according to the British National Formulary (BNF, https://bnf.nice.org.uk, i.e., simvastatin, atorvastatin, pravastatin, fluvastatin, and rosuvastatin) compared to non-exposure to a statin prescription: statin users *versus* statin non-users.

4.2.4 Outcomes of interest

Outcomes were measured at 2 months, 6 months, and 12 months from the study entry date (i.e., new diagnosis of a depressive disorder).

Efficacy outcomes were assessed via the Patient Health Questionnaire (PHQ)-9[329] as: response (50% score reduction); remission (score <5); change in depression score.

Adverse events were assessed as: number of patients who had at least one of any adverse event (Appendix A4.2); any psychiatric symptom; anxiety; sleep disturbance; memory impairment; self-harm; suicidality; completed suicide; all-cause mortality.

4.2.5 Statistical analysis

Analyses were conducted on Stata MP v16.0[317] on the QResearch server. Baseline characteristics of the study cohort were examined with descriptive statistics for the total sample, as well as separately for statin users and non-users. Outcomes of interest were explored using an intention-to-treat analysis with multivariate logistic regression, clustered by practices, to compute mean differences (MDs, for continuous outcomes) and odds ratios (ORs, for dichotomous outcomes) with 99% confidence intervals (99%CIs) for statin users *versus* statin non-users (i.e., between-subject design), both unadjusted and adjusted (aMD, aOR) for previously validated potential confounders (Appendix A4.3). Multiple imputation by chained equations was employed to impute data when values were not available: for each imputation, 10 imputed datasets were generated, and coefficient estimates across these were combined using Rubin's rule[330]. Results were calculated for both the complete case analysis (CCA) and the full set analysis (FSA, including imputed data).

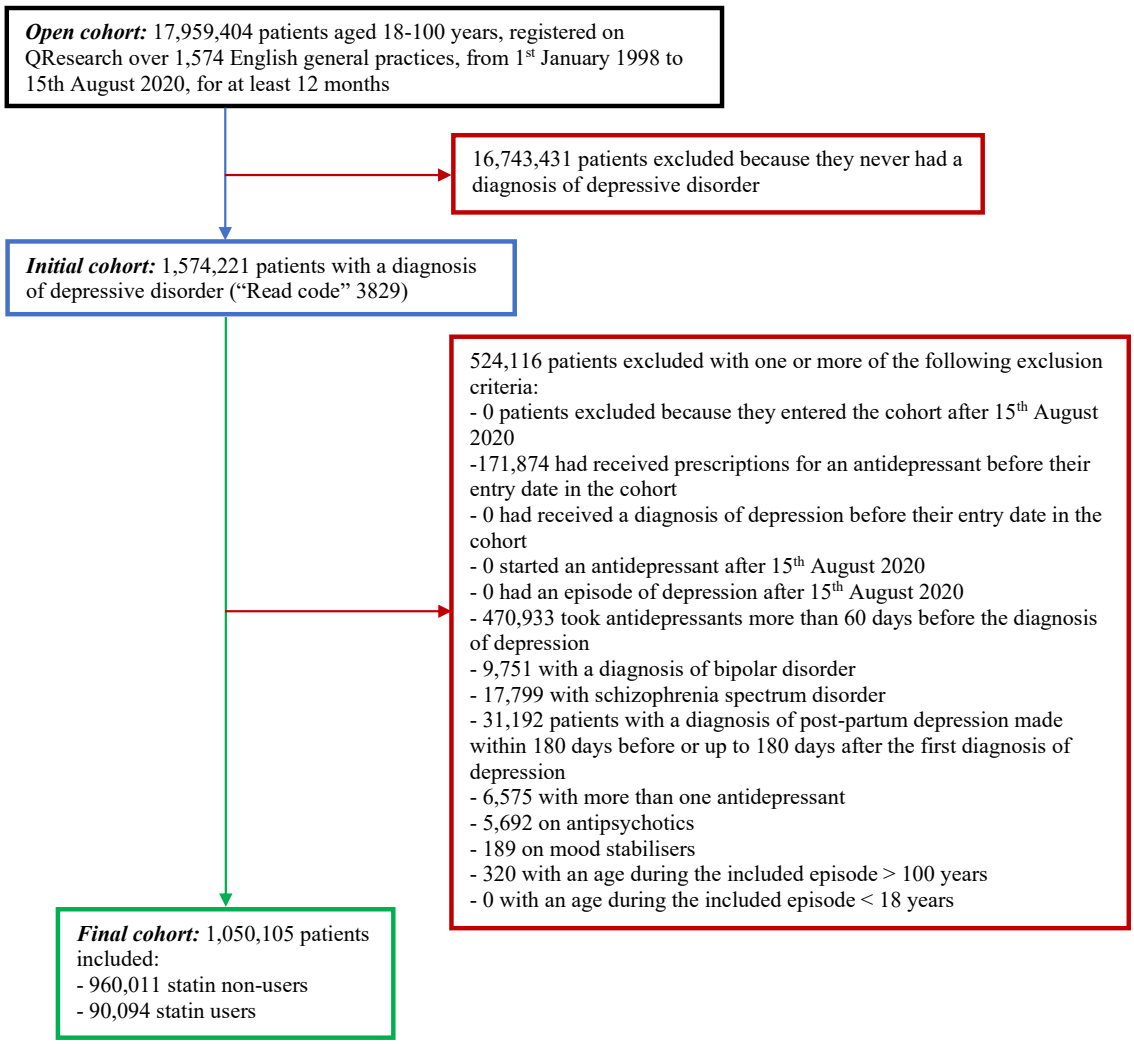
4.3 Results

The study cohort and analyses for all outcomes are reported below.

4.3.1 Study cohort

The cohort construction is in Figure 4.1. From a preliminary cohort of 1,574,221 patients with a diagnosis of depression ("Read code" 3829), following the application of inclusion/exclusion criteria, the study cohort eventually comprised 1,050,105 patients: 90,094 statin users and 960,011 statin non-users.

Figure 4.1 *Cohort construction flow chart*



Baseline characteristics of the study cohort are in Table 4.1. In summary, compared to non-users, statin users included more males (males: 41.21% *versus* 57.84%), were older (mean age: 40.90 years *versus* 68.16 years), had more physical health comorbidities

(particularly, cardiovascular, cerebrovascular, metabolic, and neoplastic), and were taking more non-psychiatric medications but fewer antidepressants (on antidepressants: 65.24% *versus* 51.99%). These expected baseline differences between the two groups were controlled for in the multivariate regression analyses, adjusted model. Missing data, which were then imputed for the FSA, are in Appendix A4.4.

Table 4.1 *Baseline characteristics of study cohort. Values are numbers (percentages) unless stated otherwise*

Characteristic	Statin users N 90,094	Statin non-users N 960,011	All sample N 1,050,105
Sex			
Male	52,112 (57.84)	395,636 (41.21)	447,748 (42.64)
Mean age (SD)	68.16 (13.76)	40.90 (16.89)	43.23 (18.32)
Read codes for depression			
Major depression	34,693 (38.51)	543,520 (56.62)	578,213 (55.06)
Minor depression	34,478 (38.27)	354,900 (36.97)	389,378 (37.08)
Other	20,923 (23.22)	61,591 (6.42)	82,514 (7.86)
PHQ-9 (SD), at baseline	N 14,996	N 181,765	N 196,761
	14.33 (6.16)	16.30 (5.30)	16.15 (5.39)
At 2 months	N 6,586	N 81,410	N 87,996
	11.45 (6.49)	12.43 (6.42)	12.36 (6.43)
At 6 months	N 1,942	N 23,754	N 25,696
	11.13 (6.90)	12.43 (6.74)	12.34 (6.76)
At 12 months	N 1,332	N 15,548	N 16,880
	11.04 (7.01)	13.10 (6.71)	12.94 (6.76)
Year of diagnosis			
1998 to 2005	9,837 (10.92)	269,200 (28.04)	279,037 (26.57)
2006 to 2010	29,087 (32.29)	199,069 (20.74)	228,156 (21.73)

2011 to 2015	27,258 (30.26)	227,977 (23.75)	255,235 (24.31)
2016 to 2020	23,912 (26.54)	263,765 (27.48)	287,677 (27.40)
Body Mass Index (SD)	N 83,962	N 748,752	N 794,459
	28.81 (6.06)	25.90 (5.82)	26.19 (5.91)
Smoking (cigarettes/day)	N 88,601	N 886,223	N 974,824
Non-smoker	42,063 (46.69)	451,484 (47.03)	493,547 (47.00)
Ex-smoker	30,120 (33.43)	158,453 (16.51)	188,573 (17.96)
Light smoker (1-9)	15,397 (17.09)	254,227 (26.48)	269,624 (25.68)
Moderate smoker (10-19)	658 (0.73)	15,676 (1.63)	16,334 (1.56)
Heavy smoker (>20)	363 (0.40)	6,383 (0.66)	6,746 (0.64)
Alcohol (daily units)	N 55,788	N 483,096	N 538,884
Non-drinker/trivial (< 1)	31,348 (34.79)	268,172 (27.93)	299,520 (28.52)
Light (1-2)	16,743 (18.58)	160,061 (16.67)	176,804 (16.84)
Medium (3-6)	6,229 (6.91)	41,133 (4.28)	47,362 (4.51)
Heavy (7-9)	791 (0.88)	6,164 (0.64)	6,955 (0.66)
Very heavy (> 9)	677 (0.75)	7,566 (0.79)	8,243 (0.78)
Ethnic group	N 75,892	N 712,011	N 787,903
White	64,052 (71.09)	604,638 (62.98)	668,690 (63.68)
Indian	2,771 (3.08)	14,280 (1.49)	17,051 (1.62)
Pakistani	1,690 (1.88)	12,297 (1.28)	13,987 (1.33)
Bangladeshi	1,805 (2.00)	9,122 (0.95)	10,927 (1.04)
Other Asian	1,452 (1.61)	11,475 (1.20)	12,927 (1.23)
Caribbean	1,428 (1.59)	11,128 (1.16)	12,556 (1.20)
Black African	1,033 (1.15)	14,707 (1.53)	15,740 (1.50)
Chinese	209 (0.23)	4,040 (0.42)	4,249 (0.40)
Other	1,452 (1.61)	30,324 (3.16)	31,776 (3.03)
Townsend deprivation score in fifths	N 89,961	N 956,639	N 1,046,600
1 (Least deprived)	21,498 (23.86)	189,459 (19.74)	210,957 (20.09)

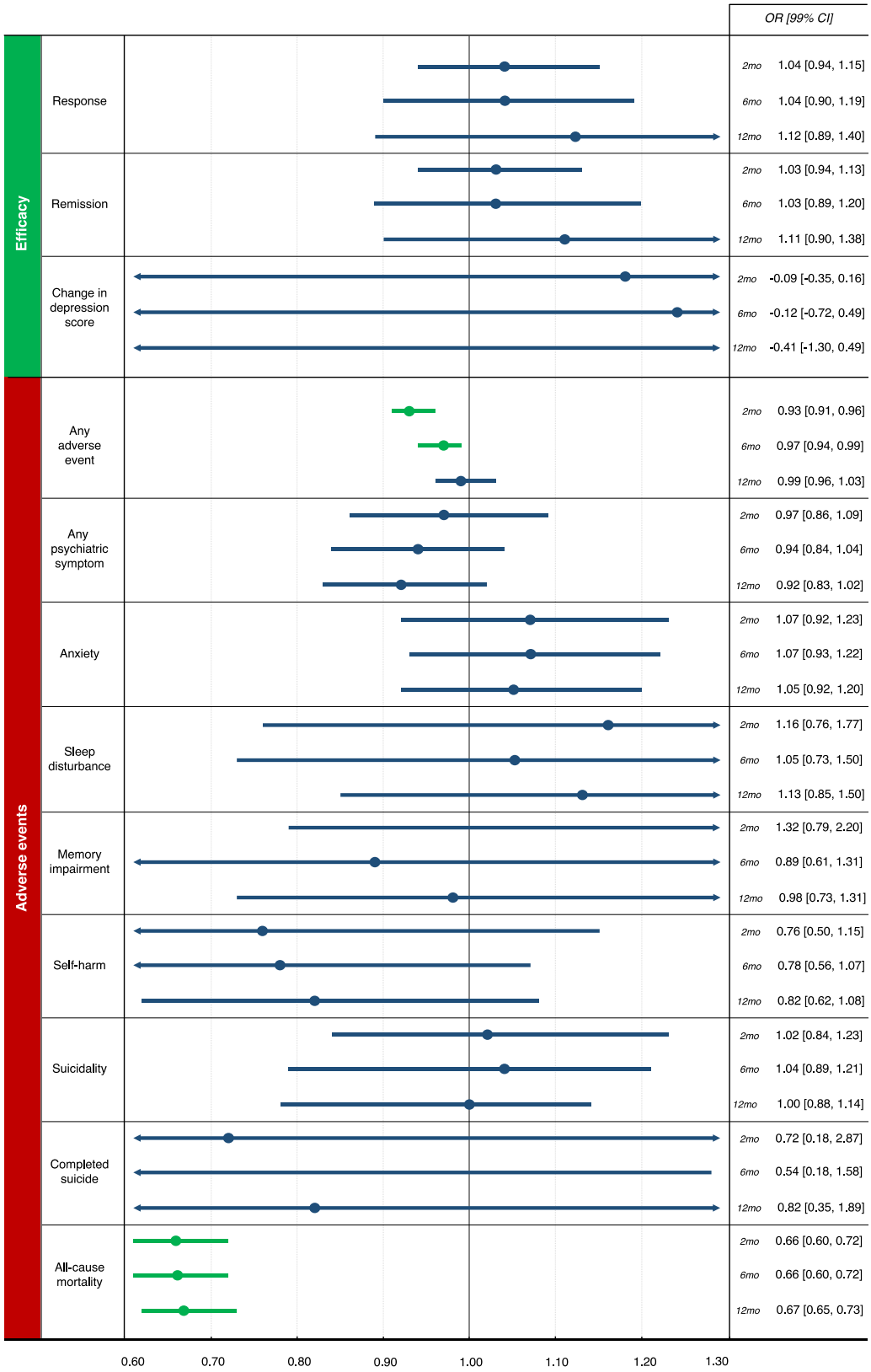
2	19,795 (21.97)	195,682 (20.38)	215,477 (20.52)
3	18,073 (20.06)	196,946 (20.51)	215,019 (20.48)
4	15,992 (17.75)	188,315 (19.62)	204,307 (19.46)
5 (Most deprived)	14,603 (16.21)	186,237 (19.40)	200,840 (19.13)
Region of England			
East Midlands	1,896 (2.10)	35,684 (3.72)	37,580 (3.58)
East of England	2,939 (3.26)	33,788 (3.52)	36,727 (3.50)
London	21,985 (24.40)	231,438 (24.11)	253,423 (24.13)
North-East	3,018 (3.35)	32,457 (3.38)	35,475 (3.38)
North-West	19,460 (21.60)	174,967 (18.23)	194,427 (18.52)
South-Central	8,542 (9.48)	116,641 (12.15)	125,183 (11.92)
South-East	9,157 (10.16)	91,659 (9.55)	100,816 (9.60)
South-West	9,471 (10.51)	110,000 (11.46)	119,471 (11.38)
West Midlands	9,309 (10.33)	85,637 (8.92)	94,946 (9.04)
Yorkshire & Humber	4,317 (4.79)	47,740 (4.97)	52,057 (4.96)
Comorbidities at baseline			
Coronary heart disease	30,215 (33.54)	16,659 (1.74)	46,874 (4.46)
Stroke	14,949 (16.59)	14,335 (1.49)	29,284 (2.79)
Diabetes	33,644 (37.34)	28,496 (2.97)	62,140 (5.92)
Epilepsy	1,906 (2.12)	13,766 (1.43)	15,672 (1.49)
Hypothyroidism	7,887 (8.75)	27,221 (2.84)	35,108 (3.34)
Arthritis	22,435 (24.90)	51,393 (5.35)	73,828 (7.03)
Anxiety	8,528 (9.47)	108,416 (11.29)	116,944 (11.14)
Migraine	4,004 (4.44)	57,199 (5.96)	61,203 (5.83)
Cancer	17,219 (19.11)	63,390 (6.60)	80,609 (7.68)
Asthma	16,543 (18.36)	142,556 (14.85)	159,099 (15.15)
Renal failure	1,820 (2.02)	2,439 (0.25)	4,259 (0.41)
Liver failure	2,329 (2.59)	6,714 (0.70)	9,043 (0.86)

Osteoporosis	3,739 (4.15)	9,731 (1.01)	13,470 (1.28)
Suicidality	1,271 (1.41)	24,497 (2.55)	25,768 (2.45)
Antidepressant category at baseline			
None	43,252 (48.01)	333,676 (34.76)	376,928 (35.89)
SSRIs	37,569 (41.70)	539,367 (56.18)	576,936 (54.94)
TCAs	3,160 (3.51)	43,420 (4.52)	46,580 (4.44)
MAOIs	8 (0.01)	77 (0.01)	85 (0.01)
other ADs	6,105 (6.78)	43,471 (4.53)	49,576 (4.72)
Use of other drugs at baseline			
Antihypertensive	50,919 (56.52)	43,572 (4.54)	94,491 (9.00)
Aspirin	35,914 (39.86)	19,277 (2.01)	55,191 (5.26)
Anticoagulants	6,945 (7.71)	7,610 (0.79)	14,555 (1.39)
NSAIDs	4,188 (4.65)	31,662 (3.30)	35,850 (3.41)
Anticonvulsants	3,975 (4.41)	13,604 (1.42)	17,579 (1.67)
Hypnotics	6,782 (7.53)	63,313 (6.60)	70,095 (6.68)
Bisphosphonates	1,219 (1.35)	2,407 (0.25)	3,626 (0.35)
Contraceptives	224 (0.25)	62,808 (6.54)	63,032 (6.00)

4.3.2 Study outcomes

For all outcomes, number of events tables are in Appendix A4.5; regression analyses in Appendix A4.6; the unadjusted and adjusted CCAs and FSAs tables in Appendix A4.7; the adjusted FSAs in Figure 4.2.

Figure 4.2 *Study outcomes, FSAs, adjusted*



Legend. Green indicates a positive result, red indicates a negative result, blue indicates no effect. 2mo: two months, 6mo: six months, 12mo: twelve months. For efficacy outcomes, an OR>1 or MD<0 favours statin users (the efficacy outcome "change in depression score" was transformed from mean difference to odds

ratio and depicted as such for the purpose of this illustration). For adverse events outcomes, an OR<1 favours statin users

On the FSA (N 1,050,105), unadjusted analyses seemed to suggest an association between statin use and better efficacy outcomes, lower psychiatric symptoms, anxiety, self-harm, suicidality, and completed suicide, but higher adverse events, sleep disturbance, memory impairment, and all-cause mortality. However, most of these results were not confirmed after being controlled for several confounders.

For efficacy, adjusted analyses, there was no evidence that statin use was associated with changes in depression score ($aMD_{2\ months}$ -0.09, 99%CI -0.35, 0.16; $aMD_{6\ months}$ -0.12, 99%CI -0.72, 0.49; $aMD_{12\ months}$ -0.41, 99%CI -1.30, 0.49), response ($aOR_{2\ months}$ 1.04, 99%CI 0.94, 1.15; $aOR_{6\ months}$ 1.04, 99%CI 0.90, 1.19; $aOR_{12\ months}$ 1.12, 99%CI 0.89, 1.40), or remission ($aOR_{2\ months}$ 1.03, 99%CI 0.94, 1.13; $aOR_{6\ months}$ 1.03, 99%CI 0.89, 1.20; $aOR_{12\ months}$ 1.11, 99%CI 0.90, 1.38) as measured on the PHQ-9 at any of the timepoints assessed.

For adverse events, adjusted analyses, statin use was associated with a lower number of people experiencing any adverse events at two and six months ($aOR_{2\ months}$ 0.93, 99%CI 0.91, 0.96; $aOR_{6\ months}$ 0.97, 99%CI 0.94, 0.99), as well as reduced all-cause mortality at all timepoints ($aOR_{2\ months}$ 0.66, 99%CI 0.60, 0.72; $aOR_{6\ months}$ 0.66, 99%CI 0.60, 0.72; $aOR_{12\ months}$ 0.67, 99%CI 0.65, 0.73). No further differences between study arms could be identified for any psychiatric symptom, anxiety, sleep disturbance, memory impairment, self-harm, suicidality, or completed suicide at any timepoint.

Results from the CCA largely overlapped with the above, though they also highlighted a reduction in self-harm episodes (N= 88,227, $aOR_{12\ months}$ 0.25, 99%CI 0.08, 0.73) and higher memory impairment (N= 88,227, $aOR_{12\ months}$ 1.13, 99%CI 1.04, 1.23) in statin users at twelve months on the adjusted analyses.

4.4 Discussion

This study has explored the potential antidepressant and neuropsychiatric effects of statins in a large cohort of 1,050,105 patients with a diagnosis of depressive disorder, registered on QResearch, and followed up for 12 months in real-world conditions. The two study groups of 90,094 statin users and 960,011 statin non-users displayed several differences at baseline (e.g., age, sex, physical comorbidities, concurrent pharmacological treatments), as expected in an observational setting. Unadjusted analyses that did not account for these variables suggested that statin use could be associated with better psychiatric outcomes (i.e., improved depression ratings and lower psychiatric symptoms including anxiety, self-harm, suicidality, completed suicide), but worse adverse events, including sleep disturbance, memory impairment, and all-cause mortality. A multivariate logistic regression analysis, which controls for such baseline differences by adjusting for numerous variables previously shown to be potential confounders for the exposure and outcomes of interest[327,328,331], was therefore performed. These variables are recorded with considerable detail on QResearch[326]; when unavailable, an elaborate imputation procedure was used to account for missing values. Nevertheless, it is possible that some variables, for examples certain medications whose impact on neuropsychiatric symptoms is currently unknown, may have gone amiss. Results were computed for two datasets: one including imputed data (i.e., FSA), and one without them (i.e., CCA), as both carry valuable information[332]. Adjusted analyses showed that statin use, compared to non-use, was not associated with better response, remission, and change in depression score as measured on the PHQ-9, nor with differences for overall psychiatric symptoms, anxiety, sleep disturbance, suicidality, or completed suicide on either dataset. Lower all-cause mortality in statin users at all timepoints was found on both the FSA and CCA. Statin use

was also associated with lower adverse events at 2 and 6 months on the FSA, and lower self-harm but higher memory impairment at 12 months on the CCA.

The observed lack of antidepressant efficacy for statin users strengthens the findings from the meta-analysis of randomised controlled trials in Chapter 2 and similar previous work[272], which could not show that statins were more efficacious than placebo for depressive symptoms in the absence of concurrent treatment with antidepressants. It should be noted that the present study assessed antidepressant efficacy in an epidemiological context by means of the PHQ-9, a validated clinical scale for measuring depressive symptom severity[329], which is recorded on QResearch during routine primary care for depression. The choice of this type of outcome measure for efficacy is more akin to a trial methodology[333], whereas previous observational studies[228,229], particularly when conducted on large nation-wide databases[236,237,257], would not usually have access to individual patients' depression ratings and so need to rely on less direct measures such as psychiatric hospitalisations or antidepressant prescriptions. These perhaps more objective outcome measures may be able to capture a more global effect that statins would express on patients functioning[205] – something that could escape measurement via a depression-specific scale. On the other hand, the several established benefits of statins on physical health may soften the general perception of one depressed patient's severity and risk, thus making them less likely to be hospitalised for their mental health or to prompt a change in antidepressant therapy[334], even though their depressive symptoms remain significant. As discussed before, it is also possible that statins would manifest either positive, negative, or neutral effects on depression in specific subsets of patients according to their demographic and clinical characteristics[111]. For example, a previous epidemiological study had shown a greater decrease in depression risk when the endpoint was in-patient

treatment, arguably reflecting more severe cases of depression, compared to primary care treatment[236] – as for patients in the current sample.

Findings for adverse events, which were lower in this large sample of statin users compared to non-users, confirm again the known safety of statins[335]. Overall, neuropsychiatric adverse outcomes were not shown to correlate with statin use in people with depression. In contrast with some pre-clinical evidence raising concerns about possible anxiogenic properties of statins[32], results for anxiety aligned with those of other clinical studies that had not found these deleterious effects[236,301]. The oftentimes reported sleep abnormalities associated with statins[336] were not seen in this subgroup of individuals diagnosed with depression, therefore more likely to experience sleep difficulties. Likewise, the broad lack of association between statins and self-harming or suicidal behaviour, in a population that is more susceptible to experience these, can curb previous concerns about increased impulsivity and suicidality in statin users[337]. In fact, tentative evidence from the CCA suggested that self-harm at 12 months was lower in depressed subjects using statins, which is in keeping with similar results previously described in patients with schizophrenia and bipolar disorder[110]. Similar caution should be applied to the isolated finding that memory impairment seemed worse for statin users on the CCA at 12 months. While more evidence is being gathered on this matter, with not always consistent results[338-340], it seems reasonable that some concerns about the potentially harmful effects of statins on cognitive function should remain.

Finally, as the tangible benefit of statins on all-cause mortality in the general population is recently being re-examined[341], a moderate reduction (i.e., between 22% and 34% across the two datasets at all timepoints) in this large sample of primary care patients with depression was observed. This finding has relevance to public health in the context of the

notorious 10-25 years "mortality gap", largely driven by physical comorbidities, in people with severe mental illness such as major depressive disorder[342,343]. Lifestyle psychosocial interventions have proved useful for improving physical health[422] and reducing mortality[423], but several barriers, including concordance with treatment, may hinder their immediate application to patients suffering from depressive symptoms. Adherence to pharmacological treatment, especially antidepressants, is another significant issue[424] that has been associated with ill-health and mortality in depression[352]. Overall, interventions aimed at improving the physical health of patients with depressive disorders are under researched and even less frequently applied to clinical practice, but if psychiatry is to fully integrate with modern medicine, it becomes paramount that psychiatrists work confidently at the interplay between physical and mental illness[425]. Here, the use of statins in depression can already provide a clinically meaningful opportunity.

Taken together with the observed lack of association between statin use and negative neuropsychiatric outcomes, these results argue against the underuse of statins in patients with mental illness, including depression[344], due to questionable concerns about adverse events. Conversely, statins might helpfully treat or prevent severe cardiometabolic conditions, thus lowering the mortality gap, exactly in those individuals needing it most because of unhealthy dietary habits and sedentary behaviour frequently seen in people at heightened risk because of depression[345].

4.4.1 Limitations

This study has several limitations that must be considered when interpreting its findings.

Due to its observational design, a lower internal validity can be anticipated as compared to an equivalent randomised controlled trial investigating the effects of statins *versus*

placebo in depression. In this population-based cohort study, I focussed on clinically relevant outcomes that have also been evaluated in comparable trials, as per Chapter 2 and Chapter 3, but measured in a real-world setting. Therefore, an in-depth assessment of antidepressant efficacy measures on the PHQ-9 and adverse events on a longer follow-up of 12 months could be included. Moreover, a larger population of patients with depression including those with multiple comorbidities and co-occurring treatments, normally excluded from clinical trials, could be involved, which increases the external validity of the results. However, patients could only be divided in an exposed (i.e., statin users) and unexposed (i.e., statin non-users) groups depending on statin use at baseline: that is, statins' prescribing was not instigated by the onset of a depressive episode, because in real-world, as compared to a corresponding clinical trial, these medications are not used for treating depression but for the management of cardiometabolic disorders. Indeed, in the UK statin prescription is usually initiated in primary care as advised by an algorithm, QRisk score, which has been developed via QResearch in the first instance. This design still allows to investigate how statin use can affect the progression of the depressive episode as well as the onset of adverse events during the study period, yet confounding by indication is a possible pitfall, which I attempted to control for through an established methodology that adjusts for a high number of potentially confounding variables[327,328,331] recorded in detail on QResearch[326]. Nevertheless, residual confounding remains a possibility in this setting[346]. Ultimately, this study design can only highlight associations, or lack thereof, between the exposure (i.e., statins) and outcomes (i.e., antidepressant effects and adverse events) of interest, but great caution should be exercised in making any causal inference.

Another methodological limitation concerns the large imputation necessary for PHQ-9 scores, which had been recorded less frequently than other data and might have

particularly affected the FSA of the efficacy outcomes. Furthermore, the imputation procedure according to Rubin's rule is liable to bias in the presence of misspecification (i.e., the imputation model's assumptions about the missing data mechanism or the complete data are incorrect) and incompatibility (i.e., one or more assumptions of the imputation model contradict with those made by the analysis procedure) of the imputation models. To reduce the potential impact of this issue, I reported results for both imputed (i.e., FSA) and non-imputed (i.e., CCA) analyses, as advised in previous studies[332]. These showed broadly overlapping results, confirming the robustness of the imputation procedure. Still, it is possible that this study was underpowered to detect the antidepressant effects of statins. Relatedly, the assessment of the effect estimates for efficacy and adverse events may be seen as too conservative, as I used 99%CIs, compared to more commonly reported 95% ones, in view of the large study sample and multiple comparisons for the outcomes of interest. The latter might have led to an inflation of falsely positive results, but this risk seems less relevant in the context of these findings generally showing no difference between study arms. Another intrinsic problem with QResearch is that adverse events may have been inconsistently recorded, as the collection of these data would rely on direct GPs' enquiring or patients' reporting of them. However, this issue would potentially apply to the whole study population, therefore any potential group's differences, or lack thereof, would not be affected overall.

Finally, as mentioned before, an intriguing avenue for further epidemiological research would explore the plausibly different biological effects of statins on neuropsychiatric outcomes according to these drugs' lipophilicity and thus brain penetrance[304]. The current study analysed the class effect for all statins on these outcomes but did not compare between individual statins as such information was not available; besides, simvastatin and atorvastatin, both lipophilic molecules, account for most of statins' prescriptions in UK

primary care[347]. It should be noted, however, that data from randomised controlled trials[283,309,320] and observational studies[236,238,239] have reported conflicting evidence on this subject.

4.4.2 Conclusion

Evidence from this real-world, population-based cohort study of primary care patients with a diagnosis of depressive disorder followed up for 12 months did not show an association between statin use and antidepressant efficacy or most neuropsychiatric adverse events. Although some concerns persist about the impact of long-term statin use and impaired memory, the broadly observed lack of neuropsychiatric adverse outcomes and the reduction in all-cause mortality in this potentially vulnerable population with depression can provide reassurance to patients, clinicians, and public health. As seen in Chapter 2 and Chapter 3 though, some evidence points towards more favourable effects of statins in depression when used as an adjunct to antidepressants. The next Chapter 5 considers this issue again, as done in Chapter 3, but from an epidemiological perspective.

Chapter 5. Effects of statin plus antidepressant in depression – a real-world epidemiological study

5.1 Introduction

The meta-analysis of randomised controlled trials in Chapter 2 and the epidemiological study in Chapter 4 have shown that statin use, compared respectively to placebo and non-use, does not seem associated to an antidepressant effect. In Chapter 3, on the other hand, the meta-analysis of randomised controlled trials – three conducted in Iran, one in Australia – in depressed participants has found that statins, in addition to any antidepressant treatment, are more efficacious than antidepressants plus placebo at 2 months [N 238, standardised mean difference (SMD) -0.48, 95% confidence interval (CI) -0.74 to -0.22] and 3 months (N 134, SMD-0.47, 95%CI -0.89 to -0.05), while also displaying comparable acceptability, tolerability, and safety. I have also discussed how such evidence appears particularly suggestive in the context of some pre-clinical[127,129,131-136] and clinical[272,298,299] studies supporting these findings. Once again though, it is important to note that small sample sizes, short lengths of follow-up, and the inclusion of highly selected populations may limit both the internal and external validity of these data – an issue that can commonly affect clinical trials[323]. Furthermore, trials are generally powered for assessing efficacy, but less so for other outcome measures[348], for which observational data represent a precious counterpart.

Only one study on a historical cohort of selective serotonin reuptake inhibitors (SSRIs) users, comparing people concurrently using a statin with those who were not, found a lower risk for psychiatric hospital contacts due to depression [N 872,216, hazard ratio (HR) 0.64, 95%CI 0.55 to 0.75] among the former[305]. This study therefore did not have a specific focus on depression since SSRI use can be linked to several other conditions, including non-psychiatric ones[349]. Besides, outcomes such as treatment discontinuations

or several adverse events, which are particularly relevant to depressed patients[13,14], had not been fully explored.

In view of this data gap, I present a real-world epidemiological study of a primary care database to investigate the acceptability, tolerability, safety, and efficacy associated with statin plus antidepressant *versus* antidepressant alone use in patients with depressive disorders.

5.2 Methods

This study was part of a larger project[325] registered on QResearch (OX3 "Choosing the right antidepressant for depressive disorder"), independently reviewed and approved by the QResearch scientific committee (18/EM/0400). The study-specific protocol is available at https://osf.io/96zcn/.

5.2.1 Database

QResearch primary care research registry (www.QResearch.org) was investigated. This database includes the anonymised electronic healthcare records of over 35 million patients registered with 1,574 practices in England, recording demographic data (e.g., age, gender), characteristics (e.g., height, weight, smoking status), symptoms and adverse events, clinical diagnoses, and prescribed medications[326]. The Egton Medical Information Systems (EMIS), managed centrally by QResearch, was used to obtain consent to provide data.

5.2.2 Cohort construction

Patients aged 18-100 years, newly diagnosed with a depressive disorder according to validated "Read codes"[327,328] already employed for the epidemiological study in Chapter 4 (Appendix A4.1), registered with English practices between 1st January 1998 and 15th August 2020 for at least 12 months were included. Compared to the previous study, initiation of first antidepressant treatment, as further detailed below, represents the study entry date. Exclusion criteria encompassed: any prescription of antidepressants or any depression diagnosis before the study entry date; any depression diagnosis made 2 months before or 2 months after starting an antidepressant; a lifetime diagnosis of schizophrenia spectrum disorder or bipolar affective disorder; any diagnosis of post-partum depression within 180 days before or up to 180 days after the first diagnosis of depression; any prescription of antipsychotics, mood stabilisers, or more than one antidepressant at baseline; no prescription of antidepressant at baseline. Participants in the study cohort were followed up for 12 months.

5.2.3 Exposure and comparison

The exposure under investigation was the use at baseline of any statin at the same time of the prescription of any antidepressant monotherapy (any statin+antidepressant) *versus* the use of any antidepressant alone (antidepressant-only) – drugs licensed in the UK according to the British National Formulary (BNF, https://bnf.nice.org.uk) (Appendix A5.1).

5.2.4 Outcomes of interest

Outcomes were measured at 2 months, 6 months, and 12 months from the study entry date (i.e., initial prescription of an antidepressant).

Acceptability was assessed as the ratio of all-cause drop-out from antidepressant treatment. Treatment drop-out was defined as: a patient with a >30-day gap between the end of a prescription of an antidepressant and the start of the next prescription (based on an average prescription length of 28 to 30 days), a patient switching to another antidepressant, or a patient prescribed any additional antidepressant, mood stabiliser, or antipsychotic.

Tolerability was assessed as the ratio of drop-out from treatment within 30 days from any adverse event, already listed for Chapter 4 (Appendix A4.2). Therefore, treatment drop-out would have to occur within 30 days of an adverse event to be assumed to be a consequence of it.

Adverse events were assessed as: number of patients who had at least one of any adverse event (Appendix A4.2); self-harm; suicidality; completed suicide; all-cause mortality.

Efficacy outcomes were assessed via the Patient Health Questionnaire (PHQ)-9[329] as: response (50% score reduction); remission (score <5); change in depression score.

5.2.5 Statistical analysis

Analyses were conducted on Stata MP v16.0[317] on the QResearch server. Baseline characteristics of the study cohort were examined with descriptive statistics for the total sample, as well as separately for any statin+antidepressant and antidepressant-only groups. Outcomes of interest were explored using an intention-to-treat analysis with multivariate logistic regression, clustered by practices, to compute mean differences (MDs, for continuous outcomes) and odds ratios (ORs, for dichotomous outcomes) with 99% confidence intervals (99%CIs) for any statin+antidepressant *versus* antidepressant-only (i.e., between-subject design), both unadjusted and adjusted (aMD, aOR) for

previously validated potential confounders (Appendix A4.3). Multiple imputation by chained equations was employed to impute data when values were not available: for each imputation, 10 imputed datasets were generated, and coefficient estimates across these were combined using Rubin's rule[330]. Results were calculated for both the complete case analysis (CCA) and the full set analysis (FSA, including imputed data).

Compared to the epidemiological study in Chapter 4, some *post-hoc* analyses were conducted to corroborate any potential positive finding. In view of the expected age difference between the group on any statin+antidepressant and the one on antidepressant-only, a sensitivity analysis on the sample of patients aged >65 years only was performed. Moreover, data for statins was compared with those of the regression analyses for aspirin to verify whether results were non-specifically associated to another medication used in a similar population.

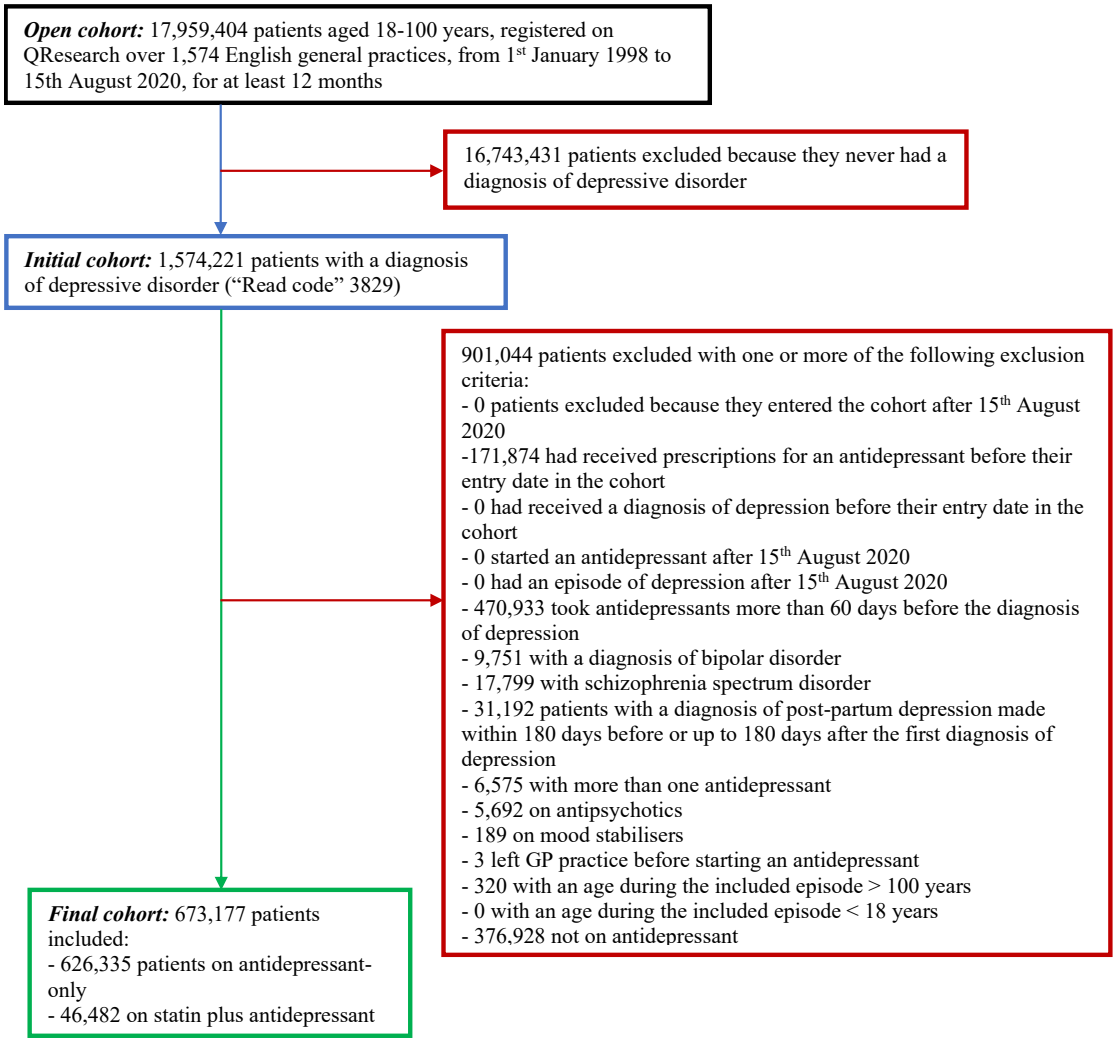
5.3 Results

The study cohort and analyses for all outcomes are reported below.

5.3.1 Study cohort

The cohort construction is in Figure 5.1. From a preliminary cohort of 1,574,221 patients with a diagnosis of depression ("Read code" 3829), following the application of inclusion/exclusion criteria, the study cohort eventually comprised 673,177 patients: 46,482 on any statin+antidepressant and 626,335 on antidepressant-only. Compared to the study cohort of the epidemiological study in Chapter 4, 376,928 patients were excluded for not being on any antidepressant, thus reducing sample size while increasing the homogeneity of the sample.

Figure 5.1 *Cohort construction flow chart*



Baseline characteristics of the study cohort are in Table 5.1. In summary, compared to the antidepressant-only group, patients on any statin+antidepressant were older (mean age 40.9 years *versus* 67.1 years), had more physical health comorbidities (particularly, cardiovascular, cerebrovascular, metabolic, and neoplastic), and were taking more non-psychiatric medications. These expected baseline differences between the two groups were controlled for in the multivariate regression analyses, adjusted model. Missing data, imputed for the FSA, are in Appendix A5.2.

Table 5.1 *Baseline characteristics of study cohort. Values are numbers (percentages) unless stated otherwise*

Characteristic	Any statin+antidepressant N 46,482	Antidepressant-only N 626,335	All sample N 673,177
Sex			
Male	26,314 (56.18)	262,431 (41.90)	288,745 (42.89)
Mean age (SD)	67.13 (14.10)	40.92 (16.54)	42.75 (17.69)
Read codes for depression			
Major depression	27,834 (59.42)	430,221 (68.69)	458,055 (68.04)
Minor depression	17,456 (37.27)	176,528 (28.18)	193,984 (28.82)
Other	1,552 (3.31)	19,586 (3.13)	21,138 (3.14)
PHQ-9, at baseline (SD)	N 10,408	N 133,939	N 144,347
	16.07 (5.22)	17.17 (4.92)	17.09 (4.95)
Year of diagnosis			
1998 to 2005	7,167 (15.30)	190,673 (30.44)	197,840 (29.39)
2006 to 2010	12,728 (27.17)	126,689 (20.23)	139,417 (20.71)
2011 to 2015	13,866 (29.60)	145,690 (23.26)	159,556 (23.70)
2016 to 2020	13,081 (27.93)	163,283 (26.07)	176,364 (26.20)
Body Mass Index (SD)	N 42,587	N 481,533	N 524,120
	28.77 (6.16)	26.01 (5.85)	26.24 (5.93)
Smoking (cigarettes/day)	N 45,707	N 574,590	N 620,297
Non-smoker	20,821 (45.55)	279,118 (48.58)	299,939 (48.35)
Ex-smoker	15,223 (33.31)	102,395 (17.82)	117,618 (18.96)
Light smoker (1-9)	9,031 (19.76)	176,979 (30.80)	186,010 (29.99)
Moderate smoker (10-19)	400 (0.88)	11,374 (1.98)	11,774 (1.90)
Heavy smoker (≥20)	232 (0.51)	4,724 (0.82)	4,956 (0.80)
Alcohol (daily units)	N 28,639	N 311,478	N 340,117

Non-drinker/trivial (< 1)	15,722 (54.90)	170,934 (54.88)	186,656 (54.88)
Light (1-2)	8,737 (30.51)	103,715 (33.30)	112,452 (33.06)
Medium (3-6)	3,299 (11.52)	27,427 (8.81)	30,726 (9.03)
Heavy (7-9)	459 (1.60)	4,233 (1.36)	4,692 (1.38)
Very heavy (> 9)	422 (1.47)	5,169 (1.66)	5,591 (1.64)
Ethnic group	N 38,483	N 454,840	N 493,323
White	34,237 (88.97)	395,624 (86.98)	429,861 (87.14)
Indian	797 (2.07)	7,104 (1.56)	7,901 (1.60)
Pakistani	767 (1.99)	7494 (1.65)	8,261 (1.67)
Bangladeshi	609 (1.58)	4,987 (1.10)	5,596 (1.13)
Other Asian	531 (1.38)	6,674 (1.47)	7,205 (1.46)
Caribbean	452 (1.17)	5,551 (1.22)	6,003 (1.22)
Black African	351 (0.91)	8,004 (1.76)	8,355 (1.69)
Chinese	59 (0.15)	2,010 (0.44)	2,069 (0.42)
Other	680 (1.77)	17,392 (3.82)	18,072 (3.66)
Townsend deprivation score in fifths	N 46,770	N 624,118	N 670,888
1 (Least deprived)	11,545 (24.68)	127,305 (20.40)	138,850 (20.70)
2	10,746 (22.98)	132,005 (21.15)	142,751 (21.28)
3	9,683 (20.70)	131,087 (21.00)	140,770 (20.98)
4	8,222 (17.58)	121,405 (19.45)	129,627 (19.32)
5 (Most deprived)	6,574 (14.06)	112,316 (18.00)	118,890 (17.72)
Region of England			
East Midlands	1,136 (2.43)	23,054 (3.68)	24,190 (3.59)
East of England	1,742 (3.72)	23,486 (3.75)	25,228 (3.75)
London	8,469 (18.08)	130,577 (20.85)	139,046 (20.66)
North-East	1,687 (3.60)	21,708 (3.47)	23,395 (3.48)
North-West	11,283 (24.09)	122,774 (19.60)	134,057 (19.91)

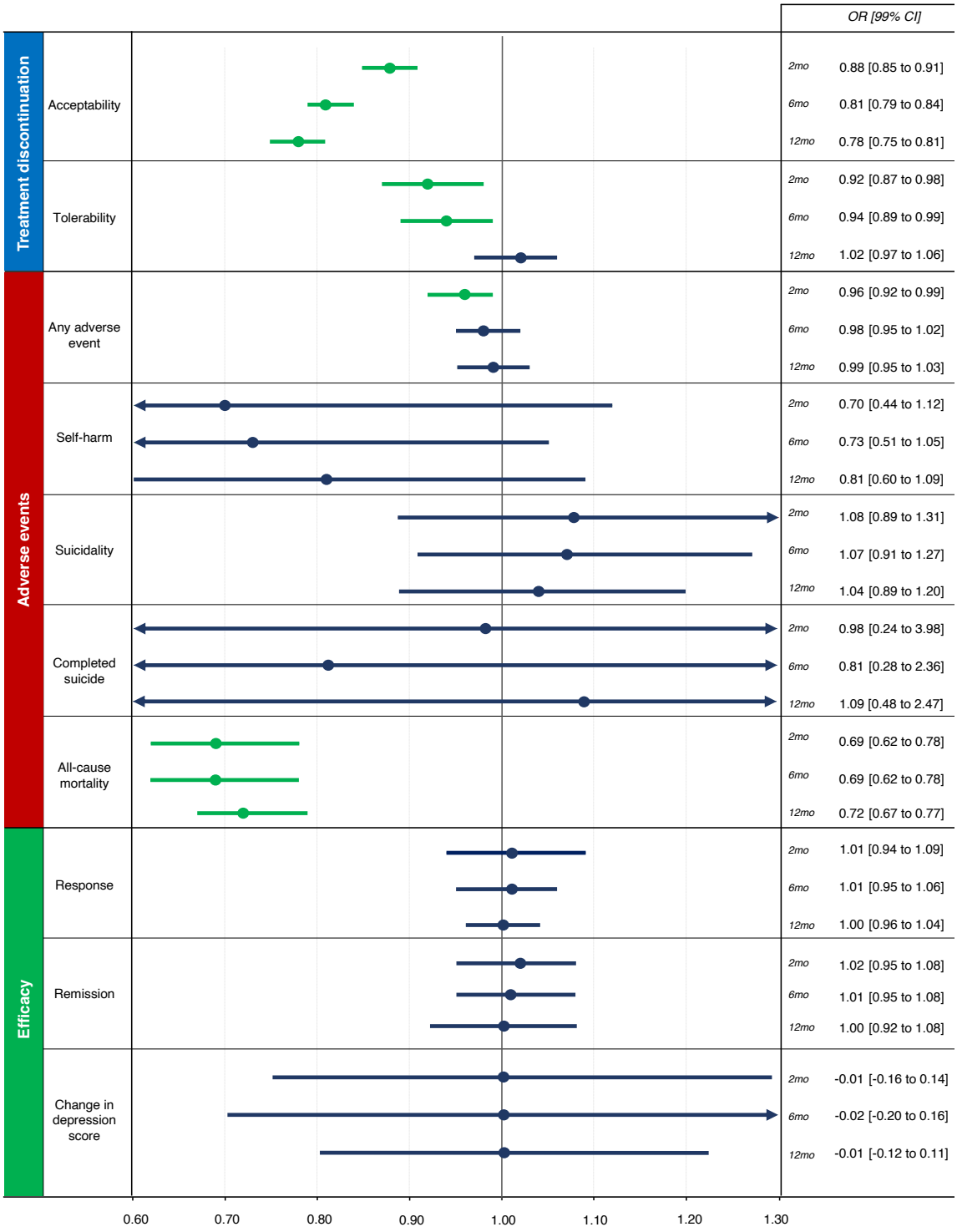
South-Central	5,183 (11.06)	80,591 (12.87)	85,774 (12.74)
South-East	4,902 (10.46)	61,619 (9.84)	66,521 (9.88)
South-West	4,706 (10.05)	71,044 (11.34)	75,750 (11.25)
West Midlands	5,490 (11.72)	60,420 (9.65)	65,910 (9.79)
Yorkshire & Humber	2,244 (4.79)	31,062 (4.96)	33,306 (4.95)
Comorbidities at baseline			
Coronary heart disease	14,068 (30.03)	9,030 (1.44)	23,098 (3.43)
Stroke	8,414 (17.96)	8,934 (1.43)	17,348 (2.58)
Diabetes	14,879 (31.76)	14,784 (2.36)	29,663 (4.41)
Epilepsy	953 (2.03)	7,911 (1.26)	8,864 (1.32)
Hypothyroidism	4,076 (8.70)	16,792 (2.68)	20,868 (3.10)
Arthritis	11,411 (24.36)	32,637 (5.21)	44,048 (6.54)
Anxiety	5,230 (11.17)	73,684 (11.76)	78,914 (11.72)
Migraine	2,287 (4.88)	37,411 (5.97)	39,698 (5.90)
Cancer	8,789 (18.76)	40,233 (6.42)	49,022 (7.28)
Asthma	8,451 (18.04)	93,238 (14.89)	101,689 (15.11)
Renal failure	803 (1.71)	1,339 (0.21)	2,142 (0.32)
Liver failure	1,122 (2.40)	4,125 (0.66)	5,247 (0.78)
Osteoporosis	1,909 (4.08)	5,939 (0.95)	7,848 (1.17)
Suicidality	677 (1.45)	16,807 (2.68)	17,484 (2.60)
Antidepressant category at baseline			
SSRIs	37,569 (80.20)	539,367 (86.11)	576,936 (85.70)
TCAs	3,160 (6.75)	43,420 (6.93)	46,580 (6.92)
MAOIs	8 (0.02)	77 (0.01)	85 (0.01)
other ADs	6,105 (13.03)	43,471 (6.94)	49,576 (7.36)
Use of other drugs at baseline			
Antihypertensives	24,753 (52.84)	26,283 (4.20)	51,036 (7.58)
Aspirin	17,203 (36.73)	11,672 (1.86)	28,875 (4.29)

Anticoagulants	3,523 (7.52)	4,492 (0.72)	8,015 (1.19)
NSAIDs	2,466 (5.26)	22,503 (3.59)	24,969 (3.71)
Anticonvulsants	2,223 (4.75)	8,247 (1.32)	10,470 (1.56)
Hypnotics	4,788 (10.22)	47,389 (7.57)	52,177 (7.75)
Bisphosphonates	661 (1.41)	1,648 (0.26)	2,309 (0.34)
Contraceptives	143 (0.31)	39,530 (6.31)	39,673 (5.89)

5.3.2 Study outcomes

For all outcomes, number of events tables are in Appendix A5.3; regression analyses in Appendix A5.4; the unadjusted and adjusted CCAs and FSAs tables in Appendix A5.5; the adjusted FSAs in Figure 5.2.

Figure 5.2 *Study outcomes, FSAs, adjusted*



Legend. Green indicates a positive result, red indicates a negative result, blue indicates no effect. 2mo: two months, 6mo: six months, 12mo: twelve months. For treatment discontinuation and adverse events outcomes, an OR<1 favours statin users. For efficacy outcomes, an OR>1 favours statin users (the efficacy outcome "change in depression score" was transformed from mean difference to odds ratio and depicted as such for the purpose of this illustration)

Patients on any statin+antidepressant showed lower all-cause discontinuation of antidepressant treatment (i.e., higher acceptability) compared to the antidepressant-only group at all time-points, which remained consistent after adjustment for both the complete CCA and the FSA (N 673,177, $aOR_{2\ months}$ 0.88, 99%CI 0.85 to 0.91; $aOR_{6\ months}$ 0.81, 99%CI 0.79 to 0.84; $aOR_{12\ months}$ 0.78, 99%CI 0.75 to 0.81).

Tolerability (i.e., discontinuation of antidepressant treatment due to adverse events) seemed worse in the statin+antidepressant group at all time-points for both datasets in the unadjusted analyses. However, after adjusting for confounders, this association was not confirmed in the CCA and suggested better tolerability for the statin+antidepressant group at 2 and 6 months in the FSA (N 673,177, $aOR_{2\ months}$ 0.92, 99%CI 0.87 to 0.98; $aOR_{6\ months}$ 0.94, 99%CI 0.89 to 0.99; $aOR_{12\ months}$ 1.02, 99%CI 0.97 to 1.06).

The number of patients with at least one adverse event was higher in the statin+antidepressant group at all time-points for both datasets in the unadjusted analyses. Adjusted analyses though showed no difference between the two groups, even though a smaller number of patients experienced adverse events in the statin+antidepressant group at 2 months in the FSA (N 673,177, aOR $0.96_{2\ months}$, 99%CI 0.92 to 0.99).

For self-harm, suicidality, and completed suicide, the overall results seemed to favour the statin+antidepressant group in the unadjusted analyses at all time-points in both datasets. However, only self-harm was lower at 12 months in the statin+antidepressant group in the CCA (N 63,801, $aOR_{12\ months}$ 0.20, 99%CI 0.06 to 0.72).

All-cause mortality was higher in patients on any statin+antidepressant at all time-points in both datasets. However, following adjustment, the statin+antidepressant group showed a lower number of deaths in the CCA at 12 months, and at all time-points in the FSA (N

673,177, $aOR_{2\ months}$ 0.69, 99%CI 0.62 to 0.78, $aOR_{6\ months}$ 0.69, 99%CI 0.62 to 0.78, $aOR_{12\ months}$ 0.72, 99%CI 0.67 to 0.77).

Though unadjusted analyses showed higher response and remission rates and lower depression scores for the statin+antidepressant group at most time-points in the two datasets, adjustment did not confirm these results for any of the efficacy outcomes measured.

Considering the age difference between the two study groups, a sensitivity analysis including only patients aged >65 years was conducted (i.e., excluding 588,196 participants with an age during the included episode <65 years). Characteristics of this subgroup (N 25,857 on any statin+antidepressant, N 59,124 on antidepressant-only) and analyses for all outcomes are in Appendix A5.6. Results largely reflected those of the whole study cohort. Specifically, people on any statin+antidepressant showed higher acceptability at all time-points ($aOR_{2\ months}$ 0.89, 99%CI 0.85 to 0.93, $aOR_{6\ months}$ 0.85, 99%CI 0.81 to 0.89, $aOR_{12\ months}$ 0.81, 99%CI 0.78 to 0.88), higher tolerability at 2 months ($aOR_{2\ months}$ 0.92, 99%CI 0.86 to 0.99), and lower all-cause mortality at all time-points ($aOR_{2\ months}$ 0.69, 99%CI 0.62 to 0.78, $aOR_{6\ months}$ 0.69, 99%CI 0.62 to 0.78, $aOR_{12\ months}$ 0.71, 99%CI 0.66 to 0.76).

To check whether the observed outcomes for the study cohort were possibly associated to the use of statins as compared to other medications prescribed in similar populations, we considered the results of the regression analyses for aspirin (Appendix A5.4). In summary, outcomes for aspirin use did not overlap with those found for statins (Appendix A5.7). Indeed, aspirin use was associated with lower tolerability ($aOR_{6\ months}$ 1.09, 95% CI 1.03 to 1.16, $aOR_{12\ months}$ 1.08, 95% CI 1.03 to 1.14) and worse safety in terms of any adverse events ($aOR_{2\ months}$ 1.07, 95% CI 1.07 to 1.11, $aOR_{6\ months}$ 1.12, 95% CI 1.07 to 1.17, aOR_{12}

$_{\text{months}}$ 1.18, 95% CI 1.12 to 1.24) and all-cause mortality ($aOR_{12\ months}$ 1.15, 95% CI 1.07 to 1.24).

5.4 Discussion

This study has explored a large cohort of 673,177 patients with a diagnosis of depressive disorder on antidepressant monotherapy, of whom 46,482 were concomitantly taking a statin, registered on QResearch, and followed up for 12 months in real-world conditions. As for Chapter 4, I had access to detailed information[326] about numerous potential confounders[327,328,331] and performed both unadjusted and adjusted analyses for each outcome via multivariate logistic regression. Results for both the CCA and FSA were reported, as per prior advice[332]. A consistent association between statin plus antidepressant, compared to antidepressant alone, and fewer treatment dropouts at 2 months, 6 months, and 12 months was found. Similarly, the statin plus antidepressant group showed better treatment tolerability at 2 months and 6 months. Despite some variability between the CCA and FSA, data supported the safety of adding a statin to antidepressant treatment, including lower adverse events at 2 months, self-harm at 12 months, and all-cause mortality at all time-points. No differences for any of the PHQ-9 efficacy outcomes was observed. Results remained robust to a sensitivity analysis in patients aged >65 years, and when compared to those for aspirin users. An infographic in lay terms, realised with the feedback of members of the public and patient groups (PPI), summarises these findings in Appendix A5.8, thus adding a PPI component to this study.

Overall, results for the statin plus antidepressant group match those of Chapter 4, which have observed a favourable profile in terms of adverse events, self-harm, and all-cause mortality for statin users. In addition, the present study highlights better antidepressant

acceptability and tolerability with concomitant use of statins. The latter findings are in line with a cohort study that highlighted a modest yet significant association between adherence to selective SSRIs and adherence to statins, which were also separately associated with lower all-cause mortality rates[350]. The observation that combined statin and antidepressant treatment increases the acceptability and tolerability of the latter can have clinically relevant implications. Consistent with previously reported data[14], a high percentage of dropouts in both the exposure and comparison arms at all time-points was observed (Appendix A5.3); however, drop-out from treatment was significantly lower in the statin plus antidepressant group. An important study in primary care patients has recently linked antidepressant treatment discontinuation to a higher risk of depressive relapse within one year[351]. Add-on statins might therefore help decreasing the risk of depressive relapses by reducing drop-out from antidepressants.

Increased adherence to antidepressant treatment has also been also associated with lower mortality[352]. This raises the possibility that the lower all-cause mortality identified in patients taking both statins and antidepressants may be partly explained by their lower rates of drop-out from treatment. Besides, such association between statin plus antidepressant use and lower mortality has significance in the context of the previously mentioned "mortality gap" in people with severe mental illness, including depression[342,343]. The observed all-cause mortality difference did not seem related to dissimilar rates of death due to suicide, as the numbers for completed suicide, though very small, did not differ between the two groups – as indeed were for suicidal behaviour more in general. Likewise, a similarly designed study on 872,216 incident SSRIs users did not show any difference in suicidal behaviour between patients on SSRI-only and those with concomitant statin use[305]. The reported data showing that suicidality, completed suicide, and self-harm are not worsened by statins in a potentially vulnerable depressed population

on antidepressants have relevance once more in addressing previous concerns that the hypercholesterolaemic effect of statins in the central nervous system (CNS) might lead to higher impulsivity and risk of suicide[337].

The absence of better antidepressant efficacy for the statin plus antidepressant group, compared to antidepressant alone, is instead more difficult to understand in the context of the available literature. In fact, the meta-analysis of randomised controlled trials of Chapter 3 and the previously discussed epidemiological study conducted in SSRIs users[305] had both indicated a more positive effect on depression when antidepressants were combined with statins. The same *caveat* extensively discussed in Chapter 4 still apply, namely that the PHQ-9 scores[329] used to determine efficacy in this study may have been underpowered or anyway unable to gauge the antidepressant benefit of statin add-on. Further, the current sample of patients affected by a first-episode depressive episode could arguably benefit from first-line antidepressant use without the need to add any statin therapy, as it may be the case instead for patients at the more severe end of the spectrum or indeed treatment-resistant. Finally, as suggested in other chapters, it is conceivable that some specific patients' characteristics, possibly related to underlying inflammatory or dysmetabolic processes, could be responsible for the differential effects of statins. Once again, two randomised controlled trials using a statin in addition to antidepressants are underway, and as they are targeting patients with treatment-resistant depression[321] or with comorbid depression and obesity[322], their results might have important implications for the usefulness of this combined treatment strategy.

5.4.1 Limitations

This study has several limitations that must be considered when interpreting its findings.

Briefly, the same methodological constraints and mitigating aspects seen for the epidemiological study in Chapter 4 are pertinent to this investigation too: confounding by indication, controlled for via multivariate logistic regression analysis; residual confounding; large imputation for missing data, dealt with the presentation of both the CCA and FSA. In this case, more explicitly, statins had not been prescribed as a supplement to antidepressant medications with the purpose of augmenting their effects, but for their primary indication of preventing or treating a range of cardiovascular, cerebrovascular, and metabolic disorders. As before though, the study design still allows to investigate how co-occurring prescriptions of statins and antidepressants can affect antidepressant adherence, adverse events, and the progression of the depressive episode.

Since the positive findings for treatment acceptability and tolerability have potential relevance to further research and even clinical practice, I have checked and robustly confirmed their validity through a sensitivity analysis in older patients only as well as a comparison with aspirin, whose prescription largely overlaps with that of statins. Still, this study had not been intended and powered to specifically assess these outcomes, therefore any conclusion based on them should be careful.

5.4.2 Conclusions

Evidence from this real-world, population-based cohort study of primary care patients with a diagnosis of depressive disorder and followed up for 12 months shows that the addition of statins to antidepressants is associated with increased treatment adherence and lower all-cause mortality on the short- and long-term, thus potentially improving both psychiatric and physical health outcomes. These findings can influence the design of future clinical trials and, if supported by further evidence from the latter, may have

implications for patients, clinicians, and public health. Meanwhile, a translational approach that can inform both further clinical studies and pre-clinical research involves the use of experimental medicine – a tool that has shown value for probing psychopharmacological interventions in depression. In the next Chapter 6 and Chapter 7, I therefore employ such methodology to investigate the neuropsychological effects relevant to depression of, respectively, atorvastatin and simvastatin.

Chapter 6. Effects of atorvastatin on emotional processing, reward learning, and verbal memory – an experimental medicine study*

*This chapter is based on the paper: "The effects of atorvastatin on emotional processing, reward learning, verbal memory and inflammation in healthy volunteers: An experimental medicine study", De Giorgi et al., 2021; DOI:10.1177/02698811211060307. Co-authors permission to reuse material is in Appendix, miscellanea

6.1 Introduction

Over Chapter 2 to Chapter 5, I have conducted four studies – two meta-analyses of randomised controlled trials, two epidemiological investigations – that add to a rich, though somewhat uncertain clinical evidence on the effects of statins in depression, as per section 1.4. In section 1.3, I have also described a wealth of pre-clinical evidence suggesting that the putative antidepressant activity of statins may be rooted in complex neurobiological, endocrine-metabolic, cardiovascular, and immunological interactions. Most of these findings, however, stem from *in vitro* or animal models, whose translation into clinically relevant applications to mental health remains challenging at best[225].

Experimental medicine trials are an innovative strategy that can probe medical conditions, such as depression in humans, while allowing to create evidence of bidirectional value: both from bedside-to-bench, and from bench-to-bedside[353]. In this respect, the cognitive neuropsychological model of depression, which uses changes in emotional processing as a biomarker for depressive disorders, has proved an invaluable tool for investigating psychopharmacological treatments with antidepressant potential[354]. Specifically, negative biases in emotional processing have long been recognised as a core feature of depression, leading to a vicious circle of negative feelings, thoughts, and behaviour which triggers and maintains depressive symptoms[355]. These emotional biases can occur across several cognitive domains, including perception, attention, and memory[356]: for example, people with depression are more likely to perceive and categorise facial expressions as negative or to attend and recall negative information in emotional word-based tasks[357]. On this basis, the cognitive neuropsychological model of depression links biological and psychological phenomena and proposes that the initial biological effect of antidepressants (e.g., increase in serotonergic neurotransmission) determine an immediate change of

negative bias in the processing of emotionally salient information, which is crucial to the later clinical response because it produces a psychological reappraisal of the interactions with the social environment, leading to new positive associations, perhaps biologically mediated by neuroplastic processes (e.g., synaptic pruning and synaptogenesis) that ultimately resolve depressive symptomatology[354].

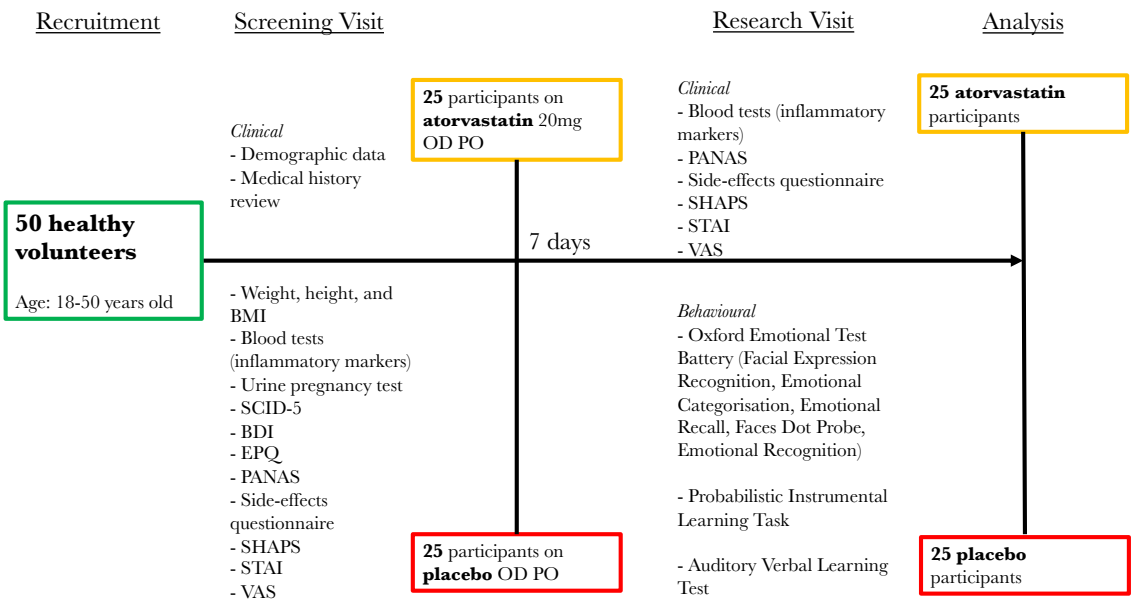
Indeed, drugs with an established antidepressant profile, such as selective serotonin reuptake inhibitors (SSRIs), lead to improvements in tasks measuring emotional biases in both healthy participants[358] and patients with depression[359]. Other molecules whose demonstrated antidepressant action does not necessarily rely on monoaminergic neurotransmission can determine positive shifts in emotional processing[15]; viceversa, some compounds of known depressogenic burden such as the cannabis receptor antagonist, rimonabant produce negative changes[360]. Intriguingly, inflammation, for which growing evidence suggests a key role in the pathophysiology of depression[207], has been shown to cause worsening of emotional biases[361], as well as deficit in reward-seeking behaviour[362], and overall cognitive impairment[363]. As discussed in section 1.3, modulation of inflammatory process is a pivotal mechanism possibly explaining the antidepressant effects of statins. Therefore, neuropsychological tasks assessing these domains would be an ideal instrument to probe these medications[364], but no studies thus far have done so in an experimental, laboratory setting. Since no previous data are available, assessing the neuropsychological effects of statins in a sample of healthy volunteers is a reasonable first step as it would exclude the impact of symptoms of depression, memory, and executive function deficits on these testing measures. Among statins, atorvastatin is the most prescribed in the UK, therefore I chose to test this molecule in the first instance. A dose of 20mg atorvastatin was successfully and safely employed in earlier trials confirming its rapid anti-inflammatory action[373] as well as its potential use in depression[296,303].

In this experimental medicine trial in healthy volunteers, my primary aim is to investigate the short-term (seven days) effects of the highly potent and moderately lipophilic atorvastatin upon a battery of emotional processing tasks previously shown to be sensitive to the early activity of standard antidepressants. Secondarily, I examine the effects of atorvastatin on reward learning, verbal memory, and inflammation as measured by the inflammatory marker high-sensitivity C-reactive protein (hs-CRP). Based on existing literature proposing that statins may exert antidepressant and anti-inflammatory effects, I hypothesise that seven-day atorvastatin would improve emotional processing, reward learning, and verbal memory, while also decreasing inflammation.

6.2 Methods

The study was a double-blind, parallel groups, randomised, gender-stratified, placebo-controlled, experimental medicine trial, approved by the University of Oxford Central University Research Ethics Committee (MS-IDREC R61966/RE001). The study protocol, which describes the methods in detail, was registered on Clinicaltrials.gov (NCT04652089). The overall study design is in Figure 6.1.

Figure 6.1 *Study design*



BDI: Beck Depression Inventory, EPQ: Eysenck Personality Questionnaire, PANAS: Positive and Negative Affect Schedule, SHAPS: Snaith-Hamilton Pleasure, STAI: State and Trait Anxiety Inventory, VAS: Visual Analogue Scales

6.2.1 Sample size calculation

Based on the primary outcome of accuracy at recognising emotional facial expressions from a previous study of antidepressants in healthy volunteers[358], a sample size of 38 would give 90% power to detect changes of a comparable magnitude (citalopram *versus* placebo effect size F 0.5, Appendix A6.1). G*Power v3.1.9.6[365] software was used for this analysis.

6.2.2 Participants

Inclusion and exclusion criteria were pre-specified in the study protocol and are reported in full in Appendix A6.2. In brief, volunteers were considered eligible if they were free from any current or past mental illness on the basis of their self-reported psychiatric history as well as the administration of the structured clinical interview for DSM-5[366] by

a study clinician. Self-reported medical and drug history were also gathered to ensure that potential participants did not suffer from any significant physical illness and were not taking any regularly prescribed medications, including any psychoactive drugs over the previous 3 months. Women who were pregnant, breast-feeding, or of child-bearing potential not using an approved contraceptive therapy were excluded. Participants who withdrew from the study were replaced as per protocol.

6.2.3 Baseline procedures

Enrolled participants completed a series of questionnaires including the Beck Depression Inventory (BDI)[367], the Eysenck Personality Questionnaire (EPQ)[368], the Positive and Negative Affect Scale (PANAS)[369], the Snaith-Hamilton Pleasure Scale (SHAPS)[370], a side-effect questionnaire (nausea, dizziness, dry mouth, headache, alertness, and agitation scored from 0 to 3), the State-Trait Anxiety Inventory (STAI)[371], and the Bond-Lader Visual Analogue Scales (BL-VAS)[372]. Also, a blood sample to determine serum hs-CRP levels was collected and frozen in two 1.0mL FluidX tubes.

A randomisation code had been designed by a researcher uninvolved in the study using an online randomisation tool (https://www.sealedenvelope.com/simple-randomiser/v1/lists). Accordingly, participants were randomised to either atorvastatin 20mg, a dose used in previous clinical trials of atorvastatin in depression[296,303] or sucrose placebo, encapsulated in identical opaque white capsules, taken orally once daily, at night, for seven days, based on previous evidence suggesting that the anti-inflammatory effects of statins are seen as early as 4 ± 3 days[373].

6.2.4 Testing visit procedures

Following the seven days of assigned treatment, participants returned for a testing visit. On this occasion, they reported their adherence and brought back their capsules' bottle to verify compliance.

Participants completed four of the previously administered questionnaires (PANAS, side-effects questionnaire, STAI-state, BL-VAS), and a second blood sample for hs-CRP: both the baseline and testing visit blood samples were assayed at the end of the study via automated immunoassay using the Architect c16000 analyser.

Finally, participants were administered a battery of neuropsychological tasks including the emotional test battery (ETB), the probabilistic instrumental learning task (PILT), and the auditory-verbal learning task (AVLT).

6.2.4.1 Emotional test battery

The ETB assesses emotional processing ("hot cognition") via five validated[359] computerised tasks: the facial expression recognition task (FERT), the emotional categorisation task (ECAT), the emotional recall task (EREC), the emotional recognition memory task (EMEM), and the faces dot-probe task (FDOT).

The FERT involves the facial expressions of six emotions: anger, disgust, fear, happy, sad, surprise, and a neutral one, adapted from the Karolinska directed emotional faces set[374], each expression depicted at a range of intensity levels[375] and randomly displayed on the computer screen for 500ms. Participants have to identify the presented facial expression as quickly and as accurately as possible by pressing a labelled key on the keyboard. Accuracy at recognising the correct emotion was the primary outcome measure for this study. A signal detection analysis is used to assess discriminability (d', perceptual

choice, a measure of sensitivity), and response bias (β, decisional choice, a measure of conservativeness)[376] for this outcome. Misclassifications and mean reaction times for each facial expression are also noted.

The ECAT involves thirty positively- (e.g., cheerful) and thirty negatively-valenced (e.g., hostile) words[377] randomly displayed on the computer screen for 500ms. These words are matched in terms of word length, ratings of frequency, and meaningfulness. Participants have to indicate as quickly and as accurately as possible whether they would like or dislike to be referred to as one of these words. Accuracy and mean reaction times for positive and negative words are noted. The EREC is an unexpected free-recall task during which participants have to write down as many self-referent words from the ECAT as possible in four minutes. Correctly- (hits) and falsely-recalled (false alarms) positive and negative words are noted. The EMEM involves the sixty self-referent words from the ECAT and sixty matched distractors (thirty positive, thirty negative) randomly displayed on the computer screen for 500ms. Participants have to recognise whether these words have been previously seen (familiar) or not (novel) in the ECAT. Accuracy, false alarms, and reaction times are noted.

The FDOT involves two faces, one neutral, and one emotional (either happy or fearful), briefly displayed at the top and at the bottom of the computer screen and then replaced by a pair of dots with two different (vertical or horizontal) orientations. Participants have to indicate whether the dots are vertically or horizontally aligned. On half the total 192 trials, faces are presented very briefly and immediately switched to a muddled face mask. Mean reaction times from trials when probes are in the same position as the emotional face (congruent trials) are subtracted from mean reaction times from trials when probes are in

the opposite position to the emotional face (incongruent trials) to calculate attentional vigilance scores.

6.2.4.2 Probabilistic instrumental learning task

The PILT, adapted from Pessiglione et al.,[378] assesses reward learning. Two pairs of symbols are displayed on the computer screen for 4,000 milliseconds: one pair is associated with win outcomes (win £0.20 or no change), the other with loss outcomes (lose £0.20 or no change). Each symbol in the pair has reciprocal probabilities (70% or 30%) of either outcome occurring and is randomly positioned either to the left or the right of a central fixation cross. Participants began the task with £1.50 and perform sixty independent trials (thirty win trials and thirty loss trials) for two runs, receiving outcome feedback (win or lose) after each trial. Participants have to use the outcome feedback to gradually learn the symbol-outcome associations over time, to consistently choose the symbol with the high-probability win whilst avoiding the symbol with the high-probability loss. End total amount, amount won, amount lost, reward and lose trials, and number of choice switches (proportion of trials in which the chosen symbol is different to the symbol chosen in the previous trial within the same condition, such that the lower the proportion of switch trials, the greater the confidence the participant is thought to have had in their choices) are noted.

6.2.4.3 Auditory-verbal learning task

The AVLT assesses verbal memory[379] ("cold cognition") through a list of fifteen nouns that participants have to recall and tell researcher. After five repetitions of free-recall, a second "interference" list of further fifteen nouns is assessed in the same manner. Then, participants have to recall the words from the first list both immediately and after a 20-

minute delay. Correct words, intrusions, and repetitions are noted. Finally, a list of fifty words containing all the words from the two lists as well as distracting words is presented. Participants have to indicate whether each word belong to the first list. Correctly-recalled (hits) and falsely-recalled (false alarms) words are noted.

6.2.5 Statistical analysis

The IBM SPSS v27 statistical package[380] was used for all the analyses.

Participants' demographics, clinical characteristics, and baseline questionnaires were reported descriptively. Repeated questionnaires (PANAS, side-effects questionnaire, STAI-state, BL-VAS), and hs-CRP were analysed via repeated measures analysis of variance (ANOVA) with group (atorvastatin *versus* placebo) as the between-subject factor and time (baseline *versus* testing visit) as the within-subject factor.

I visually checked data distributions for all neuropsychological tasks using boxplots: extreme outliers (i.e., data values that lie more than three times the interquartile range below the first quartile or above the third quartile) were excluded. The resulting data were then analysed using repeated measures ANOVA with group as the between-subject factor and emotion/valence as the within-subject factor. Significant interactions were followed up using simple main effect analyses. When assumptions of equality of variances were not fulfilled, the Greenhouse-Geisser correction was used. Partial eta squared (η^2) was reported for the main significant comparisons as a measure of effect size (η^2 0.01, small effect; η^2 0.06, medium effect; η^2= 0.14, large effect).

6.3 Results

6.3.1 Baseline measures

Participants' demographics, clinical characteristics, and baseline questionnaires (BDI, EPQ, SHAPS, STAI-trait) are in Table 6.1. Twenty-two participants were randomised to atorvastatin (eleven females) and twenty-eight (fourteen females) to placebo.

Table 6.1 *Participants' demographics, clinical characteristics, and baseline questionnaires*

		Atorvastatin	**Placebo**
Sample size		22	28
Gender		11 F/11 M	14 F/14 M
Age		24.8 (6.0)	27.6 (7.3)
English as first language		13	17
Education	High school/college	1	2
	Undergraduate	14	14
	Postgraduate	7	12
Family history of mental disorder		9	12
Smoke/day		0.1 (0.2)	0.3 (1.0)
Alcohol units per week		7.0 (6.3)	5.9 (5.3)
Caffeinated drinks per day		1.8 (1.2)	1.5 (1.2)
BMI		21.7 (2.4)	22.9 (3.1)
BDI		1 (1.4)	1.4 (1.8)
EPQ	Neuroticism/stability	5.2 (3.5)	6.0 (4.8)
	Psychoticism/socialisation	3.0 (1.9)	2.3 (2.0)

	Extroversion/introversion	14.3 (3.6)	14.7 (3.8)
	Lie/social desirability	8.9 (4.9)	9.6 (4.3)
SHAPS		0.6 (1.5)	1.0 (1.9)
STAI-t		30.9 (5.5)	28.5 (6.7)

Values are means with (standard deviations). BDI: Beck Depression Inventory, BMI: Body Mass Index, EPQ: Eysenck Personality Questionnaire, SHAPS: Snaith-Hamilton Pleasure Scale, STAI-t: Spielberger Anxiety Inventory-Trait

6.3.2 Testing visit measures

Participants' repeated questionnaires (PANAS, STAI-state, side effects questionnaire) and hs-CRP are in Table 6.2. For the PANAS-positive there was a main effect of time of visit ($F_{1,48}$ 6.28, p 0.02) due to higher scores in both groups at the testing visit in comparison to the baseline visit, but no group-time interaction (F_s <0.91, p_s >0.20). The side effects questionnaire showed a significant group-time interaction ($F_{1,48}$ 4.33, p 0.04) due to higher scores at the testing visit in comparison to the baseline visit for the placebo group and the opposite for the atorvastatin group. For the hs-CRP there was a main effect of time of visit ($F_{1,47}$ 5.91, p 0.02) due to lower scores in both groups at the testing visit in comparison to the baseline visit, but no group-time interaction (F_s <0.11, p_s >0.20). The PANAS-negative and STAI-state did not show any main effect of time of visit or group-time interaction (F_s <1.78, p_s >0.20).

Table 6.2 *Participants' repeated questionnaires and hs-CRP*

	Atorvastatin		**Placebo**			
	Screening visit	*Research visit*	*Screening visit*	*Research visit*	*Visit (p)*	*Group x Visit (p)*
PANAS-positive	31.86 (5.67)	33.23 (6.23)	33.32 (7.93)	36.36 (7.11)	0.02*	>0.20
PANAS-negative	12.14 (2.75)	13.18 (3.03)	12.14 (2.21)	12.11 (2.69)	>0.20	>0.20

Side effects	2.14 (1.13)	1.68 (1.25)	2.18 (1.72)	2.57 (1.67)	>0.20	0.04*
STAI-s	32.27 (5.53)	32.64 (6.73)	31.64 (8.91)	30.00 (6.79)	>0.20	>0.20
hs-CRP	1.44 (1.41)	1.04 (1.30)	1.55 (1.75)	1.02 (1.05)	0.02*	>0.20

Values are means with (standard deviations). An asterisk (*) represents a statistically significant difference between the atorvastatin and placebo groups. hs-CRP: high-sensitivity C-reactive protein, PANAS: Positive and Negative Affective Schedule, STAI-s: Spielberger Anxiety Inventory-state

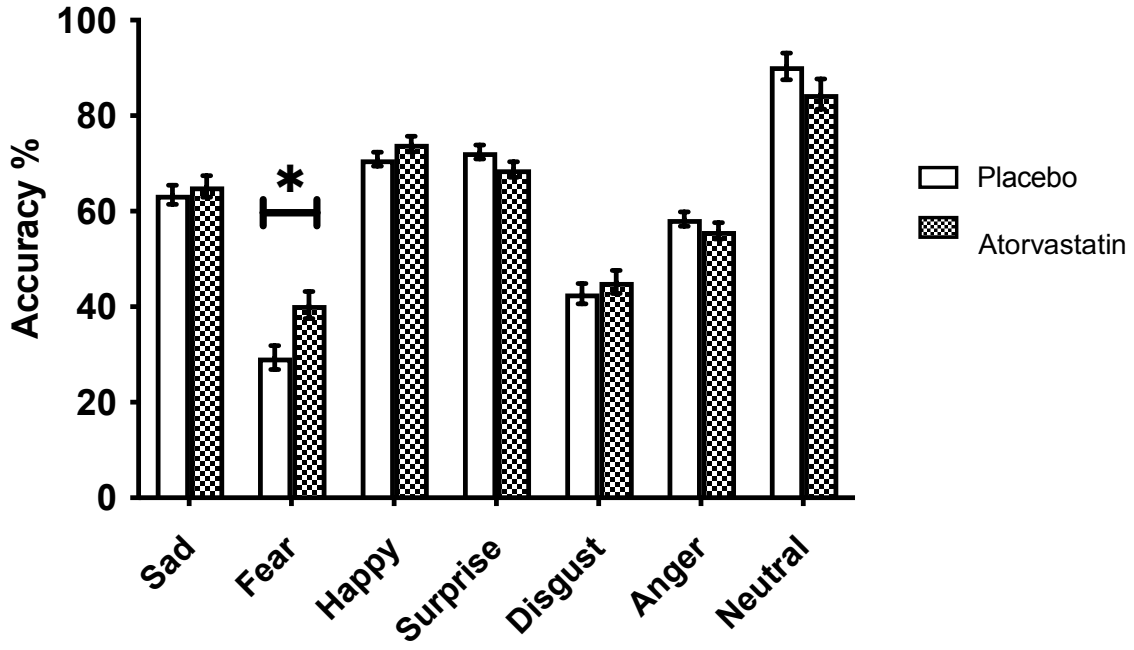
For the BL-VAS (Appendix A6.3), the items "clumsy- well coordinated" and "antagonistic-friendly" showed a main effect of time of visit (respectively $F_{1,48}$ 6.09, p 0.02 and $F_{1,48}$ 5.09, p 0.03) due to lower scores in both groups at the testing visit in comparison to the baseline visit, but no group-time interaction (F_s <0.21, p_s >0.20). The item "sad-happy" showed a significant group-time interaction ($F_{1,48}$ 6.33, p 0.01) due to higher scores at the testing visit in comparison to the baseline visit for the placebo group (scored more towards happiness) and the opposite for the atorvastatin group (scored more toward sad). All the other items on this scale did not show any main effect of time of visit or group-time interaction (F_s <0.92, p_s >0.20).

The descriptive statistics for all neuropsychological outcomes are in Appendix A6.4. No participant was excluded for being an extreme outlier.

6.3.2.1 Emotional test battery results

FERT. For facial expression recognition, a significant group-emotion interaction for total accuracy ($F_{6,288}$ 3.46, h^2 0.07, p 0.01) and sensitivity index (*d'* $F_{6,288}$ 2.61, h^2 0.06, p 0.04) was found, but no group-emotion interaction for response bias (β $F_{6,288}$ 1.20, p >0.30); pairwise comparisons revealed that participants receiving atorvastatin were more accurate at recognising fear than those on placebo ($F_{1,48}$ 8.64, p 0.006 and *d'* $F_{1,48}$ 2.62, p 0.03), but not other emotions (F_s <2.9, p_s >0.10),), as per Figure 6.2.

Figure 6.2 *Effect of atorvastatin on facial expression recognition (FERT)*



Values are means ± standard error of the mean bars, an asterisk (*) represents a statistically significant difference between the atorvastatin (grey) and placebo (white) groups

These results remained consistent when hs-CRP was used as a covariate. Because of the finding of a group-time interaction for the "sad-happy" item on the BL-VAS, this was also analysed as a covariate, but again outcome scores did not change. Moreover, there was a significant group-emotion interaction for total misclassifications ($F_{6,288}$ 3.25, h^2 0.06, p 0.02), with follow-up pairwise comparisons showing that the atorvastatin group were more likely to falsely classify faces as fearful ($F_{1,48}$ 4.38, p 0.04) and less likely to misclassify faces as surprised ($F_{1,48}$ 5.91, p 0.02) or neutral ($F_{1,48}$ 4.88, p 0.03). When positive (happy, surprise) *versus* negative (fear, disgust, anger, sadness) emotions were compared, no effect for group or group-emotion interaction was identified (F_s <2.81, p_s >0.10). Likewise, reaction times did not show any effect for group or group-emotion interaction (F_s <0.78, p_s >0.55).

Other ETB tasks. No significant group or interaction effects were observed for all measures of assessment of word valence (ECAT F_s <0.45, p_s >0.40), recall (EREC), and recognition (EMEM) of positive or negatively valenced words (F_s <1.61, p_s >0.20), and attentional vigilance towards happy or fearful faces (FDOT F_s <1.77, p_s >0.20).

6.3.2.2 Probabilistic instrumental learning task results

For reward learning, an interaction effect was observed for the total number of choice switches ($F_{1,48}$ 4.92, p 0.03); however, *post-hoc* analyses showed no significant differences between the atorvastatin and placebo groups for the losses or wins conditions (p_s >0.70). Also, no significant differences were identified for all other outcomes (F_s <1.42, p_s >0.20).

6.3.2.3 Auditory-verbal learning task results

For verbal memory, no significant differences between the atorvastatin and placebo groups were identified for any of the outcomes measured (F_s <0.82, p_s >0.20).

6.4 Discussion

This is the first experimental medicine study exploring the neuropsychological effects of a statin in healthy volunteers via an array of tasks administered under laboratory conditions. Seven-day atorvastatin, compared to placebo, led to increased recognition, sensitivity, and misclassification for fearful facial expressions, but not for other emotions, independently from subjective states of mood and anxiety and C-reactive protein level, which remained unaffected. No further changes for other tasks measuring emotional processing, nor for those assessing reward learning and verbal memory were observed.

The fact that atorvastatin affects emotional processing is of particular interest since changes in cognitive affective bias in the early stages of drug treatment can be predictive of antidepressant efficacy or alternatively adverse effects on mood even in healthy individuals[358,360]. More specifically, I found convincing evidence that atorvastatin increases the identification of fearful facial expressions, as highlighted by greater accuracy and discriminability for recognising fear as well as a higher number of fear misclassifications. The lack of any change in response bias suggests that the effect concerns sensitivity to fearful stimuli rather than a decisional choice bias. This result is in contrast with the initial prediction that seven-day atorvastatin treatment would be associated with a more positive affective bias. In fact, increased fear recognition reflects an augmented processing of threatening cues and a worsening of negative bias, both of which have been associated with acute SSRI administration[381,382]. Conversely, anxiety states have been linked to increased recognition of fearful facial expressions[383], and administration of anxiolytic drugs such as benzodiazepines have been shown to specifically counteract this effect[384]. On these bases, one could argue that atorvastatin may trigger or worsen anxiety symptoms. Some pre-clinical evidence reports both increased [147,156] and reduced[120,127,183,195] anxiety associated to statin administration; however, clinical research suggests that statin use does not affect[236,301] or even reduce[243] anxiety. The interpretation of this finding, namely that seven days of atorvastatin increases fear processing, might therefore be more complex. Changes in the recognition of fearful expressions are most frequently reported in studies exploring the effects on emotional processing of commonly prescribed antidepressants[385]. As mentioned, the administration to healthy participants of a single dose of the SSRI citalopram, respectively intravenously[381] and orally[382], was associated to higher detection of facial expressions of fear. However, when citalopram was given for seven days, a reduction in fear recognition

was observed[358]. These results parallel clinical experience with SSRIs: an early exacerbation of anxiety and agitation usually followed by anxiolytic effects on prolonged treatment[386] – something that has been similarly observed in animal models of anxiety [387]. This reversal of action seems to involve a desensitization of $5HT_{2c}$ receptors[388]; in line with this, the $5HT_{2a/c}$ receptor blocker mirtazapine immediately reduced fear recognition[389]. It is tempting to match the present finding that seven-day atorvastatin heightens fear processing in a way that is similar to the acute effect of SSRIs[381,382,387]. Then, consistently with clinical studies and experience[386], continued treatment with statins might lead to a paradoxical improvement in anxiety[243] and perhaps depressive symptoms, as reported in section 1.4. The neuropsychological and neurobiological underpinnings for such delayed effect seemingly even longer than with SSRIs[15] remain unclear. Although some of the peripheral, for example anti-inflammatory actions of statins seem relatively fast (i.e., <7 days)[373], the complex downstream effects on neurobiological, endocrine-metabolic, cardiovascular, and immunological processes, thus on anxiety and depressive symptoms, may be slower. Additional experimental medicine trials examining the effects of longer-term administration of statins on emotional processing (NCT04973800), possibly complemented by more sensitive techniques such as fMRI[354] may help clarify this issue.

The most established effects of statins on inflammation, cholesterol, and their interactions[102-105] also warrant further consideration. Seven-day atorvastatin did not change hs-CRP, whose levels did not seem to affect emotional processing. However, this exploratory analysis should be interpreted with caution, since hs-CRP was not raised for either group in this sample of healthy volunteers (Table 6.2). A mediating effect of hs-CRP on emotional processing could still become apparent in a study conducted on depressed participants with elevated baseline hs-CRP. Moreover, no measure of blood

cholesterol levels was taken in this trial, which is a limitation that other studies need to explore. In line with this, another experimental medicine trial has recently shown that a single dose of the antibiotic minocycline, which possesses putative antidepressant mechanisms, acutely decreases fear misclassifications on the ETB while increasing cholesterol and decreasing hs-CRP levels in healthy participants[390].

Impaired reward processing, related to anomalies in dopamine neurotransmission[378], is widely regarded as the foundation of the anhedonia that often characterises an inflammatory phenotype of depression[207]. In this study, I used the PILT to verify whether atorvastatin had any effect on reward learning but found no evidence of that. In view of the close relationship between inflammatory processes and the functioning of reward circuitry, it is again possible that failure to detect significant effects of atorvastatin in this task was due to the study sample not having high baseline inflammation. Also, a longer period of statin administration could have gradually affected downstream effects on the dopaminergic system that may have resulted in changes on the PILT. For example, the norepinephrine-dopamine reuptake inhibitor (NDRI), bupropion was found to cause an initial worsening of reward processing at two weeks in depressed participants which was reversed and normalised only after six weeks of treatment[391]. Furthermore, it should be noted that the current study was powered on the primary outcome of accuracy at recognising emotional facial expressions, therefore it may have been underpowered (i.e., increased false negatives) to capture changes in reward learning. The latter *caveat* applies to the apparent lack of effect of atorvastatin on verbal memory, though reports on the effect of statin on "cold" cognitive functions have less consistent[392].

6.4.1 Limitations

This study has several limitations, most of which are mentioned and put into context in the section above, and here summarised. Firstly, study participants were young, healthy volunteers – a common starting sample for early, proof-of-concept experimental medicine studies, but also one that may be less liable to show an effect of statins when compared to older populations, with raised inflammatory markers and physical comorbidity, and background diagnosis of depressive disorder. Secondly, atorvastatin 20mg for seven days was chosen as an established compound at a dose with anti-inflammatory effect over the short term, but it is possible that a more brain-penetrant molecule (e.g., simvastatin), higher doses, or longer administration periods would be necessary to cause a shift on neuropsychological measures. Finally, although there is some evidence that the latter measures may be affected by both pro-inflammatory and anti-inflammatory stimuli, it remains uncertain whether the neuropsychological tasks employed in this study can gauge the pleiotropic effects of statins. Other methodological limitations of this study consist in the conduction of several other analyses, in addition to the primary one for accuracy in recognising emotional facial expressions, which were not corrected for multiple comparisons because of the relatively small sample size. Most of these supplementary analyses did not show any statistically significant difference between the atorvastatin and the placebo group, which mitigates the potential reporting of false positives.

6.4.2 Conclusion

This study first provides evidence of the early effect of atorvastatin on emotional processing, namely an increase in the recognition of fearful facial expressions, without any concurrent changes in subjective state in healthy volunteers. Although the lack of the

potentially confounding effects of depressive and anxiety symptoms is suitable for a mechanistic study, the clinical relevance of such finding cannot be specified. Furthermore, because of the distinct pharmacology of statins, as discussed in Chapter 1, it is possible that the negative effect observed on emotional processing may be specific to atorvastatin, while other statins could behave differently. Some evidence indeed suggests that simvastatin might have a superior antidepressant profile among all statins[236,309] and especially compared to atorvastatin[238,303] – though not all studies agree[239,283]. The next Chapter 7 explores this prospect by conducting an analogous experimental medicine trial with equal design but employing simvastatin instead.

Chapter 7. Effects of simvastatin on emotional processing, reward learning, and verbal memory – an experimental medicine study*

*This chapter is based on the paper: "An experimental medicine study of the effects of simvastatin on emotional processing, reward learning, verbal memory, and inflammation in healthy volunteers", De Giorgi et al., 2022; DOI:10.1007/s00213-022-06156-y. Co-authors permission to reuse material is in Appendix, miscellanea

7.1 Introduction

In the experimental medicine trial of Chapter 6, I have found that seven-day atorvastatin, compared to placebo, increases the identification of fearful facial expressions (N 50, $F_{1,48}$ 8.64, p 0.006) independently from subjective states of mood and anxiety and the peripheral inflammatory marker, high sensitivity C-reactive protein (hs-CRP) in healthy subjects. Increased fear processing equals to an aggravation of negative affective bias, which according to a cognitive neuropsychological model of depression correlates to depressive and anxiety symptoms[354]. Among several explanations for such unexpected finding, I have considered that certain studies, though not all[239,283], have associated atorvastatin to either a depressogenic[238] or less pronounced antidepressant effect[236,303,309] compared to simvastatin. The pharmacological differences between these two statins, especially with regards to their potential to express neurobiological activity, have been reported in section 1.2 and section 1.3: in brief, simvastatin differs from atorvastatin by means of being less potent at reducing peripheral cholesterol, but it is also considerably more lipophilic (Table 1.1), thus more capable of crossing the blood-brain barrier (BBB) and expressing its effects within the central nervous system (CNS)[52].

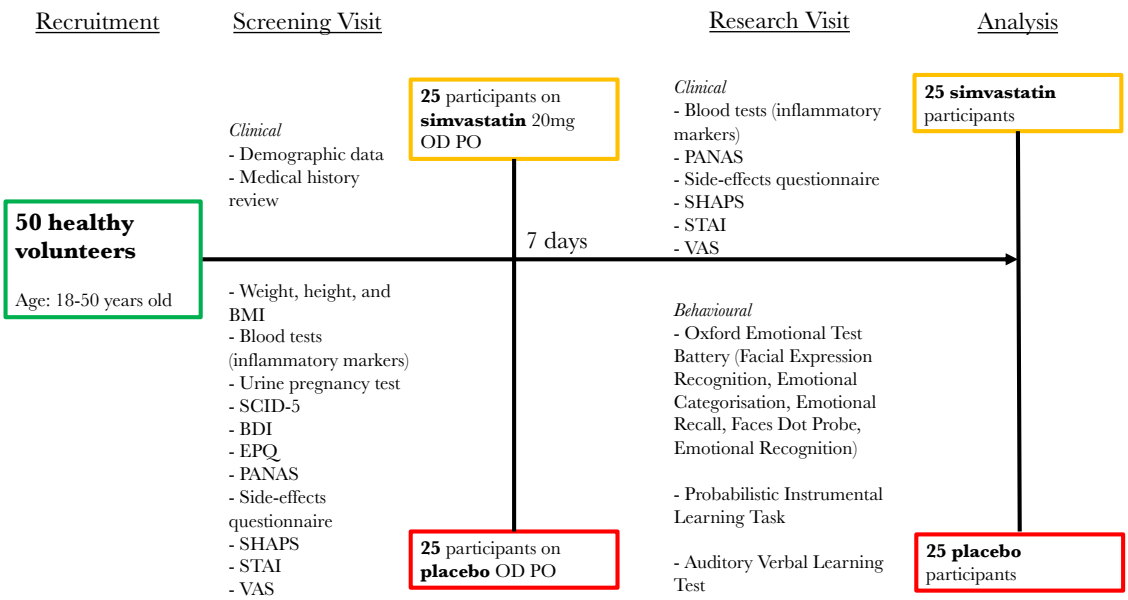
The rationale for conducting experimental medicine studies[353,354] to assess the neuropsychological effects of statins[364] has been discussed in Chapter 6. The current experimental medicine trial of simvastatin in healthy volunteers parallels the study design presented in the previous chapter, as this has already managed to detect an effect, even though negative, of atorvastatin on affective bias. Therefore, the primary aim remains the same, namely to investigate the short-term (seven days) effects of the highly lipophilic but less potent simvastatin upon a battery of emotional processing tasks that are sensitive to the early activity of typical antidepressants. Secondarily, I examine the effects of

simvastatin on reward learning, verbal memory, and inflammation as measured by the inflammatory marker hs-CRP. Conscious of the limitations and the unforeseen findings for atorvastatin in Chapter 6, but based on the reported literature supporting a better antidepressant profile for simvastatin, I hypothesise that seven-day treatment with this medication would improve emotional processing, reward learning, and verbal memory, while also decreasing inflammation.

7.2 Methods

The study was a double-blind, parallel groups, randomised, gender-stratified, placebo-controlled, experimental medicine trial, approved by the University of Oxford Central University Research Ethics Committee (MS-IDREC R69606/RE001). The study protocol, which describes the methods in detail, was registered on Clinicaltrials.gov (NCT04652089). The overall study design is in Figure 7.1, evidently replicating the one of Chapter 6. As such, the study methods remain the same, and are here reported briefly for convenience.

Figure 7.1 *Study design*



BDI: Beck Depression Inventory, EPQ: Eysenck Personality Questionnaire, PANAS: Positive and Negative Affect Schedule, SHAPS: Snaith-Hamilton Pleasure, STAI: State and Trait Anxiety Inventory, VAS: Visual Analogue Scales

7.2.1 Sample size calculation

Using G*Power v3.1.9.6[365], based on the primary outcome of accuracy at recognising emotional facial expressions from a previous study of antidepressants in healthy volunteers[358], a sample size of 38 would give 90% power to detect changes of a comparable magnitude (citalopram *versus* placebo effect size F 0.5, Appendix A7.1)

7.2.2 Participants recruitment

To account for potential difficulties in study retention due to the COVID-19 pandemic, when this study was conducted, I recruited above the number of participants estimated through the sample size calculation. Fifty-three participants [males and females, aged 18 to 50 years, body mass index (BMI) 18 to 30] were recruited for this study and provided written informed consent.

Inclusion and exclusion criteria were pre-specified in the study protocol and are reported in full in Appendix A7.2.

7.2.3 Baseline procedures

Enrolled participants completed a series of questionnaires including the Beck Depression Inventory (BDI)[367], the Eysenck Personality Questionnaire (EPQ)[368], the Positive and Negative Affect Scale (PANAS) [369], the Snaith-Hamilton Pleasure Scale (SHAPS)[370], a side-effect questionnaire (nausea, dizziness, dry mouth, headache, alertness, and agitation scored from 0 to 3), the State-Trait Anxiety Inventory (STAI)[371], and the Bond-Lader Visual Analogue Scales (BL-VAS)[372]. Also, a blood sample to determine serum hs-CRP levels was collected and frozen in two 1.0mL FluidX tubes.

A randomisation code had been designed by a researcher uninvolved in the study using an online randomisation tool (https://www.sealedenvelope.com/simple-randomiser/v1/lists). Accordingly, participants were randomised to either simvastatin 20mg, a dose used in previous clinical trials of simvastatin in depression[297,303] or sucrose placebo, encapsulated in identical opaque white capsules, taken orally once daily, at night, for seven days, based on previous evidence suggesting that the anti-inflammatory effects of statins are seen as early as 4 ± 3 days [373].

7.2.4 Testing visit procedures

Following the seven days of assigned treatment, participants returned for a testing visit.

Participants completed four of the previously administered questionnaires (PANAS, side-effects questionnaire, STAI-state, BL-VAS), and a second blood sample for hs-CRP: both the baseline and testing visit blood samples were assayed at the end of the study.

Finally, participants were administered a battery of neuropsychological tasks including the emotional test battery (ETB), the probabilistic instrumental learning task (PILT), and the auditory-verbal learning task (AVLT).

7.2.4.1 Emotional test battery

The ETB assesses emotional processing ("hot cognition") via five validated[359] computerised tasks: the facial expression recognition task (FERT), the emotional categorisation task (ECAT), the emotional recall task (EREC), the emotional recognition memory task (EMEM), and the faces dot-probe task (FDOT), described in detail in section 6.2.4.1.

The FERT involves the facial expressions of six emotions: anger, disgust, fear, happy, sad, surprise, and a neutral one, adapted from the Karolinska directed emotional faces set[374]. Accuracy at recognising the correct emotion was the primary outcome measure for this study. A signal detection analysis is used to assess discriminability (d', perceptual choice) and response bias (β, decisional choice)[376] for this outcome. Misclassifications and mean reaction times are also noted.

The ECAT involves thirty positively- (e.g., cheerful) and thirty negatively-valenced (e.g., hostile) words[377]. Accuracy and mean reaction times for positive and negative words are noted. The EREC is an unexpected free-recall task in which correctly- (hits) and falsely-recalled (false alarms) positive and negative words from the ECAT are noted. The EMEM involves the sixty self-referent words from the ECAT and sixty matched distractors (thirty positive, thirty negative). Accuracy, false alarms, and reaction times are noted.

The FDOT involves two faces, one neutral, and one emotional (either happy or fearful), briefly displayed at the top and at the bottom of the computer screen and then replaced by

a pair of dots with two different (vertical or horizontal) orientations. Mean reaction times from trials when probes are in the same position as the emotional face (congruent trials) are subtracted from mean reaction times from trials when probes are in the opposite position to the emotional face (incongruent trials) to calculate attentional vigilance scores.

7.2.4.2 Probabilistic instrumental learning task

The PILT, adapted from Pessiglione et al.,[378] assesses reward learning, described in detail in section 6.2.4.2. End total amount, amount won, amount lost, reward and lose trials, and number of choice switches (proportion of trials in which the chosen symbol is different to the symbol chosen in the previous trial within the same condition, such that the lower the proportion of switch trials, the greater the confidence the participant is thought to have had in their choices) are noted.

7.2.4.3 Auditory-verbal learning task

The AVLT assesses verbal memory[379] ("cold cognition"), described in detail in section 6.2.4.3, through a list of fifteen nouns that participants have to recall and tell researcher. Correct words, intrusions, and repetitions are noted. Then, a list of fifty words containing all the words from the previous lists as well as distracting words are presented. Correctly-recalled (hits) and falsely-recalled (false alarms) words are noted.

7.2.5 Statistical analysis

The IBM SPSS v27 statistical package[380] was used for all the analyses.

Participants' demographics, clinical characteristics, and baseline questionnaires were reported descriptively. Repeated questionnaires (PANAS, side-effects questionnaire,

STAI-state, BL-VAS), and hs-CRP were analysed via repeated measures analysis of variance (ANOVA) with group (simvastatin *versus* placebo) as the between-subject factor and time (baseline *versus* testing visit) as the within-subject factor.

I visually checked data distributions for all neuropsychological tasks using boxplots: extreme outliers (i.e., data values that lie more than three times the interquartile range below the first quartile or above the third quartile) were excluded. The resulting data were then analysed using repeated measures ANOVA with group as the between-subject factor and emotion/valence as the within-subject factor. Significant interactions were followed up using simple main effect analyses. When assumptions of equality of variances were not fulfilled, the Greenhouse-Geisser correction was used. Partial eta squared (η^2) was reported for the main significant comparisons as a measure of effect size (η^2 0.01, small effect; η^2 0.06, medium effect; η^2= 0.14, large effect). On the basis of potentially diverse physiopathological effects of simvastatin, compared to the analyses in Chapter 6, I further explored the main task (FERT accuracy, misclassifications, reaction times) in specific subgroups of participants (females *versus* males, BMI 18-25 [normoweight] *versus* 26-30 [overweight], hs-CRP <1mg/dL *versus* ≥1mg/dL, self-report of family history of mental disorder *positive* versus *negative*).

7.3 Results

7.3.1 Baseline measures

Participants' demographics, clinical characteristics, and baseline questionnaires (BDI, EPQ, SHAPS, STAI-trait) are in Table 7.1. Twenty-seven participants were randomised to simvastatin (fourteen females) and twenty-six (thirteen females) to placebo.

Table 7.1 *Participants' demographics, clinical characteristics, and baseline questionnaires*

		Simvastatin	Placebo
Sample size		27	26
Gender		14 F/13 M	13 F/13 M
Age		25.4 (3.3)	28.1 (5.7)
English as first language		20	13
Education	High school/college	3	1
	Undergraduate	13	12
	Postgraduate	11	13
Family history of mental disorder		9	11
Smoke/day		0.5 (2.0)	0.1 (0.4)
Alcohol units per week		5.5 (5.5)	4.6 (6.7)
Caffeinated drinks per day		1.6 (1.5)	1.8 (1.4)
BMI		22.3 (2.6)	22.8 (3.0)
BDI		2.7 (4.2)	2.8 (3.0)
EPQ	Neuroticism/stability	8.1 (4.3)	8.8 (3.9)
	Psychoticism/socialisation	3.4 (3.2)	2.3 (0.3)
	Extroversion/introversion	14.4 (3.9)	12.7 (5.1)
	Lie/social desirability	10.00 (4.2)	10.8 (4.2)
SHAPS		0.4 (0.6)	1.3 (1.9)
STAI-t		34.6 (7.3)	34.6 (8.3)

Values are means with (standard deviations). BDI: Beck Depression Inventory, BMI: Body Mass Index, EPQ: Eysenck Personality Questionnaire, SHAPS: Snaith-Hamilton Pleasure Scale, STAI-t: Spielberger Anxiety Inventory-Trait

7.3.2 Testing visit measures

Participants' repeated questionnaires (PANAS, STAI-state, side effects questionnaire) and hs-CRP are in Table 7.2.

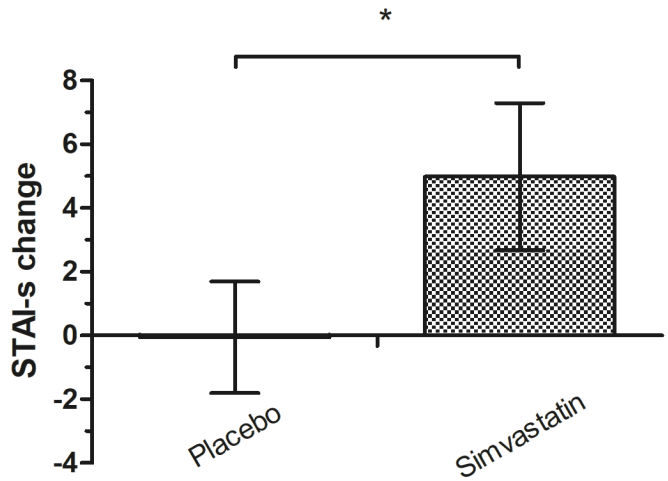
Table 7.2 *Participants' repeated questionnaires and hs-CRP*

	Simvastatin		**Placebo**			
	Screening visit	*Research visit*	*Screening visit*	*Research visit*	*Visit (p)*	*Group x Visit (p)*
PANAS-positive	30.9 (7.0)	31.9 (6.4)	31.9 (7.2)	32.5 (7.8)	>0.20	>0.20
PANAS-negative	13.0 (3.0)	14.3 (4.6)	12.4 (2.5)	12.9 (2.9)	>0.20	>0.20
Side effects	0.5 (0.9)	0.7 (0.2)	0.4 (0.6)	0.5 (0.8)	>0.20	>0.20
STAI-s	29.9 (6.4)	35.6 (9.7)	31.4 (9.0)	31.7 (8.5)	0.01*	0.02*
hs-CRP	0.7 (0.4)	0.8 (0.6)	1.0 (0.7)	1.1 (0.8)	>0.20	>0.20

Values are means with (standard deviations). An asterisk (*) represents a statistically significant difference between the atorvastatin and placebo groups. hs-CRP: high-sensitivity C-reactive protein, PANAS: Positive and Negative Affective Schedule, STAI-s: Spielberger Anxiety Inventory-state

The STAI-state showed a main effect of time ($F_{1,51}$ 6.91, p 0.01) and a group-time interaction ($F_{1,51}$ 5.37, p 0.02) due to higher scores at the testing visit for the simvastatin group (Figure 7.1). The other questionnaires and hs-CRP showed no effect of time or group-time interaction (F_s <2.97, p_s >0.20).

Figure 7.1 *Effect of simvastatin on the State-Trait Anxiety Inventory-state (STAI-s) score*



Values are mean differences between visits ± standard error of the mean bars, an asterisk (*) represents a statistically significant difference between the simvastatin (grey) and placebo (white) groups

For the BL-VAS (Appendix A7.3), several items showed a statistically significant main effect of time, but no significant group-time interaction was observed (F_s <4.10, p_s >0.20).

The descriptive statistics for all neuropsychological outcomes, including the number of outliers excluded, are in Appendix A7.4. The very low number of outliers did not compromise the conservative sample size for all tasks.

7.3.2.1 Emotional test battery results

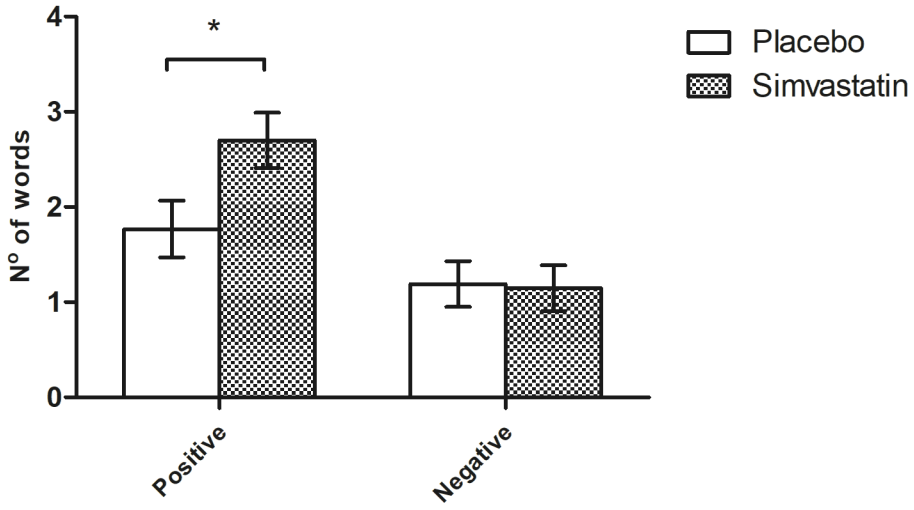
FERT. For facial expression recognition, there was no significant group-emotion interaction for any of the outcome measures (F_s< 1.40, p_s> 0.20), Appendix A7.5.

When the effect of gender was assessed on the FERT (Appendix A7.4 and Appendix A7.5), a group-gender-emotion interaction for misclassifications was observed, which approached statistical significance ($F_{6,43}$ 2.50, p 0.07). This was driven by a reduced tendency to mislabel other facial expressions as sad in the simvastatin group among female participants ($F_{1,48}$ 6.91, p 0.01, □^{2} 0.13). I therefore explored this effect within the

subgroup of females (placebo N 13, simvastatin N 14). A group-emotion interaction for misclassifications was confirmed ($F_{6,20}$ 2.57, p 0.05), which again was due to a lower number of misclassifications as sad facial expression for the simvastatin group ($F_{1,25}$ 6.60, p 0.02, $\square^2$ 0.21). A further analysis clarified that females on simvastatin misclassified fewer positive facial expressions, when compared to negative emotions, as sad facial expressions ($F_{1,25}$ 5.79, p 0.02, $\square^2$ 0.19). None of the other pre-specified variables (BMI, hs-CRP, family history of mental disorder) had any effect on this task's outcomes (F_s <3.34, p_s >0.20), Appendix A7.4.

ECAT, EREC, EMEM. For emotional recall, a group-valence interaction ($F_{1,51}$ 4.86, p 0.03) was observed, so that individuals on simvastatin recalled more positive intrusions compared to placebo ($F_{1,51}$ 4.99, p 0.03, $\square^2$ 0.09), while no effect on negatively-valenced words was identified ($F_{1,51}$ 0.02, p 0.20) (Figure 7.2). There were no further significant group-valence interactions for any of the other ECAT, EREC, and EMEM outcomes measured (F_s <2.90, p_s >0.20).

Figure 7.2 *Effect of simvastatin on emotional recall (EREC), false alarms*



Values are means ± standard errors of the mean bars, an asterisk (*) represents a statistically significant difference between the simvastatin (grey) and placebo (white) groups

FDOT. There was no significant group-emotion interaction for attentional vigilance towards happy or fearful faces on any of the FDOT outcomes (F_s<0.97, p_s>0.20).

7.3.2.2 Probabilistic instrumental learning task results

For reward learning, there was no significant effect of simvastatin on any of the outcome measures (F_s<1.43, p_s>0.20). The learning curves are in Appendix A7.6.

7.3.2.3 Auditory-verbal learning task results

For verbal memory, no significant differences between the simvastatin and placebo groups were identified for any of the outcomes measured (F_s< 2.70, p_s> 0.20).

7.4 Discussion

In this experimental medicine trial in healthy volunteers, I have assessed the neuropsychological effects of the highly lipophilic and moderately potent simvastatin through a series of tests administered under laboratory conditions. Seven-day simvastatin, compared to placebo, did not lead to any statistically significant changes on the majority of the emotional processing, reward learning, and verbal memory outcomes measured, nor it did for most of the questionnaires administered and hs-CRP. There are clues however that simvastatin may induce positive affective bias, as evidenced by the increased number of positively-valenced intrusions in the EREC task and reduced labelling of faces as sad in females on the intervention arm. At the same time, simvastatin use was associated with higher anxiety levels as measured by the STAI-s questionnaire.

A simple explanation for these results would be that simvastatin does not affect any of the cognitive domains assessed, and that the only effect observed on emotional recall was

coincidental. This conclusion once again seems to contrast with growing evidence from pre-clinical and clinical studies, presented in Chapter 1, that statins have an antidepressant and anti-anxiety potential[108]. Early changes in emotional processing produced by antidepressants can predict later effects on clinical mood states[354], but this experimental medicine trial may insinuate that statins' putative antidepressant activity may be related to physiopathological pathways that are not necessarily well-captured by the neuropsychological tasks employed.

An alternative explanation for the modest effect seen on emotional processing is that higher doses or longer periods of treatment are needed for more indicative data. Simvastatin is commonly used at doses between 10-40mg for several years and, although dosage can be increased to 80mg, its potency remains lower than atorvastatin (Table 1.1). An indication that simvastatin may express antidepressant effects only at higher doses or longer administration periods may lie in the observed dissociable effects between worsened subjective anxiety scores and mildly improved emotional processing. Comparable results were previously obtained in another experimental medicine trial where a single oral dose of the serotonergic and noradrenergic reuptake inhibitor (SNRI), duloxetine led to increased recall of false alarms for positive personality characteristics in the EREC, while also worsening anxiety and other subjective mood states[393]. This pattern seems to reflect routine clinical experience with most antidepressant medications: an initial exacerbation of anxiety followed by antidepressant and anxiolytic effects[386]. Hence in this study, seven-day simvastatin might have caused the earlier, paradoxical (and in this case, captured) increase in anxiety before showing any antidepressant activity (as suggested by a more positive outcome on the EREC). As discussed for atorvastatin in Chapter 6, the intricate pharmacological actions of simvastatin on neurobiological, endocrine-metabolic, cardiovascular, and immunological systems could require longer

administrations before showing the expected positive effects on emotional processing – and eventually on scales of mood and anxiety. Evidence from experimental medicine trials of statins with longer periods of treatment (NCT04973800) or employing more sensitive methodologies, such as neural measures of emotional processing via fMRI[354], could shed some light on this issue.

From a backward (i.e., bedside-to-bench) standpoint, simvastatin use seemed more associated with an increase in positive bias in emotional memory, similar to noradrenergic antidepressants, rather than the decrease in negative bias and emotional expression recognition that is usually seen with serotonergic drugs[394]. This observation is novel, because available evidence from pre-clinical studies has mainly implied serotonin and dopamine amongst monoamines, rather than noradrenaline, with regards to statins' activity on neurotransmitter turnover (section 1.3.1.1), though the latter effect may occur indirectly, for example via interconnected anti-inflammatory pathways[109]. Furthermore, the present findings for simvastatin appear surprising diverges from the previous experimental medicine trial of seven-day atorvastatin reported in Chapter 6, which has shown an increase in the processing of fearful faces, normally linked to anxiety states[383], in the absence of any changes for mood and anxiety questionnaire scores. On the assumption that a drug's ability to penetrate the CNS is important for expressing any activity on emotional processing, one would expect to see these same results replicated – perhaps even to a greater extent, since simvastatin is a more lipophilic molecule. Consistent with this, an increase in anxiety state on the STAI-s questionnaire was observed; however, typically more sensitive measures of fear processing (i.e., FERT, FDOT) were not affected, and indeed, a small yet positive effect on emotional recall was found. These pharmacological disputes can have meaningful translational value, as in the

context of certain evidence suggesting that peripheral, rather than central inflammation is key in the inflammatory model of depression[395].

Considerations about the observed lack of effect of simvastatin on reward learning and verbal memory are similar to those discussed for atorvastatin in Chapter 6, potentially concerning the need to target larger samples of participants with higher baseline inflammation[207] for longer follow-ups[391].

In addition to the study in Chapter 6, and on the back of the evidence from all previous chapters, the current investigation can provide useful insights about the concept of "precision" in experimental medicine. Trials conducted in healthy subjects are an essential proof-of-concept for the cognitive neuropsychological model of depression and antidepressant action, and can support go/no-go decisions for the development or repurposing of new antidepressant drugs[386]. However, in the case of statins or similar medications with complex pharmacological activity, further experimental medicine trials may need to focus on specific subpopulations that are prone to show a neuropsychological response to such interventions[396], similarly to what precision psychiatry advises for clinical studies[397]. As already mentioned, participants' baseline lipid and inflammatory profile should be taken into account when interpreting statins' effects on both neuropsychological markers and depressive scales. Participants' age and overall health status are further key issues – it is noteworthy that the current study was conducted in young, healthy volunteers. It has been speculated that older people might be more likely to experience a depressogenic reaction to statins as they would have less capacity to compensate for the wide range of neurobiological changes produced by statin treatment[111]. Nevertheless, a large observational study found an association between advancing age and lower odds of depression for statin users[238], possibly due to elderly people benefitting more

from this intervention because of age-related higher baseline inflammation[398]. For similar reasons, people who are at-risk of developing depressive episodes through factors such as obesity[399] or positive family history of mood disorders[400] might be key samples for further statin studies. However, the current simvastatin trial could not identify any interaction between statin use, BMI or psychiatric family history, and the FERT, perhaps owing to small sample size. In this context, the exploratory analysis on the subgroup of 27 female participants (i.e., lower than the number required to see an effect on the FERT according to the sample size calculation) highlights intriguing, though preliminary, findings. There is evidence of sex differences at the interface between immunity and depression[401], with inflammation seeming to be a more conspicuous driver of depressive symptomatology in females compared to males[402]. Consistent with this, a more positive effect of simvastatin was found in females as evidenced by fewer misclassifications of facial expressions and indeed specifically of positive ones as sad – another sign of induction of positive bias. This finding must be interpreted with caution because of its *post-hoc* nature on a small sample. However, it raises the possibility that these results could be replicated more convincingly in further studies including only female subjects, or with larger sample sizes and a pre-specified analysis plan focussing on females.

7.4.1 Limitations

The same methodological limitations reported for the experimental medicine trial in Chapter 6 are applicable to the current one, especially with regards to the analyses for several secondary and exploratory comparisons, which were not further corrected because of small sample size. Furthermore, although in this case I chose to explore the neuropsychological effect of a more lipophilic, and thus brain-penetrant compound (i.e.,

simvastatin), limitations regarding the population of choice, dosage and duration of the intervention, and outcome measures, as described in section 6.4.1, remain.

7.4.2 Conclusions

This experimental medicine trial in healthy volunteers shows that seven-day simvastatin administration is associated with an increase in subjective anxiety score while possibly inducing a positive bias in the recall of emotional information. No further effects on emotional processing, reward learning, and verbal memory could be detected. These findings do not have any immediate clinical application but can inform future experimental medicine and clinical trials investigating the effects of statins in depression and anxiety. A finer tailoring of the administered dose, length of treatment, molecule of choice, and target population might lead to more significative results. Together with evidence from other pre-clinical and clinical studies, including those over all the previous chapters, I would argue that further studies should focus on the highly-lipophilic simvastatin, dispensed at doses ≥20mg/day for ≥7 days, in people with depression and baseline altered lipid and inflammatory profiles.

Chapter 8.

Conclusion and future work

Depression is a common disabling disorder[1,2] for which current management strategies, including psychotherapy and pharmacotherapy[4,5], are unsatisfactory[16]. The 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase inhibitors, or statins have been eagerly proposed for repurposing in the treatment of depressive disorders[23,35-39].

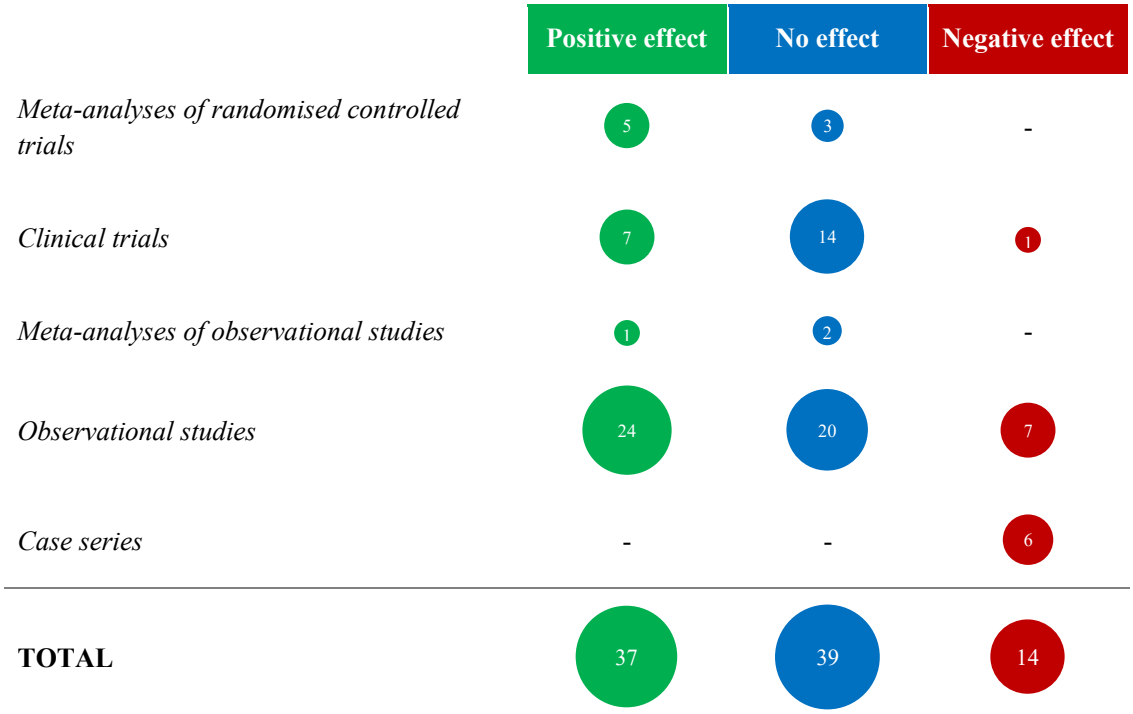
8.1 Current evidence and unresolved issues

In this dissertation, I have investigated this matter through a variety of methodologies. In Chapter 1, following an overview of the pharmacology and psychopharmacology of statins, I have described the pre-clinical evidence for several intertwined neurobiological, endocrine-metabolic, cardiovascular, and immunological mechanisms underlying the putative antidepressant and anxiolytic activity of statins (section 1.3). Then, I have reported 86 clinical studies on the effects of statins in depression (section 1.4), generally advocating for the benefit, or at least no harmful effects of these medications on depressive and anxiety symptoms, and inconsistencies likely explained by variable study designs, populations and their subgroups, interventions, and outcome measures. To help clarify some of these discrepancies, I have conducted six original investigations whose findings are here summarised.

The two meta-analyses of randomised controlled trials (RCTs) of Chapters 2-3 suggest a clinically meaningful antidepressant effect of statins only when used in add-on to traditional antidepressants and only in patients diagnosed with a depressive illness. These results expand on a subgroup analysis of the earlier work by Yatham et al.,[272], and, more specifically, included trials using statins in add-on to antidepressants in patients diagnosed with a depressive disorder, which showed a moderate beneficial effect [four RCTs, N 238, standardised mean difference (SMD) -0.48, 95% confidence interval (CI) -0.74 to -0.22]

of this intervention compared to antidepressants alone (Chapter 3), whereas statins used in monotherapy *versus* placebo in trials including people experiencing depressive symptoms, without a formal diagnosis of depression, appeared ineffective (eleven RCTs, N 2,712, SMD -0.18, 95%CI -0.41 to 0.04) (Chapter 2). Since clinical trials may be limited by relatively small sample sizes, selected populations, and short follow-ups, I have completed two large, real-world, twelve-month long epidemiological studies in patients with first-episode depression over Chapters 4-5, once again confirming a more positive profile for the combined antidepressant plus statin strategy in terms of treatment discontinuations, namely acceptability [N 673,177, adjusted odds ratio $(aOR)_{2\ months}$ 0.88, 99%CI 0.85 to 0.91; $aOR_{6\ months}$ 0.81, 99%CI 0.79 to 0.84; $aOR_{12\ months}$ 0.78, 99%CI 0.75 to 0.81] and tolerability (N 673,177, $aOR_{2\ months}$ 0.92, 99%CI 0.87 to 0.98; $aOR_{6\ months}$ 0.94, 99%CI 0.89 to 0.99). Adverse events and antidepressant efficacy do not appear different between exposures and comparisons, in either study, though reduced all-cause mortality is consistently associated with statin use, in both studies (Chapter 5, N 673,177, $aOR_{2\ months}$ 0.69, 99%CI 0.62 to 0.78, $aOR_{6\ months}$ 0.69, 99%CI 0.62 to 0.78, $aOR_{12\ months}$ 0.72, 99%CI 0.67 to 0.77; Chapter 4, $aOR_{2\ months}$ 0.66, 99%CI 0.60, 0.72; $aOR_{6\ months}$ 0.66, 99%CI 0.60, 0.72; $aOR_{12\ months}$ 0.67, 99%CI 0.65, 0.73), perhaps indicating that the value of these drugs in depression is linked to synergistic benefits on both mental and physical health. The addition of these four investigations to the available literature of clinical studies (section 1.4) on the effects of statins in depression would lead to a total of 90 studies, distributed as per Figure 8.1.

Figure 8.1 *Clinical studies investigating the effect of statins in depression*

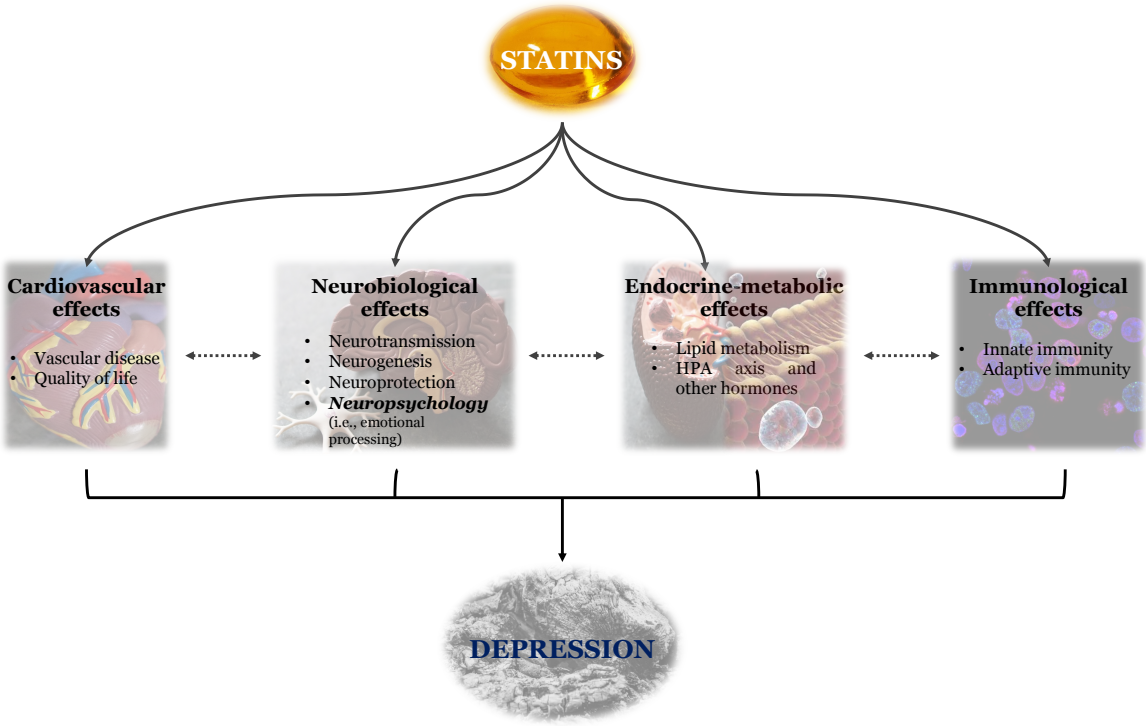


Legend. Data represent the number of studies showing either a positive, negative, or no effect per each study category; shape size is proportional to the $\log_{10}$ for the number of studies

I therefore decided to explore this subject from a different angle for Chapters 6-7, using a validated experimental medicine approach based on the cognitive neuropsychological model of depression to investigate efficacy markers of antidepressant response in healthy volunteers administered statins *versus* placebo for seven days. These two studies, however, only show modest evidence that simvastatin use leads to a slight improvement of affective bias for emotional recall ($F_{1,51}$ 4.99, p 0.03, $\square^2$ 0.09, favouring simvastatin in terms of positive intrusions), while atorvastatin seems to worsen it more robustly for facial expression recognition ($F_{1,48}$ 8.64, p 0.006 and *d'* $F_{1,48}$ 2.62, p 0.03, favouring atorvastatin in terms of identification of fearful faces), further complicating the clinical findings described above. Indeed, simvastatin treatment may have increased subjective anxiety ($F_{1,51}$ 5.37, p 0.02) in this population of young healthy volunteers. Aside from these results, these two investigations add to the existing evidence of pre-clinical studies

(section 1.3) a previously unexplored neuropsychological dimension to the effects of statins in depression, shown in the updated Figure 8.2.

Figure 8.2 *Mechanisms of statins effects in depression*



Legend. Update from Figure 1.3, in bold and italics.

Several clinical and research questions remain unanswered. Do different populations likewise show an antidepressant response to statins? For example, healthy subjects, individuals at-risk for depression, patients with first-episode or treatment-resistant depressive disorders, people with heightened risk due to clinical-demographic characteristics associated with inflammation and dyslipidaemia? Are individual statins capable of expressing distinct influences on mood and anxiety, and at which dosage and length of treatment?

In this case, simvastatin's profiling is emerging as superior to atorvastatin, both from clinical and pre-clinical studies, and indeed other statins, but dose- and time-response

relationships remain ambiguous. Do statins work better as adjunct to conventional antidepressant, rather than a standalone treatment? The clinical evidence above seems to suggest so, but the biological and psychological underpinnings of such augmenting effects must be better defined before having any meaningful medical application. Are the existing outcome measures adequate for detecting the favourable or detrimental consequences of statin use in depression? Scales measuring depressive or anxiety symptoms, neuropsychological tasks, hard outcomes such as mortality, suicidality, and psychiatric hospitalisations: all have the potential to provide clinically and translationally useful insights, but it remains uncertain which one is better tailored to gauge the effects of statins in the context of depressive illness. Overall, it is plausible that many further investigations involving a variety of research designs (i.e., clinical and experimental medicine trials, observational studies) will be required before finding an answer to all these outstanding queries. For this reason, I now outline a recently started, ongoing experimental medicine trial through which I aim to add some relevant evidence for the neuropsychological effects of statins in depression.

8.2 The OxSTEP study

People who are at-risk for developing depression due to the presence of well-known predisposing factors (e.g., temperament[403] or past depressive episodes[404]) display affective biases that resemble those seen in patients with depressive disorders, and equally improve following standard antidepressant treatment[405]. Some evidence suggests that anomalies of waking salivary cortisol, a measure of hypothalamus-pituitary-adrenal (HPA) axis functionality consistently linked to depression[406], can be seen in conjunction to deficit in the handling of emotional information in at-risk states[407]. Social isolation and loneliness,

exacerbated by the recent COVID-19 pandemic, are associated with vulnerability to depression[408], possibly via a causal mechanism involving immune and metabolic functions[409], but their consequences on emotional processing have not been examined.

Taken together, these data offer an intriguing perspective to further define the neuropsychological profile associated with statin use. Firstly, over Chapters 1-5, I have discussed the large evidence-base for the variable effects of statins in depressive disorders. Secondly, in section 1.3.2.2, I have reported that one of the mechanisms by which statins could express an antidepressant or anxiolytic effect would be mediated by their activity on the HPA axis, whose correct functioning is tightly related to lipid metabolism, stress, inflammation, and mood regulation in animals and humans[190-192]. Thirdly, in sections 1.3.2.1 and 1.3.4, both the latter and other immunological and metabolic processes, which may underlie the affective costs of social isolation and loneliness[409], appear influenced by statin administration. In the experimental medicine trials of Chapter 6 and Chapter 7, I have shown that statin use affects emotional processing in healthy subjects, something hinted at by a previous observational study examining the neuropsychological effects of these medications[364].

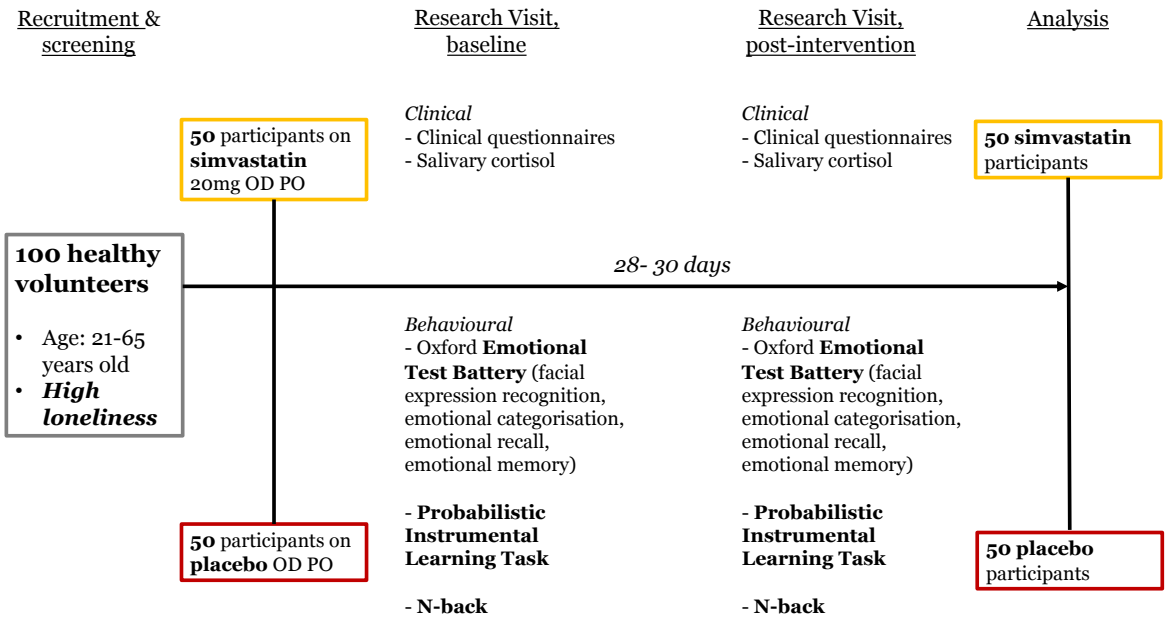
Findings between the two experimental medicine trials diverge though: seven days of the highly potent, moderately lipophilic atorvastatin (Chapter 6) seem to increase negative affective bias, while seven days of the highly lipophilic, moderately potent simvastatin (Chapter 7) would only mildly reduce it. I have hypothesised that, on the back of the complex pharmacological activity of these molecules, such discrepancy may be disentangled by investigating the neuropsychological effects of longer periods of statin treatment in vulnerable, namely at-risk for depression, individuals.

To test this hypothesis, I have designed the current experimental medicine trial: the Oxford simvaSTatin and Emotional Processing (OxSTEP) study, which assesses the effects of 28-day simvastatin administration *versus* placebo on emotional processing, reward learning, working memory, and waking salivary cortisol in people who are at-risk for depression due to high levels of loneliness. This trial benefits from an innovative approach to experimental medicine that involves a wholly online, remotely-conducted design, to resolve and capitalise on the recruitment and retention challenges due to the COVID-19 pandemic. In view of the evidence presented above, I predict that longer-term simvastatin use in a group of predisposed participants will lead to positive changes on emotional processing, and secondarily on reward learning and working memory, while also lowering waking salivary cortisol concentrations.

8.2.1 Study design

OxSTEP is an online, double-blind, parallel groups, randomised, gender-stratified, placebo-controlled, experimental medicine trial, approved by the University of Oxford Central University Research Ethics Committee (MS-IDREC R73946/RE001). The study protocol, which describes the methods in detail, is registered on Clinicaltrials.gov (NCT04973800). The overall study design is in Figure 8.2.

Figure 8.3 *Study design*



Despite several features shared with the experimental medicine trials in Chapters 6-7, OxSTEP presents some significant methodological differences.

The sample size calculation is based on the same computations of the previous studies[358] (Appendix A6.1 and A8.1). However, to account for potentially reduced sensitivity of online compared to face-to-face testing in a laboratory setting, the calculated sample size is more than doubled to a total of 100 study participants, 50 per study arm. The remote nature of the study allows for a UK-wide recruitment which makes the attainment of such number more feasible.

Eligibility criteria are in Appendix A8.1: the main difference compared to the earlier studies involves the inclusion of a population considered to be at-risk for depression due to high levels of loneliness as pre-emptively measured on the UCLA 3-item loneliness scale[410].

Enrolled participants are randomised to either simvastatin 20mg, a dose used in previous clinical trials of simvastatin in depression[297,303] or sucrose placebo. Randomised

participants are posted 30 days of the drug/placebo to their address, as well as full written instructions of how and when to take them, and secure pre-paid postal boxes, to return to the researchers with their saliva samples. To improve adherence, participants receive daily automated text messages, reminding them to take the study capsule, and make frequent contact with the study investigators.

The most significant difference between OxSTEP and the earlier experimental medicine trials is that all testing measures (i.e., questionnaires for mood and anxiety, waking salivary cortisol, neuropsychological tasks) are taken both at baseline (pre-intervention) and after 28 to 30 days of study treatment (post-intervention), to account for a potentially more heterogeneous study population in view of the nationwide recruitment. Clinical questionnaires include: the Centre for Epidemiologic Studies-Depression scale (CES-D)[411], a bespoke Covid-19-related questionnaire, the Perceived Deficit Questionnaire (PDQ)[412], the Positive and Negative Affect Scale (PANAS) [369], a bespoke side-effect questionnaire, the Snaith-Hamilton Pleasure Scale (SHAPS)[370], and the State-Trait Anxiety Inventory (STAI)[371]. Waking saliva samples are collected by the participant and sent by post, then processed and stored as per standard operating procedure, and finally analysed at the end of the study for salivary cortisol. Neuropsychological tasks involve the emotional test battery (ETB) for emotional processing[359] and the probabilistic instrumental learning task (PILT) for reward learning[378], both described in detail before (section 6.2.4.1 and section 6.2.4.2). Working memory via the N-back task[413] is also measured, in contrast to the previously assessed verbal memory. These tasks have been made suitable for remote online testing on a data protection-compliant platform and have been previously validated in a similar study with observational design[364].

8.2.2 Statistical plan

The IBM SPSS v27 statistical package[380] will be used for all the analyses.

Participants' demographics and clinical characteristics will be reported descriptively. Questionnaires (PANAS, side-effects questionnaire, STAI-state, BL-VAS), and salivary cortisol will be analysed via repeated measures analysis of variance (ANOVA) with group (simvastatin *versus* placebo) as the between-subject factor and time (pre-intervention *versus* post-intervention) as the within-subject factor. Data distributions will be visually checked for all neuropsychological tasks using boxplots: extreme outliers (i.e., data values that lie more than three times the interquartile range below the first quartile or above the third quartile) will be excluded. The resulting data will then be analysed using repeated measures ANOVA with group as the between-subject factor and emotion/valence (ETB), win or loss (PILT), and trial condition (N-back) as the within-subject factor. Significant interactions will be followed up using simple main effect analyses. When assumptions of equality of variances are not fulfilled, the Greenhouse-Geisser correction will be used. Partial eta squared (η^2) will reported for the main significant comparisons as a measure of effect size (η^2 0.01, small effect; η^2 0.06, medium effect; η^2= 0.14, large effect).

8.2.3 Strengths and limitations

The innovative design of OxSTEP presents several new opportunities and challenges.

The remote online testing allows the recruitment of a diverse study cohort, including subgroups that may have otherwise not been represented by equivalent in-person research[414]. While many studies have been severely disrupted by the COVID-19 pandemic[415], OxSTEP strategy can be robust to changes in government measures and participants testing positive or having COVID contacts. However, careful consideration

must be taken to ensure that data quality is to an equal standard of in-person testing. Furthermore, it is possible that as restrictive measures are eased if the pandemic is controlled, characteristics of the study population might vary over time too. Besides the several proactive measures to foster concordance with the study procedures mentioned above, participants known to have deviated from protocol and extreme outliers will be excluded from analysis, thus reducing the risk that results will be affected by low-effort performances.

Although most studies previously employing the ETB have used an entirely between-subject design (i.e., no pre-intervention *versus* post-intervention testing), these neuropsychological tasks have been shown to be robust to the effects of test-retest reliability[416]. Therefore, outcomes are measured before and after study treatment to allow for a more heterogenous cohort.

Certain limitations discussed for the experimental medicine trials in Chapters 6 and 7 remain applicable to the OxSTEP study. Specifically, though I can reasonably predict that the OxSTEP study population might be more liable to the anti-inflammatory effects of simvastatin as it includes older participants who might be more inflamed because of underlying psychosocial stressors (i.e., social isolation and loneliness), I am not pre-selecting participants based on objectively measured inflammatory markers, nor I am particularly including people with a confirmed current diagnosis of depression. The study intervention, namely simvastatin, is being administered for longer periods (i.e., one month versus seven days), but it remains possible that longer administrations or higher doses might be required. Finally, the neuropsychological tests employed in this study may not best tailored for capturing the pleiotropic effects of statins.

8.2.4 Summary

OxSTEP is a novel experimental medicine trial that uses a remote online study design to investigate the neuropsychological effects of 28-day simvastatin in people at-risk for depression. Its findings can have an important translational value by shedding some light upon the interactions between statins, the HPA axis, and several cognitive functions that have been linked to depressive illness. Furthermore, if this new methodology is proved to be feasible and reliable, further experimental medicine studies could be informed by it.

8.3 Designing the next randomised controlled trial

The evidence produced in this dissertation, hopefully enriched by the later OxSTEP (NCT04973800) and other ongoing studies[321,322], can inform the design of the ideal "target trial"[333,417] that can ultimately define the effects of statin treatment in depression.

Firstly, according to a precision psychiatry approach to depressive disorders[18], an appropriate study population that is more likely to benefit from the widespread neurobiological, endocrine-metabolic, cardiovascular, and immunological activities of statins (section 1.3) must be identified *a priori*. Looking at the results of Chapters 4-5, it is plausible that patients with resistant depression, who have not responded to standard treatment and are already taking an antidepressant, would be a suitable sample.

Although several markers assessing baseline dysfunctions of the abovementioned systems are potentially obtainable, widely available, easily measurable tests for peripheral inflammation [(e.g., high sensitivity C-reactive protein (hs-CRP)] and dyslipidaemia (e.g., cholesterol and its lipoproteins) might be "good enough"[207] to select those for whom statins would yield most, as it has been suggested in an earlier trial of an anti-inflammatory medication[418].

Secondly, statins are not equal, but differ in terms of their pharmacokinetics (section 1.1), especially their lipophilicity and thus ability to enter the central nervous system, hereby expressing distinct pharmacological actions (section 1.2), possibly with composite downstream effects on systems implicated in the pathophysiology of depression (section 1.3), and therefore variable clinical effects (section 1.4). Most of the evidence retrieved [236,238,303,309], including new information from Chapters 6-7, seems to favour the highly lipophilic simvastatin, which would therefore represent the molecule of choice for such "target trial", probably in add-on to an antidepressant and administered for at least two months (Chapters 2-5).

The last issue of choosing suitable outcome measures is perhaps the most problematic. A variety of outcomes has been evaluated in the studies conducted for this dissertation (Chapters 2-7), including scales for depressive symptoms, treatment discontinuations, adverse events, and neuropsychological tasks. Further useful endpoints, such psychiatric hospitalisations, have been used in other studies (section 1.4), but many specific domains that, for example, might be more likely to respond to the anti-inflammatory properties of statins (e.g., anhedonia) [207], have been rarely evaluated, while proving useful in some clinical trials[419]. Moreover, advanced techniques to assess neuropsychological outcomes, such as behavioural and neural measures of emotional processing[354], hold promise to provide further valuable translational information. Finally, physical health outcomes, such as mortality, have been shown to have great relevance in vulnerable populations affected by severe mental illness, including depressive disorders[343].

An earlier study I had started with a different, strong anti-inflammatory molecule, the JAK/STAT inhibitor tofacitinib (NCT04141904), had been designed on principles that are akin to those just described, but it was suspended due to safety concerns in the context of

the COVID-19 pandemic. Nonetheless, thanks to the skills and expertise I have gathered during this DPhil in Biomedical and Clinical Sciences, I am in the position to apply for and develop the ideal "target trial", based on the above criteria, to further investigate the effects of statins in depression as my next research step.

Abbreviations

5HT: 5-hydroxytryptamine, serotonin

ACS: acute coronary syndrome

ANOVA: analysis of variance

aOR: adjusted odds ratio

AVLT: auditory-verbal learning task

BBB: blood-brain barrier

BDI: Beck depression inventory

BDNF: brain-derived neurotrophic factor

BL-VAS: Bond-Lader visual analogue scales

CAD: coronary artery disease

CI: confidence interval

CNS: central nervous system

CRP: C-reactive protein

CSF: cerebrospinal fluid

DA: dopamine

DSM: diagnostic and statistical manual of mental disorders

EPQ: Eysenck personality questionnaire

ETB: emotional test battery

fMRI: functional magnetic resonance imaging

GABA: γ-aminobutyric acid

HDL: high-density lipoprotein

HMG-CoA: 3-hydroxy-3-methylglutaryl-Coenzyme A

HPA: hypothalamic-pituitary-adrenal

hs-CRP: high sensitivity C-reactive protein

ICD: international classification of diseases

IDO: indoleamine 2,3-dioxygenase

IL: interleukin

LDL: low-density lipoprotein

NA: noradrenaline

NMDA: N-methyl-D-aspartate

NO: nitric oxide

OR: odds ratio

PANAS: positive and negative affect scale

PILT: probabilistic instrumental learning task

PKB: protein kinase B

PPAR: peroxisome proliferator-activated receptor

PUFA: polyunsaturated fatty acids

RCT: randomised controlled trial

ROS: reactive oxygen species

SERT: serotonin transporter

SHAPS: Snaith-Hamilton pleasure scale

SMD: standardised mean difference

SNRI: serotonergic and noradrenergic reuptake inhibitor

SOD: superoxide dismutase

SSRI: selective serotonin reuptake inhibitor

STAI: state-trait anxiety inventory

TAU: treatment as usual

TNF: tumour necrosis factor

TRKB: tyrosine kinase receptor 2

References

1. Demyttenaere K, Van Duppen Z. The Impact of (the Concept of) Treatment-Resistant Depression: An Opinion Review. *Int J Neuropsychopharmacol* 2019; **22**(2): 85-92.

2. James SL, Abate D, Abate KH, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 Diseases and Injuries for 195 countries and territories, 1990-2017: A systematic analysis for the Global Burden of Disease Study 2017. *The Lancet* 2018: 1789-858.

3. Malhi GS, Mann JJ. Depression. *Lancet* 2018; **392**(10161): 2299-312.

4. Cuijpers P, Noma H, Karyotaki E, Vinkers CH, Cipriani A, Furukawa TA. A network meta-analysis of the effects of psychotherapies, pharmacotherapies and their combination in the treatment of adult depression. *World Psychiatry* 2020; **19**(1): 92-107.

5. Furukawa TA, Shinohara K, Sahker E, et al. Initial treatment choices to achieve sustained response in major depression: a systematic review and network meta-analysis. *World Psychiatry* 2021; **20**(3): 387-96.

6. American Psychological Association. Clinical practice guideline for the treatment of depression across three age cohorts. February 2019 2019. https://www.apa.org/depression-guideline (accessed 19/01/2022 2022).

7. Malhi GS, Bell E, Singh AB, et al. The 2020 Royal Australian and New Zealand College of Psychiatrists clinical practice guidelines for mood disorders: Major depression summary. *Bipolar Disord* 2020; **22**(8): 788-804.

8. Health NCCfM. Depression - The NICE guideline to the treatment and management of depression in adults (updated edition). 2020. https://www.nice.org.uk/guidance/cg90/evidence/full-guideline-pdf-4840934509 (accessed 19/01/2022 2022).

9. Lam RW, Kennedy SH, Parikh SV, MacQueen GM, Milev RV, Ravindran AV. Canadian Network for Mood and Anxiety Treatments (CANMAT) 2016 Clinical Guidelines for the Management of Adults with Major Depressive Disorder: Introduction and Methods. *Can J Psychiatry* 2016; **61**(9): 506-9.

10. Woo YS, Bahk WM, Seo JS, et al. The Korean Medication Algorithm Project for Depressive Disorder 2021: Comparisons with Other Treatment Guidelines. *Clin Psychopharmacol Neurosci* 2022; **20**(1): 37-50.

11. Fournier JC, DeRubeis RJ, Hollon SD, et al. Antidepressant drug effects and depression severity: a patient-level meta-analysis. *Jama* 2010; **303**(1): 47-53.

12. Rush AJ, Trivedi MH, Wisniewski SR, et al. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am J Psychiatry* 2006; **163**(11): 1905-17.

13. Solmi M, Miola A, Croatto G, et al. How can we improve antidepressant adherence in the management of depression? A targeted review and 10 clinical recommendations. *Braz J Psychiatry* 2021; **43**(2): 189-202.

14. Cipriani A, Furukawa TA, Salanti G, et al. Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: a systematic review and network meta-analysis. *Lancet* 2018; **391**(10128): 1357-66.

15. Harmer CJ, Duman RS, Cowen PJ. How do antidepressants work? New perspectives for refining future treatment approaches. *Lancet Psychiatry* 2017; **4**(5): 409-18.

16. Malhi GS, Bell E, Morris G, Hamilton A. The delay in response to antidepressant therapy: A window of opportunity? *Aust N Z J Psychiatry* 2020; **54**(2): 127-9.

17. Jarończyk M, Walory J. Novel Molecular Targets of Antidepressants. *Molecules* 2022; **27**(2).

18. Maj M, Stein DJ, Parker G, et al. The clinical characterization of the adult patient with depression aimed at personalization of management. *World Psychiatry* 2020; **19**(3): 269-93.

19. Rush AJ, Sackeim HA, Conway CR, et al. Clinical research challenges posed by difficult-to-treat depression. *Psychol Med* 2022; **52**(3): 419-32.

20. Ciccocioppo R. Grand Challenge in Psychopharmacology: Setting Priorities to Shape a Bright Future. *Front Psychiatry* 2017; **8**: 15.

21. Ebada ME. Drug repurposing may generate novel approaches to treating depression. Journal of Pharmacy and Pharmacology: Blackwell Publishing Ltd; 2017. p. 1428-36.

22. Berk M, Nierenberg AA. Three paths to drug discovery in psychiatry. *Am J Psychiatry* 2015; **172**(5): 412-4.

23. Mohammad Sadeghi H, Adeli I, Mousavi T, Daniali M, Nikfar S, Abdollahi M. Drug Repurposing for the Management of Depression: Where Do We Stand Currently? *Life (Basel)* 2021; **11**(8).

24. Chavez-Castillo M, Nava M, Ortega A, et al. Depression as an immunometabolic disorder: Exploring shared pharma-cotherapeutics with cardiovascular disease. *Current Neuropharmacology* 2020; **18**(11): 1138-53.

25. Jones BDM, Farooqui S, Kloiber S, Husain MO, Mulsant BH, Husain MI. Targeting Metabolic Dysfunction for the Treatment of Mood Disorders: Review of the Evidence. *Life (Basel)* 2021; **11**(8).

26. Bot M, Milaneschi Y, Al-Shehri T, et al. Metabolomics Profile in Depression: A Pooled Analysis of 230 Metabolic Markers in 5283 Cases With Depression and 10,145 Controls. *Biol Psychiatry* 2020; **87**(5): 409-18.

27. Caspani G, Turecki G, Lam RW, et al. Metabolomic signatures associated with depression and predictors of antidepressant response in humans: A CAN-BIND-1 report. *Commun Biol* 2021; **4**(1): 903.

28. Kokkeler KJE, Marijnissen RM, Wardenaar KJ, et al. Subtyping late-life depression according to inflammatory and metabolic dysregulation: a prospective study. *Psychol Med* 2022; **52**(3): 515-25.

29. Guerreiro Costa FLN, Carneiro BA, Alves GS, et al. Metabolomics of Major Depressive Disorder: A Systematic Review of Clinical Studies. *Cureus* 2022; **14**(3): e23009.

30. Fernandes B, Dai Y, Jia P, Zhao Z. W52. CHARTING THE PROTEOME LANDSCAPE IN MAJOR PSYCHIATRIC DISORDERS: FROM BIOMARKERS TO BIOLOGICAL PATHWAYS FOR PRECISION PSYCHIATRY. *European Neuropsychopharmacology* 2021; **51**: e171-e2.

31. Avan R, Sahebnasagh A, Hashemi J, et al. Update on Statin Treatment in Patients with Neuropsychiatric Disorders. *Life (Basel)* 2021; **11**(12).

32. Fracassi A, Marangoni M, Rosso P, et al. Statins and the Brain: More than Lipid Lowering Agents? *Curr Neuropharmacol* 2019; **17**(1): 59-83.

33. Tao SH, Ren XQ, Zhang LJ, Liu MY. Association between common cardiovascular drugs and depression. *Chinese Medical Journal* 2021; **134**(22): 2656-65.

34. Rahola JG. Somatic drugs for psychiatric diseases: aspirin or simvastatin for depression? *Curr Neuropharmacol* 2012; **10**(2): 139-58.

35. So HC, Chau CK, Lau A, Wong SY, Zhao K. Translating GWAS findings into therapies for depression and anxiety disorders: gene-set analyses reveal enrichment of psychiatric drug classes and implications for drug repositioning. *Psychol Med* 2019; **49**(16): 2692-708.

36. Covington HE, Vialou V, Nestler EJ. From synapse to nucleus: Novel targets for treating depression. *Neuropharmacology* 2010; **58(4-5)**: 683-93.

37. Maes M, Fisar Z, Medina M, Scapagnini G, Nowak G, Berk M. New drug targets in depression: Inflammatory, cell-mediate immune, oxidative and nitrosative stress, mitochondrial, antioxidant, and neuroprogressive pathways. and new drug candidates-Nrf2 activators and GSK-3 inhibitors. *Inflammopharmacology* 2012; **20**(3): 127-50.

38. Fabbri C, Kasper S, Zohar J, et al. Drug repositioning for treatment-resistant depression: Hypotheses from a pharmacogenomic study. *Prog Neuropsychopharmacol Biol Psychiatry* 2021; **104**: 110050.

39. Le-Niculescu H, Roseberry K, Gill SS, et al. Precision medicine for mood disorders: objective assessment, risk prediction, pharmacogenomics, and repurposed drugs. *Molecular Psychiatry* 2021; **26**(7): 2776-804.

40. Sizar O, Khare S, Jamil RT, Talati R. Statin Medications. StatPearls. Treasure Island (FL): StatPearls Publishing, Copyright © 2022, StatPearls Publishing LLC; 2022.

41. Urquhart L. Top drugs and companies by sales in 2018. *Nat Rev Drug Discov* 2019.

42. Simons J. The $10 billion pill. *Fortune* 2003; **147**(1): 58-62, 6, 8.

43. World Health Organization. WHO model list of essential medicines, 20th list (March 2017, amended August 2017). Geneva: World Health Organization, 2017.

44. Endo A, Kuroda M, Tanzawa K. Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase by ML-236A and ML-236B fungal metabolites, having hypocholesterolemic activity. *FEBS Lett* 1976; **72**(2): 323-6.

45. Iwaki Y, Lee W, Sugiyama Y. Comparative and quantitative assessment on statin efficacy and safety: insights into inter-statin and inter-individual variability via dose- and exposure-response relationships. *Expert Opin Drug Metab Toxicol* 2019; **15**(11): 897-911.

46. Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. *Science* 2001; **292**(5519): 1160-4.

47. Trapani L, Segatto M, Pallottini V. Regulation and deregulation of cholesterol homeostasis: The liver as a metabolic "power station". *World J Hepatol* 2012; **4**(6): 184-90.

48. Espenshade PJ, Hughes AL. Regulation of sterol synthesis in eukaryotes. *Annu Rev Genet* 2007; **41**: 401-27.

49. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science* 1986; **232**(4746): 34-47.

50. Ginsberg HN, Le NA, Short MP, Ramakrishnan R, Desnick RJ. Suppression of apolipoprotein B production during treatment of cholesteryl ester storage disease with lovastatin. Implications for regulation of apolipoprotein B synthesis. *J Clin Invest* 1987; **80**(6): 1692-7.

51. Grundy SM. Consensus statement: Role of therapy with "statins" in patients with hypertriglyceridemia. *Am J Cardiol* 1998; **81**(4a): 1b-6b.

52. McFarland AJ, Anoopkumar-Dukie S, Arora DS, et al. Molecular mechanisms underlying the effects of statins in the central nervous system. *Int J Mol Sci* 2014; **15**(11): 20607-37.

53. Segatto M, Manduca A, Lecis C, et al. Simvastatin treatment highlights a new role for the isoprenoid/cholesterol biosynthetic pathway in the modulation of emotional reactivity and cognitive performance in rats. *Neuropsychopharmacology* 2014; **39**(4): 841-54.

54. Yu D, Liao JK. Emerging views of statin pleiotropy and cholesterol lowering. *Cardiovasc Res* 2022; **118**(2): 413-23.

55. Shafiq N, Malhotra S, Pandhi P, Grover A. The "Statinth" wonder of the world: A panacea for all illnesses or a bubble about to burst. *Journal of Negative Results in BioMedicine* 2005; **4**.

56. Zeiser R. Immune modulatory effects of statins. *Immunology* 2018; **154**(1): 69-75.

57. Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. *Fundam Clin Pharmacol* 2005; **19**(1): 117-25.

58. Corsini A, Bellosta S, Baetta R, Fumagalli R, Paoletti R, Bernini F. New insights into the pharmacodynamic and pharmacokinetic properties of statins. *Pharmacol Ther* 1999; **84**(3): 413-28.

59. Hirota T, Fujita Y, Ieiri I. An updated review of pharmacokinetic drug interactions and pharmacogenetics of statins. *Expert Opin Drug Metab Toxicol* 2020; **16**(9): 809-22.

60. Davidson MH. Rosuvastatin: a highly efficacious statin for the treatment of dyslipidaemia. *Expert Opin Investig Drugs* 2002; **11**(1): 125-41.

61. Fong CW. Statins in therapy: understanding their hydrophilicity, lipophilicity, binding to 3-hydroxy-3-methylglutaryl-CoA reductase, ability to cross the blood brain barrier and metabolic stability based on electrostatic molecular orbital studies. *Eur J Med Chem* 2014; **85**: 661-74.

62. Irwin JC, Fenning AS, Vella RK. Statins with different lipophilic indices exert distinct effects on skeletal, cardiac and vascular smooth muscle. *Life Sci* 2020; **242**: 117225.

63. Johnson-Anuna LN, Eckert GP, Keller JH, et al. Chronic administration of statins alters multiple gene expression patterns in mouse cerebral cortex. *J Pharmacol Exp Ther* 2005; **312**(2): 786-93.

64. Lütjohann D, Stroick M, Bertsch T, et al. High doses of simvastatin, pravastatin, and cholesterol reduce brain cholesterol synthesis in guinea pigs. *Steroids* 2004; **69**(6): 431-8.

65. Botti RE, Triscari J, Pan HY, Zayat J. Concentrations of pravastatin and lovastatin in cerebrospinal fluid in healthy subjects. *Clin Neuropharmacol* 1991; **14**(3): 256-61.

66. Parker BA, Polk DM, Rabdiya V, et al. Changes in memory function and neuronal activation associated with atorvastatin therapy. *Pharmacotherapy* 2010; **30**(6): 625.

67. Hsiang B, Zhu Y, Wang Z, et al. A novel human hepatic organic anion transporting polypeptide (OATP2). Identification of a liver-specific human organic anion transporting polypeptide and identification of rat and human hydroxymethylglutaryl-CoA reductase inhibitor transporters. *J Biol Chem* 1999; **274**(52): 37161-8.

68. Lee G, Dallas S, Hong M, Bendayan R. Drug transporters in the central nervous system: brain barriers and brain parenchyma considerations. *Pharmacol Rev* 2001; **53**(4): 569-96.

69. Tsuji A, Saheki A, Tamai I, Terasaki T. Transport mechanism of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors at the blood-brain barrier. *J Pharmacol Exp Ther* 1993; **267**(3): 1085-90.

70. Farooqui AA, Ong WY, Horrocks LA, Chen P, Farooqui T. Comparison of biochemical effects of statins and fish oil in brain: The battle of the titans. *Brain Research Reviews* 2007; **56**(2): 443-71.

71. Cartocci V, Servadio M, Trezza V, Pallottini V. Can Cholesterol Metabolism Modulation Affect Brain Function and Behavior? *Journal of Cellular Physiology* 2017; **232**(2): 281-6.

72. Andersson M, Elmberger PG, Edlund C, Kristensson K, Dallner G. Rates of cholesterol, ubiquinone, dolichol and dolichyl-P biosynthesis in rat brain slices. *FEBS Lett* 1990; **269**(1): 15-8.

73. Nieweg K, Schaller H, Pfrieger FW. Marked differences in cholesterol synthesis between neurons and glial cells from postnatal rats. *J Neurochem* 2009; **109**(1): 125-34.

74. Dietschy JM, Turley SD. Cholesterol metabolism in the brain. *Curr Opin Lipidol* 2001; **12**(2): 105-12.

75. Locatelli S, Lütjohann D, Schmidt HH, Otto C, Beisiegel U, von Bergmann K. Reduction of plasma 24S-hydroxycholesterol (cerebrosterol) levels using high-dosage simvastatin in patients with hypercholesterolemia: evidence that simvastatin affects cholesterol metabolism in the human brain. *Arch Neurol* 2002; **59**(2): 213-6.

76. Ostrowski SM, Johnson K, Siefert M, et al. Simvastatin inhibits protein isoprenylation in the brain. *Neuroscience* 2016; **329**: 264-74.

77. Cibičková L. Statins and their influence on brain cholesterol. *J Clin Lipidol* 2011; **5**(5): 373-9.

78. Schneider M, Levant B, Reichel M, Gulbins E, Kornhuber J, Müller CP. Lipids in psychiatric disorders and preventive medicine. Neuroscience and Biobehavioral Reviews: Elsevier Ltd; 2017. p. 336-62.

79. Kosowski M, Smolarczyk-Kosowska J, Hachuła M, et al. The Effects of Statins on Neurotransmission and Their Neuroprotective Role in Neurological and Psychiatric Disorders. *Molecules* 2021; **26**(10).

80. Roy A, Jana M, Kundu M, et al. HMG-CoA Reductase Inhibitors Bind to PPARα to Upregulate Neurotrophin Expression in the Brain and Improve Memory in Mice. *Cell Metab* 2015; **22**(2): 253-65.

81. Robin NC, Agoston Z, Biechele TL, James RG, Berndt JD, Moon RT. Simvastatin promotes adult hippocampal neurogenesis by enhancing Wnt/β-catenin signaling. *Stem Cell Reports* 2014; **2**(1): 9-17.

82. Pooler AM, Xi SC, Wurtman RJ. The 3-hydroxy-3-methylglutaryl co-enzyme A reductase inhibitor pravastatin enhances neurite outgrowth in hippocampal neurons. *J Neurochem* 2006; **97**(3): 716-23.

83. Mailman T, Hariharan M, Karten B. Inhibition of neuronal cholesterol biosynthesis with lovastatin leads to impaired synaptic vesicle release even in the presence of lipoproteins or geranylgeraniol. John Wiley & Sons, Ltd (10.1111); 2011. p. 1002-15.

84. Lu D, Qu C, Goussev A, et al. Statins increase neurogenesis in the dentate gyrus, reduce delayed neuronal death in the hippocampal CA3 region, and improve spatial learning in rat after traumatic brain injury. *J Neurotrauma* 2007; **24**(7): 1132-46.

85. Wu H, Lu D, Jiang H, et al. Simvastatin-mediated upregulation of VEGF and BDNF, activation of the PI3K/Akt pathway, and increase of neurogenesis are associated with therapeutic improvement after traumatic brain injury. *J Neurotrauma* 2008; **25**(2): 130-9.

86. McFarland AJ, Davey AK, Anoopkumar-Dukie S. Statins Reduce Lipopolysaccharide-Induced Cytokine and Inflammatory Mediator Release in an In Vitro Model of Microglial-Like Cells. *Mediators Inflamm* 2017; **2017**: 2582745.

87. Mancini G, Martins WC, de Oliveira J, de Bem AF, Tasca CI. Atorvastatin Improves Mitochondrial Function and Prevents Oxidative Stress in Hippocampus Following Amyloid-β(1-40) Intracerebroventricular Administration in Mice. *Mol Neurobiol* 2020; **57**(10): 4187-201.

88. Fujimoto T, Morofuji Y, Kovac A, et al. Pitavastatin Ameliorates Lipopolysaccharide-Induced Blood-Brain Barrier Dysfunction. *Biomedicines* 2021; **9**(7).

89. Zheng X, Liao Y, Wang J, et al. The Antineuroinflammatory Effect of Simvastatin on Lipopolysaccharide Activated Microglial Cells. *Evid Based Complement Alternat Med* 2018; **2018**: 9691085.

90. Yadollah-Damavandi S, Sharifi ZN, Arani HZ, et al. Atorvastatin Prevents the Neuron Loss in the Hippocampal Dentate Gyrus Region through its Anti-Oxidant and Anti-Apoptotic Activities. *CNS Neurol Disord Drug Targets* 2021; **20**(1): 76-86.

91. Tibrewala A, Jivan A, Oetgen WJ, Stone NJ. A Comparative Analysis of Current Lipid Treatment Guidelines: Nothing Stands Still. *J Am Coll Cardiol* 2018; **71**(7): 794-9.

92. Sahebkar A, Rathouska J, Simental-Mendía LE, Nachtigal P. Statin therapy and plasma cortisol concentrations: A systematic review and meta-analysis of randomized placebo-controlled trials. *Pharmacological Research* 2016; **103**: 17-25.

93. Xu J, Lecanu L, Han Z, Yao Z, Greeson J, Papadopoulos V. Inhibition of Adrenal Cortical Steroid Formation by Procaine Is Mediated by Reduction of the cAMP-Induced 3-Hydroxy-3-methylglutaryl-coenzyme A Reductase Messenger Ribonucleic Acid Levels. *Journal of Pharmacology and Experimental Therapeutics* 2003; **307**(3): 1148-57.

94. Abbasi F, Lamendola C, Harris CS, et al. Statins Are Associated With Increased Insulin Resistance and Secretion. *Arterioscler Thromb Vasc Biol* 2021; **41**(11): 2786-97.

95. Grunwald SA, Haafke S, Grieben U, Kassner U, Steinhagen-Thiessen E, Spuler S. Statins Aggravate the Risk of Insulin Resistance in Human Muscle. *Int J Mol Sci* 2022; **23**(4).

96. Wang CY, Liu PY, Liao JK. Pleiotropic effects of statin therapy: molecular mechanisms and clinical results. *Trends Mol Med* 2008; **14**(1): 37-44.

97. Milajerdi A, Larijani B, Esmaillzadeh A. Statins influence biomarkers of low grade inflammation in apparently healthy people or patients with chronic diseases: A systematic review and meta-analysis of randomized clinical trials. *Cytokine* 2019; **123**: 154752.

98. Ferro D, Parrotto S, Basili S, Alessandri C, Violi F. Simvastatin inhibits the monocyte expression of proinflammatory cytokines in patients with hypercholesterolemia. *J Am Coll Cardiol* 2000; **36**(2): 427-31.

99. Weitz-Schmidt G, Welzenbach K, Brinkmann V, et al. Statins selectively inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site. *Nat Med* 2001; **7**(6): 687-92.

100. Bu DX, Tarrio M, Grabie N, et al. Statin-induced Krüppel-like factor 2 expression in human and mouse T cells reduces inflammatory and pathogenic responses. *J Clin Invest* 2010; **120**(6): 1961-70.

101. Kim YC, Kim KK, Shevach EM. Simvastatin induces Foxp3+ T regulatory cells by modulation of transforming growth factor-beta signal transduction. *Immunology* 2010; **130**(4): 484-93.

102. Morris G, Puri BK, Olive L, et al. Endothelial dysfunction in neuroprogressive disorders-causes and suggested treatments. *BMC Medicine* 2020; **18**(1): 305.

103. Morris G, Berk M, Walder K, O'Neil A, Maes M, Puri BK. The lipid paradox in neuroprogressive disorders: Causes and consequences. *Neuroscience and Biobehavioral Reviews* 2021; **128**: 35-57.

104. van Diepen JA, Berbée JF, Havekes LM, Rensen PC. Interactions between inflammation and lipid metabolism: relevance for efficacy of anti-inflammatory drugs in the treatment of atherosclerosis. *Atherosclerosis* 2013; **228**(2): 306-15.

105. Jain MK, Ridker PM. Anti-inflammatory effects of statins: clinical evidence and basic mechanisms. *Nat Rev Drug Discov* 2005; **4**(12): 977-87.

106. Swiger KJ, Manalac RJ, Blaha MJ, Blumenthal RS, Martin SS. Statins, mood, sleep, and physical function: a systematic review. *Eur J Clin Pharmacol* 2014; **70**(12): 1413-22.

107. You H, Lu W, Zhao S, Hu Z, Zhang J. The relationship between statins and depression: a review of the literature. *Expert Opin Pharmacother* 2013; **14**(11): 1467-76.

108. De Giorgi R, Rizzo Pesci N, Quinton A, De Crescenzo F, Cowen PJ, Harmer CJ. Statins in Depression: An Evidence-Based Overview of Mechanisms and Clinical Studies. *Front Psychiatry* 2021; **12**: 702617.

109. Walker AJ, Kim Y, Borissiouk I, et al. Statins: Neurobiological underpinnings and mechanisms in mood disorders. *Neurosci Biobehav Rev* 2021; **128**: 693-708.

110. Hayes JF, Lundin A, Wicks S, et al. Association of Hydroxylmethyl Glutaryl Coenzyme A Reductase Inhibitors, L-Type Calcium Channel Antagonists, and Biguanides With Rates of Psychiatric Hospitalization and Self-Harm in Individuals With Serious Mental Illness. *JAMA Psychiatry* 2019; **76**(4): 382-90.

111. Kim SW, Kang HJ, Jhon M, et al. Statins and Inflammation: New Therapeutic Opportunities in Psychiatry. *Front Psychiatry* 2019; **10**: 103.

112. Bortolato B, Miskowiak KW, Köhler CA, et al. Cognitive remission: a novel objective for the treatment of major depression? *BMC Med* 2016; **14**: 9.

113. Russell AAM, Sutherland BA, Landowski LM, Macleod M, Howells DW. What has preclinical systematic review ever done for us? *BMJ Open Sci* 2022; **6**(1): e100219.

114. Bannach-Brown A, Przybyła P, Thomas J, et al. Machine learning algorithms for systematic review: reducing workload in a preclinical review of animal studies and reducing human screening error. *Syst Rev* 2019; **8**(1): 23.

115. Munn Z, Peters MDJ, Stern C, Tufanaru C, McArthur A, Aromataris E. Systematic review or scoping review? Guidance for authors when choosing between a systematic or scoping review approach. *BMC Med Res Methodol* 2018; **18**(1): 143.

116. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. *Syst Rev* 2016; **5**(1): 210.

117. Kilic FS, Ozatyk Y, Kaygisiz B, Baydemyr C, Erol K. Focused Conference Group: P17 - Newapproaches and targets in psychiatry investigation of antidepressant and anxiolytic effects of simvastatine in rats. *Basic and Clinical Pharmacology and Toxicology* 2010; **107**(SUPPL. 1): 374.

118. Gudadappanavar AM, Hiremath SV, Shashikant T. Evaluation of antidepressant activity of simvastatin, lovastatin and atorvastatin in male swiss mice - An experimental study. *International Journal of Drug Development and Research* 2013; **5**(2): 102-8.

119. Iqubal A, Iqubal MK, Kumar R, Ahmad S. A step closer to lookup alternate or adjuvant therapy for epilepsy: Intranasal delivery of solid-lipid nanoparticles of pitavastatin to appraise antiepileptic properties in mice. *International Research Journal of Pharmacy* 2015; **6**(12): 820-4.

120. Jyothsna VC, Balaji O, Chogtu B. Effect of different statins in animal model of anxiety in wistar rats. *Asian Journal of Pharmaceutical and Clinical Research* 2018; **11**(10): 369-71.

121. Pytka K, Podkowa K, Rapacz A, et al. The role of serotonergic, adrenergic and dopaminergic receptors in antidepressant-like effect. *Pharmacol Rep* 2016; **68**(2): 263-74.

122. Pytka K, Dziubina A, Młyniec K, et al. The role of glutamatergic, GABA-ergic, and cholinergic receptors in depression and antidepressant-like effect. *Pharmacol Rep* 2016; **68**(2): 443-50.

123. Shrivastava S, Pucadyil TJ, Paila YD, Ganguly S, Chattopadhyay A. Chronic Cholesterol Depletion Using Statin Impairs the Function and Dynamics of Human Serotonin 1A Receptors †. *Biochemistry* 2010; **49**: 5426-35.

124. Nothdurfter C, Tanasic S, Di Benedetto B, et al. Impact of lipid raft integrity on 5-HT3 receptor function and its modulation by antidepressants. *Neuropsychopharmacology* 2010; **35**(7): 1510-9.

125. Deveau CM. A new mechanism of serotonin transporter regulation by simvastatin and the isoprenylation pathway. *Dissertation Abstracts International: Section B: The Sciences and Engineering* 2022; **83**(2-B): No-Specified.

126. Deveau CM, Rodriguez E, Schroering A, Yamamoto BK. Serotonin transporter regulation by cholesterol-independent lipid signaling. *Biochem Pharmacol* 2021; **183**: 114349.

127. Kilic FS, Ozatik Y, Kaygisiz B, Baydemir C, Erol K. Acute antidepressant and anxiolytic effects of simvastatin and its mechanisms in rats. *Neurosciences (Riyadh)* 2012; **17**(1): 39-43.

128. ElBatsh MM. Antidepressant-like effect of simvastatin in diabetic rats. *Can J Physiol Pharmacol* 2015; **93**(8): 649-56.

129. Ludka FK, Constantino LC, Kuminek G, et al. Atorvastatin evokes a serotonergic system-dependent antidepressant-like effect in mice. *Pharmacology Biochemistry and Behavior* 2014; **122**: 253-60.

130. Delva NJ, Matthews DR, Cowen PJ. Brain serotonin (5-HT) neuroendocrine function in patients taking cholesterol-lowering drugs. *Biological Psychiatry* 1996; **39**(2): 100-6.

131. Casarotto PC, Girych M, Fred SM, et al. Antidepressant drugs act by directly binding to TRKB neurotrophin receptors. *Cell* 2021; **184**(5): 1299.

132. Renshaw PF, Parsegian A, Yang CK, et al. Lovastatin potentiates the antidepressant efficacy of fluoxetine in rats. *Pharmacology Biochemistry and Behavior* 2009; **92**(1): 88-92.

133. Santos T, da Cruz JGP, Baungratz MM, da Cruz JN, Dal Magro DD, da Cruz JGP. Behavioral interactions of simvastatin and fluoxetine in tests of anxiety and depression. 2012. p. 413.

134. Al-Asmari AK, Ullah Z, Al Masoudi AS, Ahmad I. Simultaneous administration of fluoxetine and simvastatin ameliorates lipid profile, improves brain level of neurotransmitters, and increases bioavailability of simvastatin. *J Exp Pharmacol* 2017; **9**: 47-57.

135. Bhattarai B, Swamy S, Baral SR, Acharya CP. A study on the effect of atorvastatin on the pharmacokinetic and antidepressant activity of fluoxetine. *National Journal of Physiology, Pharmacy and Pharmacology* 2017; **7**(11): 1224-9.

136. Li F, Ling ZL, Wang ZJ, et al. Differential effects of pravastatin on the pharmacokinetics of paroxetine in normal and diabetic rats. *Xenobiotica* 2017; **47**(1): 20-30.

137. Rana DG, Patel AK, Joshi CG, Jhala MK, Goyal RK. Alteration in the expression of exon IIC transcripts of brain-derived neurotrophic factor gene by simvastatin [correction of simvastain] in chronic mild stress in mice: a possible link with dopaminergic pathway. *Canadian journal of physiology and pharmacology* 2014; **92**(12): 985-92.

138. Rana DG, Patel A, Joshi CG, Goyal RK. Possible involvement of altered expression of BDNF exon II gene and specific dopamine receptors in simvastatin induced beneficial effects in depression. *Molecular Cytogenetics* 2014; **7**(SUPPL. 1).

139. Rana DG, Upadhyay UM, Goyal RK. The preferential effect of simvastatin on dopaminergic agents induced behavioral effect in experimental models of depression. *International Journal of Pharmaceutical Research* 2014; **6**(2): 55-60.

140. Sehar N, Agarwal NB, Vohora D, Raisuddin S. Atorvastatin prevents development of kindling by modulating hippocampal levels of dopamine, glutamate, and GABA in mice. *Epilepsy and Behavior* 2015; **42**: 48-53.

141. Yan J, Xu Y, Zhu C, et al. Simvastatin prevents dopaminergic neurodegeneration in experimental parkinsonian models: the association with anti-inflammatory responses. *PLoS One* 2011; **6**(6): e20945.

142. da Cruz JN, Dal Magro DD, de Lima DD, da Cruz JGP. Simvastatin treatment reduces the cholesterol content of membrane/lipid rafts, implicating the N-methyl-D-aspartate receptor in anxiety: A literature review. *Braz J Pharm Sci* 2017; **53**(1).

143. Wang Q, Zengin A, Deng C, et al. High dose of simvastatin induces hyperlocomotive and anxiolytic-like activities: The association with the up-regulation of NMDA receptor binding in the rat brain. *Exp Neurol* 2009; **216**(1): 132-8.

144. Ludka FK, Zomkowski AD, Cunha MP, et al. Acute atorvastatin treatment exerts antidepressant-like effect in mice via the L-arginine-nitric oxide-cyclic guanosine monophosphate pathway and increases BDNF levels. *Eur Neuropsychopharmacol* 2013; **23**(5): 400-12.

145. Dolatshahi M, Davoudi S, Paridar Y, Naserzadeh R, Ghorbanzadeh B. Pharmacological evidence for the involvement of the opioid system in the antidepressant-like effect of simvastatin in mice: Without tolerance and withdrawal syndrome. Elsevier Ireland Ltd; 2020. p. 134578.

146. Wang H, Zhou J, Liu QZ, Wang LL, Shang J. Simvastatin and Bezafibrate ameliorate Emotional disorder Induced by High fat diet in C57BL/6 mice.: Nature Publishing Group; 2017. p. 2335.

147. Eriksson M, Eklundh T, Sjoberg S, Angelin B, Nordin C. Cholesterol lowering and cerebrospinal fluid neurotransmitters: Increased levels of the anxiogenic cholecystokinin-tetrapeptide during simvastatin administration to healthy male volunteers. *Biological Psychiatry* 1996; **40**(4): 302-4.

148. Yang T, Nie Z, Shu H, et al. The Role of BDNF on Neural Plasticity in Depression. *Front Cell Neurosci* 2020; **14**: 82.

149. Tartt AN, Mariani MB, Hen R, Mann JJ, Boldrini M. Dysregulation of adult hippocampal neuroplasticity in major depression: pathogenesis and therapeutic implications. *Mol Psychiatry* 2022.

150. Tsai S-J. Statins may enhance the proteolytic cleavage of proBDNF: Implications for the treatment of depression. Churchill Livingstone; 2007. p. 1296-9.

151. Ludka FK, Cunha MP, Dal-Cim T, et al. Atorvastatin Protects from Aβ1–40-Induced Cell Damage and Depressive-Like Behavior via ProBDNF Cleavage. Springer US; 2017. p. 6163-73.

152. Binder LB, Ludka FK, Cunha MP, et al. Atorvastatin prevents cell death and depressive-like behaviour induced by Abeta1-40 peptide via BDNF cleavage. *Journal of Neurochemistry* 2015; **134**(SUPPL. 1): 332-3.

153. Tang C-R, Yu X-B, Zhang H-N, et al. Lovastatin Prevents Depressive Behaviors and Increased Hippocampal Neurogenesis in Streptozotocin-Induced Diabetic Mice. S. Karger AG; 2020. p. 339-48.

154. Yang C, Ni HY, Yin JJ, et al. Atorvastatin ameliorates depressive behaviors via regulation of alpha7nAChR expression by PI3K/Akt-BDNF pathway in mice. *Biochemical and Biophysical Research Communications* 2022; **593**: 57-64.

155. Rahangdale S, Fating R, Gajbhiye M, et al. Involvement of agmatine in antidepressant-like effect of HMG-CoA reductase inhibitors in mice. Elsevier B.V.; 2021.

156. Okudan N, Belviranli M. High dose simvastatin and rosuvastatin impair cognitive abilities of healthy rats via decreasing hippocampal neurotrophins and irisin. *Brain Res Bull* 2020; **165**: 81-9.

157. Olloquequi J, Cornejo-Córdova E, Verdaguer E, et al. Excitotoxicity in the pathogenesis of neurological and psychiatric disorders: Therapeutic implications. *J Psychopharmacol* 2018; **32**(3): 265-75.

158. Bhatt S, Nagappa AN, Patil CR. Role of oxidative stress in depression. Elsevier Ltd; 2020. p. 1270-6.

159. Eisel ULM, Dolga A, Granic I, et al. Inflammation as a target in neurodegenerative disease and related depression. *Acta Physiologica Hungarica* 2010; **97**(4): 437-8.

160. Naserzadeh R, Abad N, Ghorbanzadeh B, Dolatshahi M, Mansouri MT. Simvastatin exerts antidepressant-like activity in mouse forced swimming test: Role of NO-cGMP-KATP channels pathway and PPAR-gamma receptors. Elsevier; 2019. p. 92-100.

161. Shahsavarian A, Javadi S, Jahanabadi S, et al. Antidepressant-like effect of atorvastatin in the forced swimming test in mice: The role of PPAR-gamma receptor and nitric oxide pathway. Elsevier; 2014. p. 52-8.

162. Kuhad A, Kishor S, Chadha R. Psychopharmacological studies on atrovastatin and its solvatomorphs in experimental paradigm of depression. *International Journal of Neuropsychopharmacology* 2012; **15**(SUPPL. 1): 188.

163. Taniguti EH, Ferreira YS, Stupp IJV, et al. Atorvastatin prevents lipopolysaccharide-induced depressive-like behaviour in mice. Elsevier; 2019. p. 279-86.

164. Ludka FK, Dal-Cim T, Binder LB, Constantino LC, Massari C, Tasca CI. Atorvastatin and Fluoxetine Prevent Oxidative Stress and Mitochondrial Dysfunction Evoked by Glutamate Toxicity in Hippocampal Slices. Springer US; 2017. p. 3149-61.

165. Ludka FK, Constantino LC, Dal-Cim T, et al. Involvement of PI3K/Akt/GSK-3β and mTOR in the antidepressant-like effect of atorvastatin in mice. Pergamon; 2016. p. 50-7.

166. Walther A, Cannistraci CV, Simons K, et al. Lipidomics in major depressive disorder. Frontiers in Psychiatry: Frontiers Media S.A.; 2018.

167. Gulbins E, Palmada M, Reichel M, et al. Acid sphingomyelinase-ceramide system mediates effects of antidepressant drugs. *Nature Medicine* 2013; **19**(7): 934-8.

168. Park YM, Lee BH, Lee SH. The association between serum lipid levels, suicide ideation, and central serotonergic activity in patients with major depressive disorder. *J Affect Disord* 2014; **159**: 62-5.

169. Sun S, Yang S, Mao Y, Jia X, Zhang Z. Reduced cholesterol is associated with the depressive-like behavior in rats through modulation of the brain 5-HT1A receptor. *Lipids Health Dis* 2015; **14**: 22.

170. Patel JS, Polanka BM, Stewart JC. Association of blood lipids fractions with depressive symptom clusters and individual symptoms in men and women in the United States: National health and nutrition examination survey (NHANES) 2005-2012. *Psychosomatic Medicine* 2016; **78**(3): A25.

171. Lütjohann D. Brain cholesterol and suicidal behaviour. *International Journal of Neuropsychopharmacology* 2007; **10**(2): 153-7.

172. Wagner CJ, Musenbichler C, Böhm L, et al. LDL cholesterol relates to depression, its severity, and the prospective course. *Prog Neuropsychopharmacol Biol Psychiatry* 2019; **92**: 405-11.

173. Goldstein MR, Mascitelli L, Pezzetta F. Is the increase in LDL cholesterol induced by selective serotonin reuptake inhibitor therapy a blessing in disguise? *Medical Hypotheses* 2010; **74**(5): 955-6.

174. Chien IC, Lin CH, Chou YJ, Chou P. Increased risk of hyperlipidemia in patients with major depressive disorder: a population-based study. *J Psychosom Res* 2013; **75**(3): 270-4.

175. Fabre LF, Smith LC. Total cholesterol levels in subjects with major depression. *Journal of Clinical Lipidology* 2012; **6**(3): 267-8.

176. Persons JE, Fiedorowicz JG. Depression and serum low-density lipoprotein: A systematic review and meta-analysis. *J Affect Disord* 2016; **206**: 55-67.

177. Shin JY, Suls J, Martin R. Are cholesterol and depression inversely related? A meta-analysis of the association between two cardiac risk factors. *Ann Behav Med* 2008; **36**(1): 33-43.

178. Lin PY, Huang SY, Su KP. A meta-analytic review of polyunsaturated fatty acid compositions in patients with depression. *Biol Psychiatry* 2010; **68**(2): 140-7.

179. Flicker L, Almeida O, Yeap B, Hankey G, Golledge J. HDL cholesterol and the risk of depression over 5 years among older men. *International Psychogeriatrics* 2013; **25**(SUPPL. 1): S70.

180. Ancelin ML, Carrière I, Boulenger JP, et al. Gender and genotype modulation of the association between lipid levels and depressive symptomatology in community-dwelling elderly (the ESPRIT study). *Biol Psychiatry* 2010; **68**(2): 125-32.

181. Coutu MF, Dupuis G, D'Antono B. The Impact of Cholesterol Lowering on Patients' Mood. *Journal of Behavioral Medicine* 2001; **24**(6): 517-36.

182. Parekh A, Smeeth D, Milner Y, Thure S. The Role of Lipid Biomarkers in Major Depression. *Healthcare (Basel)* 2017; **5**(1).

183. Can ÖD, Ulupinar E, Özkay ÜD, Yegin B, Öztürk Y. The effect of simvastatin treatment on behavioral parameters, cognitive performance, and hippocampal morphology in rats fed a standard or a high-fat diet. 2012. p. 582-92.

184. Ferris HA, Davis F, Kahn CR. Insulin action at astrocytes is important for regulation of circadian rhythm and metabolism. *Diabetes* 2016; **65**(Supplement 1): A13.

185. Citraro R, Chimirri S, Aiello R, et al. Protective effects of some statins on epileptogenesis and depressive-like behavior in WAG/Rij rats, a genetic animal model of absence epilepsy. *Epilepsia* 2014; **55(8)**: 1284-91.

186. Persons JE, Robinson JG, Coryell WH, Payne ME, Fiedorowicz JG. Longitudinal study of low serum LDL cholesterol and depressive symptom onset in postmenopause. *Journal of Clinical Psychiatry* 2016; **77**(2): 212-20.

187. Huffman JC, Smith FA, Fricchione GL, Januzzi JL, Nadelman S, Pirl WF. Depression and failure of cholesterol lowering after acute myocardial infarction. *Primary Care Companion to the Journal of Clinical Psychiatry* 2010; **12**(1).

188. Horowitz MA, Zunszain PA, Anacker C, Musaelyan K, Pariante CM. Glucocorticoids and inflammation: a double-headed sword in depression? How do neuroendocrine and inflammatory pathways interact during stress to contribute to the pathogenesis of depression? *Mod Trends Pharmacopsychiatry* 2013; **28**: 127-43.

189. Kennis M, Gerritsen L, van Dalen M, Williams A, Cuijpers P, Bockting C. Prospective biomarkers of major depressive disorder: a systematic review and meta-analysis. *Mol Psychiatry* 2020; **25**(2): 321-38.

190. Pellosmaa H, Dougall AL. A systematic review of the role statins and mood on plasma lipids in animal models. *Psychosomatic Medicine* 2018; **80**(3): A17.

191. Pellosmaa H, Orsak G, Tebbe D, et al. Managing high cholesterol: Relationships among stress, depression, adherence and inflammatory markers. *Psychosomatic Medicine* 2015; **77**(3): A136.

192. Pellosmaa H, Dougall AL. Examining the role of statins and mood on plasma lipids and health outcomes: A systematic review. *Psychosomatic Medicine* 2016; **78**(3): A94.

193. Sheets WJ, Brooks SD, Chantler P, Frisbee J. Interventions blunting systemic inflammation and oxidant stress improve depressive symptoms and vascular function in a model of metabolic syndrome. FASEB Journal: FASEB; 2016.

194. Lin P-Y, Chang AYW, Lin T-K. Simvastatin treatment exerts antidepressant-like effect in rats exposed to chronic mild stress. Elsevier; 2014. p. 174-9.

195. Kumar A, Vashist A, Kumar P, Kalonia H, Mishra J. Protective effect of HMG CoA reductase inhibitors against running wheel activity induced fatigue, anxiety like behavior, oxidative stress and mitochondrial dysfunction in mice. *Pharmacol Rep* 2012; **64**(6): 1326-36.

196. Ji H, Guo WZ, Yan ZH, Li D, Lu CL. Influence of drug treatment on glucocorticoid receptor levels in patients with coronary heart disease. *Chinese Medical Journal* 2010; **123**(13): 1685-9.

197. Menolascino M, Nelson-Hawks J. Potential mood effect of statin medications. *Journal of Alternative and Complementary Medicine* 2012; **18**(6): 635-6.

198. Pereira J, Massardo T, Saez C, et al. Endothelial dysfunction, oxidative stress and activation of Rho A/Rho kinase pathway in major depressive disorder. *Research and Practice in Thrombosis and Haemostasis* 2019; **3**(Supplement 1): 488.

199. Chrysohoou C, Kollia N, Tousoulis D. The link between depression and atherosclerosis through the pathways of inflammation and endothelium dysfunction. *Maturitas* 2018; **109**: 1-5.

200. Taylor WD, Aizenstein HJ, Alexopoulos GS. The vascular depression hypothesis: Mechanisms linking vascular disease with depression. Mol Psychiatry; 2013. p. 963-74.

201. van Agtmaal MJM, Houben A, Pouwer F, Stehouwer CDA, Schram MT. Association of Microvascular Dysfunction With Late-Life Depression: A Systematic Review and Meta-analysis. *JAMA Psychiatry* 2017; **74**(7): 729-39.

202. Massardo T, Quintana J, Pereira J, et al. Effect of Statins on Brain Perfusion in Major Depression as an Addition to Standard Therapy. SPM Analysis of Inflammation, Platelet Activation, Endothelial Dysfunction and Neurocognitive Parameters. *European Journal of Nuclear Medicine and Molecular Imaging* 2020; **47(SUPPL 1)**: S446-S7.

203. Massardo T, Quintana JC, Risco L, et al. Effect of Low-Dose Statins in Addition to Standard Therapy on Brain Perfusion and Neurocognitive Performance in Patients with Major Depressive Disorder. *Neuropsychobiology* 2022: 1-15.

204. Ishak WW, Edwards G, Herrera N, et al. Depression in Heart Failure: A Systematic Review. *Innov Clin Neurosci* 2020; **17**(4-6): 27-38.

205. Yang C-C, Jick SS, Jick H. Lipid-Lowering Drugs and the Risk of Depression and Suicidal Behavior. American Medical Association; 2003. p. 1926.

206. Downs JR, Oster G, Santanello NC. HMG CoA reductase inhibitors and quality of life. *Jama* 1993; **269**(24): 3107-8.

207. Miller AH, Haroon E, Felger JC. Therapeutic Implications of Brain-Immune Interactions: Treatment in Translation. *Neuropsychopharmacology* 2017; **42**(1): 334-59.

208. Lamers F, Milaneschi Y, Vinkers CH, Schoevers RA, Giltay EJ, Penninx BWJH. Depression profilers and immuno-metabolic dysregulation: Longitudinal results from the NESDA study. *Brain, Behavior, and Immunity* 2020; **88**: 174-83.

209. Wu H, Lv W, Pan Q, et al. Simvastatin therapy in adolescent mice attenuates HFD-induced depression-like behavior by reducing hippocampal neuroinflammation. Elsevier B.V.; 2019. p. 83-95.

210. Zhang HN, Xu H, Yu XB, et al. Effect of simvastatin on lipopolysaccharides induced depressive behaviors and neuroinflammation in mice. *Chinese Pharmacological Bulletin* 2017; **33**(3): 373-8.

211. Lim S-W, Shiue Y-L, Liao J-C, et al. Simvastatin Therapy in the Acute Stage of Traumatic Brain Injury Attenuates Brain Trauma-Induced Depression-Like Behavior in Rats by Reducing Neuroinflammation in the Hippocampus. Springer US; 2017. p. 122-32.

212. Yu X-B, Zhang H-N, Dai Y, et al. Simvastatin prevents and ameliorates depressive behaviors via neuroinflammatory regulation in mice. Elsevier; 2019. p. 939-49.

213. Menze ET, Ezzat H, Shawky S, et al. Simvastatin mitigates depressive-like behavior in ovariectomized rats: Possible role of NLRP3 inflammasome and estrogen receptors' modulation. *Int Immunopharmacol* 2021; **95**: 107582.

214. Hai-Na Z, Xu-Ben Y, Cong-Rong T, et al. Atorvastatin ameliorates depressive behaviors and neuroinflammatory in streptozotocin-induced diabetic mice. Springer; 2020. p. 695-705.

215. Ma W, Shen D, Pan J, et al. Statin function as an anti-inflammation therapy for depression in patients with coronary artery disease by downregulating interleukin-1beta. *Journal of Cardiovascular Pharmacology* 2016; **67**(2): 129-35.

216. Kang HJ, Bae KY, Kim SW, et al. Effects of interleukin-6, interleukin-18, and statin use, evaluated at acute stroke, on post-stroke depression during 1-year follow-up. *Psychoneuroendocrinology* 2016; **72**: 156-60.

217. Kim S-W, Kang H-J, Bae K-Y, et al. Interactions between pro-inflammatory cytokines and statins on depression in patients with acute coronary syndrome. Elsevier; 2018. p. 250-4.

218. Lesperance F, Frasure-Smith N, Theroux P, Irwin M. The Association Between Major Depression and Levels of Soluble Intercellular Adhesion Molecule 1, lnterleukin-6, and C-Reactive Protein in Patients With Recent Acute Coronary Syndromes. *The American Journal of Psychiatry* 2004; **161**(2): 271-7.

219. Ait Tayeb AEK, Becquemont L, El-Asmar K, et al. SOD2 genetic polymorphism (rs4880) has no impact on 6-month response to antidepressant treatment and inflammatory biomarkers in depressed patients. *Basic and Clinical Pharmacology and Toxicology* 2020; **126(3)**: 289-95.

220. Haapakoski R, Ebmeier KP, Alenius H, Kivimäki M. Innate and adaptive immunity in the development of depression: An update on current knowledge and technological advances. *Prog Neuropsychopharmacol Biol Psychiatry* 2016; **66**: 63-72.

221. Neurauter G, Wirleitner B, Laich A, Schennach H, Weiss G, Fuchs D. Atorvastatin suppresses interferon-gamma -induced neopterin formation and tryptophan degradation in human peripheral blood mononuclear cells and in monocytic cell lines. *Clin Exp Immunol* 2003; **131**(2): 264-7.

222. Wirleitner B, Rudzite V, Neurauter G, et al. Immune activation and degradation of tryptophan in coronary heart disease. *Eur J Clin Invest* 2003; **33**(7): 550-4.

223. Wirleitner B, Sperner-Unterweger B, Fuchs D. Statins to reduce risk of depression. *Journal of the American College of Cardiology* 2004; **43**(6): 1132-3.

224. van der Naald M, Wenker S, Doevendans PA, Wever KE, Chamuleau SAJ. Publication rate in preclinical research: a plea for preregistration. *BMJ Open Sci* 2020; **4**(1): e100051.

225. Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. *Nat Neurosci* 2010; **13**(10): 1161-9.

226. Parsaik AK, Singh B, Murad MH, et al. Statins use and risk of depression: a systematic review and meta-analysis. *J Affect Disord* 2014; **160**: 62-7.

227. Lindberg G, Hallas J. Cholesterol-lowering drugs and antidepressants - A study of prescription symmetry. *Pharmacoepidemiology and Drug Safety* 1998; **7**(6): 399-402.

228. Lee MC, Peng TR, Lee CH, et al. Statin use and depression risk: A systematic review and meta-analysis. *J Affect Disord* 2021; **282**: 308-15.

229. Zhang L, Bao Y, Tao S, Zhao Y, Liu M. The association between cardiovascular drugs and depression/anxiety in patients with cardiovascular disease: A meta-analysis. *Pharmacol Res* 2022; **175**: 106024.

230. Glaus J, Vandeleur CL, Lasserre AM, et al. Aspirin and statin use and the subsequent development of depression in men and women: Results from a longitudinal population-based study. *Journal of Affective Disorders* 2015; **182**: 126-31.

231. Pasco JA, Jacka FN, Williams LJ, et al. Clinical Implications of the Cytokine Hypothesis of Depression: The Association between Use of Statins and Aspirin and the Risk of Major Depression. *Psychotherapy and Psychosomatics* 2010; **79**(5): 323-5.

232. Williams LJ, Pasco JA, Mohebbi M, et al. Statin and Aspirin Use and the Risk of Mood Disorders among Men. *The international journal of neuropsychopharmacology* 2016; **19**(6).

233. Mansi I, Frei CR, Pugh MJ, Mortensen EM. Psychologic disorders and statin use: a propensity score-matched analysis. *Pharmacotherapy* 2013; **33**(6): 615-26.

234. Smeeth L, Douglas I, Hall AJ, Hubbard R, Evans S. Effect of statins on a wide range of health outcomes: a cohort study validated by comparison with randomized trials. *British Journal of Clinical Pharmacology* 2009; **67**(1): 99-109.

235. Köhler-Forsberg O, Gasse C, Petersen L, Nierenberg AA, Mors O, Østergaard SD. Statin treatment and the risk of depression. *J Affect Disord* 2019; **246**: 706-15.

236. Molero Y, Cipriani A, Larsson H, Lichtenstein P, D'Onofrio BM, Fazel S. Associations between statin use and suicidality, depression, anxiety, and seizures: a Swedish total-population cohort study. *Lancet Psychiatry* 2020; **7**(11): 982-90.

237. Kessing LV, Rytgaard HC, Gerds TA, Berk M, Ekstrøm CT, Andersen PK. New drug candidates for depression - a nationwide population-based study. *Acta Psychiatr Scand* 2019; **139**(1): 68-77.

238. Redlich C, Berk M, Williams LJ, Sundquist J, Sundquist K, Li X. Statin use and risk of depression: a Swedish national cohort study. *BMC Psychiatry* 2014; **14**: 348.

239. Dave CV, Winterstein AG, Park H, Cook RL, Hartzema AG. Comparative risk of lipophilic and hydrophilic statins on incident depression: A retrospective cohort study. *J Affect Disord* 2018; **238**: 542-6.

240. Chuang CS, Yang TY, Muo CH, Su HL, Sung FC, Kao CH. Hyperlipidemia, statin use and the risk of developing depression: a nationwide retrospective cohort study. *Gen Hosp Psychiatry* 2014; **36**(5): 497-501.

241. Wium-Andersen IK, Wium-Andersen MK, Jørgensen MB, Osler M. Anti-inflammatory treatment and risk of depression in 91,842 patients with acute coronary syndrome and 91,860 individuals without acute coronary syndrome in Denmark. *Int J Cardiol* 2017; **246**: 1-6.

242. Stafford L, Berk M. The Use of Statins After a Cardiac Intervention Is Associated With Reduced Risk of Subsequent Depression. *The Journal of Clinical Psychiatry* 2011; **72**(09): 1229-35.

243. Young-Xu Y, Chan KA, Liao JK, Ravid S, Blatt CM. Long-term statin use and psychological well-being. *Journal of the American College of Cardiology* 2003; **42**(4): 690-7.

244. Otte C, Zhao S, Whooley MA. Statin Use and Risk of Depression in Patients With Coronary Heart Disease. *The Journal of Clinical Psychiatry* 2012; **73**(05): 610-5.

245. Kang J-H, Kao L-T, Lin H-C, Tsai M-C, Chung S-D. Statin use increases the risk of depressive disorder in stroke patients: A population-based study. *Journal of the Neurological Sciences* 2015; **348**(1-2): 89-93.

246. Li Y, Guo Y, Zhou M, Ma M, Fang J, He L. Paradoxical effect of statin medication on depressive disorder in first-ever ischemic stroke patients: possible antidepressant-like effect prestroke and the opposite in continuous medication poststroke. *Int Clin Psychopharmacol* 2021; **36**(3): 147-53.

247. Asplund K, Eriksson M. Inflammation, poststroke depression and statins. *International Journal of Stroke* 2011; **6**(6): 567-8.

248. Kim JM, Stewart R, Kang HJ, et al. A prospective study of statin use and poststroke depression. *J Clin Psychopharmacol* 2014; **34**(1): 72-9.

249. Katzan I, Thompson N, Itrat A. High-sensitivity C-reactive protein and depression in patients with ischemic stroke. *Stroke* 2017; **48**(Supplement 1).

250. Wang Z, Lam B, Wong A, Xiong Y, Mok V. Predictors for post-stroke depression-three-year longitudinal study. *Cerebrovascular Diseases* 2017; **44**(Supplement 1): 47.

251. Wium-Andersen IK, Wium-Andersen MK, Jørgensen MB, Osler M. Anti-inflammatory treatment and risk for depression after first-time stroke in a cohort of 147 487 Danish patients. *J Psychiatry Neurosci* 2017; **42**(5): 320-30.

252. Khokhar BR, Simoni-Wastila L, Slejko JF, Perfetto E, Zhan M, Smith GS. Mortality and secondary injury following traumatic brain injury in older medicare stat in users. *Pharmacoepidemiology and Drug Safety* 2017; **26(Supplement 2)**: 161-2.

253. Wee H-Y, Ho C-H, Fu, et al. Increased Risk of New-Onset Depression in Patients With Traumatic Brain Injury and Hyperlipidemia: The Important Role of Statin Medications. *J Clin Psychiatry* 2016; **77**(4): 505-11.

254. Yeh J-J, Syue S-H, Lin C-L, Hsu CY, Shae Z, Kao C-H. Effects of statins on anxiety and depression in patients with asthma-chronic obstructive pulmonary disease overlap syndrome. *Journal of Affective Disorders* 2019; **253**: 277-84.

255. Huang CI, Lin LC, Tien HC, et al. Hyperlipidemia and statins use for the risk of new-onset anxiety/depression in patients with head and neck cancer: A population-based study. *PLoS ONE* 2017; **12**(3).

256. Medici CR, Gradus JL, Pedersen L, Sorensen HT, Ostergaard So D, Christiansen CF. No impact of preadmission anti-inflammatory drug use on risk of depression and anxiety after critical illness. *Critical Care Medicine* 2017; **45**(10): 1635-41.

257. Leutner M, Matzhold C, Kautzky A, et al. Major Depressive Disorder (MDD) and Antidepressant Medication Are Overrepresented in High-Dose Statin Treatment. *Front Med (Lausanne)* 2021; **8**: 608083.

258. Boumendil E, Tubert-Bitter P. Depression-induced absenteeism in relation to antihyperlipidemic treatment: A study using GAZEL cohort data. *Epidemiology* 1995; **6(3)**: 322-5.

259. Alghamdi J, Matou-Nasri S, Alghamdi F, Alghamdi S, Alfadhel M, Padmanabhan S. Risk of Neuropsychiatric Adverse Effects of Lipid-Lowering Drugs: A Mendelian Randomization Study. *Int J Neuropsychopharmacol* 2018; **21**(12): 1067-75.

260. Feng L, Yap KB, Kua EH, Ng TP. Statin use and depressive symptoms in a prospective study of community-living older persons. *Pharmacoepidemiol Drug Saf* 2010; **19**(9): 942-8.

261. Williams ED, Eastwood SV, Tillin T, Stewart R, Chaturvedi N, Hughes AD. Statin use is associated with reduced depressive symptoms in Europeans, but increased symptoms in ethnic minorities in the UK: an observational study. *British journal of clinical pharmacology* 2015; **80**(1): 172-3.

262. Agustini B, Mohebbi M, Woods RL, et al. Association Between Statin Use and Depressive Symptoms in a Large Community-Dwelling Older Population Living in Australia and the USA: A Cross-Sectional Study. *CNS Drugs* 2019; **33(7)**: 685-94.

263. Asuzu K, Aneni E, Esposito AP, et al. Statin use is associated with prevalent depression in asymptomatic octogenarians. *Circulation* 2015; **131**(SUPPL. 1).

264. Tatley M, Savage R. Psychiatric Adverse Reactions with Statins, Fibrates and Ezetimibe. *Drug Safety* 2007; **30**(3): 195-201.

265. Cham S, Koslik HJ, Golomb BA. Mood, Personality, and Behavior Changes During Treatment with Statins: A Case Series. *Drug Safety - Case Reports* 2016; **3**(1): 1-.

266. Davidson KW, Reddy S, McGrath P, Zitner D, MacKeen W. Increases in depression after cholesterol-lowering drug treatment. *Behavioral Medicine* 1996; **22**(2): 82-4.

267. Duits N, Bos FM. Depressive symptoms and cholesterol-lowering drugs. *The Lancet* 1993; **341**(8837): 114-.

268. Lechleitner M, Hoppichler F, Konwalinka G, Patsch J, Braunsteiner H. Depressive symptoms in hypercholesterolaemic patients treated with pravastatin. *The Lancet* 1992; **340**(8824): 910-.

269. Rosenson RS, Goranson NL. Lovastatin-associated sleep and mood disturbances. *The American journal of medicine* 1993; **95**(5): 548-9.

270. O'Neil A, Sanna L, Redlich C, et al. The impact of statins on psychological wellbeing: 12A systematic review and meta-analysis. *BMC Medicine* 2012; **10**: 154.

271. Köhler-Forsberg O, C NL, Hjorthøj C, Nordentoft M, Mors O, Benros ME. Efficacy of anti-inflammatory treatment on major depressive disorder or depressive symptoms: meta-analysis of clinical trials. *Acta Psychiatr Scand* 2019; **139**(5): 404-19.

272. Yatham MS, Yatham KS, Ravindran AV, Sullivan F. Do statins have an effect on depressive symptoms? A systematic review and meta-analysis. *J Affect Disord* 2019; **257**: 55-63.

273. Stewart RA, Sharples KJ, North FM, Menkes DB, Baker J, Simes J. Long-term assessment of psychological well-being in a randomized placebo-controlled trial of cholesterol reduction with pravastatin. *Archives of Internal Medicine* 2000; **160(20)**: 3144-52.

274. Hyyppä MT, Kronholm E, Virtanen A, Leino A, Jula A. Does simvastatin affect mood and steroid hormone levels in hypercholesterolemic men? A randomized double-blind trial. *Psychoneuroendocrinology* 2003; **28(2)**: 181-94.

275. Morales K, Wittink M, Datto C, et al. Simvastatin causes changes in affective processes in elderly volunteers. *Journal of the American Geriatrics Society* 2006; **54(1)**: 70-6.

276. Carlsson CM, Papcke-Benson K, Carnes M, McBride PE, Stein JH. Health-related quality of life and long-term therapy with pravastatin and tocopherol (vitamin E) in older adults. *Drugs and Aging* 2002; **19(10)**: 793-805.

277. Santanello NC, Barber BL, Applegate WB, et al. Effect of pharmacologic lipid lowering on health-related quality of life in older persons: Results from the Cholesterol Reduction in Seniors Program (CRISP) pilot study. *Journal of the American Geriatrics Society* 1997; **45(1)**: 8-14.

278. Robertson CS, McCarthy JJ, Miller ER, Levin H, McCauley SR, Swank PR. Phase II Clinical Trial of Atorvastatin in Mild Traumatic Brain Injury. *Journal of Neurotrauma* 2017; **34(7)**: 1394-401.

279. Gengo F, Cwudzinski D, Kinkel P, Block G, Stauffer L, Lines C. Effects of treatment with lovastatin and pravastatin on daytime cognitive performance. *Clinical Cardiology* 1995; **18(4)**: 209-14.

280. Harrison RWS, Ashton CH. Do cholesterol-lowering agents affect brain activity? A comparison of simvastatin, pravastatin, and placebo in healthy volunteers. *British Journal of Clinical Pharmacology* 1994; **37(3)**: 231-6.

281. Muldoon MF, Barger SD, Ryan CM, et al. Effects of lovastatin on cognitive function and psychological well-being. *American Journal of Medicine* 2000; **108(7)**: 538-46.

282. Wardle J, Armitage J, Collins R, Wallendszus K, Keech A, Lawson A. Randomised placebo controlled trial of effect on mood of lowering cholesterol concentration. *British Medical Journal* 1996; **313(7049)**: 75-8.

283. Lee MC, Peng TR, Chen BL, et al. Effects of various statins on depressive symptoms: A network meta-analysis. *J Affect Disord* 2021; **293**: 205-13.

284. Sparks DL, Sabbagh MN, Connor DJ, et al. Atorvastatin for the treatment of mild to moderate Alzheimer disease: Preliminary results. *Archives of Neurology* 2005; **62(5)**: 753-7.

285. Chan D, Binks S, Nicholas JM, et al. Effect of high-dose simvastatin on cognitive, neuropsychiatric, and health-related quality-of-life measures in secondary progressive multiple sclerosis: secondary analyses from the MS-STAT randomised, placebo-controlled trial. *Lancet Neurology* 2017; **16**(8): 591-600.

286. Sheridan DA, Bridge SH, Crossey MME, et al. Depressive symptoms in chronic hepatitis C are associated with plasma apolipoprotein E deficiency. *Metabolic brain disease* 2014; **29**(3): 625-34.

287. Visseren FLJ, Bouter PK, Van Loon BJP, Erkelens WD. Treatment of dyslipidaemia with fluvastatin in patients with type 2 diabetes mellitus: Effects on lipids, mental state and fibrinolysis. *Clinical Drug Investigation* 2001; **21(10)**: 671-8.

288. Ormiston T, Wolkowitz OM, Reus VI, Manfredi F. Behavioral implications of lowering cholesterol levels: a double-blind pilot study. *Psychosomatics* 2003; **44**(5): 412-4.

289. Krysiak R, Drosdzol-Cop A, Skrzypulec-Plinta V, Okopień B. The effect of atorvastatin on sexual function and depressive symptoms in young women with elevated cholesterol levels — a pilot study. *Endokrynologia Polska* 2018; **69**(6): 688-94.

290. Al Badarin FJ, Spertus JA, Gosch KL, Buchanan DM, Chan PS. Initiation of statin therapy after acute myocardial infarction is not associated with worsening depressive symptoms: Insights from the Prospective Registry Evaluating Outcomes After Myocardial Infarctions: Events and Recovery (PREMIER) and Translational R. *American Heart Journal* 2013; **166**(5): 879-86.

291. Hoogwegt MT, Theuns DAMJ, Kupper N, Jordaens L, Pedersen SS. Relation of Statin Therapy to Psychological Functioning in Patients With an Implantable Cardioverter Defibrillator. *The American Journal of Cardiology* 2013; **111**(8): 1169-74.

292. Agostini JV, Tinetti ME, Han L, McAvay G, Foody JM, Concato J. Effects of Statin Use on Muscle Strength, Cognition, and Depressive Symptoms in Older Adults. *Journal of the American Geriatrics Society* 2007; **55**(3): 420-5.

293. Olson MB, Kelsey SF, Matthews KA, et al. Lipid-lowering medication use and aggression scores in women: a report from the NHLBI-sponsored WISE study. *Journal of women's health (2002)* 2008; **17**(2): 187-94.

294. Mandas A, Congiu MG, Abete C, et al. Cognitive decline and depressive symptoms in late-life are associated with statin use: evidence from a population-based study of Sardinian old people living in their own home. *Neurological Research* 2014; **36**(3): 247-54.

295. Ghanizadeh A, Hedayati A. Augmentation of fluoxetine with lovastatin for treating major depressive disorder, a randomized double-blind placebo controlled-clinical trial. *Depress Anxiety* 2013; **30**(11): 1084-8.

296. Haghighi M, Khodakarami S, Jahangard L, et al. In a randomized, double-blind clinical trial, adjuvant atorvastatin improved symptoms of depression and blood lipid values in patients suffering from severe major depressive disorder. *J Psychiatr Res* 2014; **58**: 109-14.

297. Gougol A, Zareh-Mohammadi N, Raheb S, et al. Simvastatin as an adjuvant therapy to fluoxetine in patients with moderate to severe major depression: A double-blind placebo-controlled trial. *J Psychopharmacol* 2015; **29**(5): 575-81.

298. Salagre E, Fernandes BS, Dodd S, Brownstein DJ, Berk M. Statins for the treatment of depression: A meta-analysis of randomized, double-blind, placebo-controlled trials. *J Affect Disord* 2016; **200**: 235-42.

299. Bai S, Guo W, Feng Y, et al. Efficacy and safety of anti-inflammatory agents for the treatment of major depressive disorder: a systematic review and meta-analysis of randomised controlled trials. *J Neurol Neurosurg Psychiatry* 2020; **91**(1): 21-32.

300. Hang X, Li J, Li Z, et al. Comparative Efficacy and Acceptability of Anti-inflammatory Agents on Major Depressive Disorder: A Network Meta-Analysis. *Frontiers in Pharmacology* 2021; **12**.

301. Berk M, Mohebbi M, Dean OM, et al. Youth Depression Alleviation with Anti-inflammatory Agents (YoDA-A): a randomised clinical trial of rosuvastatin and aspirin. *BMC Med* 2020; **18**(1): 16.

302. Fotso Soh J, Almadani A, Beaulieu S, et al. The effect of atorvastatin on cognition and mood in bipolar disorder and unipolar depression patients: A secondary analysis of a randomized controlled trial. *Journal of Affective Disorders* 2020; **262**: 149-54.

303. Abbasi SH, Mohammadinejad P, Shahmansouri N, et al. Simvastatin versus atorvastatin for improving mild to moderate depression in post-coronary artery bypass graft patients: A double-blind, placebo-controlled, randomized trial. *J Affect Disord* 2015; **183**: 149-55.

304. Kim SW, Bae KY, Kim JM, et al. The use of statins for the treatment of depression in patients with acute coronary syndrome.: Nature Publishing Group; 2015. p. e620.

305. Köhler O, Gasse C, Petersen L, et al. The Effect of Concomitant Treatment With SSRIs and Statins: A Population-Based Study. *Am J Psychiatry* 2016; **173**(8): 807-15.

306. Dubovsky SL. What is new about new antidepressants? Psychotherapy and Psychosomatics: S. Karger AG; 2018. p. 129-39.

307. Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A. Inflammaging: a new immune-metabolic viewpoint for age-related diseases. *Nat Rev Endocrinol* 2018; **14**(10): 576-90.

308. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol* 2016; **16**(10): 626-38.

309. De Giorgi R, De Crescenzo F, Rizzo Pesci N, et al. Statins for major depressive disorder: A systematic review and meta-analysis of randomized controlled trials. *PLoS One* 2021; **16**(3): e0249409.

310. Broncel M, Gorzelak-Pabiś P, Sahebkar A, et al. Sleep changes following statin therapy: a systematic review and meta-analysis of randomized placebo-controlled polysomnographic trials. *Archives of medical science : AMS* 2015; **11**(5): 915-26.

311. Köhler-Forsberg O, Otte C, Gold SM, Østergaard SD. Statins in the treatment of depression: Hype or hope? *Pharmacol Ther* 2020; **215**: 107625.

312. Rohatgi A. WebPlotDigitizer 4.5. 2021.

313. Higgins JPT, Thomas J, Chandler J, et al. Cochrane Handbook for Systematic Reviews of Interventions version 6.2 (updated February 2021). Cochrane, 2021; 2021.

314. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol* 2014; **14**: 135.

315. Sterne JAC, Savović J, Page MJ, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ* 2019; **366**: l4898.

316. GRADEpro GDT. GRADEpro Guideline Development Tool [Software]. 2021.

317. StataCorp. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC.; 2021.

318. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Bmj* 2021; **372**: n71.

319. De Giorgi R. The wh-questions of network meta-analyses. *BJPsych Advances* 2019; **25**(6): 396-8.

320. De Giorgi R, De Crescenzo F, Cowen PJ. Effects of various statins on depressive symptoms: is there enough evidence? *J Affect Disord* 2021; **295**: 1093-4.

321. Husain MI, Chaudhry IB, Khoso AB, et al. Adjunctive simvastatin for treatment-resistant depression: study protocol of a 12-week randomised controlled trial. *BJPsych Open* 2019; **5**(1): e13.

322. Otte C, Chae WR, Nowacki J, et al. Simvastatin add-on to escitalopram in patients with comorbid obesity and major depression (SIMCODE): study protocol of a multicentre, randomised, double-blind, placebo-controlled trial. *BMJ Open* 2020; **10**(12): e040119.

323. Kennedy-Martin T, Curtis S, Faries D, Robinson S, Johnston J. A literature review on the representativeness of randomized controlled trial samples and implications for the external validity of trial results. *Trials* 2015; **16**: 495.

324. Tuccori M, Montagnani S, Mantarro S, et al. Neuropsychiatric adverse events associated with statins: epidemiology, pathophysiology, prevention and management. *CNS Drugs* 2014; **28**(3): 249-72.

325. De Crescenzo F, Garriga C, Tomlinson A, et al. Real-world effect of antidepressants for depressive disorder in primary care: protocol of a population-based cohort study. *Evid Based Ment Health* 2020; **23**(3): 122-6.

326. Hippisley-Cox J, Vingradova J, Coupland C, Pringle M. Comparison of key practice characteristics between general practices in England and Wales and general practices in the QRESEARCH database. 2005. https://www.qresearch.org/Public_Documents/Characteristics%20of%20QRESEARCH%20practices%20_database%20version%208_%20v1.0.pdf (accessed 19/01/2022 2022).

327. Coupland C, Dhiman P, Morriss R, Arthur A, Barton G, Hippisley-Cox J. Antidepressant use and risk of adverse outcomes in older people: population based cohort study. *Bmj* 2011; **343**: d4551.

328. Coupland C, Hill T, Morriss R, Moore M, Arthur A, Hippisley-Cox J. Antidepressant use and risk of adverse outcomes in people aged 20-64 years: cohort study using a primary care database. *BMC Med* 2018; **16**(1): 36.

329. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med* 2001; **16**(9): 606-13.

330. Rubin DB. Multiple Imputation after 18+ Years. *Journal of the American Statistical Association* 1996; **91**(434): 473-89.

331. Vinogradova Y, Coupland C, Brindle P, Hippisley-Cox J. Discontinuation and restarting in patients on statin treatment: prospective open cohort study using a primary care database. *Bmj* 2016; **353**: i3305.

332. Hughes RA, Heron J, Sterne JAC, Tilling K. Accounting for missing data in statistical analyses: multiple imputation is not always the answer. *Int J Epidemiol* 2019; **48**(4): 1294-304.

333. Hernán MA. Methods of Public Health Research - Strengthening Causal Inference from Observational Data. *N Engl J Med* 2021; **385**(15): 1345-8.

334. Šprah L, Dernovšek MZ, Wahlbeck K, Haaramo P. Psychiatric readmissions and their association with physical comorbidity: a systematic literature review. *BMC Psychiatry* 2017; **17**(1): 2.

335. Collins R, Reith C, Emberson J, et al. Interpretation of the evidence for the efficacy and safety of statin therapy. *Lancet* 2016; **388**(10059): 2532-61.

336. Takada M, Fujimoto M, Yamazaki K, Takamoto M, Hosomi K. Association of statin use with sleep disturbances: data mining of a spontaneous reporting database and a prescription database. *Drug Saf* 2014; **37**(6): 421-31.

337. Kułak-Bejda A, Bejda G, Lech M, Waszkiewicz N. Are Lipids Possible Markers of Suicide Behaviors? *J Clin Med* 2021; **10**(2).

338. Iadecola C, Parikh NS. Statins and Cognitive Impairment: Not a Culprit, Protective in Some? *J Am Coll Cardiol* 2019; **74**(21): 2569-71.

339. Roy S, Hyman D, Ayyala S, et al. Cognitive Function Assessment in Patients on Moderate- or High-Intensity Statin Therapy. *J Clin Med Res* 2020; **12**(4): 255-65.

340. Samaras K, Makkar SR, Crawford JD, et al. Effects of Statins on Memory, Cognition, and Brain Volume in the Elderly. *J Am Coll Cardiol* 2019; **74**(21): 2554-68.

341. Byrne P, Demasi M, Jones M, Smith SM, O'Brien KK, DuBroff R. Evaluating the Association Between Low-Density Lipoprotein Cholesterol Reduction and Relative and Absolute Effects of Statin Treatment: A Systematic Review and Meta-analysis. *JAMA Intern Med* 2022.

342. Weye N, Momen NC, Christensen MK, et al. Association of Specific Mental Disorders With Premature Mortality in the Danish Population Using Alternative Measurement Methods. *JAMA Netw Open* 2020; **3**(6): e206646.

343. Fiorillo A, Sartorius N. Mortality gap and physical comorbidity of people with severe mental disorders: the public health scandal. *Ann Gen Psychiatry* 2021; **20**(1): 52.

344. Ljung R, Köster M, Björkenstam E, Salmi P. Associations between statin use and suicidality, depression, anxiety, and seizures. *Lancet Psychiatry* 2021; **8**(2): e2.

345. Vancampfort D, Firth J, Schuch FB, et al. Sedentary behavior and physical activity levels in people with schizophrenia, bipolar disorder and major depressive disorder: a global systematic review and meta-analysis. *World Psychiatry* 2017; **16**(3): 308-15.

346. Nørgaard M, Ehrenstein V, Vandenbroucke JP. Confounding in observational studies based on large health care databases: problems and potential solutions - a primer for the clinician. *Clin Epidemiol* 2017; **9**: 185-93.

347. Curtis HJ, Walker AJ, MacKenna B, Croker R, Goldacre B. Prescription of suboptimal statin treatment regimens: a retrospective cohort study of trends and variation in English primary care. *Br J Gen Pract* 2020; **70**(697): e525-e33.

348. Phillips R, Hazell L, Sauzet O, Cornelius V. Analysis and reporting of adverse events in randomised controlled trials: a review. *BMJ Open* 2019; **9**(2): e024537.

349. Jannini TB, Lorenzo GD, Bianciardi E, et al. Off-label Uses of Selective Serotonin Reuptake Inhibitors (SSRIs). *Curr Neuropharmacol* 2022; **20**(4): 693-712.

350. Avrahamy H, Shoval G, Hoshen M, et al. Association between Adherence to SSRI Treatment and Mortality among Individuals with Metabolic Syndrome Components. *Pharmacopsychiatry* 2021; **54**(5): 232-9.

351. Lewis G, Marston L, Duffy L, et al. Maintenance or Discontinuation of Antidepressants in Primary Care. *N Engl J Med* 2021; **385**(14): 1257-67.

352. Krivoy A, Balicer RD, Feldman B, et al. Adherence to antidepressants is associated with lower mortality: a 4-year population-based cohort study. *J Clin Psychiatry* 2016; **77**(5): e566-72.

353. Dawson GR. Experimental Medicine in Psychiatry New Approaches in Schizophrenia, Depression and Cognition. *Curr Top Behav Neurosci* 2016; **28**: 475-97.

354. Godlewska BR, Harmer CJ. Cognitive neuropsychological theory of antidepressant action: a modern-day approach to depression and its treatment. Psychopharmacology: Springer; 2020.

355. Elliott R, Zahn R, Deakin JF, Anderson IM. Affective cognition and its disruption in mood disorders. *Neuropsychopharmacology* 2011; **36**(1): 153-82.

356. Roiser JP, Sahakian BJ. Hot and cold cognition in depression. *CNS Spectr* 2013; **18**(3): 139-49.

357. Roiser JP, Elliott R, Sahakian BJ. Cognitive mechanisms of treatment in depression. *Neuropsychopharmacology* 2012; **37**(1): 117-36.

358. Harmer CJ, Shelley NC, Cowen PJ, Goodwin GM. Increased positive versus negative affective perception and memory in healthy volunteers following selective serotonin and norepinephrine reuptake inhibition. *American Journal of Psychiatry* 2004; **161**(7): 1256-63.

359. Harmer CJ, O'Sullivan U, Favaron E, et al. Effect of acute antidepressant administration on negative affective bias in depressed patients. *American Journal of Psychiatry* 2009; **166**(10): 1178-84.

360. Horder J, Browning M, Di Simplicio M, Cowen PJ, Harmer CJ. Effects of 7 days of treatment with the cannabinoid type 1 receptor antagonist, rimonabant, on emotional processing. *J Psychopharmacol* 2012; **26**(1): 125-32.

361. Cooper CM, Godlewska B, Sharpley AL, Barnes E, Cowen PJ, Harmer CJ. Interferon-α induces negative biases in emotional processing in patients with hepatitis C virus infection: a preliminary study. *Psychol Med* 2018; **48**(6): 998-1007.

362. Felger JC, Treadway MT. Inflammation Effects on Motivation and Motor Activity: Role of Dopamine. *Neuropsychopharmacology* 2017; **42**(1): 216-41.

363. Paine NJ, Bosch JA, Ring C, Drayson MT, Veldhuijzen van Zanten JJ. Induced mild systemic inflammation is associated with impaired ability to improve cognitive task performance by practice. *Psychophysiology* 2015; **52**(3): 333-41.

364. Gillespie AL, Wigg C, van Assche I, Murphy SE, Harmer CJ. Associations between statin use and negative affective bias during COVID-19: an observational, longitudinal UK study investigating depression vulnerability. *Biological Psychiatry* 2022.

365. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 2007; **39**(2): 175-91.

366. First MB, Williams JBW, Karg RS, Spitzer RL. User's guide for the SCID-5-CV Structured Clinical Interview for DSM-5® disorders: Clinical version. Arlington, VA, US: American Psychiatric Publishing, Inc.; 2016.

367. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An Inventory for Measuring Depression. Arch Gen Psychiatry; 1961. p. 561-71.

368. Eysenck SBG, Eysenck HJ, Barrett P. A revised version of the psychoticism scale. Pergamon; 1985. p. 21-9.

369. Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: The PANAS scales.: American Psychological Association (APA); 1988. p. 1063-70.

370. Snaith RP, Hamilton M, Morley S, Humayan A, Hargreaves D, Trigwell P. A scale for the assessment of hedonic tone. The Snaith-Hamilton Pleasure Scale. Royal College of Psychiatrists; 1995. p. 99-103.

371. Spielberger C, RL G, RD L. STAI manual. *Consulting Psychologists Press* 1970.

372. Bond A, Lader M. The use of analogue scales in rating subjective feelings. John Wiley & Sons, Ltd; 1974. p. 211-8.

373. Macin SM, Perna ER, Farías EF, et al. Atorvastatin has an important acute anti-inflammatory effect in patients with acute coronary syndrome: Results of a randomized, double-blind, placebo-controlled study. *American Heart Journal* 2005; **149**(3): 451-7.

374. Lundqvist E, Flykt A, Öhman A. The Karolinska Directed Emotional Faces *KDEF, CD ROM from Department of Clinical Neuroscience, Psychology section, Karolinska Institutet* 1998.

375. Young AW, Rowland D, Calder AJ, Etcoff NL, Seth A, Perrett DI. Facial expression megamix: Tests of dimensional and category accounts of emotion recognition. Elsevier; 1997. p. 271-313.

376. Grier JB. Nonparametric indexes for sensitivity and bias: Computing formulas. *Psychological Bulletin* 1971; **75**(6): 424-9.

377. Anderson NH. LIKABLENESS RATINGS OF 555 PERSONALITY-TRAIT WORDS. J Pers Soc Psychol; 1968. p. 272-9.

378. Pessiglione M, Seymour B, Flandin G, Dolan RJ, Frith CD. Dopamine-dependent prediction errors underpin reward-seeking behaviour in humans. *Nature* 2006; **442**(7106): 1042-5.

379. Rey A. L'examen clinique en psychologic [The clinical examination in psychology]. 1964.

380. IBM. SPSS Statistics 27 Documentation. 2020.

381. Harmer CJ, Bhagwagar Z, Perrett DI, Völlm BA, Cowen PJ, Goodwin GM. Acute SSRI administration affects the processing of social cues in healthy volunteers. *Neuropsychopharmacology* 2003; **28**(1): 148-52.

382. Browning M, Reid C, Cowen PJ, Goodwin GM, Harmer CJ. A single dose of citalopram increases fear recognition in healthy subjects. *Journal of Psychopharmacology* 2007; **21**(7): 684-90.

383. Mogg K, Bradley BP. Selective orienting of attention to masked threat faces in social anxiety. *Behaviour Research and Therapy* 2002; **40**(12): 1403-14.

384. Zangara A, Blair R, Curran VH. A comparison of the effects of a β-adrenergic blocker and a benzodiazepine upon the recognition of human facial expressions. *Psychopharmacology* 2002; **163**(1): 36-41.

385. Harmer CJ, Cowen PJ. 'It's the way that you look at it'-a cognitive neuropsychological account of SSRI action in depression. *Philosophical Transactions of the Royal Society B: Biological Sciences* 2013; **368**(1615).

386. Harmer CJ, Cowen PJ, Goodwin GM. Efficacy markers in depression. Journal of Psychopharmacology; 2011. p. 1148-58.

387. Burghardt NS, Sullivan GM, McEwen BS, Gorman JM, Ledoux JE. The selective serotonin reuptake inhibitor citalopram increases fear after acute treatment but reduces fear with chronic treatment: A comparison with tianeptine. *Biological Psychiatry* 2004; **55**(12): 1171-8.

388. Deakin JFW, Graeff FG. 5-HT and mechanisms of defence. *Journal of Psychopharmacology* 1991; **5**(4): 305-15.

389. Arnone D, Horder J, Cowen PJ, Harmer CJ. Early effects of mirtazapine on emotional processing. *Psychopharmacology* 2009; **203**(4): 685-91.

390. Chan SY, Capitão L, Probert F, et al. A single administration of the antibiotic, minocycline, reduces fear processing and improves implicit learning in healthy volunteers: analysis of the serum metabolome. *Translational Psychiatry* 2020; **10**(1): 148-.

391. Walsh AEL, Huneke NTM, Brown R, Browning M, Cowen P, Harmer CJ. A dissociation of the acute effects of bupropion on positive emotional processing and reward processing in healthy volunteers. *Frontiers in Psychiatry* 2018; **9**(OCT): 482-.

392. Schultz BG, Patten DK, Berlau DJ. The role of statins in both cognitive impairment and protection against dementia: a tale of two mechanisms. *Transl Neurodegener* 2018; **7**: 5.

393. Harmer CJ, Heinzen J, O'Sullivan U, Ayres RA, Cowen PJ. Dissociable effects of acute antidepressant drug administration on subjective and emotional processing measures in healthy volunteers. *Psychopharmacology (Berl)* 2008; **199**(4): 495-502.

394. Pringle A, McCabe C, Cowen PJ, Harmer CJ. Antidepressant treatment and emotional processing: can we dissociate the roles of serotonin and noradrenaline? *J Psychopharmacol* 2013; **27**(8): 719-31.

395. Bullmore E. Inflamed depression. *The Lancet* 2018; **392**(10154): 1189-90.

396. Cotter J, Barnett JH. Using Affective Cognition to Enhance Precision Psychiatry. *Front Psychiatry* 2018; **9**: 288.

397. Fernandes BS, Borgwardt S, Carvalho AF, Steiner J. Editorial: Back to the Future: On the Road Towards Precision Psychiatry. *Front Psychiatry* 2020; **11**: 112.

398. Ferrucci L, Fabbri E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. *Nat Rev Cardiol* 2018; **15**(9): 505-22.

399. Capuron L, Lasselin J, Castanon N. Role of Adiposity-Driven Inflammation in Depressive Morbidity. *Neuropsychopharmacology* 2017; **42**(1): 115-28.

400. Monroe SM, Slavich GM, Gotlib IH. Life stress and family history for depression: the moderating role of past depressive episodes. *J Psychiatr Res* 2014; **49**: 90-5.

401. Rainville JR, Tsyglakova M, Hodes GE. Deciphering sex differences in the immune system and depression. *Front Neuroendocrinol* 2018; **50**: 67-90.

402. Köhler-Forsberg O, Buttenschøn HN, Tansey KE, et al. Association between C-reactive protein (CRP) with depression symptom severity and specific depressive symptoms in major depression. *Brain Behav Immun* 2017; **62**: 344-50.

403. Maniglio R, Gusciglio F, Lofrese V, Belvederi Murri M, Tamburello A, Innamorati M. Biased processing of neutral facial expressions is associated with depressive symptoms and suicide ideation in individuals at risk for major depression due to affective temperaments. *Compr Psychiatry* 2014; **55**(3): 518-25.

404. Hayward G, Goodwin GM, Cowen PJ, Harmer CJ. Low-dose tryptophan depletion in recovered depressed patients induces changes in cognitive processing without depressive symptoms. *Biol Psychiatry* 2005; **57**(5): 517-24.

405. Di Simplicio M, Norbury R, Harmer CJ. Short-term antidepressant administration reduces negative self-referential processing in the medial prefrontal cortex in subjects at risk for depression. *Mol Psychiatry* 2012; **17**(5): 503-10.

406. Nandam LS, Brazel M, Zhou M, Jhaveri DJ. Cortisol and Major Depressive Disorder-Translating Findings From Humans to Animal Models and Back. *Front Psychiatry* 2019; **10**: 974.

407. Le Masurier M, Cowen PJ, Harmer CJ. Emotional bias and waking salivary cortisol in relatives of patients with major depression. *Psychol Med* 2007; **37**(3): 403-10.

408. Blanco C, Wall MM, Olfson M. Psychological Aspects of the COVID-19 Pandemic. *J Gen Intern Med* 2020; **35**(9): 2757-9.

409. Pourriyahi H, Yazdanpanah N, Saghazadeh A, Rezaei N. Loneliness: An Immunometabolic Syndrome. *Int J Environ Res Public Health* 2021; **18**(22).

410. Russell DW. UCLA Loneliness Scale (Version 3): reliability, validity, and factor structure. *J Pers Assess* 1996; **66**(1): 20-40.

411. Radloff LS. The CES-D Scale: A Self-Report Depression Scale for Research in the General Population. *Applied Psychological Measurement* 1977; **1**(3): 385-401.

412. Strober LB, Binder A, Nikelshpur OM, Chiaravalloti N, DeLuca J. The Perceived Deficits Questionnaire: Perception, Deficit, or Distress? *Int J MS Care* 2016; **18**(4): 183-90.

413. Redick TS, Lindsey DR. Complex span and n-back measures of working memory: a meta-analysis. *Psychon Bull Rev* 2013; **20**(6): 1102-13.

414. Clark LT, Watkins L, Piña IL, et al. Increasing Diversity in Clinical Trials: Overcoming Critical Barriers. *Curr Probl Cardiol* 2019; **44**(5): 148-72.

415. McDermott MM, Newman AB. Remote Research and Clinical Trial Integrity During and After the Coronavirus Pandemic. *Jama* 2021; **325**(19): 1935-6.

416. Thomas JM, Higgs S, Dourish CT. Test-retest reliability and effects of repeated testing and satiety on performance of an Emotional Test Battery. *J Clin Exp Neuropsychol* 2016; **38**(4): 416-33.

417. Chevance A, Ravaud P, Cornelius V, Mayo-Wilson E, Furukawa TA. Designing clinically useful psychopharmacological trials: challenges and ways forward. *Lancet Psychiatry* 2022.

418. Raison CL, Rutherford RE, Woolwine BJ, et al. A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression: the role of baseline inflammatory biomarkers. *JAMA Psychiatry* 2013; **70**(1): 31-41.

419. Salvadore G, Nash A, Bleys C, al. e. A double-blind, placebo-controlled, multicenter study of sirukumab as adjunctive treatment to a monoaminergic antidepressant in adults with major depressive disorder. American College of Neuropsychopharmacology Annual Meeting. Hollywood, FL, USA; 2018.

420. Harrison H, Griffin SJ, Kuhn I, Usher-Smith JA. Software tools to support title and abstract screening for systematic reviews in healthcare: an evaluation. *BMC Med Res Methodol*. 2020 Jan 13;20(1):7. doi: 10.1186/s12874-020-0897-3

421. Clougherty JE, Kinnee EJ, Cardet JC, Mauger D, Bacharier L, Beigelman A, Blake KV, Cabana MD, Castro M, Chmiel JF, Covar R, Fitzpatrick A, Gaffin JM, Gentile D, Israel E, Jackson DJ, Kraft M, Krishnan JA, Kumar HV, Lang JE, Lazarus SC, Lemanske RF Jr, Lima J, Martinez FD, Morgan W, Moy J, Myers R, Naureckas ET, Ortega VE, Peters SP, Phipatanakul W, Pongracic JA, Ross K, Sheehan WJ, Smith LJ, Solway J, Sorkness CA, Wechsler ME, Wenzel S, White SR, Holguin F. Geography, generalisability, and susceptibility in clinical trials. *Lancet Respir Med*. 2021 Apr;9(4):330-332. doi: 10.1016/S2213-2600(21)00046-1.

422. Luciano M, Sampogna G, Amore M, Andriola I, Calcagno P, Carmassi C, Del Vecchio V, Dell'Osso L, Di Lorenzo G, Gelao B, Giallonardo V, Rossi A, Rossi R, Siracusano A, Fiorillo A; LIFESTYLE Working Group. How to improve the physical health of people with severe mental illness? A multicentric randomized controlled trial on the efficacy of a lifestyle group intervention. *Eur Psychiatry*. 2021 Nov 23;64(1):e72. doi: 10.1192/j.eurpsy.2021.2253.

423. Fiorillo A, Luciano M, Pompili M, Sartorius N. Editorial: Reducing the Mortality Gap in People With Severe Mental Disorders: The Role of Lifestyle Psychosocial Interventions. *Front Psychiatry*. 2019 Jun 20;10:434. doi: 10.3389/fpsyt.2019.00434.

424. Dell'Osso B, Albert U, Carrà G, Pompili M, Nanni MG, Pasquini M, Poloni N, Raballo A, Sambataro F, Serafini G, Viganò C, Demyttenaere K, McIntyre RS, Fiorillo A. How to improve adherence to antidepressant treatments in patients with major depression: a psychoeducational consensus checklist. *Ann Gen Psychiatry*. 2020 Oct 12;19:61. doi: 10.1186/s12991-020-00306-2.

425. Fiorillo A, Maj M. The role of psychiatry in modern medicine. *Int Rev Psychiatry*. 2018 Apr;30(2):169-175. doi: 10.1080/09540261.2018.1436675.

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Appendix