Vascular mechanisms in late-life depressive disorder

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Abstract:

There is growing evidence to suggest that vascular disease plays an important role in late life depressive disorder. The aim of this study was to characterize vascular impairment in late life depression. Assessment of endothelial function, arterial stiffness, and atherosclerosis in a variety of vessel beds was carried out in 25 subjects with late life depression and 21 nondepressed control subjects. All study subjects underwent wave velocity, pulse wave analysis, carotid intima media thickness analysis, and magnetic resonance imaging of the brain and a subset gluteal fat biopsy and direct assessment of small artery endothelial function.

There were no baseline differences in demographics or a range of vascular risk factors between the groups. There was a generalised vascular dysfunction in depressed subjects with significantly more atherosclerosis, poorer endothelial function and increased arterial stiffness. On neuroimaging, depressed subjects had significantly more dilated Virchow–Robin spaces in the basal ganglia. White matter lesion volumes in all regions were higher in depressed subjects but not significantly so. Furthermore, subjects with late onset depression (onset >60years) had greater vascular impairment when compared to those with early onset illness. Lastly, depressed subjects who did not respond to antidepressant monotherapy showed more vascular dysfunction compared to responders.

The study has a number of limitations including the small sample size and as the study was cross sectional, the observed relationship between vascular dysfunction and depression is associative rather than causal. Further research in larger samples is required to address the methodological limitations of this study. If the study results are confirmed, the use of vasoprotective drugs to improve vascular function or retard atherosclerosis as disease modifying agents in late life depression would be a rational development.
Declaration:

Chapter 4 in the thesis has been submitted to the University of Manchester as part of PhD of Dr. Adam Greenstein and it has also been published as a paper in the journal Hypertension. However both Dr. Greenstein and I made equal contributions to the published paper as reflected in the joint first authorship.

As the thesis is presented in alternative format as a series of papers, it is necessary for me to acknowledge the contribution of my collaborators over the whole research project. Gluteal biopsy and small artery analysis was done by Dr. Adam Greenstein. Dr. Marietta Scott conducted the analysis of the white matter hyperintensity volumes. Prof. Alan Jackson performed the semiquantitative magnetic resonance imaging evaluations. Carotid ultrasound scans were performed by vascular technologists. Prof. Baldwin along with Prof. Anthony Heagerty, Prof. Alistair Burns and Prof. Rayaz Malik developed the initial study hypotheses.

Some study data

Contribution:

This is to confirm that I was primarily responsible for the development and writing of this thesis. I was actively involved in the design and development of the study protocol and ethical submission process. I recruited and consented all of the study participants. I coordinated the study and supported the study participants during all the study assessments. I administered all the psychiatric and physical health rating scales. I performed pulse wave analysis and pulse wave velocity measures in all subjects. I collated all the data and performed all the statistical analysis with advice from Barbara Tomenson. I wrote the initial manuscript of all the four papers that have been published or submitted for publication from the study.
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Acknowledgements:

I am greatly indebted to Prof. Bob Baldwin for his exceptional advice and encouragement throughout the research project. I have been very lucky to find such a kind and supportive supervisor. He was generous with his time and patiently guided me throughout the project. I also wish to thank Prof. Burns my co supervisor for providing me with expert guidance, mentorship and direction throughout the project.

I thoroughly enjoyed working with my collaborator Dr. Adam Greenstein and am thankful to his indispensable contribution. I also thank Prof. Rayaz Malik, Prof. Anthony Heagerty, Prof. Kennedy Cruickshank from the department of Medicine; Prof. Alan Jackson, Dr. Marietta Scott, from the department of Radiology for their contributions. I am grateful to Barbara Tomenson for giving me outstanding statistical advice. I would like to express my sincere gratitude to the staff at Wellcome Trust Research Facility for their help and assistance with the conduct of the study. I would like to express my sincere gratitude to all the study participants who gave their time willingly and without renumeration. This study was supported by a grant from the Translational Imaging Unit of the University of Manchester and by the Wellcome Clinical Research Facility at Manchester Royal Infirmary.

On a personal note, I am grateful to my brothers Karthik and Sridar for their unwavering support through the years. Finally, I would like to thank my wife, Deepa, for her patience, encouragement, support and for bringing so much happiness into my life.

Dedication:

This thesis is dedicated to my parents, Paranthaman & Jayalakshmi.
About the Author:

I graduated from The Tamilnadu Dr MGR Medical University, India with a MBBS degree in 2000. I completed my basic psychiatric training in Liverpool and Oxford and gained my Membership of Royal College of Psychiatrists in 2003. I did my higher training in general adult and old age Psychiatry in Manchester. I gained my Diploma in Geriatric Medicine from Royal College of Physicians of London in 2006. I have been working as a consultant Old Age Psychiatrist in Bolton, Greater Manchester since 2008.
Chapter 1: Introduction

Depression is the most common psychiatric syndrome of later life. Depression is not a normal part of the ageing process. Depression already ranks alongside the most disabling medical conditions including heart disease in later life (Baldwin et al., 2002a). A number of misconceptions such as depression in late life is understandable or realistic leads to under-diagnosis and as a result is frequently overlooked by non-specialist medical staff (Clarke et al., 1995). In the community probably fewer than 20% of cases are detected or treated (Cole et al., 2003). Usually depression begins in younger adult life and recurs periodically but it can arise for the first time in later life. Recently, late life depression has gained increased attention in research due to the ageing population and the proposed links between depression and vascular disease.

1.1.1 Epidemiology:

Life expectancy is increasing worldwide. The percentage of older adults (aged 65 and above) is reported to rise steadily to as much as 30% of the population in some societies (Baldwin et al., 2002a). The reported prevalence rates of late life depression varies enormously (0.9- 42%) with rates higher in older people living in institutional settings than those living in the community (Djernes, 2006). Studies have found a weighted average prevalence of 1.8% for major depression, 9.8% for minor depression and 13.5% for all clinically relevant depressive syndromes and the rates are consistent across the world (Beekman et al., 1999). There is limited data on the incidence of late life depression. Based on the Baltimore Epidemiologic catchment study, Eaton et al. (1989,1997) have reported an estimated incidence 1.25 per 100 persons for major depression among adults age 65 years or older and 1.5 per 1000 per year for late-onset depression relative to early-onset depression. Women are more commonly affected than men. As many as three quarters of patients who present to old age Psychiatrists may have late onset
form of illness (Baldwin et al., 2002a). The natural course of depression is one to two years if left untreated.

1.1.2 Clinical Features:

Both ICD-10 (World Health Organization 1992) and DSM-IV (American Psychiatric Association 1994) stipulate that that either depressed mood or loss of interest or pleasure must be present to make a diagnosis of depression. Other common symptoms are weight loss, change in appetite, disturbed sleep, reduced concentration or attention. Neither classification has separate criteria for diagnosing late onset depression. The clinical presentation of depression in later life is very similar to early life. However, it is widely believed that there is a distinction between early onset and late onset depression but there is no consensus on what the cut off age should be (typically between 50 and 65 years).

In contrast to depression earlier in life, the presentation and treatment of late life depression is coloured by co-morbidity with medical illness and the presence of cognitive impairment. Low mood may be less prominent and patients may complain of non specific symptoms such as insomnia, fatigue and anorexia. Compared to younger adults, certain symptoms of depressive disorder become more frequent with advancing years. These include a preponderance of anxiety, agitation, somatic complaints (Baldwin et al., 1995), insomnia (Husain et al., 2005), hypochondriasis (Gurland, 1976) and a greater likelihood of psychotic symptoms (Brodaty et al., 1997). Some symptoms may reduce in frequency or intensity. In a recent study of individuals between 18 and 75 years of age, older depressed adults reported less irritability and hypersomnia and were less likely to hold negative views of themselves or of their future (Husain et al., 2005). Brodaty et al., (2005) examined the effect of age at onset on depression phenomenology in a sample of 810 patients referred to a specialist mood disorders unit in Australia. Some clinical
types, such as melancholia and psychosis, and certain clinical features, such as psychomotor agitation, retardation, non-interactiveness, hypochondriasis, and severe guilt were more common in older patients and were associated more with age than with age at onset, although this effect was more pronounced in females.

Prince et al., (1999) used the ‘EURO-D’ scale in 14 countries to compare symptoms of depression and identified two factors under which the majority of depressive symptoms clustered. One they called ‘affective suffering’, characterised by depression, tearfulness and a wish to die and the second was a ‘motivation’ factor, comprising loss of interest, poor concentration and lack of enjoyment. It was the motivation factor which tended to increase with age rather than affective suffering, which remained constant across age-groups. The motivational factor shows some similarity to the symptom profile of vascular depression, discussed later. Others have, however noted few differences in symptom profiles (Blazer, 2003; Musetti et al., 1989) and it is possible that study bias (for example studying only inpatients or patients from tertiary referral centres) favouring those with more severe illness may account for some of this. However detection of depression in older people remains complicated because of the presence of co morbid medical illness, multiple medications, overlap of some symptoms of depression with normal aging changes and cognitive impairment.

Van der Berg et al., (2001) compared the differences in aetiological and vulnerability factors between early and late onset depression in a study of 132 depressed older persons by studying a number of factors such as neuroticism, physical health, vascular risk factors and life stress. They found that early onset depression was associated with neuroticism and late onset subjects fell into two categories, those without life stress had higher vascular risk factors compared to subjects whose depression was preceded by a life stressor. Based on this, they concluded that late life
depression can be broadly divided into the following subgroups with different etiological pathways: (1) early-onset with longstanding psychobiological vulnerability; (2) late-onset as reaction to severe life stress; and (3) late-onset with vascular risk factors.

1.1.3 Prognosis:

There is good evidence for the efficacy of both pharmacological and psychological treatments in late-life depression (Baldwin, 2003). Antidepressants are as effective in late life as in other age groups although remission may take longer to achieve. Although short term response to treatment is good, long term prognosis of late life depression remains poor when compared to depression in adult life with high relapse rates (Mitchell et al., 2005). A systematic review of prognostic studies in late life depression found that two years after study enrolment, 33% were well, 33% were depressed, and 21% had died and prognostic factors included age, social support, life events, physical illness, cognitive impairment and severity of index episode (Cole et al., 1999) (Table 1).

Chronicity affects at least a third of patients and this can lead to wide range of negative outcomes. Depression is associated increased health care utilisation, serious morbidity and mortality (Baldwin et al., 2002a; Unutzer., 1997), impaired psychosocial functioning and is the leading cause of suicide among older people. Moreover the risk of subsequent disability is increased leading to decline in quality of life (Bruce et al., 1994). There are also considerable effects on the carers with increased caregiver dissatisfaction and distress (Soldato et al., 2008). Early recognition, diagnosis, and treatment can minimize suffering, prevent premature death and improve overall functioning (Lebowitz et al., 1997). When depression is adequately treated, quality of life improves (Shmuely et al., 2001).
Table 1: Prognosis of depression in older people (Cole et al.63,)

<table>
<thead>
<tr>
<th>Prognosis Category</th>
<th>Number of Studies</th>
<th>Percent of Subjects in Category</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well</td>
<td>12</td>
<td>6-46</td>
<td>33.1</td>
</tr>
<tr>
<td>Depressed</td>
<td>12</td>
<td>17-47</td>
<td>32.7</td>
</tr>
<tr>
<td>Dead</td>
<td>9</td>
<td>8-38</td>
<td>20.6</td>
</tr>
<tr>
<td>Other</td>
<td>7</td>
<td>4-20</td>
<td>11.8</td>
</tr>
</tbody>
</table>

1.2 Risk factors for late life depression:

Clearly, late life depression is a heterogeneous entity with different aetiologies. A wide range of risk factors have been identified for late life depression including lower income, cognitive impairment, medical illness and social support (Heun et al., 2005). Cole et al. (2003) noted that bereavement, sleep disturbance, disability, previous depression, and female gender were significant risk factors for depression in older people in the community. Older individuals with late-onset major depression generally, when compared with those with early-onset major depression, have a less frequent family history of mood disorders, greater impairment in neuropsychological tests, more neurosensory hearing impairment and more white-matter hyperintensities on brain imaging (Alexopoulos, 2005).

Psychosocial and social risk factors may also play a role but those who survive into later life may actually be characterized by psychological resilience (Hickie & Scott, 1998). Therefore, research into late life depression over the last few years has placed particular emphasis on biological factors especially vascular risk factors. Determining risk factors accurately is important for precise treatment and prevention.

1.2.1 Medical illness and depression:

There is substantial evidence demonstrating that depression is co-morbid with many severe and chronic medical conditions such as Parkinson’s disease, cancer, epilepsy, arthritis and chronic obstructive pulmonary disease (Bisschop et al., 2004; Evans et al., 2005; Yohannes et al., 2003).
Even though depression can be considered as an understandable reaction to disabling medical conditions, recent research suggests that depression may play a causal role in illnesses such as stroke and ischaemic heart disease.

1.2.2 Cardiovascular disease and depression:

There is compelling evidence to show that depression is an independent risk factor for cardiovascular disease and it affects the course and severity of heart disease. The two conditions in later life may well be synergistic (Glassman et al., 1998). It has been shown in many studies that depression is a risk factor for coronary heart disease (Anda et al., 1993; Pratt et al., 1996; Barefoot et al., 1996). Specifically in older people, depressive symptoms have been shown to be an independent risk factor for coronary heart disease in a 6 year prospective study of 5888 subjects living in the community (Ariyo et al., 2000). (Figure 1). It also predicts morbidity and mortality in patients with existing cardiac disease (Barefoot et al., 1996; Herrmann et al., 2000). The risk of cardiac death in the 6 months after an acute MI is approximately four times greater in patients with depression compared to controls (Frasure-Smith et al., 1993). Conversely, prevalence of depression is increased in coronary heart disease (Rudisch & Nemeroff, 2003) and this has been shown in older patients as well (Romanelli et al., 2002). The prevalence of major depression is also increased in patients with heart failure (Freedland et al., 2003).
A number of mechanisms have been proposed for the association between cardiovascular disease and depression but they all appear to be indirect. These include changes in autonomic nervous system function associated with depression, such as ventricular tachycardia, decreased heart rate variability and elevated levels of proinflammatory cytokines which are causal factors in development and progression of atherosclerosis (Evans et al., 2005).

1.2.3 Vascular risk factors and depression:

A number of classic vascular risk factors have been linked with depression. Depression is three times more likely in those with hypertension than those without (Rabkin et al., 1983), but discrepant studies also exist showing no such association (Jones-Webb et al., 1996). The converse is also true with hypertension being more likely in older depressed patients (Adamis et al., 2000). Hypotension has also been shown to associated with depression (Paterniti et al., 2000) and being a causative factor of brain white matter lesions (WML) in older people (Thomas et al., 2000). Hypotension may be due to WML lesions affecting the brain stem leading to autonomic failure (Ballard et al., 2000) or is more likely to be part of a systemic disease such
as diabetes or a degenerative process such as Parkinson’s disease or perhaps due to some drugs prescribed in older people such as levodopa and improper use of antihypertensives.

A literature review to determine the prevalence of depression in diabetes found that it is at least three times more prevalent relative to the general population (Gavard et al., 1993). Moreover, depression is associated with hyperglycaemia in patients with established type 1 or type 2 diabetes (Lustman et al., 2000). Conversely, Eaton et al., (1996) using data from the epidemiological catchment area study revealed that depression may be a risk factor for developing diabetes and this has been replicated in other studies (Kawakami et al., 1999). Depressive symptoms are also more common in patients with peripheral arterial disease compared to controls (Arseven et al., 2001). Surprisingly, low rather than high serum total cholesterol has been shown to be associated with depression in some studies (Partonen et al., 1999, Horsten et al., 1997) but not in all (Blazer et al., 2002). Other vascular risk factors such as smoking and high Body Mass index (BMI) have also been linked with depression (Almeida et al., 2005; Yang et al., 2007) as well as predicting a poorer prognosis (Baldwin et al., 2006).

1.2.4 Cerebrovascular disease and depression:

The association between cerebrovascular disease and depression has been known for more than a century. The German Psychiatrist, Gaupp (1905) put forward a concept of arteriosclerotic depression to refer to patients in whom the depression was attributed to hardening of the arteries (quoted by Amore et al., 2007). Felix Post (1962) suggested that the ‘subtle cerebral changes may make ageing persons increasingly liable to affective disturbance …’ (Post, 1962).

Although this link has been known for a long time, it has been only possible in the last two decades to study the subtle brain changes in depression systematically due to advances in brain imaging techniques such as computer tomography (CT) scan and Magnetic Resonance (MR)
scans. Sub clinical cerebrovascular disease is generally accepted to be visualised as hyperintensities in MR scans, though some disagreement exists regarding their aetiology. The hyperintensities appear as areas of increased signal intensity bright regions and are best visualised in T2 weighted and fluid attenuated inversion recovery (FLAIR) images. The hyperintensities are seen either in the sub cortical white matter or deep gray matter. Sub-cortical hyperintensities can be either peri-ventricular hyperintensities (PVH), surrounding the ventricular system, or Deep white matter hyperintensities (DWMH’s). Collectively, they are often referred to as White matter lesions (WML) but this overall designation may obscure important differences in causation (Thomas et al, 2002b).

Several lines of evidence support a link between cerebrovascular disease and depression. First, depression is unquestionably associated with acute stroke and affects 20–50% of patients within a year after stroke (Paranthaman & Baldwin, 2006). Depression appears to be specific complication of stroke and is significantly more likely after stroke than in other illnesses with comparable disability (Folstein et al, 1977). There is some suggestion that depression is more common after left hemispheric lesions (Robinson et al., 1984, Starkstein et al., 1987) but negative studies also exist (Schwartz et al., 1993; Agrell & Dehlin, 1994). Silent cerebral infarction has been reported in a majority of older patients with major depression with the rates higher in late onset compared to early onset (Fujikawa et al., 1993). The corollary seems to be true as well, with depression being a risk factor for stroke that appears to be independent of traditional cardiovascular risk factors. In a prospective epidemiological study, subjects with a history of depressive disorder were 2.6 times more likely to report stroke than those without (Larson et al., 2001).
Second, subtle cerebrovascular disease in the form of WML have also been reported to be more frequent in late life depression in several studies. WML are more common in older depressed patients compared to healthy controls (O’Brien et al., 1996, Dahabra et al., 1998; de Groot et al., 2000; Taylor et al., 2003a) and in late onset compared to early onset cases (Coffey et al., 1989; Figiel et al., 1991a; Salloway et al., 1996; Murata et al., 2001) but this has not been confirmed in all studies (Zebenko et al., 1990; Rabins et al., 1991; Greenwald et al., 1996). It would also appear that WML severity, volume and location (frontal deep white matter and basal ganglia) are also important in late life depression (de groot et al 2000; Tupler et al., 2002; Greenwald et al., 1998).

The increased frequency of WML in late life depression has also been shown in large population studies. The cardiovascular health study (n=3660) found that basal ganglia lesions were predictive of depressive symptoms even after controlling for a range of demographic and medical variables (Steffens et al., 1999). Follow up data of the above study demonstrated that increase in depression over time was related to white matter and basal ganglia lesions but only the latter remained significant after controlling for confounders (Steffens et al., 2002).

In the Rotterdam scan study (n=1077), individuals with severe white matter lesions were 3 to 5 times more likely to have depressive symptoms compared to those with mild or no WML. Also, persons with a history of late-onset depression were more likely to have severe sub cortical WML (de Groot et al., 2000). The recent pan European multicentre LADIS study (n=629) partly replicated the results of the Rotterdam study by showing that WML severity was linked to depressive symptoms (Firbank et al., 2005). Furthermore, a recent meta analysis has shown that WML are more severe and frequent in late-onset depression compared to healthy controls and
early-onset cases (Herrmann et al., 2008). Table 2 illustrates the numbers involved in the analysis by grouping.

<table>
<thead>
<tr>
<th>Presence and absence of WMH (grouped as absent or minimal vs moderate or large)</th>
<th>Severity of WMH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio (95% CI)</td>
</tr>
<tr>
<td>Periventricular WM</td>
<td></td>
</tr>
<tr>
<td>LLD/controls</td>
<td>2.15 (1.5–3.1)**</td>
</tr>
<tr>
<td>LOD/controls</td>
<td>2.57 (1.6–4.2)**</td>
</tr>
<tr>
<td>LOD/EOD</td>
<td>4.51 (2.2–9.2)**</td>
</tr>
<tr>
<td>Deep WM</td>
<td></td>
</tr>
<tr>
<td>LLD/controls</td>
<td>1.92 (1.2–3.0)**</td>
</tr>
<tr>
<td>LOD/controls</td>
<td>2.64 (1.3–5.5)*</td>
</tr>
<tr>
<td>LOD/EOD</td>
<td>4.33 (2.7–6.9)**</td>
</tr>
<tr>
<td>Combined WM</td>
<td></td>
</tr>
<tr>
<td>LLD/controls</td>
<td>2.64 (1.3–5.5)*</td>
</tr>
<tr>
<td>LOD/controls</td>
<td>4.33 (2.7–6.9)**</td>
</tr>
</tbody>
</table>

\*p<0.05; \**p<0.01; \***p<0.001.

Table 2: WML in late life depression (Herrmann et al., 2008).

Third, a higher prevalence of depression in vascular dementia compared to Alzheimer’s disease has been reported in several studies (Newman et al., 1999; Ballard et al., 2000).

Fourth, some studies have shown that WML are associated with cardiovascular risk factors. Breteler et al., (1994) found that the prevalence of white matter lesions was independently associated with history of stroke or myocardial infarction, factor VIIc activity (a thrombogenic factor) and fibrinogen level in an age and gender-stratified random sample drawn from the general population in the Rotterdam study. Furthermore, significant relations with blood pressure level, hypertension, and plasma cholesterol with WML were present for subjects aged 65 to 74 years. Liao et al., (1996) found that WML were related to duration of hypertension, its treatment and control in a community study of 1920 participants. In another longitudinal community study,
carotid plaques at baseline were significantly associated with severe WMLs four years later and this effect remained even after controlling for age and hypertension (Pico et al., 2002).

Finally, the vascular basis of WML has been shown in neuropathological studies. Thomas et al., (2002a) investigated the neuropathological basis of WML in 20 older depressed subjects and 20 older controls and found that all deep white matter hyperintensities were due to ischemia in depressed subjects compared to less than one third in controls. The lesions were also more frequently located at Dorsolateral Prefrontal Cortex (DLPFC) in depressed subjects. They also noted that larger lesions were usually ischemic in both groups but the punctate lesions were predominantly ischemic in depressed elderly but not in control subjects. WML were also linked to large vessel atheromatous disease in another study (Thomas et al., 2001). In addition a significant increase in Intercellular adhesion molecule-1 (ICAM-1) which is considered to be marker of ischemia has been shown in DLPFC of depressed older patients (Thomas et al., 2000).

Further evidence of the vascular aetiology of WML and evidence of white matter tract disruption has been demonstrated using diffusion Tensor imaging (DTI) in a small study of geriatric depressed subjects (Taylor et al., 2001). Reduction in the density of pyramidal neurons in the orbitofrontal cortex in older depressed patients compared to controls has also been reported (Rajkowska et al., 2005).

In summary, WML located in deep white matter and basal ganglia have been more consistently associated with late life (especially late-onset) depression but some discrepant studies do exist. Studies of PVH have yielded mixed results, some showing increased frequency in late life depression (Coffey et al., 1993) while others have not (de Groot et al., 2000; Rabins et al., 1991). Whilst clearly linked to vascular risk factors, the mechanism of WML may represent microvascular disease rather than damage from major cerebral vessel territory.
In addition to WML, other structural abnormalities have been reported in late life depression. These include smaller frontal lobe volumes, reductions in volume of prefrontal cortex and orbitofrontal cortex (Almeida et al., 2003; Kumar et al., 1998; Lai et al., 2000). Complementing the structural imaging data, functional imaging (SPECT) studies have revealed reduced perfusion in frontal region (Navarro et al., 2004) but not in all studies (Ebmeier et al., 1998).

1.2.5 Virchow Robin Spaces:

Virchow-Robin spaces (VRS) are perivascular spaces that surround the arteries and arterioles as they course from the subarachnoid space through the brain tissue. They are visible on neuroimaging especially when dilated and are frequently found in the basal ganglia, internal capsule, thalamus, and brain stem (Bokura et al., 1998). They are associated with and are markers of the severity of cerebral small vessel disease (Braffman et al., 1988, Rouhl et al., 2008). On MRI, dilated VRSs are easily identified as small linear structures with signal intensity equal to that of CSF on all pulse sequences and have a distinctive anatomical distribution (Selvarajah et al., 2009). Dilated VRS in the basal ganglia are associated with treatment resistance in late-onset depression (Patankar et al., 2007).

1.2.6 Cerebrovascular disease and depression: pathophysiology

Several mechanisms can explain the association between cerebrovascular disease and depression. It has been suggested that atherosclerosis can contribute to depression. Tiemeier et al., (2004) found extra coronary atherosclerosis (including carotid intima media thickness) was linked to depressive disorders in the large population based Rotterdam study (n=3747) and furthermore the results suggested a dose-response relationship. The same authors had earlier shown lower vasomotor reactivity which is a risk factor for cerebrovascular disease in the middle cerebral artery using CO2 inhalation and increased arterial stiffness using carotid-femoral pulse wave...
velocity in older depressed patients (Tiemeier et al., 2002a, 2003), suggesting that atherosclerosis contributes to depression. Atherosclerosis can contribute to depression in later life by the effects of cytokines on central monoamine systems (Lyness et al., 2001).

So, the proposed pathway for vascular disease causing depression is that: vascular risk factors such as hypertension, diabetes mellitus, and ischaemic heart disease contribute to cerebral small vessel disease causing cerebral ischaemia (WML), leading to disruption of key frontal subcortical circuits (striato-pallido-thalomo-cortical) resulting in depression. Alexopolus et al., (1997) proposed that this can happen, either through small strategically placed lesions or an accumulation of lesions, eventually having a ‘threshold effect’. The fact that WML in late life depression are more often found in deep white matter and basal ganglia provides some support for this view. Taylor et al., (2003b) using Statistical Parametric Mapping (SPM) showed that older depressed subjects exhibited WMLs in bilateral frontal and left parieto-temporal regions, whereas such lesions were located bilaterally in parieto-temporal regions in control subjects suggesting that that frontostriatal disconnection plays a role in late-life depression. Finally, neurological signs consistent with sub cortical-frontal dysfunction were more frequently present in late onset depressed subjects compared to healthy controls in another study (Baldwin et al., 2005b).

It must also be recognized that depression itself can cause or exacerbate preexisting vascular disease though various mechanisms such as hypothalamic-pituitary-adrenal axis over-activity (Musselman et al., 1998, O’Brien et al., 2004), increased platelet activation and hypercoagulability (Laghrissi-Thode et al., 1997), abnormal folate or homocysteine mechanisms (Hickie et al., 2005), impaired arterial endothelial function and sub clinical atheroma formation (Jones et al., 2003). Also, depressed patients often adopt unhealthy life styles (smoking,
sedentary life style) and are known to comply poorly with medical treatment (Di Matteo et al., 2000). Other mechanisms that have been proposed linking vascular disease and depression include a common pathophysiological process such as inflammation (Katz et al., 2004) and common genetic factors. Apolipoprotein-E (APO-E) is known to play a role in lipid metabolism and cholesterol transport important in cardiovascular and cerebrovascular disease (Lavretsky et al., 2000). The ApoE E4 allele has been linked to late onset depression (Krishnan et al., 1996), gray-matter lesion volume (Steffens et al., 2003a) and deep white matter hyperintensities (Nebes et al, 2001). However, this has not been replicated in other studies (Hickie et al., 2001; Rigaud et al., 2002; Blazer et al., 2002). The two-way interaction of depression and vascular disease is illustrated in Figure 2.

Figure 2: Association between Depression and vascular disease (courtesy of Dr.Baldwin)
The pathology underlying cerebral ischaemia leading to WML is unclear. It is possible both large vessel atherosclerosis and small vessel arteriosclerosis play a role along with other contributory factors such as transient decreases in local perfusion because of auto regulatory dysfunction and micro embolic disease. Earlier studies showed that large vessel disease in the form of carotid stenosis is associated with severity of WML (Fazekas et al., 1988; Waterston et al., 1990). More recent studies have failed to find an association between severity of WML and the degree of carotid stenosis suggesting that WML is linked to diseases of small arteries and arterioles (Streifler et al., 1995; Adachi et al., 1997). Indeed there is substantial evidence for cerebral small vessel disease playing a causative role in WML (Pantoni & Garcia, 1997).

Van swieten et al., (1991) performed Magnetic resonance imaging (MRI) and neuropathological examination postmortem on the brains of 40 patients aged over 60 yrs and found that demyelination of the white matter was always accompanied by arteriolosclerosis and normal arterioles were always found in brains with normal white matter. This suggests that arteriolosclerosis is the primary factor in the pathogenesis of diffuse white matter lesions in the elderly. Other histopathological studies have confirmed this close relationship between arteriosclerosis and WML (Fazekas et al., 1993; Leifer et al., 1990). It has also been demonstrated that areas with WML have normal blood volume but reduced autoregulatory capacity in keeping with a small vessel disease model (Marstrand et al., 2002; Matsushita et al., 1994). A recent study did not find a correlation between severity of WML and cerebral blood flow providing further evidence that local micro vascular abnormality in the form of cerebral arteriosclerosis of the penetrating vessels could be the dominant pathogenic factor (Patankar et al., 2006). Pathophysiologically, it is thought that chronic diffuse arteriopathy of the cerebral small vessels leads to hypo perfusion and impaired auto regulation resulting in WML (Hassan et
al., 2003). It has been postulated that endothelial dysfunction may underlie cerebral small vessel disease and this will be discussed in detail later.

The pathological process that leads to cerebral white matter lesions may begin in or before midlife. Population based studies have demonstrated that mid-life atherosclerosis and vascular risk factors are associated with WML in later life (De Leeuw et al., 2000a; Swan et al., 1998). Similarly, depression at all ages seems to increase later vascular disease. Indeed long term follow up studies have shown that clinical depression is an independent risk factor for incident coronary artery disease for several decades after onset even after adjusting for multiple cardiovascular risk factors (Ford et al., 1998).

The above data provide convincing evidence that the relationship between vascular disease and depression is bi-directional and there are plausible biological explanations to explain it in both directions (Fig 2). Vascular disease can contribute to the development of depression, hypertension, diabetes, cerebrovascular disease including stroke and coronary artery disease are all associated with high rates of depression. Conversely, depression is a risk factor for stroke, coronary artery disease and probably diabetes. The direction of causality between cerebrovascular disease and depression remains to be established. Indeed, ultimately this question may be unanswerable given the bi-directional nature of the interaction between the two (Baldwin et al., 2005a).

1.3 Vascular depression (VaD):

The above data linking vascular disease and depression led to the vascular depression (VaD) hypothesis. First described as ‘arteriosclerotic depression’ (Krishnan & McDonald, 1995), the model is of small vessel disease of the penetrating arteries supplying brain white matter and the basal ganglia. The clinical symptoms and signs of vascular depression have been described by
Alexopoulos et al., (1997) and the neuro radiodological features by Krishnan et al., (1997). Primarily, VaD postulates that vascular disease contributes significantly to the pathogenesis of a subtype of late life depression with a distinct clinical presentation. The main features of VaD are late onset, association with vascular disease or hyperintensities on MRI and cognitive impairment such as executive dysfunction which include planning, sequencing, organization, and abstract thinking. The latter has been well characterised and the term “depression executive dysfunction syndrome of late life” has been proposed (Alexopoulos et al., 2002). More recently, the term “Subcortical ischemic depression (SID)” has been proposed as a more accurate term to describe vascular depression due to sub cortical ischaemic lesions (Krishnan et al., 2004).

Two different sets of diagnostic criteria have been proposed for the diagnosis of VaD. One based on clinical and/or neuro imaging evidence for cerebrovascular disease, cognitive impairment (including executive dysfunction) as primary features and a number of secondary features including age at onset after 50 years (Steffens& Krishnan., 1998). The other set of criteria is based on clinical and/or laboratory evidence of vascular disease and age at onset after 65 years as primary features and secondary features including neuro psychological impairment (Alexopoulos et al., 1997). However currently there is no consensus and because of the different methodologies used in measuring MR scan findings, clearly defined criteria with good reliability may be difficult to achieve (Baldwin& O’Brien, 2002b).

1.3.1 Clinical features of VaD:

It has been suggested that symptoms of VaD may vary from that of non vascular/endogenous depression. Alexopolous et al., (1997) found that patients with vascular depression had greater disability, less agitation and guilt feelings, greater lack of insight and greater overall cognitive impairment. Other features that are more frequent in VaD include late age at onset, less
psychosis and more anhedonia (Krishnan et al., 1997). Psychomotor retardation is also quite common and is thought to correlate independently with the degree of white-matter change (Simpson et al 2000). Nebes et al., (2001) found depressive symptoms such as impaired motivation and concentration were related to deep white matter hyper intensities in a community study. Another recent study found that SID patients had less family history of depression and more lassitude and more often a history of hypertension (Krishnan et al., 2004). Cognitive impairment notably executive functioning which includes impairments in planning, sequencing, organizing, and abstracting are attributed to disruption of the integrity of striatofrontal pathways and is thought to more frequent in vascular depression. A recent study of stroke patients found that patients with MRI-defined sub cortical ischemic vascular disease (SIVD) were more likely to be depressed than patients with other types of stroke, and that executive dysfunction was the main cognitive domain that differentiated SIVD patients from those without (Pohjasvaarra et al., 2003), providing some evidence for the above. Other neuropsychological deficits include reduced performance on new learning tasks (Simpson et al., 1998). Even though several of the symptoms described in vascular depression such as psychomotor change, apathy and dysexecutive symptoms may be linked to vascular disease, the symptomatic differences may be ‘too non-specific to be diagnostically useful’ (Baldwin, 2005b).

1.3.2 VaD Outcomes:

Severity of WML has been linked to poor response to ECT in two studies (Hickie et al., 1995, Steffens et al., 2001) but not in an earlier one (Coffey et al., 1988). Poor outcome (O’Brien., 1998) and chronicity of depressive symptoms at 5 years follow-up (Heiden et al., 2005) have also been reported. Greater progression of WML volume has also been associated with poor outcomes in a longitudinal study (Taylor et al., 2003b). In contrast, Simpson et al., (1997) found
that the location of lesions and not the severity was associated with resistance to antidepressant therapy. Recent studies have shown that Virchow Robin Space dilatation and abnormalities in Cerebro spinal fluid flow patterns which are biomarkers for cerebral micro vascular abnormality can predict treatment response in late life depression (Patankar et al., 2007; Naish et al., 2006). WML have been linked to cognitive impairment and possibly later dementia (Baldwin et al., 2000) but this has not been replicated in community studies (Schmidt et al., 1999). Deep white matter lesions have been shown to be significantly associated with mortality in depressed older patients, even after controlling for potential confounders (Levy et al., 2003). Executive dysfunction which is a specific feature of VaD has been found to be associated with higher relapse rates, residual symptoms and disability (Alexopoulos et al., 2000; Kiosses et al., 2000) and resistance to treatment (Baldwin et al., 2004).

However, Krishnan et al., (1998), using amalgamated data from antidepressant efficacy trials failed to find any significant difference in outcome in depressed patients with MRI defined vascular changes compared to those without in a 6 month cohort study. Similarly Sallaoway et al. (2002) did not find a difference in antidepressant treatment response between groups of geriatric depressed patients with high or low sub cortical hyperintensity scores. In an earlier prospective study Baldwin et al., (1993) found that 1 year outcomes were similar between elderly depressed patients with diagnosed cerebral pathology compared to those without. So, the influence of neuroimaging findings such as WML on treatment outcomes and the underlying mechanisms remain unclear at the moment.

1.3.3 Treatment implications:
Although many new antidepressants have become available and are prescribed in late life depression, the evidence suggests that that the prognosis has not substantially altered since the
introduction of the original heterocyclic antidepressants in the late 1950s (Cole et al., 1999). If VaD is found to be a subtype of late life depression with specific aetiology it would have implications for treatment and prognosis since rational therapies for depressive disorder based on vasoprotection as well as antidepressants could be used.

In a double blind, placebo controlled randomized trial, Taragano et al., (2005) tested this approach using the calcium channel blocker nimodipine, which has vasoprotective properties. 101 patients with VaD according to Alexopoulos (1997) criteria received either fluoxetine and Nimodipine or fluoxetine and placebo (fig 3). More patients on the active combination achieved full remission of depression and had lower rates of relapses over eight months compared to those on antidepressant alone, with a number-need-to-treat of four. The same group had shown similar results in an earlier study (Taragano et al., 2001). However this needs replication.

Figure 3: Remission rates with Nimodipine as augmentation agent (Taragano et al, 2005)
If frontostriatal pathways are dysfunctional in vascular depression, new treatments might include a range of new possibilities. These include drugs such as dopamine D$_3$ receptor agonists, cholinesterase inhibitors and opiate receptor agonists and antagonists which modify the neurotransmitters in the above pathways (Alexopoulos et al, 2001). Ameliorating the effect of vascular risk factors such as hypertension, high cholesterol by appropriate treatment is another approach.

1.3.4 Limitations of the vascular depression hypothesis:

The relationship between vascular disease and depression is complex. Our understanding of it remains limited and significant gaps in knowledge remain. The majority of published studies on vascular depression are cross sectional and therefore the link is associative rather than causative. It could be that unknown confounders are the cause of the association between vascular disease and depression. It has also been suggested that these two disorders could coexist based on common etiological factors such as genetic vulnerability, alcoholism and personality traits (Ramasubbu, 2000).

First, it is not yet clear whether vascular depression is a specific subtype of late life depression with characteristic clinical features. A recent systematic review of studies comparing depressed patients with and without vascular etiology concluded that sufficient evidence is lacking at the moment to support a clinically-recognisable subtype of vascular depression (McDougall et al., 2007).

Second, if sub clinical cerebrovascular disease caused depression, then the prevalence of cerebrovascular risk factors (CVRFs) would be expected to be increased in depressed patients but evidence for this is weak. Some clinical studies have shown that CVRF’s are increased in late life depression (Baldwin & Tomenson, 1995) but other clinical studies comparing depressed
subjects with controls have been negative (Baldwin et al, 2004; Kumar et al., 1997; Lyness et al., 1998). Other cross sectional studies based in Primary care and in the community have also yielded mixed results (Lyness et al., 1999, 2000; Stewart et al., 2001; Rainer et al., 2006; Yochim et al., 2006). The results have been largely negative in longitudinal community based studies too (Lyness et al., 2000; Cervilla et al., 2004; Nuyen et al., 2007) apart from a study of geriatric rehabilitation patients which found that CVRFs at baseline were related to depressive symptoms at 6 and 18 months follow up (Mast et al., 2004). Unexpectedly, in the Nuyen study, CVRFs appeared to be associated with depression with onset between ages 50 and 69 years, but not for onset ≥70 years, although another cross sectional study found that CVRFs burden was positively related to depressive symptoms independent of other co morbidity in those over the age of 85 but not in other age groups (Mast et al., 2005).

The lack of association between CVRF’s and depression might be explained by a number of factors. It could be because the vascular risk is being modified as patients are usually actively treated for vascular diseases such as hypertension and high cholesterol while under study and studies have shown that pharmacological control of hypertension reduces progression of WML (Dufouil et al., 2001). Also, vascular factors examined in studies have been those used to assess stroke risk, and these might not be sensitive to broader and more subtle cerebrovascular disease (Thomas et al., 2004).

Third, it has not been convincingly shown that WML precede the onset of depression. Several studies have examined the longitudinal association between WML and late life depression. In the large Cardiovascular Health study of 3236 subjects, baseline severity of WML was not predictive of incident depression (Steffens et al., 2002). Likewise, Versluis et al. (2006.), in the PROSPER study, did not find any association between baseline WML and development of depressive
symptoms during a mean follow up of 33 months. In the Rotterdam study of older adults, baseline atherosclerosis as assessed by a composite measure including carotid IMT did not predict incident depression over 6 years of follow up. However, two other recent longitudinal studies have found a link between baseline WML and incident depression. Godin et al., (2008) found in the three city (3C)-Dijon study in France that in subjects who were free of depression at study enrolment, baseline WML volume predicted incident depression over 4 years of follow up. In the European multi-centre Leukoariosis and Disability in the Elderly (LADIS) study, 639 elderly subjects had baseline WML assessment by MR brain scan and clinical follow up for upto 3 years (Teodorczuk et al., 2010). The results showed that baseline WML severity independently predicted both depressive symptoms at 2 and 3 years and incident depression over the 3 years. However, the study population became more significantly disabled over the study period and baseline severity of WML no longer predicted depressive symptoms at 3 years or incident depression suggesting that disability may be a mediator of depressive symptoms. Further longitudinal studies are needed to accurately elucidate the temporal relationship between WML and emergence of depressive symptoms.

Fourth, mild to moderate WML are commonly seen in normal ageing and they increase with age (Schmidt et al., 1999; Wahlund et al., 1996) and their clinical importance is not fully understood at present. WML in normal ageing has been associated with cardiovascular risk factors, including hypertension and diabetes (Longstreth et al., 1996). WML are not specific for late life depression. Indeed they have been found to be more common in middle-aged unipolar (Krishnan et al., 1988) and young bipolar patients relative to controls (Figiel et al., 1991b).

Fifth, the neuropathological basis of WML has not yet been established definitively. More than one mechanism could account for the WML and they could represent dilated perivascular spaces,
minute brain cysts, necrosis, and arteriolar hyalinization, (Ramasubbu, 2000) but as noted earlier (Thomas et al., 2001; 2002a) larger lesions are usually of ischaemic origin. Curiously, WML were not linked to athermatous disease or cerebral micro vascular disease in a neuropathological study suggesting that hypotensive disease may be an important cause of WML (Thomas et al., 2002b).

Finally, neuroimaging studies in late life depression often have reached divergent and inconsistent conclusions with regards to lesion location and severity. For example in the Cardiovascular Health Study (Steffens 1999) basal ganglia lesions but not of deep white matter were related to depression. This could be perhaps due to the heterogeneous nature of late life depression or probably due to the different methodologies employed in the studies, including the rating of WMLs. It is known that visual rating scales display ceiling effects and poor discrimination of absolute lesion volumes and consequently, they may be less sensitive in differentiating clinical groups (Van Straaten et al., 2006). In fact in a recent study, common visual rating scales (Fazekas, Schelten) had little correlation with volumetric analysis of WML which are considered the most objective, when assessing white matter change over time (Prins et al., 2004). It has also been suggested that the vascular depression hypothesis may understate the role of non-biological and genetic factors (Carmelli et al., 2002).

Despite these limitations, there is adequate evidence to support vascular disease as aetio-logically important in late-life depression and supportive evidence for a vascular depression subtype associated with cognitive dysfunction and often but not exclusively with a late age at onset. Further research is needed to disentangle to the complex relationship between vascular disease and depression but as yet there are no studies of the vasculature in late-life depression.
1.4 Measures of vascular function and pathology:

1.4.1 Endothelial function:

The endothelium is a single layer of endothelial cells lining the inner surface of all blood vessels and represents the barrier between the vessel wall and the blood. The endothelium synthesizes and releases a number of vasoactive substances including Nitric oxide (NO). The endothelium plays a key role in vascular homeostasis regulating vascular tone, smooth muscle cell proliferation, fibrinolysis as well as interactions between the vessel wall, platelets, and leukocytes (Wilson et al., 2006). Vascular homeostasis depends upon a fine balance between contracting and relaxing factors released by the endothelium and NO plays a pivotal role (Moncada et al., 1993).

NO promotes vasodilatation and is synthesized from L-arginine by the action of endothelial NO synthase (eNOS). NO is the one of the principal factors behind the antiatherosclerotic properties of the endothelium. Nitric oxide (NO) plays a critical role in the maintenance of vascular tone and reactivity, maintains the vascular smooth muscle in a non proliferative state, inhibits platelet and white cell activation and negates the actions of potent endothelium-derived contracting factors such as angiotensin II and endothelin-1 (Verma et al., 2003).

A single layer of endothelial cells is the only constituent of capillaries, which form the microcirculation, differing from other vessels, which contain smooth muscle cells and adventitia (Li et al., 2007). It is known that intact cerebral endothelium plays a crucial role in cerebral blood flow and in the blood-brain barrier. Endothelium-dependent NO mediates basal cerebral blood flow and cerebral auto regulation, a mechanism which ensures a constant blood flow to the brain over a wide range of perfusion pressures (White et al., 1998; 2000).
So, not surprisingly, chronic endothelial dysfunction plays a pivotal role in cerebral small vessel disease (SVD) and this is partly due to decreased production of NO (Hassan et al., 2003; 2004). Also, augmented release of superoxide anion (O$_2^-$) may annihilate NO radical, neutralizing its vasodilator capacity (Di Napoli et al., 2005). Atherothrombosis could be another way by which endothelial dysfunction may contribute to ischemic cerebrovascular events. Endothelial dysfunction has been demonstrated in cerebral arteries of animal models of hypertension (Brunner et al., 2005). Neuropathological studies have demonstrated damage to the microvascular endothelium in cerebral SVD in humans (Lin et al., 2000). Genetic studies also indicate that endothelial function may be important in mediating cerebral small vessel disease (Ellbaz et al., 2000). As noted earlier cerebral SVD probably underpins WML.

Endothelial dysfunction, a systemic disturbance, is the impairment of the normal functions of the endothelium and this plays a critical role in the initiation of atherosclerosis (Ross, 1999) and occurs in response to wide range of cardiovascular risk factors (Verma & Anderson, 2002). Endothelial dysfunction is typified by a reduction of the bioavailability of vasodilators, in particular NO. In addition to the impaired vasodilatation, endothelial dysfunction comprises a specific state of “endothelial activation,” which is characterized by a pro inflammatory, proliferative, and pro coagulatory milieu that favors all stages of atherogenesis (Bonetti et al., 2003). Endothelial dysfunction results in both functional and structural alterations in the arterial wall. In larger arteries, the effect is more structural with endothelial dysfunction contributing to thickening, remodeling, and atherosclerosis which results in stiffening. In the smaller arterioles and resistance vessels, alteration in tone results as due to reductions in endothelium dependent NO (Cohn et al., 2001). Endothelium is an important regulator of arterial stiffness and studies have directly related increased stiffness with impaired endothelial function (Oliver & Webb,
2003). In fact, it has been suggested that arterial stiffness may be involved in the pathogenesis of atherosclerosis and growing evidence indicates that arterial stiffness may be an important additional and independent risk factor for cardiovascular disease (Arnett et al., 1994). Given the close relationship between endothelial dysfunction and atherosclerosis, testing of endothelial function is a useful biomarker of atherosclerosis and a number of tests have been developed.

Endothelial function can be assessed in several ways including invasively by gluteal skin biopsy, quantitative coronary angiography, and non invasively by laser doppler flowmetry, Pulse wave analysis (PWA) and Flow-mediated dilation (FMD) of the brachial artery. FMD is the one of the most widely used research tool but it requires ultrasonographic expertise and is operator dependent which decreases its feasibility as a screening tool in clinical practice (Bonetti, 2003). Rajagopalan et al., (2001) were the first to study endothelial function in depression. 15 young patients with major depression, not on antidepressants, with no known cardiac risk factors were compared with 15 age and gender matched controls. Endothelial function as measured by flow mediated dilatation was significantly impaired in the patient group. Lipid profiles, body mass index (BMI), blood pressure were similar between the groups and the only predictor of endothelial dysfunction by multivariate and univariate analysis was the presence of depression. Broadley et al., (2002) found similar results in a study of young to middle aged subjects aged 18-55 but in this study depressed patients were being treated with antidepressants. The persistence of altered endothelial function despite effective treatment suggests that impaired endothelial function may contribute to depression. Conversely, Sherwood et al., (2005) showed that vascular endothelial function was impaired in coronary heart disease patients with significant depressive symptoms compared to those without suggesting that endothelial dysfunction could be another pathway through which depression may increase cardiovascular risk.
Endothelial dysfunction is reversible and a number of strategies such as cholesterol lowering, antihypertensive therapy, smoking cessation, supplementation with folic acid, and physical exercise, have been shown to lead to an improvement in endothelial health (Bonetti et al., 2003). Currently there is much interest in the beneficial effects of statins, Angiotensin Converting Enzyme (ACE) inhibitors and Angiotensin II Receptor Blockers (ARB) on promoting endothelial function, a property which appears to be independent of the primary prescribing indication for such drugs (Bertrand, 2004). Large clinical trials over the last few years have shown that statins reduce the incidence of cerebrovascular events and this is partly due to improvements in endothelial function (Cimino et al., 2007). As described earlier, two recent studies have demonstrated that nimodipine (a drug with vasoprotective properties) when combined with antidepressant medication led to better outcomes in patients with late-life depression (Tarangano et al., 2001; 2005).

A direct assessment of cerebral vasculature in patients with depressive disorder would be ideal but, unlike some other end-organs, is clearly not feasible currently. If WML in late-life depression are atherosclerotic they are likely to be part of a widespread process not confined to the brain, since the risk factors for endothelial dysfunction are conditions known to have widespread effects such as hypertension, diabetes, smoking, exposure to high levels of low-density lipoprotein, homocysteine and fibrinogen (Di Napoli & Papa, 2005). Besides, it is well known that endothelial dysfunction represents a systemic syndrome affecting multiple vascular beds including the cerebral vasculature (Elesber et al., 2006). Therefore evidence of endothelial dysfunction can be expected in the periphery as well as centrally in conditions such as vascular depression. The presence of coronary endothelial dysfunction has been shown to be associated with an increased risk of cerebrovascular events (Targonski et al., 2003).
Indirect (non-invasive) measures which correlate with endothelial dysfunction, atherosclerosis and arterial stiffness include intima-media thickness in the carotid arteries using ultrasonography (Teimeier et al, 2004), pulse wave analysis (PWA) (Klocke et al., 2003) and pulse wave velocity (PWV) (Oliver & Webb, 2003; Broadley et al., 2005). In this study, it was proposed to undertake a direct assessment of endothelial function by gluteal skin biopsy with pharmacological testing in the laboratory and indirect assessment by assessing arterial stiffness using PWA, PWV and atherosclerosis using carotid ultrasound. As there are known no studies of endothelial function in depressed subjects using gluteal biopsy, we considered it important to have both direct and well known indirect measures to allow comparison with published research. Although not confined to older subjects, abnormalities in some of these measures have been positively associated with depressive disorder.

1.4.2 Pulse Wave Velocity (PWV):

PWV is a simple, indirect and non-invasive measure of arterial stiffness and is an established marker of vascular disease. PWV is probably the best measure of arterial stiffness and is widely validated (O’Rourke et al., 2002). The loss of arrangement of elastic fibres and laminae in the media of central elastic arteries with an increase in collagenous material and proliferation of smooth muscle cells leads to arterial stiffening. A number of factors such as endothelial dysfunction, age, and hypertension are involved in arterial stiffening (Tropeano et al., 2006; Laurent et al., 2005).

An arterial pressure wave is created when blood is ejected from the left ventricle during systole and is transmitted toward the periphery. The velocity of pulse wave travelling a given distance between two sites is the PWV and this can be measured in different ways and in different sites. Aortic PWV has emerged as an important independent predictor of cardiovascular events (Oliver
PWV is inversely related to the arterial vessel wall compliance and so stiffer the wall, higher is the PWV.

In aortic PWV, the arterial pulse wave is measured at the carotid and the femoral artery. As both these arteries are located quite superficially, their pulse waveforms can be easily measured noninvasively. The pulse wave has to travel through most of the aorta in between these 2 sites. The time delay between the arrival pulse wave at these 2 points is obtained either by simultaneous measurement, or by gating separate recordings to a fixed point in the cardiac cycle usually to the peak of the R-wave of the ECG. The distance traveled by the pulse wave is measured over the body surface and PWV is then calculated as distance /time (metres/second). Arterial pulse waves can be detected by using pressure-sensitive transducers, or applanation tonometry.

Arterial stiffness is associated with atherosclerosis as shown by Van Popele et al., (2001) and colleagues in the Rotterdam study of more than 3000 elderly subjects. In this study aortic stiffness was measured by carotid-femoral pulse wave velocity and atherosclerosis by carotid ultrasound, ankle brachial index and aortic calcifications by x-rays. The relationship between arterial stiffness and atherosclerosis could be bidirectional or both could be independent processes. In the Health, Aging and Body Composition (Health ABC) study of 2488 generally healthy, community-dwelling older adults, aortic PWV was associated with higher cardiovascular mortality, coronary heart disease, and stroke even after adjustment for demographic variables, blood pressure, known CV disease, and other variables related to events (Sutton-Tyrrell, et al., 2005). Other studies have also shown an association between increased arterial stiffness and cerebrovascular disease independent of atherosclerosis (Van Dijk et al., 2005; Lehmann et al., 1999).
Tiemeier et al. (2003) studied the relationship between aortic stiffness as measured by carotid femoral PWV and depressive symptoms in 3704 subjects aged over 60 in the population based Rotterdam study. Depressive symptoms were considered to be significant if subjects scored > 16 in the Centre for Epidemiology studies –Depression scale and those who screened positive underwent a psychiatric assessment using the Dutch version of the Present State Examination. Participants with increased pulse wave velocity were more likely to have depressive symptoms even after controlling for demographic factors and atherosclerosis and the association was stronger for major depression meeting DSM-IV criteria. Odds ratios (ORs) for depressive symptoms were 1.17 per standard deviation increase in aortic pulse wave velocity and 1.48 for depression meeting DSM-IV criteria.

So, it appears that arterial stiffness may contribute to the relationship between vascular factors and depression through its association with atherosclerosis. An alternate possibility is that arterial stiffness could be a risk factor of cerebrovascular disease in itself (Dijk et al., 2004). Or it could be that arterial stiffness contributes to depression via increase pulse pressure. Arterial stiffness leads to increased systolic pressure and decreased diastolic pressure and increase pulse pressure has been associated with cerebrovascular disease (Domanski et al., 1999) but in the Tiemeier study there was no association between pulse pressure and depressive symptoms. The efficacy of antihypertensive drugs such as ACE inhibitors and calcium channel blockers such as in lowering arterial stiffness has been well established in number of studies and in a meta analysis (Laurent et al., 2002). Furthermore the effect of ACE inhibitors in reducing arterial stiffness appears to be independent of reduction in blood pressure (Tropeano et al., 2006). If it is established arterial stiffness plays a role in the aetiology of late life depression, these drugs and others that improve endothelial function may have a preventative role. Other non
pharmacological treatments such as exercise, weight loss also improve arterial compliance (Laurent et al., 2002).

1.4.2 Pulse Wave Analysis (PWA):

Pulse Wave Analysis is a new technique through which stiffness of the central aorta can be measured non invasively. It is a simple and reproducible technique with high within-observer and between-observer reproducibility (Wilkinson et al., 1998). A pulse wave is produced when the left ventricle contracts in systole. When the wave front encounters a change in resistance i.e. when passing from large arteries to peripheral arteries a reflected wave is produced. The resulting combination of the two waves i.e. wave generated by the ejection and the reflected wave is the aortic pulse waveform. If the aorta is perfectly elastic, it absorbs the entire wave produced by the ventricular contraction where as if it is rigid a large proportion of the wave is reflected.

O’Rourke and colleagues (1996) developed the central pulse wave analysis (PWA) system which uses applanation tonometry to record pressure waves from the radial artery accurately. In this technique, a micro manometer-tipped probe detects the pulse pressure wave in the radial artery and the pressure waveform is digitised to be viewed on a computer screen. From this, the corresponding central aortic waveform can be generated by using a validated generalized transfer function using computer software. The generalised transfer function has been derived from studies recording the peripheral waveform at the same time as the central ascending aortic waveform obtained invasively (Davies et al., 2003).

The aortic stiffness can be measured by calculating Augmentation index (AIx) from the central aortic waveform. As aortic stiffness increases, transmission velocity of both forward and reflected waves increases, which causes the reflected wave to arrive earlier in the central aorta.
and augment the pressure in late systole (Weber et al., 2004). Therefore, augmentation of the central aortic pressure is a manifestation of early wave reflection and the augmented pressure divided by pulse pressure expressed as a percentage is the Augmentation index. In addition to the augmentation index, aortic blood pressure values can also be calculated from the waveform. Recently findings have demonstrated that central pressure and not the brachial pressure, directly affects the target organs and that latter does not always reflect the former (Hirata et al., 2006). Moreover, the shape of the central waveform is an important, independent predictor of cardiovascular risk (Wilkinson et al., 2001). Larger AIx values indicate increased wave reflection from the periphery because of greater arterial stiffness.

Normal ageing is accompanied by arterial stiffening and this is caused by an increase in arterial wall thickness secondary to hyperplasia of the intima and additionally by loss of elastin in the media. Not surprisingly, augmentation index also increases with age (Vaitkevicius et al., 1993). Increased AIx has been associated with cardiovascular risk factors (Nichols et al., 2002) and with coronary artery disease (Weber et al., 2004).

Duprez et al., (2001) studied the relationship between cerebral WML and large and small artery compliance in 24 apparently healthy older subjects (mean age of 84) White matter load was assessed by magnetic resonance imaging and arterial compliance by PWA. Severity of WML was associated with significantly decreased arterial elasticity indices irrespective of blood pressure and cholesterol levels. A recent case control study looked at endothelial function using PWA in 31 subjects’ depressed subjects and 18 healthy volunteers (Rybakowski et al., 2006). Patients and controls did not have any known cardiovascular risk factors such as hypertension and diabetes and smoking was equally prevalent in the groups. Arterial endothelial function was found to be impaired in patients compared to control subjects, both during a depressive episode
and in remission after pharmacological treatment. Severity of depression did not influence the results suggesting that endothelial dysfunction may be a trait marker of depression. Aortic Augmentation index can be reduced by exercise and drugs such as nitrates, ACE inhibitors and calcium channel blockers (O’Rourke et al., 2005; Nichols et al., 2002).

1.4.3 Carotid Intima Media Thickness (IMT):

B wave ultrasound is a non invasive method of visualizing superficial arterial walls in high resolution and in real time. A characteristic B mode image of an arterial wall is composed of two parallel echogenic lines separated by a hypo echoic space. The two lines correspond to lumen–intima and the media–adventitia interface and the distance between them is the intima media thickness.

Carotid intima-media thickness (IMT) can be measured by this method. The B-mode ultrasonographic measurement of IMT does not differ significantly from IMT as measured by histopathologic examination (Cheng et al., 1993). Carotid B-mode ultrasonography also allows for estimating lumen diameter, presence and extent of plaques. It is considered to be the closest investigation to an arterial biopsy and is accurate and is reproducible (Cheng et al., 2002). Carotid IMT correlates significantly with endothelial dysfunction (Juonala et al, 2004; Corrado et al., 2005). Carotid IMT increases with age and is generally greater in men compared to women (Ando et al., 2000; Allan et al., 1997). Others have shown that IMT is related to cardiovascular risk factors such as body mass index, blood pressure, total cholesterol, glucose, smoking, diabetes and hypertension (Gariepy et al., 1998; Guvener et al., 2000; Pauletto et al., 1999). Carotid atherosclerosis is an index of generalized atherosclerosis and has been positively linked with cardio and cerebro vascular disease. There is substantial evidence demonstrating that elevated carotid IMT raises the relative risk of cardiovascular events such as myocardial
infarction (Cheng et al., 2002). By using data from the population based Rotterdam study, Bots and co-workers (1997) showed that Carotid IMT was associated with incident cerebrovascular disease. The odds ratio for stroke per standard deviation increase in the carotid IMT (0.16mm) was 1.41. Moreover, investigators from the same study found a dose-dependent relation between carotid plaques and the risk of stroke and cerebral infarction with the highest risk for lacunar infarctions (Hollander et al., 2002). This was replicated in the large Atherosclerosis Risk in Communities (ARIC) Study of 15792 subjects in which baseline IMT was correlated with stroke incidence over a median follow up period of 7 years (Chambless et al., 2000). There was a clear increase in stroke event rate with every incremental increase in mean IMT.

Several studies have found an association between increased IMT and WML. In the prospective population based EVA study, 640 elderly subjects underwent ultrasound measurement of IMT at baseline and were followed up for 4 years (Pico et al., 2002). At follow up WML was assessed by MRI and a repeat IMT measurement was performed. Baseline IMT was significantly associated with increased risk of severe WML at 4 years with an odds ration of 1.70 and the effect remained even after controlling age, gender and hypertension. In addition, increase in IMT was associated with severe WML’s but this did not reach significance.

Bots et al., (1993) examined WML and carotid IMT in 111 randomly selected subjects aged over 65, stratified by age and gender from the Rotterdam study. The common carotid IMT was significantly higher in those with WML compared to those without with the mean difference being 0.13mm after adjustment for age and gender. Further logistic regression analysis indicated that a 0.1mm increase in common carotid IMT resulted in 50% greater probability of WML.

Another large study, Rotterdam scan study, with participants randomly selected from two large cohort studies, the Zoetermeer Study and the Rotterdam Study reported associations between
WML and IMT. In this cross sectional study, 1077 subjects aged between 60-90 years underwent MR brain scan and carotid ultrasonography. Carotid plaques and IMT were associated with periventricular WML (de Leeuw et al., 2000b). Other cross sectional studies have found similar associations between IMT and WML (Manolio et al., 1999).

Carotid IMT has also been found to be correlated with depression in a number of studies. Recently, Faramawi and colleagues (2007) explored the association between depressive symptoms and carotid artery atherosclerosis in 3781 subjects aged ≥65 years in the Cardiovascular Health Study. Depressive symptoms were assessed using Center for Epidemiologic Studies Depression Scale (CES-D) and atherosclerosis by ultrasound carotid IMT. Multivariate analysis after adjusting for potential confounders showed that depressive symptoms were associated with larger carotid IMT’s. Likewise, in a statistically significant relationship between IMT’s and depressive symptoms was found in the Rotterdam study (Tiemeier et al, 2004).

Chen et al (2006) studied 11 patients and 14 controls in a small case control study to assess the relationship between carotid atherosclerosis and late onset depression. The mean age of study subjects was 75 and investigations included IMT and MR brain scan. Total IMT was significantly higher in the depressed group. Interestingly there was a significant difference in IMT only in the Common carotid and bifurcation segments and not in the internal carotid. Severity of WML also correlated positively with IMT.

The direction of causality in the relationship between IMT and depression remains unclear or there could be a common pathophysiological process behind both. As discussed earlier depression can contribute to atherosclerosis in several ways. Early-onset depression and recurrent episodes have been linked with increased IMT (Elovainio et al., 2005; Jones et al.,
2003). But in the above discussed Chen study, the mean age of onset of depression was 75 years. So, the shorter duration of depression is unlikely to have caused the increased IMT suggesting that atherosclerosis may underlie late onset depression.

Treatment of vascular factors can reduce carotid IMT and more importantly decrease the incidence of vascular events. In a double blind placebo controlled multi centre Regression Growth Evaluation Statin Study (REGRESS), Pravastatin 40mg/day significantly reduced mean carotid IMT over 2 years (de Groot, 1998) and other studies have reached similar conclusions (MacMahon et al., 1998; Furburg et al., 1994). Treatment of hypertension also leads to an attenuation of IMT as demonstrated by a recent meta analysis with calcium channel blockers being better than ACE inhibitors or beta blockers (Wang et al., 2006). In addition to IMT regression treatment of hypertension with Verapamil led to lower cardiovascular event rate in another study (Zanchetti, 1998).

1.4.4 Carotid Plaques:

IMT and plaque are different ultrasonographic phenotypes of atherosclerosis. They are biologically distinct and represent different stages of atherogenesis which is a complex multistep process that has many physical, molecular, and genetic determinants (Spence & Hegel, 2004; Simon et al., 2010). IMT probably represents smooth muscle cell proliferation, whereas plaque formation reflects a later stage of atherogenesis related to platelet aggregation, hypercoagulability and attenuated fibrinolysis (Willeit et al., 2000). Although both IMT and plaque are predictors future vascular events, some studies have suggested that the latter, a carotid plaque, may be the more powerful predictor of vascular outcomes (Ebrahim et al., 1999; Spence et al., 2002). Carotid plaques have also been linked to WML (De Leeuw et al., 2000; Pico et al.,
2002) and depressive symptoms (Haas et al., 2005). Lifetime history of major depression has been linked to carotid plaques (Jones et al., 2003).

In the population-based Rotterdam Scan study of community dwelling older people, increasing number of plaques in the carotid artery the severity of periventricular white matter lesions increased (de Leeuw et al., 2000). The relationship between carotid plaques and depression appears to be directional. The risk of plaque was higher in depressed diabetic men subjects relative to non-depressed participants after adjustment for age, smoking status, systolic blood pressure, dyslipidaemia and body mass index with an odd ratio of 5.9 (Spitzer et al., 2008). On the contrary, Mlekusch, et al. (2006) showed in a fairly large of study if older people that significant decline in depressive symptoms in patients with carotid artery stenosis following coronary artery stenting.

1.4.5 Gluteal Skin biopsy:

Blood vessels measuring <400 micrometer are usually called as small or resistance arteries. Nitric oxide release plays a significant influence on tone in small resistance vessels (Cohn et al., 2001). So, the earliest manifestations of endothelial dysfunction could be found in the small arteries. Indeed impairment of endothelium-dependent dilation of small arteries has been shown in diabetes and hypercholesterolemia (Schofield et al., 2002; Goode et al., 1997; Malik et al., 2005). Increased wall–lumen ratio of small arteries is consistently found in hypertension and besides it is a strong predictor of increased mortality and morbidity from coronary heart disease and stroke (Cruickshank et al., 2005). Hypertension is also associated with similar structural alterations in cerebral vessels, over a large range of arterial sizes (Johansson et al., 1985).

The functioning of small arteries can be assessed by non invasive techniques such as Laser Doppler imaging of blood flow in the skin or in the eye by fundus photography or fluorescein
angiography. However, to study the structure in addition to function of such vessels, a small but invasive biopsy of skin and subcutaneous fat is the only method currently available (Heagerty, 2007). The study of resistance vessels by this method was developed by Aalkjaer et al., (1987). The biopsy is usually done in the gluteal region though some have used the distal anterior forearm. Gluteal biopsy has been shown to be safe and well tolerated. A resistance artery is isolated from the biopsy tissue and the vessel can be mounted to study the structure and functioning of the endothelium by either the wire myograph method or the pressurized artery preparation (Schiffrin et al., 1997). Endothelial function can be assessed by challenging the vessel with various chemical agents such as L-NG-monomethylarginine (L-NMMA), an inhibitor of nitric oxide synthase and agonists such as acetylcholine.

Currently, there are no published studies of gluteal biopsy in the psychiatric literature. However, other studies have found associations between WML and retinal micro vascular abnormalities which are presumed to reflect cerebral micro vascular disease. In the prospective population based Atherosclerosis Risk in Communities (ARIC) Study, 1684 persons with a mean age of around 60 had MRI brain scan and micro vascular assessment by retinal photography (Wong et al., 2002). The photographs were digitised and the diameters of individual retinal vessels coursing through a specified area were measured on the computer and this was summarised as the arteriole-to-venule ratio (AVR). A strong association between retinal micro vascular abnormalities such as focal arteriolar narrowing and WML lesion was found and this was independent of age and vascular factors. Ikram et al., (2006) went one step further and measured the retinal vessels diameter using a semi automated system in the Rotterdam scan study and found that retinal venular dilatation was related to progression of WML suggesting the hypoxia could play a role.
1.5 Other factors:

1.5.1 Inflammatory markers:

Levels of inflammatory markers in the periphery are raised in depression. There is growing interest in the relationship between accumulated injury, stress and inflammation and age-related increases in inflammatory markers, with the suggestion that depression is one marker of frailty (Katz, 2004). Inflammation may promote atherosclerosis and thrombosis by elevating serum levels of fibrinogen, leukocytes, clotting factors, and by altering the metabolism and functions of endothelial cells (Rost et al, 2001) and this may play a crucial role in both the initiation and progression of cerebral small vessel disease (Di Napoli et al, 2005).

C-reactive protein (CRP) is an acute-phase reactant and is a sensitive blood marker of inflammation. It is involved in the endothelial inflammatory response (Khreiss et al, 2004) and has been shown to be associated with ischemic stroke (Woodward et al, 2005). CRP has also been linked to depression (Penninx et al, 2003) and WML in later life.

The relationship between C-reactive protein (CRP) and WML was studied by van Dijk and colleagues (2005) in the population-based Rotterdam Scan study of 1033 participants. The mean age of study subjects was around 70. Higher CRP levels were associated with presence and progression of white matter lesions, particularly with marked lesion progression even after adjusting for cardiovascular risk factors and carotid atherosclerosis. But in the Austrian Stroke Prevention Study of 505 subjects CRP was associated with carotid atherosclerosis but not with WML. However the subjects in this study were younger, healthier and a different methodology was used to measure white matter change compared to the Rotterdam study (Schmidt et al., 2006).
ESR is widely used as a non-specific marker of inflammation and it has been associated with poorer outcome in late life depression (Baldwin et al., 2006). This study will therefore measure CRP and ESR and assess its relationship to measures of endothelial function.

Lastly, a range of other factors such as Vitamin B12 and folate levels, metabolic syndrome, haemoglobin levels have been linked to vascular risk, WML and depression or its treatment. Vitamin B12 and Folate play an essential role in maintaining the integrity of the neurological systems involved in mood regulation (Penninx et al., 2000). Several population-based studies have found low Vit B12 and folate levels in late life depressed subjects (Tiemeier et al., 2002b; Dimopoulos et al., 2007). WML in depression has been linked to low B12 and folate (Hickie et al., 2005; Iosifescu et al., 2005; Scott et al., 2004) and there is some support for folate augmentation in depression treatment (Taylor et al., 2003c), although not specifically in vascular depression.

1.5.2 Metabolic Syndrome

The metabolic syndrome refers to a cluster of closely related cardiovascular risk factors such as central obesity, dyslipidemia (raised triglycerides, low levels of high-density lipoprotein), raised blood glucose and hypertension. Currently there are two major definitions for metabolic syndrome provided by International Diabetes Federation (Alberti et al., 2005) and the revised National Cholesterol Education Program: Adult Treatment Panel III (NCEP ATP III, 2001). Both the definitions are quite similar but the IDF definition uses ethnicity specific cut-off points for waist circumference. The IDF definition is based on simple clinical and biochemical parameters such as waist circumference, cholesterol levels and blood pressure. The metabolic syndrome is a risk factor for cerebral atherosclerosis (Bushnell & Guzick, 2005) and
cerebrovascular disease (Solenski, 2007). It has also been linked to abnormalities in small vessel structure and function (Wiernsperger et al., 2007).

High density lipoprotein (HDL) is protective of vascular disease and lower HDL has been associated with depression and outcome (Baldwin et al, 2006; Kim et al, 2004). Cholesterol lowering medications have been associated with decrease in relapse rates in late life depressed subjects (Steffens et al., 2003b). Depressive symptoms have been associated with anaemia in a large study of around 1000 older persons living in the community (Onder et al., 2005). We therefore measured these peripheral markers (Vit B12, folate, full blood count & cholesterol levels) and assessed subjects for the presence of metabolic syndrome.
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Chapter 2: Methods

2.1 Study Aim:

The main aim was to investigate atherosclerosis and endothelial function in late-life depression by indirect and direct methods. The major intention was to assess whether vascular function was impaired in late life depression compared to non depressed controls (Please see Figure.1 for an overview of study measures). I also explored correlations between measures of vascular function and neuroimaging markers suggestive of cerebrovascular disease. In addition, in this research I compared vascular function and MRI defined brain ischaemic changes between early-onset depressed and late-onset depressed subjects. Finally, I explored whether any of the vascular measures can help distinguish patients who have responded to antidepressant monotherapy from those requiring more intensive management (‘resistant’ cases). These aims are encompassed within discrete hypotheses outlined in the ensuing studies (Chapters 3 -6).

2.2 Objectives:

(1) Direct assessment of endothelial function by skin biopsy and laboratory testing.

(2) Indirect assessment of endothelial function/vasculature using intima-media thickness measures in the carotid arteries, Pulse Wave analysis of the radial artery, and aortic pulse wave velocity using the carotid femoral method.

(3) Assessment of cerebral atrophy and imaging biomarkers of microvascular disease (white matter lesion load, cerebral lacunes, basal ganglia hyperintensity and Virchow Robin space dilatation) using MRI scanning.

(4) Investigation of relevant peripheral biochemical markers

   Inflammatory markers (ESR, CRP)

   Full blood count
B12 and folate
Lipid profile
Blood glucose

(5) Clinical assessment of vascular risk factors, metabolic syndrome and medical morbidity

**Figure 1: Vascular pathway in late life depression**
2.3 Statistical power:

As endothelial function was the one of the main outcomes of interest in the study, we used a previous study by Rajagopalan et al., (2001) to base the power calculation. The effect size on the Rajagopalan study was extremely large at 3.04 (mean difference of 5.78 divided by the standard deviation of 1.9). The power calculation based on this study showed that a sample size of 4 in each group will have 80% power to detect a difference in endothelial function at a 0.05 two-sided significance level. The endothelial function effect size on the only other study in the psychiatric literature was even larger (Broadley et al., 2002). However, both these studies were done in adults with a mean age of around 30. Extrapolating these data to geriatric patients may be problematic because of age-associated decrements in vascular performance and greater inter individual difference with age (Cruickshank et al., 2002). Therefore, pragmatically, sample sizes of 25 per group were chosen. It was assumed that not all subjects would consent to gluteal biopsy. Using the previously described data and those from other work in our laboratory (Malik et al., 2005), it was estimated that 15 per group would be sufficient to demonstrate group difference in endothelial function.

2.4 Study design:

A case control study involving 25 patients with depression and 21 non-depressed control subjects. Patients with depression were recruited from two clinical secondary care sites in Greater Manchester. Control subjects were recruited from spouses or partners of depressed subjects or via advert in day and community centres within the same geographical area.
Inclusion criteria for patients:

1) Age over 60 years at the time of assessment

2) Satisfy criteria for past or present history of Depressive Episode, moderate or severe, psychotic or non-psychotic (World Health Organisation, ICD10, 1992)

3) Stable medication

Exclusion criteria:

1) History or neurological evidence of stroke, space occupying lesion, neuro-degenerative disorders including Parkinson's disease

2) Dementia (World Health Organisation, ICD-10, 1992)

3) Past head injury with loss of consciousness; history of another psychiatric disorder besides depression

4) Inability to co-operate with testing schedules

5) Electroconvulsive Treatment (ECT) within 3 months of initial recruitment.

6) Arrhythmic disorders such as atrial fibrillation and severe valvular disease due to the difficulties in getting proper data from the pulse wave analysis system

7) Patients on warfarin because of the gluteal biopsy

8) Contraindications for MR brain scan such as heart valves, pace maker.

Inclusion criteria for control subjects:

1) No previous history of psychiatric disturbance

2) Stable medical health.

The same exclusion criteria as patients were applied.

Psychiatrists in the Manchester Mental Health & Social Care Trust were approached by one of the project team and invited to submit names of patients aged over 60 with depression. A review
of the clinical notes was undertaken to determine whether the entry criteria (above) are likely to be met. If so, and provided the treating clinician is satisfied that their patient is well enough to participate, the patient was approached to assess mental capacity in relation to participating in the study and explain its purpose if capacity is present. Those interested in participating were given an information sheet and a letter in a pre-paid envelope to return if they wish to take part. After receipt of the letter, the participant was revisited to obtain informed consent.

The diagnosis of past or present history of Depression was made using ICD-10 symptom checklist for Depressive Episode based on information from the following sources: direct patient interview, review of hospital psychiatric records and confirmation by treating psychiatrist. This meant that depression diagnosis was made retrospectively at least for some cases. The study had to balance its aims with the resources available and the demands placed on potential subjects, bearing in mind the extensive protocol. Since all subjects were under the care of specialist old age psychiatric services it was felt that this balance could be achieved using the significant amount of clinical data available without using time-consuming tools such as the Structured Clinical Interview (SCID) to confirm the diagnosis of depression. All the depressed subjects also completed the Massachusetts General Hospital Antidepressant Treatment Response Questionnaire (MGH ATRQ) (Chandler et al., 2010), a valid and reliable self-rated scale, to gather data on dose and duration of antidepressant treatment. This data was supplemented by data from the treating psychiatrist and review of medical records to assess patient’s response to treatment and degree of treatment resistance. Various other scales to measure psychiatric and physical illnesses as described below were also completed.

All studies were performed during a visit to the Manchester Wellcome Trust Clinical Research Facility (CRF). Subjects, arrived fasted, and underwent blood testing, physical evaluation, pulse
wave velocity and analysis. After breakfast, they moved to the adjacent 1.5 Tesla MR scanning facility for brain scanning. Then they underwent gluteal biopsy followed by carotid ultrasonography. Most participants underwent all the tests over the course of a single morning, however in some cases, it was carried out over 2 visits.

2.5 Study Measures:

2.5.1 Demographic information:
Age, gender, civil status, smoking status, alcohol intake, thorough medical history and current prescribed medication was recorded for each subject

2.5.2 Psychiatric measures:

a) WHO ICD-10 symptom checklist for Depressive Episode, moderate or severe
b) Montgomery Asberg Depression Rating scale for severity (Montgomery & Asberg, 1979): It is a frequently used observer rated depression rating scale. It is composed of 10 items and each item scored on a scale of seven points (from 0 to 6) (Appendix 1)
c) The Mini-Mental Status Examination (MMSE) (Folstein et al., 1975) (Appendix 2)
d) Treatment resistance measures:
This is a five stage model and gives a categorical value for the degree of resistance. The stages are: 0: response to monotherapy; 1: non-response to antidepressant monotherapy; 2: non-response to two trials of monotherapy from drugs of different classes; 3: Stage 2 plus failure to respond to one augmentation strategy; 4: Stage 3 plus failure of a second augmentation strategy; 5: Stage 4 plus failure to respond to one course of ECT.
(ii) Massachusetts General Hospital staging method (MGH-S) (Fava, 2003):
This is based on self report and is accompanied by Antidepressant Treatment Response Questionnaire [ATRQ] (Appendix 4) to facilitate the staging of treatment resistance. The scale is scored as follows: Non-response to each adequate (at least 6 weeks of an adequate dose of antidepressant) trial is given a point (1 point per trial); Optimisation of dose/duration, and augmentation/combination of each trial is given half a point (0.5 point per trial per optimisation/strategy); ECT increases the overall score by 3 points. The scale generates a continuous variable which represents degree of treatment resistance with higher scores indicating greater treatment resistance.

2.5.3 Physical measures

i) Waist circumference

ii) Weight and height (to calculate Body Mass Index)

iii) Cumulative Illness Rating Scale – Geriatrics (Miller et al., 1992) (Appendix 5): CIRS-G quantifies medical burden in the elderly and has been validated in several studies (Hudon et al., 2005; Rochon et al., 1996). It includes the following organ-system specific categories: cardiac, vascular, haematological, respiratory, otorhinolaryngological, ophthalmological, upper gastrointestinal, lower gastrointestinal, hepatic and pancreatic, renal, genitourinary, musculoskeletal, neurological, psychiatric and endocrine, metabolic and breast. The scoring system is from 0 to 4 for each category according to the level of impairment that is present, with: 0, no problem; 1, minor current problem or significant history; 2, morbidity or moderate discomfort, requiring primary care treatment; 3, severe problem: constant significant discomfort, chronic problem difficult to control; 4, extremely severe problem, requiring immediate treatment: organ failure or severe functional impairment. The availability of a manual with operationalized criteria facilitates consistent scoring of the scale (Miller et al., 1991)
2.5.4 Vascular disease measures:

i) Framingham stroke risk scale (Wolf et al., 1991) (Appendix 6): Computed for each patient based on the history and physical findings, this score comprises a weighted composite measure of the following factors: age, systolic blood pressure, treatment with antihypertensive, diabetes, cigarette consumption, evidence of cardiovascular disease, atrial fibrillation and left ventricular hypertrophy. The score gives a likelihood of stroke within the next 10 years, expressed as a percentage. Separate scores are provided for males and females. Although not strictly linear, the higher the score, the greater the risk.

ii) Metabolic syndrome (Appendix 7) as defined by International Diabetes Federation (Alberti et al., 2005)

2.5.5 Blood testing:

Inflammatory markers, CRP, ESR

Fasting lipids

Fasting blood glucose

Plasma B12 and folate

Full blood count

Renal function tests

Liver function tests.

2.5.6 Neuroimaging assessment:

MRI Imaging protocol:

Magnetic resonance imaging was performed on the 1.5 Tesla scanner at the Clinical Research Facility. Imaging protocol consisted of the following sequence is:
1. T1 weighted multi slice inversion recovery- to assess Virchow-Robin spaces

2. Fluid attenuated inversion recovery (FLAIR) – to assess white matter lesions

3. Single slice quantitative phase contrast images of the following to assess cerebral blood flow:
   a. internal carotid arteries
   b. basilar artery
   c. internal cerebral vein and superior sagittal sinus
   d. cerebral aqueduct
   e. foramen magnum

4. T2 sagittal inversion recovery – To assess white matter lesions

Analysis was performed in the Division of Imaging Science and Biomedical Engineering (ISBE). The following potential biomarkers of vascular abnormality were assessed.

1. Evidence of vascular injury including cerebral atrophy, white matter lesion load, presents of cerebral lacunes, established infarcts, basal ganglia hyperintensities and dilated Virchow Robin spaces

2. Volumetric Analysis of WML was undertaken using locally developed software (TINA) (http://www.tina-vision.net). WML were also assessed using the Scheltens scoring system (Scheltens et al., 1993)

3. Assessment of dilated Virchow Robin Spaces

2.5.7 Non-invasive physiological procedures to assess endothelial function, atherosclerosis and arterial stiffness:

2.5.7.1 Pulse Wave Velocity:

This was performed by the same investigator in all patients. The test was carried out in the morning with the subjects having fasted from midnight. After having rested supine for at least 5 minutes, brachial blood pressure was measured using the sphygmomanometer. Aortic PWV was measured using an automatic device (Micro Medical Ltd, UK) with a 5MHz Doppler probe. The sites used were right carotid and right femoral sites. The carotid (at the base of the neck) and femoral artery (inguinal region) pressure waves were recorded non invasively and ECG gating allowed the sites to be measured consecutively. The distance traveled by the pulse wave was measured over the surface of the body with a tape measure and it was defined as: (distance from the sternal notch to femoral arterial measurement site) – (distance from carotid artery measurement site to the sternal notch). PWV was calculated as the distance: transit time ratio and is expressed as meters per second. A minimum of 10 beats were averaged for each site using the R wave of the ECG for synchorinisation.

2.5.7.2 Pulse Wave Analysis:

SphygmoCor (PWV Medical, Sydney, Australia), a computerized and simple-to-use device was used to assess pulse waveforms in the right radial artery. The system is validated and is based on the principle of applanation tonometry and comes with appropriate acquisition and analysis software for recording and analysis of the arterial pulse. Peripheral artery pressure waveforms were acquired from the right radial artery noninvasively. The probe was placed over the radial artery at the point of maximum pulse amplitude, and real time arterial waveform was displayed on a laptop computer screen. The quality of the waveform
was optimised by careful repositioning of the probe. The system has built-in quality-control measures and recordings were taken when a reproducible signal was obtained (usually two screens or 10 consecutive beats). AIX is influenced by heart rate, an index normalized for heart rate of 75 bpm (AIX@75) was used in the analysis (Wilkinson et al., 2000)

2.5.7.3 Carotid Ultrasonography:

The carotid arteries were evaluated with high-resolution B-mode ultrasonography. Detailed B-mode images of the right and left common carotid artery, common carotid bifurcation, and the first centimetre of the internal carotid artery were obtained using a Philips/ATL HDI 5000 ultrasound system. All the ultrasound scans were done by experienced vascular technologists blinded to clinical data. Intima-media wall thickness and assessment of the degree of focal plaque were calculated giving two primary measures of carotid disease.

Studies have shown that posterior (far) wall IMT seen with ultrasound reflects the anatomical intima/medial layer (Bots et al., 1993; Pignoli et al., 1986). The intima-media thickness was measured by two-dimensional ultrasound at 3 sites (far wall) on both sides:

1. Common Carotid artery (CCA) 1cm proximal to the beginning of the carotid bulb.
2. Within the carotid bulb.
3. Internal Carotid artery (ICA) 0.5cm distal to the flow divider.

The measurements were repeated thrice at each site and the average was calculated (Bots et al., 1993). IMT of the each segment was calculated as the mean of the average IMT of the both the left and right sides i.e. (average IMT right far wall + average IMT left far wall)/2. An overall measure of IMT was calculated as the average of the CCA IMT, bifurcation IMT, and ICA IMT. Plaque measurements were also taken at these sites if present and/or at the site of the thickest plaque by taking a diameter reduction measurement and, if necessary, a velocity measurement.
The carotid arteries were also assessed in cross section to check for any significant plaques. The degree of plaque was graded using the following criteria:

- **Grade 0**: no observable plaque
- **Grade 1**: one small plaque (<30% lumen diameter loss)
- **Grade 2**: one medium plaque (30-50% lumen diameter loss) or multiple small plaques
- **Grade 3**: one large plaque (50-69% lumen diameter loss) or multiple plaques with at least one medium plaque
- **Grade 4**: one severe plaque (>70% lumen diameter loss) or multiple plaque with at least one large plaque
- **Grade 5**: occluded

Four segments are graded according to these criteria: CCA, Carotid bulb, ICA and ECA. These grades are then summed for each side of the neck to give a plaque index, a measure of focal plaque. It has been found to be a valid and reproducible measure of carotid atherosclerosis (Talbot et al., 2000)

### 2.6 Direct assessment of endothelial function with Gluteal Skin biopsy:

A single subcutaneous gluteal fat biopsy was obtained from each subject. 3-5mls of 1% lignocaine will be infiltrated allowing tissue (2cm x 1.5cm x 1.5cm) to be harvested. The fat biopsy was placed immediately in ice cold physiological saline solution (PSS). Sutures were removed 7-10 days following the procedure by the participant’s General Practitioner. Alternatively some participants returned to the Wellcome Trust Clinical Research Facility for suture removal by the nursing staff. Analysis was done in the Department of Medicine, Manchester Royal Infirmary.
2.6.1 Pressure arteriography:

Small arteries 65µm-230µm were isolated and carefully cleaned under a dissecting microscope and transferred to an arteriographic bath chamber (Living Systems Instruments, Burlington, VT.) and cannulated. Wall thickness and lumen diameter are recorded using a Video Dimension Analyser (Living Systems Instruments, Burlington, Vermont) and connected to a chart recorder. Vessels are connected to a pressure servo (Living Systems Instruments, Burlington, Vermont) pressurised to 50mmHg, and any vessel exhibiting a leak is discarded.

For pharmacological experiments and passive structure assessment, vessels are allowed to equilibrate to 37°C for 1 hr and then challenged serially with 60mmol KPSS until a steady vasoconstriction >50% is achieved. For experiments to measure myogenic responses and passive structure, arteries will be allowed to equilibrate for 1-2 hours to develop spontaneous tone of >20% constriction from a relaxed passive state at 37°C.

2.6.2 Vascular function:

Following viability assessment with KPSS, each vessel was stimulated with the following:

1) Nor epinephrine - to assess vasoconstrictor response

2) Acetylcholine (Ach) on a preconstricted vessel- to assess endothelium dependent vasodilatation.

3) Ach on a preconstricted vessel after pre-treatment with L-NO^{G}-monomethyl-arginine (L-NMMA) an inhibitor of nitric oxide (NO) synthase (NO mediated vasodilatation)- to assess whether reduced vaosdilation is due to just downregulation of NO synthase or due to additional alternations in endothelium-derived hyperpolarizing factor release and the cyclooxygenase pathway.

4) Nitro donor sodium nitroprusside (SNP)- to assess Endothelium independent
vasodilatation.

2.6.3 Myogenic Tone:

To determine myogenic responsiveness, the intra-luminal distending pressure was reduced to 20mmHg and then increased to 40mmHg, with subsequent increments of 40mmHg to 200mmHg. At each pressure step the vessel is allowed to stabilize over 5-10 minutes. The pressure was again reduced to 50mmHg and the vessel is superfused with Ca\(^{2+}\)-free PSS containing EDTA for 20 minutes. The pressure steps were repeated to obtain passive pressure/diameter relations.

2.6.4 Vascular Structure:

1. Cross-sectional wall area (CSA) was calculated as CSA = \(\pi (D+2WT/2)^2 - \pi (D/2)^2\)

   where D is inner diameter and WT the wall thickness.

2. Wall/lumen ratio (W/L) = WT/D x 100.

2.7 Statistical Analysis:

Statistical analysis was performed using SPSS 16.0 software. Parametric and where relevant non-parametric testing was used to assess group differences and logistic regression to determine which baseline variables predict group membership (control/depressed, Late onset depression/Early onset depression). Full statistical methods are described in subsequent chapters.
References:


10. Miller MD TA. *A Manual of Guidelines for Scoring the Cumulative Illness Rating Scale*


Chapter 3: Vascular function in older adults with depressive disorder

Paranthaman R et al.,

Vascular function in older adults with depressive disorder

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3.1 Abstract

**Background:** Cerebrovascular disease plays an important role in depressive disorder, especially in older adults. An understanding of vascular function in depression is important aetiologically and for innovative treatments designed which may improve prognosis through amelioration of vascular damage.

**Methods:** Assessment of endothelial function, arterial stiffness and atherosclerosis in a variety of vessel beds in 25 elderly subjects with depressive disorder compared with 21 non-depressed controls. Subjects underwent Pulse Wave Velocity (PWV), Pulse Wave Analysis (PWA), carotid Intima Media Thickness (IMT) and Magnetic Resonance Imaging (MRI). A subset (16 patients and 15 controls) had assessment of biopsied small artery dilatation to acetylcholine to further assess endothelial function.

**Results:** The mean sample age was 72.9 years with an average age at onset of 60 years for depression. Mean Carotid IMT was significantly higher in depressed subjects (p<0.01). PWV was 1.6 m/s higher in depressed subjects (borderline significance). There was a significant reduction in the dilatation response to acetylcholine in pre-constricted small arteries (p=0.01). On MRI, depressed subjects had significantly more dilated VRS in the basal ganglia (p=0.01). Depressed subjects had greater volume of white matter lesions in all regions but this did not reach statistical significance. There were no baseline differences in vascular risk.
Conclusion: Depression in the elderly is associated with poorer endothelial function and more atherosclerosis. This is associated with a greater WMH lesion load and basal ganglia microangiopathy. The use of vasoprotective drugs to improve endothelial function or retard atherosclerosis as depression-modifying agents should be explored.
3.2 INTRODUCTION

In older patients with depression, cerebrovascular disease may predispose to, precipitate or perpetuate depression, which is the basis of the vascular depression hypothesis (1). An important component of the hypothesis is a consistent finding of an increased white matter hyperintensities (WMH) on Magnetic Resonance Imaging (MRI) in depressed subjects compared with non-depressed control subjects (2, 3). These hyperintensities are taken to indicate white matter damage (4). A range of pathological changes can lead to WMH but post-mortem studies support ischaemia as an important cause (5, 6). However, ischaemia is an end product of a range of potential vascular mechanisms, including systemic (endothelial dysfunction, atheroma and inflammation (7, 8, 9)), haemodynamic (hypotension (10, 11)) and localized (vascular damage to fronto-striatal circuitry and subcortical regions (12, 13)). Clarifying vascular processes which may underlie visualized WMH is of relevance both to aetiology and to potential new treatments approaches aimed at ameliorating brain injury associated with depression (14).

The literature regarding vascular mechanisms in depressive disorder is small compared to that for WMH but suggests that endothelial dysfunction and atherosclerosis occur. Endothelial dysfunction is the first step in atherogenesis and the pathway to atherosclerosis. In two studies of younger depressed adults (under 60 years), Flow-Mediated Dilatation (FMD), an indirect measure of endothelial function, was impaired (15, 16). Old age depression (60 and above) has been shown to be associated with increased pulse wave velocity (PWV), a measure of arterial stiffness (17), and increased intima-media thickness (IMT) (7, 18), a measure of atherosclerosis. Augmentation index as measured by Pulse Wave Analysis (PWA) is another measure of arterial stiffness and correlates with endothelial function (19).
The aim of the current study was to perform measures of vascular function in older patients with depression and concomitantly measure structural brain abnormalities on MRI. Drawing upon the above literature, the following vascular measures were chosen: PWV and PWA for arterial stiffness and IMT for atherosclerosis. FMD when used in small samples is considered not to be reliable (20), so it was decided to use a novel technique successfully applied to small groups of subjects as an assessment of endothelial function. This involves the acquisition of small vessels from gluteal fat biopsy and analysis in vitro (8).

Although other imaging modalities exist to assess white matter integrity (for example Diffusion Tensor Imaging (4)), most work exploring links between depression and cerebrovascular disease has been conducted using measures of WMH, either with visual rating scales (3) or automated or semi-automated assessment of volume (21, 12, 13). Additionally, we have demonstrated abnormally dilated Virchow-Robins spaces (VRS) notably in the basal ganglia in geriatric depression (22). Basal ganglia lesions are known to be predictive of depression (23). VRS are perivascular spaces which when abnormally dilated are thought to indicate cerebral microangiopathy (24 - 26).

As discussed, if WMH is the product of cerebral ischaemia, there are several possible mechanisms. Two of these, endothelial dysfunction and atherosclerosis are explored in this study. The main hypothesis was that in late life depressive disorder there is impairment of vascular function (as assessed by endothelial function, arterial stiffness and atherosclerosis) compared to a non-depressed control group. Secondary hypotheses were first that neuroimaging markers suggestive of ischaemia as assessed by WMH and cerebral microangiopathy as measured by VRS occur more commonly in depressed subjects and second that there is a correlation between WMH and peripheral vascular function.
3.3 MATERIALS AND METHODS

3.3.1 Subjects

Following Ethics Committee approval, patients with depressive disorder were recruited from the case registers of two clinical secondary care sites in Greater Manchester. Approximately 40% of those eligible declined and 25% had contraindications (below). There were no age or gender differences between those who refused and those who agreed to participate. Control subjects could be spouses or partners of depressed subjects or were recruited via advert in day and community centres. All participants gave full informed consent.

3.3.2 Inclusion and exclusion criteria

Patients were aged 60 and over at the time of assessment and fulfilled criteria for past or present history of Depressive Episode, moderate or severe, psychotic or non-psychotic (27) and were on stable medication. Patients with a history of stroke, space occupying lesion, neuro-degenerative disorders including Parkinson's disease, Dementia (27), previous head injury with loss of consciousness or a history of another psychiatric disorder besides depression were excluded as were those with severe valvular heart disease or on warfarin (because of contraindications to scan or biopsy) or atrial fibrillation (because of difficulty in interpreting pulse wave measures). Control participants had no previous history of psychiatric disturbance and had stable medical health. All studies were performed during a visit to the Manchester Wellcome Trust Clinical Research Facility.

3.3.3 General study Measures

Age, gender, smoking status, medical history and current medication were recorded for each subject along with waist circumference, weight, height and fasting blood for blood sugar and
lipids. Psychiatric measures included WHO ICD-10 symptom checklist for Depressive Episode, Montgomery Asberg Depression Rating scale (MADRS) for severity (28) and the Mini-Mental Status Examination (MMSE) (29). Physical health was measured by the Cumulative Illness Rating Scale – Geriatrics (30) and the metabolic syndrome by operational criteria (31).

3.3.4 Vascular measures

Pulse Wave Analysis

The SphygmoCor (PWV Medical, Sydney, Australia) system was used to acquire peripheral artery pressure waveforms noninvasively from the right radial artery. The corresponding central arterial waveform was then generated using a validated transfer function (32) from which Augmentation index (AIx) normalized to a heart rate of 75, a measure of systemic arterial stiffness was calculated.

Pulse Wave Velocity (PWV)

Aortic PWV was measured from Doppler flow signals obtained sequentially from the right carotid and right femoral arteries using a noninvasive device (Micro Medical Ltd, Rochester, Kent, UK). A minimum of 10 beats were averaged for each site using the R wave of the ECG for synchronisation. Using the distance traveled by the pulse wave over the surface of the body with a tape measure (from the sternal notch to the femoral artery and carotid artery to the sternal notch), PWV was calculated as the distance: transit time ratio and is expressed as meters per second.

Intima Media Thickness (IMT)

The carotid arteries were evaluated with high-resolution B-mode ultrasonography using a Philips/ATL HDI 5000 ultrasound system. IMT was measured by two-dimensional ultrasound at 3 sites (far wall) on both sides: (1) Common Carotid artery (CCA) 1cm proximal to the
beginning of the carotid bulb. (2) Within the carotid bulb; (3) Internal Carotid artery (ICA) 0.5cm distal to the flow divider. The measurements were repeated thrice at each site and the average was calculated (33). An overall measure of IMT was calculated as the average of the CCA IMT, bifurcation IMT, and ICA IMT of both sides.

*Gluteal fat biopsy of resistance vessels*

A single subcutaneous gluteal fat biopsy was obtained using 3 to 5 ml of 2% lignocaine, allowing tissue (2x1.5x1.5cm) to be harvested. Small arteries 200 to 250μm in diameter were dissected from the tissue and isolated vessels were then transferred to an arteriographic bath chamber and cannulated. Lumen diameter was recorded with the use of a Video Dimension Analyser (Living Systems Instrumentations). After viability assessment with potassium enriched physiological saline small arteries were preconstricted with $10^{-5}$ Norepinephrine. Endothelial function in the small arteries was then studied by constructing a cumulative dose response to Acetylcholine. The maximal vasodilation in response to a concentration of $10^{-5}$M of Acetylcholine was taken as a surrogate marker for endothelial function.

PWV and PWA were measured by the same investigator, who was not blind to group status, after appropriate training. Carotid ultrasound scans were conducted by an experienced vascular technologist blinded to clinical data. Gluteal fat biopsy was performed by an investigator who was unaware of the subject’s group status.

**3.3.5 Neuroimaging evaluation**

*Protocol:* Magnetic Resonance Imaging of the brain was carried out at 1.5T on a Philips Intera Achieva using a SENSE head coil. FLAIR (fluid-attenuated inversion recovery) and T1-weighted inversion Recovery (T1-IR) images were acquired as part of the imaging protocol. For both scans, 45 transverse slices, 3.0mm thick with no slice gap were obtained using a field-of-
view of 230x230mm, providing full coverage of the brain. Images were reconstructed using a matrix size of 256x256, yielding pixels of 0.9x0.9mm. Imaging parameters specific to the FLAIR sequence were: TR/TE/TI=11000/140/2800ms, echo train length = 53 and parameters specific to the T1-IR were: TR/TE/TI=3198/15/400ms, echo train length = 5.

*Visual ratings (WMH and VRS)*

All the ratings were conducted by an experienced neuroradiologist (A.J.) who was blind to patient group. The assessment of white matter lesion load was performed on matched T1-weighted inversion recovery and T2-weighted FLAIR images using a modified Scheltens scale which has four sub-scales: cortical deep white matter (DWMH) (0–24); periventricular hyperintensities (PVH) (0–6), basal ganglia changes (0–24) and infratentorial changes (0–24) (34). Heavily T1 weighted inversion recovery sequences were used to score the presence of visible Virchow Robin spaces (VRS) using a locally developed scoring scheme, which reflects their distribution and number, as previously described (25). Previously we have reported weighted Cohen’s kappa values in the range 0.52–0.89 for inter- and intra-observer variation for the modified Schelten’s scale (35) and the VRS scales (25).

*Volumetric analysis of white matter hyperintensities (WMH)*

Quantitative measures of WMH were undertaken using locally developed software ([www.tina-vision.net](http://www.tina-vision.net)). FLAIR images were registered to T1-IR images and re-sliced to correct for patient motion. In order to assign WMH voxels the resulting images were registered to a Talairach atlas (36) and segmented to produce a mask containing only voxels within grey and white matter. This was applied to the median smoothed FLAIR maps and the resulting images used to produce maps of voxels containing WMH. The maps were quality controlled and manually edited where necessary to remove non-WMH voxels. Volume of WMH (number of voxels x voxel volume)
was calculated for the Frontal, Temporal, Parietal, Occipital and Limbic Lobes. These volumes were normalised to head size by dividing by the scale factors between the T1-IR dataset and the Talairach atlas brain.

### 3.4 Statistical Analysis

The sample size was estimated on the basis of existing data. Using subjects with major depression, Rajagopalan et al (16) found significant differences in endothelial function in groups of 15 controls and 15 depressed. The equivalent figures for Broadley et al (15) were 10 and 12. Extrapolating this data to geriatric patients may be problematic because of age-associated decrements in vascular performance and greater inter-individual difference with age (37). Therefore, pragmatically, sample sizes of 25 per group were chosen. It was envisaged not all subjects would consent to gluteal biopsy. Using the above data and from other work in our laboratory (8), it was estimated that 15 per group would be sufficient to demonstrate group difference in endothelial function.

Statistical analysis was performed using Statistical Package for the Social Sciences database (version 14.0, [www.spss.com](http://www.spss.com)). Continuous variables are expressed as means ±SD and categorical variables as frequency and percentage. Comparison between two groups (depressed and controls) was performed using t test for parametric data and Mann-Whitney test for non parametric data and Chi-squared tests for categorical data. Binary Logistic regression with forward stepwise selection of variables was used to predict variables significantly associated with group membership. For WMH quantitative data, median (interquartile range) are reported as the distribution of data was skewed and could not be converted to a normal distribution even with mathematical transformation. Association between peripheral vascular measures and neuroimaging data was assessed using Pearson’s or Spearman correlation as appropriate.
3.5 RESULTS

3.5.1 General data

The baseline characteristics of the 46 study participants, 25 patients with depression and 21 controls are presented in Table 1. The target of 25 per group was not reached because of time restraints in the study. Some neuroimaging data was lost because of technical problems (n=20 per group for the WMH volumetric calculations were complete). Two thirds were women and the mean age was 72 years. Most patients had late onset depression with mean age of onset of first episode of depression being 60 (range 41 to 76 years). There were no significant differences between the groups apart from the MADRS score being higher in depressed group and control subjects being more likely to be married. Twenty two (88%) subjects in the depressed group were on antidepressants (14 were on Selective Serotonin Reuptake inhibitors (SSRIs) alone or in combination with Noradrenergic and Specific Serotonin Agonists (NaSSAs); four on NaSSAs only; two on Selective Noradrenaline Reuptake inhibitors (SNRIs); two on tricyclics). One patient was taking lithium and four were on anxiolytic/hypnotic drugs. Three subjects in the depressed group and all subjects in the control group were on no psychotropic medication. Small artery data using gluteal fat biopsy was available for 31 subjects (16 patients and 15 controls). The main reason for no biopsy data was technical difficulty in the laboratory.

3.5.2 Vascular Findings

The augmentation index (AIx@75) was higher in the depressed group than in the control group but not significantly so (p=0.14; Table 2). Pulse Wave velocity (PWV) was higher in the depressed group compared to controls (11.56±3.60 vs. 9.99±2.05) at a borderline statistically significant level (p=0.08). Mean IMT was significantly higher in the carotid arteries in depressed subjects (Depressed=0.12± 0.05 v Controls 0.09 ± 0.01), p<0.01). Detailed data for small vessel
structure and function are reported elsewhere (Greenstein et al submitted for publication). Here we present data for relaxation of pre-constricted vessels with acetylcholine. Maximal responses of preconstricted gluteal fat arteries to the vasodilator Acetylcholine demonstrated a significant difference between the two groups of patients studied. Thus, in control participants small arteries dilated to 96 ± 1.4% of the original resting diameter while in depressed patients the maximal dilation was to 82 ± 4.5% (p=0.012).

3.5.3 Neuroimaging findings

Scheltens and VRS measures were available for all subjects. As stated, WMH volumetric data was available for 40 subjects. There were no group differences in Schelten’s measures (Table 3). VRS scores in the basal ganglia and sub insular region were significantly higher in the depressed group (Table 3). There were no statistically significant differences in the total WMH volumes or individual regional scores although there were greater volumes in all regions in the depressed group (Table 4).

3.5.4 Logistic regression to predict group membership

Logistic regression with forward stepwise selection of variables was used to predict variables significantly associated with group membership in 43 subjects who had the full data excluding small artery biopsy measures. Variables were included that were significant or borderline significant in univariate analyses and included mean IMT, PWV, VRS basal ganglia and VRS sub insular region along with other potential predictors such as age, gender, metabolic syndrome and total WMH volumes. Mean IMT alone accurately predicted 73.7% of group membership (Confidence Interval: 1.1-2.0). PWV also significantly and slightly improved the prediction to 78.9% (Confidence Interval: 1.1-1.9).
Small artery data in 31 subjects obtained by gluteal biopsy was also assessed using logistic regression by including endothelial function (percentage relaxation to Acetylcholine $10^{-5}$M) as a predictor variable in addition to the other variables described above. Endothelial function alone accurately predicted 76.7% of group membership with an OR of 1.09 (Confidence interval:1.01 to 1.16; p=0.01) for lower endothelial function suggesting that for a decrease of 1 in endothelial function, the probability of being depressed is raised by a factor of 1.09. No other variables were selected. The analyses were repeated including antidepressant use as a covariate and this did not alter the results.

3.5.5 Correlations between vascular and imaging measures

Correlations were done in the whole sample. Small artery endothelial function was inversely correlated with mean IMT (Pearson's r = -0.44; p=0.02) indicating that endothelial dysfunction was associated with higher IMT scores. Mean IMT correlated with VRS in the basal ganglia (r= 0.36; p=0.02) and sub insular region (r= 0.36; p=0.02) suggesting a link between a peripheral atheroma and cerebral microvascular angiopathy. This must be interpreted with caution due to multiple comparisons in a small sample. None of the correlations between WMH measures and peripheral vascular measures was significant.

3.6 DISCUSSION

3.6.1 Main Findings

Subjects with depressive disorder had impaired endothelial function and more atherosclerosis compared to control subjects as evidenced by statistically significant differences in resistance vessel endothelial function and carotid IMT; each predicted about 75% of group membership. The findings for Pulse Wave Velocity and Pulse Wave Analysis were in the same direction but

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1 The observed associations may simply reflect previously stated group differences as the correlations were done in the whole sample.
group difference was not significant. Together the data support the main hypothesis of vascular impairment in old age depressive disorder.

The VRS score, notably in the basal ganglia, was significantly higher in the depressed group. With volumetric measures of WMH volumes, greater volumes were found in all four regions but not to a statistically significant degree. There were no significant group differences on the Scheltens score. There was therefore only limited support for the first of the subsidiary hypotheses, of more white matter changes suggestive of ischaemia in the depressed group. WMH scores did not correlate with any of the peripheral vascular measures. The second subsidiary hypothesis was therefore not supported.

3.6.2 Relevance to the literature

The findings are consistent with research on depressive disorder showing endothelial dysfunction and atherosclerosis in depressive disorder (15, 16). These studies were of non-geriatric subjects, so the data presented extends these finding to older adults.

Depression and vascular disease have a two-way interaction. Atherosclerosis can contribute to depression onset in several ways such as hypothalamic-pituitary-adrenal axis over-activity and increased platelet activation (2, 10). Tiemeier et al (7, 17) reported that carotid IMT and PWV were associated with depression in older subjects. Alternately, depression can trigger atherosclerosis. In a study of mid-life subjects, a lifetime history of depression was associated with atheromatous plaque in the carotid arteries (38). Although the current study cannot address whether vascular dysfunction causes depression or depression directly interferes with vascular function, the differences between depressed and control subjects could not be explained by underlying group differences in physical health burden or a range of vascular risk factors including type 2 diabetes, metabolic syndrome, elevated blood pressure, serum lipids or blood
glucose. Additionally, treatment with agents which are known to influence endothelial function such as angiotensin converting enzyme (ACE) inhibitors or statins (8) was similar between the two groups of patients studied. This suggests that the condition of depression itself is linked to endothelial dysfunction and atherosclerosis in old age. Duration of depression and the presence and severity of depressive symptoms could be determinative but were not addressed in this study. The limited research to date suggests that the state of depression versus non-depression does not explain differences in vascular function in non-geriatric subjects (15, 16, 39).

Antidepressants may alter vascular function. Although controversial, recent studies have shown that SSRIs, the most commonly used antidepressants in this study, may protect or improve vascular endothelial function in healthy volunteers and in depression (40-43). Tricyclic antidepressants have cardiotoxic (largely arrhythmia) effects (44) but were prescribed to only two subjects; removing them from the dataset made no difference to the results. Nevertheless, the role of antidepressants as a mediator of adverse vascular function cannot be ruled out as it was not fully evaluated in this study.

Turning to imaging findings, the absence of difference in Scheltens measure of WMH seems surprising. However not all studies have reported group differences (45, 46). The discrepancy could be because the Scheltens scale gives weight to small punctate lesions, whereas research suggests that it is the larger lesions that are most significantly associated with geriatric depression (47). Accordingly, automated volumetric measures are now preferred (4, 48). The volumetric WMH data in this study supports a higher lesion burden in depressed subjects. Recently, specific localisation of WMH burden in white matter underlying the dorsolateral prefrontal cortex was reported in geriatric depressed subjects (13). This group highlighted the importance of using segmentation techniques which permit atlas localisation. Our technique did
this but small numbers and skewing did not permit reliable estimates of highly specific regions. Unlike the Scheltens scale, the basal ganglia VRS data in this study do provide evidence of regional damage in an area likely to be critical to fronto-striatal circuits and is consistent with other studies (23).

Without carefully designed epidemiological studies the question of whether depression causes vascular dysfunction or vascular dysfunction causes depression may be difficult to disentangle, given the bi-directional nature of the interaction between them. Although there are other potential underlying mechanisms such as inflammation (49), this perspective suggests that optimal antidepressant treatment may reduce vascular damage in depression or that vasoprotective interventions may reduce depressive symptomatology. With regard to the first proposition, in patients averaging 60 years of age following a heart attack, an SSRI reduced later re-infarction and mortality (50). Also, statins can improve endothelial function (51) and both ACE inhibitors and Angiotensin II Receptor Blockers promote endothelial function, a property which appears to be independent of the primary prescribing indication (52). With regard to the second proposition, a controlled study has shown that nimodipine, a drug with vasoprotective properties, in combination with fluoxetine reduced time to remission of late-life depression (53). Further research is needed to explain whether endothelial dysfunction associated with depressive disorder is reversible and whether vasoprotective drugs can alter the course or severity of major depression (54, 14).

This is not a study specifically of vascular depression. However, the concept is not without its critics (55) and may prove too restrictive in terms of a general understanding of the associations between vascular disease and depressive disorder (2, 55). Recent findings of the importance of
lesion localisation (12, 13) rather than lesion load alone may explain why the evidence linking vascular risk and depression is contradictory (2, 10).

**3.6.3 Limitations**

This is a cross-sectional study and so the observed relationship between vascular function and depression is associative rather than causal. A type 2 error due to small numbers also likely accounts for limited statistical significance on some of the measures (including the volumetric WMH measure). Small artery data from gluteal biopsy were available in only a sub-set of patients and there were no significant differences in vascular risk factors between the groups. Although lifestyle factors such as smoking and alcohol were recorded in both groups and did not differ, we had no information about levels of physical fitness which may have affected vascular function. The study population was predominantly white Caucasian which may limit the generalizability of the findings (56). Selection bias towards recruitment of depressed subjects with more subtle vascular disease cannot be ruled out but both patients and control subjects were from the same geographical location, although spouses of patients with depression may suffer stress which itself could promote atherosclerosis. The use of ICD criteria may have broadened criteria for entry compared to DSM (57) and reduced homogeneity and thus the chances of detecting group differences. Alternatively, this may have resulted in subjects more representative of clinical practice.

Although most depressed subjects in this study had a late-onset depression, some had recurrent depression from earlier adulthood and late-onset cases show the greatest amount of WMH (3). This may have lessened group differences. This is therefore a study of depression in old age rather than specifically of late-onset depression. Lastly, although it is only an assumption that extra-cerebral endothelial dysfunction is likely to be associated with a similar process in the
brain, it is consistent with current opinions which view endothelial dysfunction and atherosclerosis as systemic processes (58).

3.7 Conclusions

The finding of significant differences in endothelial function and atherosclerosis in older depressed patients compared to non-depressed control subjects is consistent broadly with the vascular depression hypothesis. That this could not be explained by differences in vascular risk suggests that depression itself may contribute to poorer vascular function, either directly or via proximal factors such as inflammation or lifestyle. The disturbances affected large, medium and small vessels and suggests that in geriatric depression there is a systemic disturbance in vascular function reflected in localized cerebral changes affecting front-striatal circuitry. The results are preliminary and need to be replicated in larger samples. Another potential area of further research is whether treatment of endothelial dysfunction improves outcomes for major depression.

3.8 Acknowledgements

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Conflict of Interests: Marietta Scott was concurrently employed by AstraZeneca UK plc whilst working on this study. Whilst Dr. Scott was not working in the neuroscience area, AstraZeneca is actively employed in discovering, developing, manufacturing and marketing prescription medicines in six areas of healthcare including the neurosciences. All other authors report no biomedical financial interests or potential conflicts of interest.
Contribution of Authors:
RCB was the Principal Investigator and along with AMH, RAM and ASB developed the study hypotheses. RCB is responsible for the integrity of the data. JKC assisted in the design, the large vessel measures and provided the pulse-wave analyzer. AJ conducted the semi-quantitative MRI evaluations. MLJS conducted the analysis of the white matter hyperintensity volumes. AG carried out the gluteal biopsies and small vessel analysis. RP recruited the subjects, conducted the large and medium artery analyses and co-ordinated the study whilst on the Higher Specialist Training scheme for Psychiatry of the North West Deanery, England.
References:


27. World Health Organisation. (1992): The ICD-10 Classification of Mental and Behavioural Disorders. WHO.Geneva


Table 1

Characteristics of study sample (t test for parametric data and Mann-Whitney test for non-parametric data and Chi-squared tests for categorical data)

<table>
<thead>
<tr>
<th>Variable</th>
<th>All Subjects (n=46)</th>
<th>Depressed (n=25)</th>
<th>Controls (n=21)</th>
<th>Dep vs. control P value</th>
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<td>Age, yrs</td>
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<td>72.6(4.4)</td>
<td>72.2(6.5)</td>
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</tr>
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<td>17(68)</td>
<td>13(61)</td>
<td>NS</td>
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<td>6(24)</td>
<td>15(71.4)</td>
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<td>Ethnicity-European, n (%)</td>
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<td>24(96)</td>
<td>20(95.2)</td>
<td>NS</td>
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<td>14.8(10.2)</td>
<td>2.6(1.9)</td>
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<tr>
<td>MMSE&lt;sup&gt;2&lt;/sup&gt; Score</td>
<td>29.1(1.0)</td>
<td>29.1(1.0)</td>
<td>29.1(1.1)</td>
<td>NS</td>
</tr>
<tr>
<td>CIRS-G score&lt;sup&gt;3&lt;/sup&gt;</td>
<td>6.6(3.2)</td>
<td>7.2(3.6)</td>
<td>6(2.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Alcohol units/week</td>
<td>6.22(11.6)</td>
<td>5.7(13.7)</td>
<td>6.8(8.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking status†, n (%)</td>
<td>6(13)</td>
<td>4(16)</td>
<td>2(9.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking pack years‡</td>
<td>16.35(23.7)</td>
<td>19.8(25.8)</td>
<td>12.2(20.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Taking statin, n (%)</td>
<td>18(39.1)</td>
<td>12(48)</td>
<td>6(28.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension&lt;sup&gt;4&lt;/sup&gt;, n (%)</td>
<td>31(67.3)</td>
<td>15(60)</td>
<td>16(76.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Taking anti hypertensive’s, n (%)</td>
<td>31(67.3)</td>
<td>15(60)</td>
<td>16(76.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes&lt;sup&gt;5&lt;/sup&gt;, n (%)</td>
<td>5(10.9)</td>
<td>2(8)</td>
<td>3(14.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index, kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>28.8(4.9)</td>
<td>28.6(4.8)</td>
<td>29(5.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Waist circumference, cms</td>
<td>96.8(12.3)</td>
<td>96.5(13.8)</td>
<td>97.1(10.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Metabolic syndrome&lt;sup&gt;6&lt;/sup&gt;, n (%)</td>
<td>27(58.7)</td>
<td>15(60)</td>
<td>12(57.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic Blood Pressure, mmHg</td>
<td>144.6(18.2)</td>
<td>143(17.8)</td>
<td>146.3(18.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic Blood Pressure, mmHg</td>
<td>76.4(10.8)</td>
<td>76.1(12.0)</td>
<td>76.7(9.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean Arterial blood pressure, mmHg</td>
<td>99.1(10.6)</td>
<td>98.5(11.8)</td>
<td>100.0(9.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Resting pulse rate</td>
<td>70.9(11.6)</td>
<td>73.2(10.7)</td>
<td>68.2(12.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>181.7(31.7)</td>
<td>185.6(34.8)</td>
<td>181.7(28.6)</td>
<td>NS</td>
</tr>
<tr>
<td>High Density Lipoprotein(HDL), mg/dL</td>
<td>58.0(19.7)</td>
<td>58.0(24.7)</td>
<td>54.1(11.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>124.0(57.6)</td>
<td>124.0(44.3)</td>
<td>124.0(70.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>100.8(25.2)</td>
<td>97.2(25.2)</td>
<td>106.2(23.4)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are mean (Standard deviation) unless specified

1. MADRS, Montgomery Asberg Depression Rating scale
2. MMSE, Mini Mental State Examination
3. CIRS-G, Cumulative Illness Rating Scale – Geriatrics
4. Hypertension defined according to British Hypertension Society Guidelines (http://www.bhsoc.org)
5. Diabetes mellitus defined according to WHO criteria
6. Metabolic syndrome- Based on the definition provided by provided by International Diabetes Federation Epidemiology Task Force Consensus Group (Alberti et al, 2005)

†Current smokers; ‡Pack–years of smoking are for those who had ever smoked
Italics conversion factors: To convert total cholesterol to millimoles/liter multiply by 0.0259, High Density Lipoprotein to millimoles/liter multiply by 0.0259, Triglycerides to millimoles/liter multiply by 0.0113, Glucose to millimoles/liter multiply by 0.0555
Table 2  
Measures of arterial function (Parametric and non parametric tests as appropriate)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Depressed (n=25) Mean(SD)</th>
<th>Controls (n=21) Mean(SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV (Pulse wave velocity)</td>
<td>11.56(3.60)</td>
<td>9.99(2.05)</td>
<td>0.08</td>
</tr>
<tr>
<td>PWA (Pulse Wave Analysis) Augmentation index at HR75</td>
<td>30.6(9.1)</td>
<td>26.9(7.5)</td>
<td>0.14</td>
</tr>
<tr>
<td>Mean aortic systolic pressure</td>
<td>118.2(18.0)</td>
<td>119.1(15.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean aortic diastolic pressure</td>
<td>91.2(14.4)</td>
<td>88.8(11.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean IMT(^1)</td>
<td>0.12(0.05)</td>
<td>0.09(0.01)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

1. IMT- Intima Media thickness
Data was missing for 2 subjects in PWV and PWA and 3 subjects in IMT due to technical difficulty or non availability of vascular technician
See text for gluteal biopsy data
### Table 3
Neuroimaging: Scheltens and Virchow Robin Spaces (Non parametric tests)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Depressed (n=25) Mean(SD)</th>
<th>Controls (n=21) Mean(SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal Ganglia VRS</td>
<td>3.88(1.45)</td>
<td>2.76(1.55)</td>
<td>P=0.01</td>
</tr>
<tr>
<td>Centrum semiovale VRS</td>
<td>1.17(0.83)</td>
<td>1.05(0.97)</td>
<td>NS</td>
</tr>
<tr>
<td>Sub-insular VRS</td>
<td>1.25(0.68)</td>
<td>0.76(0.89)</td>
<td>0.05</td>
</tr>
<tr>
<td>Mesencephalon VRS</td>
<td>0.50(0.51)</td>
<td>0.29(0.46)</td>
<td>NS</td>
</tr>
<tr>
<td>Scheltens PVH score</td>
<td>2.28(1.90)</td>
<td>2.33(2.48)</td>
<td>NS</td>
</tr>
<tr>
<td>Scheltens Deep white matter score</td>
<td>6.16(4.85)</td>
<td>6.67(6.29)</td>
<td>NS</td>
</tr>
<tr>
<td>Scheltens infratentorial score</td>
<td>0.52(1.41)</td>
<td>0.86(1.80)</td>
<td>NS</td>
</tr>
<tr>
<td>Scheltens Basal ganglia score</td>
<td>0.80(1.22)</td>
<td>1.52(3.23)</td>
<td>NS</td>
</tr>
<tr>
<td>Scheltens Total score</td>
<td>9.76(7.27)</td>
<td>11.38(12.30)</td>
<td>NS</td>
</tr>
</tbody>
</table>

VRS- Virchow Robin Spaces
PVH- Periventricular Hyperintensities
### Table 4
**Neuroimaging: White Matter Hyperintensity Volumes (Non parametric tests)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Depressed (n=20)</th>
<th>Controls (n=20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cerebral WML</td>
<td>342.2(748.1)</td>
<td>202.0(881.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>98.7(212.6)</td>
<td>69.4(284.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Temporal lobe</td>
<td>13.7(111.2)</td>
<td>5.9(135.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Parietal lobe</td>
<td>9.7(61.2)</td>
<td>1.2(55.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>4.2(16.9)</td>
<td>1.6(30.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Limbic lobe</td>
<td>16.7(66.8)</td>
<td>12.4(65.0)</td>
<td>NS</td>
</tr>
</tbody>
</table>

All WML volumes are corrected for head size and are expressed in mm$^3$. Data are presented as median (interquartile range) as the distribution was skewed and could not be converted to a normal distribution after mathematical transformation. Comparisons were performed by Mann-Whitney non parametric test.
Chapter 4: Cerebrovascular damage in late-life depression is associated with structural and functional abnormalities of subcutaneous small arteries

Greenstein A, Paranthaman R et al (Joint first author)

Cerebrovascular damage in late-life depression is associated with structural and functional abnormalities of subcutaneous small arteries

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4.1 Abstract:
Late-life depression is increasingly viewed as a vascular illness due to patients exhibiting characteristic white matter brain lesions and in-vivo large artery endothelial dysfunction. However, the ‘vascular depression’ hypothesis pertains to the microvasculature and this circulation has not been studied in this context. Our objective was to examine structure and function of small subcutaneous arteries in patients with late-life depression. Thus, 16 patients aged 71.8±4 years with late-life depression were compared with 15 control participants aged 72.1±5.9 years. There were similar cardiovascular profiles between the two groups. All participants underwent magnetic resonance imaging brain scans and subcutaneous gluteal fat biopsy from which small arteries were isolated and studied using pressure myography. Cerebral microvascular damage in depressed patients was confirmed by assessment of basal ganglia Virchow Robin Space scores (Depressed patients (Patients:3.9±1.7 vs Controls:2.5±1.6, p=0.01). Contractility to norepinephrine was equivalent in both groups but relaxation of the small arteries to acetylcholine was significantly reduced in depressed patients (84±4%) compared to control participants (96±1.4%, p=0.012). This difference in arterial relaxation was reduced but not entirely eliminated when nitric oxide synthase was inhibited. Depressed patients also exhibited hypertrophic wall growth with an increase in medial cross sectional area (p=0.035, multiple analysis of variance and wall thickness (p=0.04, multiple analysis of variance). In conclusion, despite similar cardiovascular profiles, depressed patients with cerebral microvascular damage show abnormalities of subcutaneous small artery structure and function.

Keywords: Ageing, endothelium, cerebrovascular disorders, remodeling, small artery
4.2 Introduction

Depressive disorder in later life is common and associated with disability, increased health care utilization and significant morbidity and mortality\textsuperscript{1, 2}. Although the aetiology of this disorder is heterogeneous\textsuperscript{3}, several lines of evidence support a link between vascular disease and late life depression. Both stroke and myocardial infarction are linked with depression in a bi-directional manner, each contributing to the risk of occurrence of the other\textsuperscript{4}. Further, lesions in brain white matter and basal ganglia (referred to here collectively as ‘WML’) visualised on Magnetic Resonance Imaging (MRI) and known to be associated with ischaemic damage\textsuperscript{3} occur more often in depressed compared to non-depressed older people\textsuperscript{5}. This has led to the ‘vascular depression’ hypothesis which proposes distinct clinical\textsuperscript{6} and neuroradiological\textsuperscript{7} appearances in such patients. The presence of Virchow Robin Spaces (VRS) in patients with depression\textsuperscript{8} lends further weight to this. These are perivascular spaces which when abnormally dilated are indicative of cerebral microangiopathy\textsuperscript{9}. They are seen as small linear structures with signal intensity equal to that of cerebrospinal fluid and have a distinctive anatomical distribution\textsuperscript{10}. A rapidly growing body of work has also demonstrated in-vivo vascular changes in depressive disorder. Thus, endothelial dysfunction occurs in the large arteries of untreated patients\textsuperscript{11} and those taking antidepressant therapy\textsuperscript{12}. Compared to matched participants without depression there is also a greater prevalence\textsuperscript{13} and incidence\textsuperscript{14} of increased carotid intima media thickness and an increase in aortic pulse wave velocity\textsuperscript{15}, even after adjustment for traditional risk factors. The studies to date however all pertain to large arteries and whilst endothelial dysfunction may be indicative of a pro-atherogenic state\textsuperscript{16}, this does not explain the development of microvascular pathology in the brain. We therefore designed a study to investigate small artery changes in late life depression. Arteries studied were taken from subcutaneous gluteal biopsies. Although clearly
from a different circulatory bed to that implicated in the development of cerebral microvascular damage, structural changes in human cerebral small arteries in response to hypertension have been shown to be similar to those found taken from a subcutaneous gluteal biopsy\textsuperscript{17}. 
4.3 Methods:

4.3.1 Subjects:

Following approval from the National Research Ethics committee, patients with depression were recruited from secondary care sites in Greater Manchester. Control subjects were recruited from spouses or partners of depressed subjects or via an advert in community centres. All participants gave full informed consent. All procedures followed were in accordance with institutional guidelines and the guidelines from the Declaration of Helsinki.

4.3.2 Inclusion criteria for patients and control subjects:

Patients were included if they were aged over 60 years at the time of assessment, on stable medication and satisfied criteria for past or present history of Depressive Episode, moderate or severe, psychotic or non-psychotic (World Health Organisation, International classification of diseases 10 (WHO ICD10), 1992). Patients with history of stroke, space occupying lesion, neuro-degenerative disorders including Parkinson's disease, Dementia, previous head injury with loss of consciousness, history of another psychiatric disorder besides depression, atrial fibrillation or severe valvular heart disease were excluded. Patients who had undergone Electroconvulsive Treatment within 3 months of initial recruitment were also excluded. Control participants had no previous history of psychiatric disturbance.

All studies were performed at the Manchester Wellcome Trust Clinical Research Facility. Age, gender, civil status, smoking status, alcohol intake, medical history (including cardiovascular disease) and current medication were recorded for each subject. Additionally, waist circumference, weight and height were measured and fasting blood was taken for estimation of glycemia and lipids. Psychiatric measures included WHO ICD-10 symptom checklist for Depressive Episode, Montgomery Asberg Depression Rating scale (MADRS) for severity and
The Mini-Mental Status Examination (MMSE)\textsuperscript{19}. Blood pressure was measured sitting, after 15 minutes of rest, by a semiautomatic machine (Omron\textsuperscript{TM} 705 CP, White Medical) with the mean of 3 readings recorded.

4.3.3 Neuroimaging evaluation:

This was conducted using a 1.5T Phillips Gyroscan scanner (Phillips Medical Systems, Best, NL) and the imaging protocol used was the axial FLAIR (fluid-attenuated inversion recovery), T1-weighted inversion recovery and T2-weighted FLAIR sequence. Slices were 3.0 mm thick with no interslice gap.

All ratings were done by an experienced neuroradiologist (A.J.) who was blind to patient group. The assessment of white matter lesion load was performed on matched T1-weighted inversion recovery and T2-weighted FLAIR images, using a scoring system based on the modified Scheltens scale which has four sub-scales: cortical deep white matter (DWMH) (0–24); periventricular hyperintensities (PVH) (0–6), basal ganglia changes (0–24) and infratentorial changes (0–24). Heavily T1 weighted inversion recovery sequences were used to score the presence of visible Virchow Robin spaces (VRS) using a locally developed scoring scheme, which reflects their distribution and number\textsuperscript{20}. VRS number were scored as follows: Centrum semiovale and in the external and extreme capsule: 0= none, 1=less than 5 in either hemisphere, and 2 ≥ 5 in either hemisphere; mesencephalon: 0 = absent and 1= present; basal ganglia: 0= only in the substantia innominata and <5 on either side, 1= only in the substantia innominata and >5 dilated VRS on either side; 2=>0≤5 in lentiform nucleus on either side, 3=5–10 in lentiform or >0<5 in caudate nucleus on either side, 4=≥10 in lentiform nucleus and ≤5 in caudate nucleus on either side, 5=≥10 in lentiform nucleus and >5 in caudate nucleus on either side. These
findings are also reported (in more detail) in a parallel paper (Paranthaman et al Biological Psychiatry in press)

4.3.4 Gluteal fat biopsy:

A single subcutaneous gluteal fat biopsy was obtained from each subject by using 3 to 5 ml of 2% lignocaine, allowing tissue (2x1.5x1.5cm) to be harvested and placed immediately in ice-cold physiological saline solution (PSS). Small arteries 100 to 150μm in diameter were dissected from the tissue and carefully cleaned under a dissecting microscope. Isolated vessels were then transferred to an arteriographic bath chamber (Living systems Instrumentation) and cannulated as described previously\(^{21}\). The chamber was placed on the stage of an inverted microscope and superfused with PSS, gassed with 5% CO\(_2\)/95% air (pH 7.4 to 7.45) at 37°C, at a superfusion rate of 20mL/min. PSS composition was (mM) 139NaCl, 4.7KCl, 25NaHCO\(_3\), 1.17KH\(_2\)PO\(_4\), 1.17MgSO\(_4\), 0.026EDTA, 1.6CaCl\(_2\) and 5.5glucose. Lumen diameter was recorded with the use of a Video Dimension Analyser (Living Systems Instrumentations) connected to a chart recorder. Vessels were connected to a pressure servo system (Living Systems Instrumentation) and pressurised to 60mmHg; any vessel with a leak was discarded. Vessels were allowed to equilibrate to 37°C for 1 hour and then challenged with 60mM KPSS until a steady vasoconstriction was attained.

4.3.5 Pressure myography: Pharmacological assessment

After viability assessment with KPSS, each vessel was stimulated as follows: (1) Cumulative addition of norepinephrine (Sigma-Aldrich), \(10^9,3\times10^9,10^8,3\times10^8,10^7,3\times10^7,10^6,3\times10^6,10^5\)M with 3 to 5 minutes incubation per concentration. (2) Endothelial function was assessed via the cumulative response to Acetylcholine (Ach) (Sigma-Aldrich) achieved by adding serial
concentrations (M) 10^{-9}, 3 \times 10^{-9}, 10^{-8}, 3 \times 10^{-8}, 10^{-7}, 3 \times 10^{-7}, 10^{-6}, 3 \times 10^{-6}, 10^{-5} to a preconstricted vessel with 10^{-5} norepinephrine. (3) After 1 hour of incubation with 5 \times 10^{-5} M L-NG-monomethyl-arginine (L-NMMA) (Sigma-Aldrich), an inhibitor of nitric oxide synthase, the response to Ach was repeated as in (2).

4.3.6 Pressure myography: Passive structure assessment

Passive structure was determined for each vessel after completion of the functional studies. The vessel was superfused for 20 minutes with Ca-free PSS containing 2mmol/l ethylene glycol-bis (-amino ethyl ether)-N,N,N',N'-tetraacetic acid to ensure the vessels were devoid of active tone. To determine the structural and mechanical properties of the arteries, the intraluminal pressure was reduced to 3mmHg to determine the unstressed diameter and then increased in steps to 20, 40, 60, 80, 100, 120, 140, 160 and 180mmHg.

Calculations

The wall/lumen ratio was calculated as WT/D x 100, where WT is wall thickness and D is lumen diameter. Wall cross sectional area (CSA) was calculated as: CSA=\pi(D + 2WT /2)^2 - \pi(D/2)^2.

Stress (\sigma) = P \times D / 2WT, where P is pressure and 1mmHg = 1334dyn/cm^2.

Strain (\varepsilon) = (D – D_0) / D_0, where D_0 is the lumen diameter at 3mmHg.

4.4 Statistical analysis

Statistical analysis was performed using SPSS™ for Windows. Data are expressed as the Mean ± standard error of the mean. Statistical comparisons were made using the Student unpaired t-test or, for multiple readings across a range of intraluminal pressures or a cumulative dose response, a multiple analysis of variance (ANOVA). p<0.05 was considered significant. Simple linear
univariate analysis was performed using statsdirect™ programme to assess the potential influence of confounding variables (age, smoking, civic status, alcohol intake, cholesterol subfractions, systolic and diastolic blood pressure, fasting glucose, history of diabetes, hypertension or ischaemic heart disease and treatment with Angiotensin converting enzyme (ACE) inhibitors, Angiotensin II Receptor I blockers (ARB) or Calcium channel blockers (CCB)) on endothelial function and wall structure (wall thickness and cross sectional area).

**Statement of responsibility**

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.
4.5 Results

The baseline characteristics of the study participants are presented in Table 1. There were no significant differences between the two groups apart from MADRS score which was higher in the depressed group and a greater degree of married participants in the control group. Blood pressure tended to be higher in the control group, which also had a lesser degree of statin use, but neither difference was significant. 3 (18%) patients in the depressed group had a history of ischaemic heart disease compared with 2 (13%) control participants. None of the participants had a history of stroke. 14 (88%) of the participants in the depressed group were on antidepressants. Of these, eight participants were on Selective Serotonin Reuptake inhibitors (SSRIs) alone or in combination with tricyclics (two subjects). Four participants were on Noradrenergic and specific serotonergic antidepressants (NaSSAs) and two were taking Selective Noradrenaline Reuptake inhibitors (SNRIs). One participant was prescribed Lithium and four participants were taking anxiolytic/hypnotic drugs.

4.5.1 Neuroimaging

VRS in the basal ganglia and the sub insular region were significantly higher in the depressed group (Table 2) compared with controls. There were no statistically significant differences in the regional Scheltens scores.

4.5.2 Subcutaneous small artery function (Figures 1 and 2)

There were no differences in contractility to norepinephrine when patients with depression were compared with control participants. Endothelial function, assessed by measuring the ability of Ach to dilate a preconstricted vessel, was significantly reduced in depressed patients (p=0.028 for multiple ANOVA comparing multiple points of two cumulative dose responses between depressed patients and control participants). Final resting diameter after $10^{-5}$M Ach also differed
significantly between the groups: Depressed patients: 84±4% vs Control participants 96±1.4%, p=0.012). 7 patients with depression and 12 control participants underwent assessment of endothelial function following LNMMA (inhibitor of nitric oxide synthase) incubation. There was a small reduction in relaxation following this protocol in both groups but arteries from depressed patients still exhibited less relaxation than the controls (p=0.015, multiple ANOVA). A difference in final resting diameter following maximal acetylcholine dilation after incubation with LNMMA was also seen (Depressed patients: 67±9% vs Control participants: 83±3%, p=0.17) but this was not significant.

Vasodilatory responses to acetylcholine within groups were also compared before and after incubation with LNMMA (Figure 2). In the control group there was a significant difference in final resting diameter after 10⁻⁵ M Ach (Before LNMMA incubation: 96±1.5% vs after LNMMA incubation: 83±3%, p=0.01). This difference was not significant when the cumulative dose response curves were compared using a multiple ANOVA (p=0.08). In the depressed patients there was a reduction in final resting diameter to 10⁻⁵ M Ach following LNMMA incubation but this was not significant (Before LNMMA incubation: 85±3% vs after LNMMA incubation: 67±9%, p=0.14). At lower doses of acetylcholine the cumulative dose responses appeared to approximate and overall the difference between the curves using a multiple ANOVA in this group was not significant (p=0.06).

4.5.3 Small artery structure and distensibility (Figures 3 and 4)

Small artery structure from subcutaneous fat was compared in 16 patients with depression and 14 control participants. Compared to arteries from control participants, those from patients with depression showed a significant increase in wall thickness (p=0.04, multiple ANOVA across pressure range 3mmHg to 180mmHg. At 100mmHg of intraluminal pressure: Control group wall
thickness: 24±1.4μm vs Depressed group wall thickness: 27±1.6μm, p=0.07) and medial cross sectional area (p=0.035, multiple ANOVA across pressure range 3mmHg to 180mmHg. At 100mmHg of intraluminal pressure: Control group wall cross sectional area: 10184±1029μm² vs Depressed group wall cross sectional area: 13295±1108μm², p=0.05). There were no significant differences in lumen diameter, wall to lumen ratio or small artery distensibility.

4.5.4 Univariate analysis

One-by-one univariate analysis was performed to evaluate the contribution of each variable which may be considered to have been a confounding factor in the differences seen in endothelial function and wall structure (for example: history of ischaemic heart disease, smoking history, age, etc). With regards to wall thickness there were no significant correlations between the potential confounding factors (including separation into depressed or non-depressed groups). Pertaining to endothelial function, separation into depressed or non-depressed groups was the only factor with a significant correlation co-efficient (r=0.58, p<0.001). There were a number of variables which were significantly associated with wall cross sectional area: Diastolic blood pressure (r=-0.45, p=0.02), triglycerides (r=-0.41, p=0.03), fasting plasma glucose (r=0.4, p=0.04). The correlation of group membership (depressed or non-depressed) with wall cross sectional area was r=0.34 with a significance of p=0.06.
4.6 Discussion:

This case control study has demonstrated that patients with late-life depression have impaired small subcutaneous fat arterial function and abnormal wall structure compared with control subjects. The findings cannot be explained by differences in traditional risk factors such as type 2 diabetes, ischaemic heart disease, HDL cholesterol, triglycerides or blood glucose. Blood pressure was higher in control participants, but this was not significant and indeed, the structural pattern of wall growth traditionally associated with hypertension was observed in depressed patients. Treatment with angiotensin converting enzyme inhibitors or calcium channel blockers which are known to influence small artery endothelial function\textsuperscript{22} or wall remodeling\textsuperscript{23-25} was similar between the two groups of patients studied. Depressed patients were more likely to be taking statins which can improve small artery endothelial function\textsuperscript{26}, but despite this, relaxation to acetylcholine was impaired compared with control participants.

The vascular findings are consistent with previous research highlighting impaired vascular function in late-life depressive disorder\textsuperscript{11-14}, although ours is the first study to examine structure and function in small arteries. In type 2 diabetes or in patients with metabolic syndrome, small artery endothelial dysfunction is caused by down-regulation of nitric oxide synthase\textsuperscript{27, 28}. In this study a reduction in NO bioavailability appeared to largely, but not exclusively, account for the observed endothelial dysfunction and this may reflect additional alterations to endothelium derived hyperpolarising factor release or the cyclo-oxygenase pathway.

We also report significant structural alterations to the small arteries in depression. The changes are characterized by hypertrophic growth of the small artery wall, more commonly seen in patients with type 2 diabetes\textsuperscript{28-30}. Thus in depressed patients, despite arterial remodeling there was preservation of the lumen with an increase in wall thickness and wall cross sectional area.
This finding may be particularly relevant as hypertrophy of the small artery wall is one of the most sensitive prognostic indicators for subsequent incidence of cardiovascular events\textsuperscript{31}.

There are a number of explanations for our small artery findings. The nature of the gluteal fat biopsy does not allow a large number of participants to be studied and thus the findings may be unique to this cohort. However, our findings of endothelial dysfunction concur with previous studies which have identified late-life depression as a risk factor for this defect. The structural changes to the small arteries may reflect an additional abnormality of small artery autoregulation, as hypertrophic remodeling has previously been found in tandem with damage to autoregulatory capacity\textsuperscript{28, 32}. In patients with Type 2 diabetes a dual process of loss of autoregulation and hypertrophic remodeling has been proposed as a contributory factor in the development of target organ damage\textsuperscript{33, 34}. Clearly, the small arteries studied were from a different circulation to those which may be involved in the pathogenesis of vascular depression, which are presumably small penetrating pial arteries. As such the findings are associative, but it should be noted that the structural and functional changes which occur in human subcutaneous small arteries in response to hypertension and diabetes\textsuperscript{28} are mirrored in mesenteric\textsuperscript{35}, coronary\textsuperscript{36} and cerebral\textsuperscript{17} arteries.

In this study the Scheltens measure of white matter lesions (WML) did not differentiate depressed subjects from controls. This is at odds with the majority of studies in this area\textsuperscript{37}, but our findings are not unprecedented\textsuperscript{38, 39} and are in keeping with a general trend to analyse white matter damage using automated functions\textsuperscript{40} or VRS scores. Excessive VRS in the basal ganglia are associated with treatment resistance in late-onset depression\textsuperscript{8}, vascular dementia\textsuperscript{20} and asymptomatic subjects at risk of stroke\textsuperscript{10} and in these studies the amount of dilated VRS predicted diagnosis (depression or stroke) better than WML scores.
This study has a number of limitations. The small numbers of participants limits the interpretative value of the findings in respect of the WML, VRS and the small artery data. There is absence of reliable data regarding duration of symptoms and this also limits discussion of potential interactions between WML and chronicity of depression. We also acknowledge that confounding by unmeasured variables could play a role. For example, nutrition might be different (inferior) in depressed people and adversely affect microvascular function. There was an inverse correlation between wall cross sectional area and both diastolic blood pressure and triglycerides. Triglyceride levels were lower in the depressed group and while this may be due to greater statin use, neither difference (triglyceride level or use of statin) was statistically significant. As such it is difficult to interpret these observations and there are no published studies on the effect of triglycerides and small artery structure. There was also a positive correlation between wall cross sectional area and fasting glucose, which was equivalent between the two groups. Overall the cross-sectional nature of the study limits understanding of causal relationships and treatment with antidepressants is a potential confounder. However, although an initial study with paroxetine suggested that this drug may cause reduction in nitric oxide bioavailability\textsuperscript{41}, subsequent studies have shown that the SSRIs most commonly used in this study improve vascular endothelial function\textsuperscript{42-45}. Thus, both paroxetine\textsuperscript{42,43} and citalopram\textsuperscript{45} increase plasma nitric oxide metabolic end products in control participants and in patients with depression. Further, sertraline improves brachial artery flow mediated dilation in depressed patients with coronary artery disease\textsuperscript{44}.

Despite these limitations, this study has demonstrated altered vascular function and structure in subcutaneous small arteries from patients with late life depression. Our findings are in agreement with an emerging consensus that depression in later life is associated with vascular
abnormalities, not explained by traditional cardiovascular risk factors. Impaired subcutaneous small artery endothelial function and abnormal wall growth were seen in tandem with dilated Virchow Robins Spaces in the basal ganglia which are a marker of cerebral microangiopathy. Thus, this study provides a tentative physiological basis for the findings that vasoprotective drugs can improve the prognosis of late-life depression, and if replicated, could lead to a dual approach to treatment based on both depression management and vasoprotective agents.

Endothelial dysfunction can be reversed by treatment with statins, angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs). Perhaps even more pertinently, ACE inhibitors, ARBs and calcium channel blocking drugs are also able to reverse hypertrophic remodeling in Type 2 Diabetes, a similar small artery phenotype to that which we have found in the depressed patients. Beyond the implications for the development of therapies to lessen the burden of vascular disease in depression, this work also establishes a basis for additional mechanistic and functional studies into the effects of depression on the microcirculation.

4.7 Perspective:

Late-life depression is increasingly viewed as a vascular illness due to patients exhibiting characteristic microvascular white matter brain lesions and in-vivo large artery endothelial dysfunction. We undertook the first small artery study in this patient population and used magnetic resonance imaging to demonstrate the presence of microvascular lesions in the brain. Although risk factors associated with small artery damage were comparable between the groups studied, depressed patients showed both abnormal growth of the subcutaneous gluteal small artery wall and endothelial dysfunction. The functional damage was not entirely explained by damage to nitric oxide bioavailability which is usually responsible for vascular dysfunction,
suggesting additional pathogenic mechanisms. The arteries studied were from a different
circulation to that involved in the disease process, which are presumably penetrating pial arteries.
However, previous studies of the subcutaneous gluteal small artery have shown that this
microcirculation undergoes similar adaptive processes to disease to those in arteries from
cardiac, cerebral and mesenteric circulatory beds. As such, our findings are therefore in support
of a generalized microvascular pathology in late-life depression.
Sources of Funding

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Disclosures

The authors declare no conflict of interest.
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Figure 1: A, Contraction to Noradrenaline in small arteries from control subjects (□, n=15) and patients with depression (♦, n=16) B, Cumulative dose responses of pre-constricted arteries to increasing doses of Acetylcholine in control subjects (□) and patients with depression (♦) (p = 0.028, multiple ANOVA). C Cumulative dose responses of pre-constricted arteries to increasing doses of Acetylcholine following incubation with LNMMA in control subjects (□, n = 12) and patients with depression (♦, n = 7) (p=0.015, multiple ANOVA).

Figure 2: A, Cumulative dose responses of pre-constricted arteries to increasing doses of Acetylcholine in control subjects before incubation with LNMMA (□, n=15) and after incubation with LNMMA (■, n=12) (p = 0.08, multiple ANOVA). B Cumulative dose responses of pre-constricted arteries to increasing doses of Acetylcholine in depressed patients before incubation with LNMA (◊, n = 16) and after incubation with LNMMA (♦, n = 7) (p = 0.06, multiple ANOVA).

Figure 3: Patients with depression have significantly greater wall thickness (B) and cross sectional area (D) of the wall when compared with control participants. There is no significant difference in lumen diameter (A) or the wall to lumen ratio (C) □:control subjects (n=14); ♦: patients with depression (n=16). * p < 0.05 (comparison of depressed patients against control participants: Wall thickness: p = 0.04. Cross sectional area: p = 0.035, multiple ANOVA)

Figure 4: A: Strain-pressure relations in small arteries from control subjects(□, n=14) and patients with depression (♦, n=16). B: Wall stress vs intraluminal pressure in small arteries from control subjects (□) and patients with depression (♦) C: Stress-strain relations in small arteries
from control subjects (□) and patients with depression (♦). All points are shown ± standard error bars. There is no statistical significance between the groups.

Table 1: Characteristics of study sample (t test for parametric data and Mann-Whitney test for non parametric data and Chi-squared tests for categorical data). Data are mean (Standard deviation) unless specified

* Montgomery Asberg Depression Rating Scale
†: Mini-Mental State Examination
‡: Number of smokers per group
§: Pack–years of smoking are for those who had ever smoked

Table 2: Neuroimaging: Virchow Robin Spaces (VRS) Scores and Scheltens scores. Data are mean ± Standard deviation
1A

Percentage of Resting diameter (%)

Log [NA] (M)

□: Control participants
♦: Depressed patients

1B

Percentage relaxation (%)

Log [Ach] (M)

*: Significant difference
Control participants
□: Before LNMMA
■: After LNMMA

Depressed patients
◊: Before LNMMA
♦: After LNMMA
3A

3B

□: Control participants
♦: Depressed patients
3C

Intraluminal pressure (mmHg) vs. wall to lumen ratio (%).

3D

Intraluminal pressure (mmHg) vs. cross-sectional area (μm²).
4A

4B

4C

□: Control participants
◆: Depressed patients
<table>
<thead>
<tr>
<th>Demographic variable</th>
<th>Depressed (n=16)</th>
<th>Controls (n=15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>71.8 ± 4</td>
<td>72.1 ± 5.9</td>
<td>0.92</td>
</tr>
<tr>
<td>Women, n(%</td>
<td>12(75)</td>
<td>9(60)</td>
<td>0.46</td>
</tr>
<tr>
<td>Married, n(%)</td>
<td>2(12.5)</td>
<td>11(73.3)</td>
<td>0.001</td>
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<tr>
<td>MADRS * Score</td>
<td>14.8 ± 11.1</td>
<td>2.5 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MMSE† Score</td>
<td>28.9 ± 1</td>
<td>29.2 ± 1.1</td>
<td>0.35</td>
</tr>
<tr>
<td>Alcohol units/week</td>
<td>5.3 ± 12.6</td>
<td>8.1 ± 9.8</td>
<td>0.48</td>
</tr>
<tr>
<td>Smoking status‡ ,n(%)</td>
<td>4(16)</td>
<td>2(9.5)</td>
<td>0.65</td>
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<tr>
<td>Smoking pack years §</td>
<td>25.9 ± 27.8</td>
<td>17 ± 23</td>
<td>0.34</td>
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<td>Taking statin, n(%)</td>
<td>10(62.5)</td>
<td>4(26.7)</td>
<td>0.07</td>
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<tr>
<td>Hypertension, n(%)</td>
<td>11(68.8)</td>
<td>12(80)</td>
<td>0.69</td>
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<tr>
<td>Diabetes, n(%)</td>
<td>2(12.5)</td>
<td>3(20.0)</td>
<td>0.65</td>
</tr>
<tr>
<td>History of Ischaemic Heart Disease, n(%)</td>
<td>3 (18)</td>
<td>2(15)</td>
<td>1.0</td>
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<td>Body mass index (kg/m²)</td>
<td>28.9 ± 4.6</td>
<td>32.2 ± 17</td>
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<td>Waist circumference (cm)</td>
<td>97.9 ± 13.3</td>
<td>97.3 ± 10.4</td>
<td>0.89</td>
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<td>Systolic Blood Pressure (mm Hg)</td>
<td>139 ± 14.7</td>
<td>148 ± 18.1</td>
<td>0.15</td>
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<tr>
<td>Diastolic Blood Pressure (mm Hg)</td>
<td>75.1 ± 12.1</td>
<td>76.7 ± 9.4</td>
<td>0.9</td>
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<td>Total cholesterol (mg/dL)</td>
<td>181 ± 34</td>
<td>174 ± 27</td>
<td>0.6</td>
</tr>
<tr>
<td>High Density Lipoprotein (mg/dL)</td>
<td>61 ± 27</td>
<td>54 ± 11</td>
<td>0.48</td>
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<tr>
<td>Triglycerides (mg/dL)</td>
<td>132 ± 45</td>
<td>141 ± 79</td>
<td>0.64</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>104 ± 31</td>
<td>107 ± 27</td>
<td>0.68</td>
</tr>
<tr>
<td>Treatment with ACE,ARB or CCB</td>
<td>(%)</td>
<td>6(37.5)</td>
<td>8(53.3)</td>
</tr>
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</table>
Table 2

<table>
<thead>
<tr>
<th>Region</th>
<th>Depressed (n=16)</th>
<th>Controls (n=15)</th>
<th>Between group statistic</th>
<th>P value</th>
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<tr>
<td>Basal Ganglia VRS</td>
<td>3.9 ± 1.7</td>
<td>2.5(1.6)</td>
<td>Mann Whitney U=143.5</td>
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<td>Centrum semiovale VRS</td>
<td>1.1 ± 0.9</td>
<td>0.9(0.9)</td>
<td>Mann Whitney U=226.0</td>
<td>0.69</td>
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<tr>
<td>Sub-insular VRS</td>
<td>1.3 ± 0.8</td>
<td>0.5(0.8)</td>
<td>Mann Whitney U=169.5</td>
<td>0.05</td>
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<tr>
<td>Mesencephalon VRS</td>
<td>0.4 ± 0.5</td>
<td>0.3(0.5)</td>
<td>Mann Whitney U=198.0</td>
<td>0.86</td>
</tr>
<tr>
<td>Scheltens PVH score</td>
<td>2.4 ± 1.8</td>
<td>2.3(2.6)</td>
<td>Mann Whitney U=252.5</td>
<td>0.54</td>
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<tr>
<td>Scheltens deep white matter score</td>
<td>5.5 ± 4.4</td>
<td>6.5(6.4)</td>
<td>Mann Whitney U=258.0</td>
<td>0.92</td>
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<tr>
<td>Scheltens infratentorial score</td>
<td>0.52 ± 1.4</td>
<td>0.86 ± 1.8</td>
<td>Mann Whitney U=211.5</td>
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<td>Scheltens basal ganglia score</td>
<td>0.8 ± 1.2</td>
<td>1.52 ± 3.2</td>
<td>Mann Whitney U=255.5</td>
<td>0.95</td>
</tr>
</tbody>
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Chapter 5: Age at onset and vascular pathology in late life depression

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Age at onset and vascular pathology in late life depression

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Key words: Age at onset, depression, vascular
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Conflict of Interests: Marietta Scott was concurrently employed by AstraZeneca UK plc whilst working on this study. Whilst Dr. Scott was not working in the neuroscience area, AstraZeneca is actively employed in discovering, developing, manufacturing and marketing prescription medicines in six areas of healthcare including the neurosciences. All other authors report no biomedical financial interests or potential conflicts of interest.

Previous Presentations: A preliminary version of this study was presented as a free communication at the International Psychogeriatric Association 2010 International meeting.
5.1 Abstract:

**Objective:** There is considerable evidence to suggest that late onset depression may be aetiologically distinct from early onset depression. The aim of this study was to compare vascular function and MRI defined brain ischaemic changes between early-onset depressed (EOD) and late-onset depressed (LOD) subjects.

**Design:** Case control study

**Participants:** 25 subjects with late life depression recruited from secondary care were divided into groups with EOD (<60 years, 11 subjects) and LOD (>60 subjects, 14 subjects).

**Measures:** All subjects underwent a variety of vascular assessments including Pulse Wave Analysis (PWA), Pulse Wave Velocity (PWV), Carotid Intima Media Thickness (IMT) and Magnetic Resonance Imaging (MRI) to assess white matter hyperintensities (WMH).

**Results:** The mean age of LOD subjects was 71.3 ± 4.0 years and EOD was 73.6 ± 4.7 years (p=NS). There were no baseline differences in vascular risk or sociodemographic variables. LOD subjects had significantly higher common carotid IMT (EOD 0.06 (0.01); LOD 0.09 (0.02), P = 0.02), carotid plaques (EOD 2.1 (1.1); LOD 5.4 (3.9), P=0.02) and peripheral augmentation index (EOD 81.7 (7.9); LOD 96.2 (21.6), P= 0.04) when compared to early onset subjects, indicating more vascular pathology. There were no group differences in WMH. Age at onset of depression was positively correlated with peripheral augmentation index, common carotid IMT and plaque index.

**Conclusions:** This study suggests that elderly subjects with LOD have greater vascular impairment when compared to those with an early onset illness. Whether
preventing vascular disease at an earlier age may decrease the risk of last onset depression is a potential area for future research.
5.2 Introduction:

Researchers have been interested in the concept of age at onset of mood disorders as it is thought that aetiology may vary by age of onset. It has even been suggested that classifying mood disorders by age at onset may be more useful than classification by polarity (Benazzi, 2009). Studies have shown not only differences between early and late onset late life depression but also between early and late onset adult depression (Bukh et al., 2009).

The aetiology of late life depressive disorder is heterogeneous. There is considerable evidence to show that late onset depression (LOD) may be different from early onset depression (EOD). LOD patients have lower familial loading but have more medical comorbidity and vascular risk factors. These give rise to considerations that LOD and EOD depression may have different aetiologies and pathophysiology. For example, in LOD cerebrovascular disease may play a greater role compared to EOD, where neurodegeneration may be a long-term consequence.

A recent community-based study did not demonstrate any clinical differences between late and early onset depression and the distinction between EOD and LOD subtypes remains controversial. However, the evidence for cerebrovascular disease playing an important role in the pathogenesis of late life depression is abundant. White matter hyperintensities (WMH) which are surrogate markers for cerebrovascular damage are frequently increased in elderly depressed subjects, especially in those with LOD compared to EOD. This has led to the vascular depression hypothesis which proposes distinct clinical and neuroradiological appearances in such patients. Besides measurement of WMH, another approach is to assess the vasculature in depressed subjects directly. This is not possible with the cerebral circulation but there is a growing literature concerning systemic vascular changes in depressive disorder such
that a reasonable premise is brain changes in depressed patients, such as WMH, are likely to be due to a systemic vascular atheromatous process. Recent evidence supports a trait rather than state approach to the vascular depression hypothesis. Smith et. al.\textsuperscript{12} showed in a recent study of middle age and older adults with a mean age of 52 that higher carotid IMT, a measure of atheroma, was associated with a later onset of first depressive episode rather than the severity of current depressive symptomatology.

In older community living subjects, Tiemeier et al\textsuperscript{13} showed that carotid Intima Media Thickness (IMT), a measure of atherosclerosis was linked to late-life depressive disorder in a dose-response manner. The same investigators have also demonstrated that increased pulse wave velocity (PWV), a measure of arterial stiffness, is associated with late life depression\textsuperscript{14}. Augmentation index as measured by Pulse Wave Analysis (PWA) is another measure of arterial stiffness and is an independent predictor of vascular events\textsuperscript{15}.

The relationship between age of onset of depression and extra-cerebral vascular function in late life depression has not yet been examined and was the aim of this study. This study was part of a wider case control study of vascular factors in late life depression\textsuperscript{16} and in this report we present new data and some secondary analysis of the original data. We hypothesised that subjects with late onset depression will have more systemic vascular pathology and greater WMH compared to those with early onset illness. In addition, we predicted that there will be a correlation between peripheral vascular measures and WMH.

5.3 Methods:

Following Ethical Committee approval, patients with depressive disorder were recruited from the case registers of two sites in Greater Manchester, England.
(National Health Service secondary care providers, each participant being under the
care of a consultant psychiatrist). Approximately 25% of those eligible declined, and
20% had contraindications (discussed subsequently). There were no age or gender
differences between those who refused and those who agreed to participate. All
participants gave full informed consent. Early-onset illness (EOD) was defined as
having the first episode of major depression before age of 60 years and those who had
their first episode at or above the age of 60 years were defined as having a late onset
illness\textsuperscript{17} (LOD). Age of onset of first major depressive episode was determined by
patient interview, confirmation by treating psychiatrist and review of the hospital
psychiatric records. For some LOD subjects, this was their first episode of major
depression.

Subjects aged 60 and over at the time of assessment were included if they satisfied
criteria for past or present history of Depressive Episode, moderate or severe,
psychotic or non-psychotic (World Health Organisation, ICD10, 1992) as judged by
an ICD10 checklist and with confirmation by his or her psychiatrist; all were on stable
medication. Subjects with history of stroke, space occupying lesion, neuro-
degenerative disorders including Parkinson's disease, Dementia (World Health
Organisation, ICD-10, 1992), previous head injury with loss of consciousness, history
of another psychiatric disorder besides depression were excluded. Subjects with atrial
fibrillation (because of difficulty in interpreting pulse wave measures) or severe
valvular heart disease were also excluded. All studies were performed during a visit to
the Manchester Wellcome Trust Clinical Research Facility (CRF). Psychiatric
measures included WHO ICD-10 symptom checklist for Depressive Episode,
Montgomery Asberg Depression Rating scale (MADRS) for severity\textsuperscript{18} and the Mini-
Mental Status Examination (MMSE)\textsuperscript{19}. Physical health burden was measured by
Cumulative Illness Rating Scale – Geriatrics\textsuperscript{20} and vascular risk factors were rated using the Framingham stroke risk scale\textsuperscript{21}. Presence of metabolic syndrome was assessed according to the International Diabetes Federation criteria\textsuperscript{22}. Due to the complex protocol, subjects’ mental state might have changed between acceptance into the study and the last study measures and we did not have data on proportion of subjects currently or recently depressed.

5.3.1 Neuroimaging evaluation:

Protocol: Magnetic Resonance Imaging of the brain was carried out at 1.5T on a Philips Intera Achieva using a SENSE head coil. FLAIR (fluid-attenuated inversion recovery) and T1-weighted inversion Recovery (T1-IR) images were acquired as part of the imaging protocol. For both scans, 45 transverse slices (except in the case of one subject where 40 slices were used), 3.0mm thick with no slice gap were obtained using a field-of-view of 230x230mm, providing full coverage of the brain. Images were reconstructed using a matrix size of 256x256, yielding pixels of 0.9x0.9mm. Imaging parameters specific to the FLAIR sequence were: TR/TE/TI=11000/140/2800ms, echo train length = 53 and parameters specific to the T1-IR were: TR/TE/TI=3198/15/400ms, echo train length = 5.

Analysis:
Quantitative measures of WMH were undertaken using locally developed software (www.tina-vision.net). FLAIR images were registered to T1-IR images and re-sliced to correct for patient motion. In order to assign WMH voxels the resulting images were registered to a Talairach atlas\textsuperscript{23} and segmented to produce a mask containing only voxels within grey and white matter. This was applied to the median smoothed FLAIR maps and the resulting images used to produce maps of voxels containing WMH. The maps were quality controlled and manually edited where necessary to
remove non-WMH voxels. Volume of WMH (number of voxels x voxel volume) was calculated for the frontal, temporal, parietal and occipital Lobes. These volumes were normalised to head size by dividing by the scale factors between the T1-IR dataset and the Talairach atlas brain.

5.3.2 Vascular measures:

A) Pulse Wave Velocity:

This was performed after appropriate training by the same investigator who was not blind to participant’s group status in all cases. After resting supine for at least 5 minutes, aortic PWV was measured from Doppler flow signals obtained sequentially from the right carotid and femoral arteries using a noninvasive device (Micro Medical Ltd, Rochester, Kent, UK) with a 5MHz Doppler probe. The distance traveled by the pulse wave was measured over the surface of the body with a tape measure from the sternal notch to femoral arterial measurement site and from carotid artery measurement site to the sternal notch. PWV was calculated as the distance: transit time ratio and is expressed as meters per second.

B) Pulse Wave Analysis

SphygmoCor (PWV Medical, Sydney, Australia), a computerized and simple-to-use device was used to acquire peripheral artery pressure waveforms from the right radial artery noninvasively. The corresponding central aortic waveform was generated by using a generalized transfer function using computer software from which augmentation index (AIx) was derived. The peripheral pressure waveform was used to determine the peripheral augmentation index (pAIx), which is primarily determined by the intensity and timing of reflected pressure waves, thereby providing a measure of both arterial stiffness and vasodilation of small muscular arteries. This was computed by dividing the difference between the second systolic peak and the
diastolic pressure by the difference between the first systolic peak and the diastolic pressure and then multiplying by 100\textsuperscript{26}. The same investigator, who was aware of the subject’s group status, performed the assessment in all patients after appropriate training.

C) Carotid Ultrasonography:

The carotid arteries were evaluated with high-resolution B-mode ultrasonography using a Philips/ATL HDI 5000 ultrasound system. All the ultrasound scans were conducted by experienced vascular technologists blind to clinical data. Studies have shown that posterior (far) wall IMT seen with ultrasound reflects the anatomical intima /medial layer\textsuperscript{27}. The intima-media thickness was measured at 3 sites (far wall) on both sides: (1) Common Carotid artery (CCA) 1cm proximal to the beginning of the carotid bulb; (2) Within the carotid bulb; (3) Internal Carotid artery (ICA) 0.5cm distal to the flow divider. The measurements were repeated thrice at each site and the average was calculated\textsuperscript{27}. IMT of the each segment was calculated as the mean of the average IMT of the both the left and right sides.

Plaque measurements were also taken at these sites if present and/or at the site of the thickest plaque by taking a diameter reduction measurement and, if necessary, a velocity measurement. The degree of plaque was graded using the following criteria: Grade 0: no observable plaque; Grade 1: one small plaque (<30% lumen diameter loss); Grade 2: one medium plaque (30-50% lumen diameter loss) or multiple small plaques; Grade 3: one large plaque (50-69% lumen diameter loss) or multiple plaques with at least one medium plaque; Grade 4: one severe plaque (>70% lumen diameter loss) or multiple plaque with at least one large plaque; Grade 5: occluded. Four segments are graded according to these criteria: CCA, Carotid bulb, ICA and ECA. These grades are then summed for each side of the neck to give a plaque index, a
measure of focal plaque which has been found to be a valid and reproducible measure of carotid atherosclerosis\textsuperscript{28}.

5.4 Statistical analysis:

Statistical analysis was performed using Statistical Package for the Social Sciences (version 14.0, www.spss.com). Continuous variables are expressed as means ±SD and categorical variables as frequency and percentage. Comparison between two groups (late and early onset subjects) was performed using t tests for normally distributed, Mann Whitney for non normally distributed and Chi-squared tests for categorical data. Association between peripheral vascular measures and neuroimaging data was assessed using Pearson’s or Spearman correlation as appropriate.

5.5 Results:

11 patients with early onset depression and 14 patients with late onset depression were recruited. The baseline characteristics of the 25 study participants are presented in Table 1. As expected, the age of onset of depression significantly differed between the groups (EOD: 48.3 ± 6.0) vs. LOD: 72.0 ±4.2; p<0.001). Participants were mainly white, two thirds (68%) were women and the mean age was 72.4 years. MADRS and MMSE were similar between the two groups. There were no statistical differences between the early and late onset groups in terms of age, ethnicity, gender, smoking status, physical illness burden, and vascular risk factors (Table 1). Twenty two subjects were prescribed antidepressants with the majority prescribed SSRI’s .There was no difference in the proportion or the type of antidepressants that subjects were taking between the two groups.

The peripheral augmentation index as measured by pulse wave analysis was significantly higher in LOD group than EOD (96.2±21.6 vs. 81.7±7.9; p=0.04). There was no difference in central augmentation index and Pulse Wave velocity (PWV)
between the two groups (Table 2). Late onset patients had significantly higher common carotid IMT (p=0.006, p=0.018 after Bonferroni correction) and total atheromatous plaque (p=0.02; Table 2). The association between age at onset and common carotid IMT and plaque index remained significant after adjusting for potential confounders such as age, systolic blood pressure, body mass index and metabolic syndrome but the association with peripheral augmentation index disappeared.

Some neuroimaging data was lost because of technical problems. WMH volumetric data was available for all subjects in the early onset group and 9 subjects in the late onset group. There were no significant differences in vascular risk factors or demographic variables between those who had the WMH data and those who did not. There was no statistically significant difference in total WMH volumes or individual regional scores between the late and early onset groups (Table 3). WMH data was limited by small numbers and conclusions are hard to draw.

Age at onset positively correlated with PWA peripheral augmentation index (r=0.45; p=0.03), common carotid IMT (r=0.62; p=0.001) and plaque index (r=0.42; p=0.04). Likewise total WMH was positively significantly associated with common carotid IMT (r=0.53; p=0.04) and plaque index approached significant level (r= 0.42; p=0.07).

5.6 Discussion:

We found that even in the small numbers studied, elderly patients with LOD had poorer vascular function compared to those with EOD. This was seen most significantly for common carotid intima media thickness, carotid plaque index and PWA peripheral augmentation index. As far we know, our study is the first to compare vascular pathology by age at onset specifically in late life depression.
Although all the WMH volumes were greater in the LOD group, there were no significant group differences. Therefore the hypothesis of more white matter changes in the LOD was not supported. Total WMH score showed a moderate positive correlation with common carotid IMT and plaque index, suggesting a link between a peripheral atherosclerosis and cerebral ischaemia. However, this needs to be interpreted with caution as a large number of comparisons were done in a small sample.

5.6.1 White matter hyperintensities:

The lack of group differences in WMH lesion volumes is surprising as the extent of such lesions in late-life depression is known to be greater in late- than early-onset cases\textsuperscript{9}. However not all studies have reported group differences\textsuperscript{29,30}. The study is therefore not sufficiently powered to address WMH findings and another consideration is that strategic location rather than counts of WML may be particularly relevant in late-life depression\textsuperscript{31}.

5.6.2 Vascular findings:

We have previously shown that late life depression is associated with endothelial dysfunction and atherosclerosis\textsuperscript{16}. The present study adds to the above findings by showing a difference in vascular pathology between elderly subjects with early and late onset depression. Given the central role accorded to age at onset in the vascular depression hypothesis the results support the concept of vascular depression. We found that age at onset was associated only with common carotid IMT and not the other two carotid segments. This is consistent with the results of the study by Chen et al\textsuperscript{32} who found that CCA IMT was more strongly associated with depression than bifurcation or ICA IMT. Several studies have shown that vascular risk factors influence the wall thickening of the different carotid segments differentially due to
factors such as bifurcation geometry and differences in hemodynamics. However, and total plaque score was significantly higher in LOD subjects. It has been shown that irrespective of location carotid plaques increase the risk of stroke. In our study, common carotid IMT was associated with WMH which is consistent with the literature.

5.6.3 Potential mechanisms underlying study findings:

As this is a cross sectional study, we were not able to assess the temporal relationship between depressive symptoms and atherosclerosis. We cannot determine whether vascular dysfunction causes late onset depression or late onset depression directly interferes with vascular function. Atherosclerosis can contribute to depression onset in several ways such as hypothalamic-pituitary-adrenal axis over-activity, increased platelet activation and hypercoagulability and altered inflammatory responses. Depression independently contributes to the cause and progression of vascular disease by various mechanisms such as raised cortisol levels and impaired endothelial function.

The relationship between vascular disease and depression is bi-directional. In the Cardiovascular Health Study of 3,781 participants aged > 65 years, the presence of depressive symptoms at baseline was associated with increased CCA-IMT over 3 years. SSRI’s have been shown to reduce later re-infarction and mortality in patients averaging 60 years of age following a heart attack. Also, statins and calcium channel blockers reduce the progression of carotid IMT and lower cardiovascular events. It is also possible that another factor such as inflammation may be involved in both vascular dysfunction and depression. In this study, the groups were well matched in terms of demographics, illness burden and a range of vascular risk factors suggesting that late onset depression itself is linked to vascular dysfunction. This was further
supported by the positive relationship found between CCA IMT, peripheral augmentation index, plaque index and age at onset. However, only longitudinal epidemiological studies can determine whether vascular dysfunction precedes late onset depression. The finding of vascular impairment in late onset depression not explained by traditionally assessed vascular risk factors is of clinical importance whatever the direction of cause.

Research suggesting that vasoprotective drugs can improve the prognosis of late-life depression, if replicated, could lead to a dual approach to treatment based on both depression management and vasoprotective agents. Although preliminary, our study finding of more pronounced vascular pathology in late onset depression adds to the increasing evidence that vascular risk factors may be an important treatment target warranting further investigation for prevention of late life depression.

5.6.4 Study limitations:

The small sample size limited our statistical power as well as limiting the generalisability of the findings. The observed relationship between arterial function and age at onset of depression is associative rather than the causal. Data were not available on factors such as illness duration or number of episodes which could be determinative. Further, the evaluation of age at onset of depression could be subject to recall bias or omission in the clinical record, although all were under the care of specialist old age psychiatric services with a significant amount of available clinical data. Nevertheless a degree of inaccuracy in the determination of age at onset cannot be ruled out. Also, it may be wrong to assume that underlying aetiology varies by a specific cut off age when age at onset is used a dichotomous variable. Although using age at onset as a continuous variable is an alternative approach, it was decided to use a specific age cut-off age in this study in line with the majority of research in this field.
in order to allow comparison\textsuperscript{45}. Median age as a cut off has its own limitations\textsuperscript{46}. A cut off age of 60 is in line with consensus guidelines\textsuperscript{47} and previous research\textsuperscript{48}. We included subjects with past depression in the study as previous research has shown that the state of depression versus nondepression does not explain differences in vascular function in non-geriatric subjects\textsuperscript{49,50,51}. Whilst it is possible that vascular measures can be influenced by current mood, as discussed in the Introduction, a recent study by Smith et al\textsuperscript{12}, supports the view that vascular pathology (as assessed by carotid IMT) is associated more with onset of first depressive episode rather than severity of current depressive episode. Nevertheless we acknowledge the lack of data about actual mood at the time of the evaluation. Treatment with antidepressants is another potential confounder. Recent studies have shown that the SSRIs most commonly used in this study may protect or improve vascular endothelial function\textsuperscript{52,53}. Tricyclic antidepressants have cardiovascular effects including being arrhythmic, but only two of our subjects were taking them and removing them from the dataset made no difference to the results. Although current antidepressant use did not differ between the groups, we had no information on lifetime use of these medications. There could be other potential cofounders such as level of physical activity that was not measured in this study.

5.7 Conclusions:

Vascular pathology may be more closely associated with late than early-onset depression. Our sample size was small and so our findings should be regarded as preliminary and need to be replicated in studies with larger number of participants. Clarification of the association between vascular function and late life depression may lead to potentially new treatment paradigms such as use of vasoprotective drugs in depressive disorder. If replicated, the results suggest a new hypothesis for future
studies, that preventing vascular disease related to aging may decrease the risk of last onset depression.
References:


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Table 1: Demographic characteristics of study sample

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Early onset subjects (age at onset &lt;60) (n=11)</th>
<th>Late onset subjects (age at onset &gt;60) (n=14)</th>
<th>Between-group statistic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>71.3(4.0)</td>
<td>73.6(4.7)</td>
<td>df=23, t=-1.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>17(68)</td>
<td>13(61)</td>
<td>Fisher exact test</td>
<td>1.0</td>
</tr>
<tr>
<td>Age at onset</td>
<td>48.3(6.0)</td>
<td>72.0(4.2)</td>
<td>df=23, t=-11.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MADRS' Score</td>
<td>15.1(10.8) Range 11-34</td>
<td>14.6(10.2) Range 14-35</td>
<td>df=21, t=0.11</td>
<td>0.9</td>
</tr>
<tr>
<td>MMSE' Score</td>
<td>29.1(1.0)</td>
<td>29.1(1.1)</td>
<td>Mann Whitney U=64.5</td>
<td>0.5</td>
</tr>
<tr>
<td>CIRS-G score</td>
<td>7.9(4.0)</td>
<td>6.8(3.4)</td>
<td>df=20, t=0.75</td>
<td>0.5</td>
</tr>
<tr>
<td>Framingham score</td>
<td>12.3(2.5)</td>
<td>13.9(2.7)</td>
<td>df=22, t=-1.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Alcohol units/week</td>
<td>5.9(15.0)</td>
<td>5.6(13.3)</td>
<td>Mann Whitney U=68.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Smoking status†, n (%)</td>
<td>1(9.1)</td>
<td>3(21.4)</td>
<td>df=2, χ² =0.54</td>
<td>0.5</td>
</tr>
<tr>
<td>Smoking pack years‡</td>
<td>16.6(26.6)</td>
<td>22.5(25.9)</td>
<td>Mann Whitney U=64</td>
<td>0.5</td>
</tr>
<tr>
<td>Alcohol units/week</td>
<td>5.9(15.0)</td>
<td>5.8(14.0)</td>
<td>Mann Whitney U=68.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Taking statin, n (%)</td>
<td>6(54.5)</td>
<td>6(42.9)</td>
<td>Fisher exact test</td>
<td>0.7</td>
</tr>
<tr>
<td>Hypertension‡, n (%)</td>
<td>7(63.6)</td>
<td>8(57.1)</td>
<td>Fisher exact test</td>
<td>1.0</td>
</tr>
<tr>
<td>Diabetes‡, n (%)</td>
<td>1(9.1)</td>
<td>1(7.1)</td>
<td>Fisher exact test</td>
<td>1.0</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>30.4(4.1)</td>
<td>27.3(5.0)</td>
<td>df=23, t=1.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Waist circumference, cms</td>
<td>101.2(10.3)</td>
<td>92.9(15.4)</td>
<td>df=23, t=1.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Metabolic syndrome§, n (%)</td>
<td>9(81.8)</td>
<td>6(42.8)</td>
<td>Fisher exact test</td>
<td>0.1</td>
</tr>
<tr>
<td>Systolic Blood Pressure, mm Hg</td>
<td>139.6(11.6)</td>
<td>144.6(18.8)</td>
<td>df=23, t=-0.96</td>
<td>0.3</td>
</tr>
<tr>
<td>Diastolic Blood Pressure, mm Hg</td>
<td>77.1(9.5)</td>
<td>77.0(13.1)</td>
<td>df=23, t=0.70</td>
<td>0.5</td>
</tr>
<tr>
<td>Resting pulse rate</td>
<td>75.3(12.3)</td>
<td>71.6(9.1)</td>
<td>df=23, t=0.87</td>
<td>0.4</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.6(0.8)</td>
<td>5.2(0.8)</td>
<td>df=23, t=-2.8</td>
<td>0.1</td>
</tr>
<tr>
<td>High Density Lipoprotein (HDL), mmol/L</td>
<td>1.3(0.3)</td>
<td>1.7(0.8)</td>
<td>df=23, t=0.11</td>
<td>0.1</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.4(0.6)</td>
<td>1.5(0.4)</td>
<td>df=23, t=0.67</td>
<td>0.5</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.9(2.0)</td>
<td>5.1(0.5)</td>
<td>Mann Whitney U=43.5</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Data are mean (Standard deviation) unless specified (t tests for normally distributed data; Mann Whitney for non normally distributed; Chi-squared tests for categorical data)

1. MADRS, Montgomery Asberg Depression Rating scale
2. MMSE, Mini Mental State Examination
3. CIRS-G, Cumulative Illness Rating Scale – Geriatrics
4. Hypertension defined according to British Hypertension Society Guidelines (http://www.bhsoc.org)
5. Diabetes mellitus defined according to WHO criteria
6. Metabolic syndrome- Based on the definition provided by provided by International Diabetes Federation Epidemiology Task Force Consensus Group (Alberti et al. 2005)
†Current smokers; ‡ Pack-years of smoking are for those who had ever smoked
SI conversion factors: To convert total cholesterol to millimoles/liter multiply by 0.0259, High Density Lipoprotein to millimoles/liter multiply by 0.0259, Triglycerides to millimoles/liter multiply by 0.0113, Glucose to millimoles/liter multiply by 0.0555
Table 2 Vascular function measures (t tests for normally distributed data; Mann Whitney for non normally distributed data)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Early onset depression (n=11) Mean(SD)</th>
<th>Late onset depression (n=14) Mean(SD)</th>
<th>Between-group statistic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulse Wave analysis peripheral augmentation index</td>
<td>81.7(7.9)</td>
<td>96.2(21.6)</td>
<td>df=23, t=-2.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Pulse Wave analysis central augmentation index</td>
<td>30.6(7.8)</td>
<td>35.2(11.6)</td>
<td>df=23, t=-0.68</td>
<td>0.5</td>
</tr>
<tr>
<td>Pulse wave velocity</td>
<td>11.0(4.1)</td>
<td>12.0(3.3)</td>
<td>df=22, t=-0.69</td>
<td>0.5</td>
</tr>
<tr>
<td>Common carotid IMT</td>
<td>0.06(0.01)</td>
<td>0.09(0.02)</td>
<td>df=22, t=-3.1</td>
<td>0.018*</td>
</tr>
<tr>
<td>Bulb IMT</td>
<td>0.14(0.06)</td>
<td>0.15(0.06)</td>
<td>df=21, t=-0.36</td>
<td>0.7</td>
</tr>
<tr>
<td>Internal carotid IMT</td>
<td>0.10(0.05)</td>
<td>0.16(0.05)</td>
<td>df=20, t=-1.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Common carotid plaque</td>
<td>0.0(0.0)</td>
<td>0.6(1.0)</td>
<td>Mann Whitney U=45.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Internal carotid plaque</td>
<td>0.9(0.7)</td>
<td>1.9(1.7)</td>
<td>Mann Whitney U=43.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Bulb plaque</td>
<td>1.1(0.8)</td>
<td>2.0(1.3)</td>
<td>df=22, t=-1.8</td>
<td>0.08</td>
</tr>
<tr>
<td>External carotid plaque</td>
<td>0.1(0.3)</td>
<td>0.6(1.0)</td>
<td>Mann Whitney U=55.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Plaque Index(overall measure)</td>
<td>2.1(1.1)</td>
<td>5.4(3.9)</td>
<td>df=22, t=-2.3</td>
<td>0.02</td>
</tr>
</tbody>
</table>

1. IMT- Intima Media thickness
2. P value after Bonferroni correction

1 subject had data missing for PWV, 1 for plaque index and 3 had part of IMT data missing due to technical difficulty or non availability of vascular technician

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Table 3 WMH volumetric data (t test and Mann Whitney test as appropriate)

<table>
<thead>
<tr>
<th>White Matter Lesion Volume</th>
<th>Early onset depression (n=9) Mean(SD)</th>
<th>Late onset depression (n=11) Mean(SD)</th>
<th>Between-group statistic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cerebral WML volume</td>
<td>400.0(506.4)</td>
<td>810.4(856.3)</td>
<td>df=18, t=-1.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Frontal lobe WML volume</td>
<td>168.4(282.3)</td>
<td>335.1(482.7)</td>
<td>df=18, t=-0.97</td>
<td>0.3</td>
</tr>
<tr>
<td>Temporal lobe WML volume</td>
<td>31.7(47.6)</td>
<td>84.2(68.3)</td>
<td>df=18, t=-2.0</td>
<td>0.06</td>
</tr>
<tr>
<td>Parietal lobe WML volume</td>
<td>18.7(24.6)</td>
<td>85.8(110.5)</td>
<td>df=18, t=-1.8</td>
<td>0.1</td>
</tr>
<tr>
<td>Occipital lobe WML volume</td>
<td>5.7(8.1)</td>
<td>28.3(44.3)</td>
<td>Mann Whitney U=28.0</td>
<td>0.1</td>
</tr>
</tbody>
</table>

All white matter lesions (WML) volumes are corrected for head size and are expressed in mm$^3$
Chapter 6: Relationship of endothelial function and atherosclerosis to treatment response in late life depression

R. Paranthaman et al.,
(Accepted for publication in International Journal of Geriatric Psychiatry)
Relationship of endothelial function and atherosclerosis to treatment response in late life depression

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Key words: mood disorders, depression, old age, treatment response, endothelial function, atherosclerosis

Conflict of Interest: None

Number of words in abstract: 250

Number of words in text: 2575

Number of figures: 0

Number of Tables: 4
6.1 Abstract:

Objective: Treatment response in late-life depression has been linked to cerebrovascular disease notably via the vascular depression hypothesis. This study investigated the relationship between endothelial function and atherosclerosis and treatment response to antidepressant monotherapy.

Methods: Twenty five patients with late life depression were compared with 21 non-depressed control subjects in a case control study. Nine of the depressed subjects were responders to antidepressant monotherapy and 16 were not. Vascular measures included assessment of carotid intima media thickness (IMT) representing atherosclerosis, and biopsied small artery dilatation to acetylcholine to assess endothelial function in a subset of subjects.

Results: There were no group differences in vascular risk or sociodemographic variables. There was a significant group difference (Responders v Non-responders v Controls) on both IMT and endothelial function (P<0.01 and P<0.05 respectively) with a significant difference between controls and non-responders (P<0.001) on IMT and between controls and responders (P<0.05) and control versus non-responders (P<0.05) on endothelial function but no significant difference between responders and non-responders. On both IMT and endothelial function, there was a gradient across groups, with control subjects having best vascular structure or function, non-responders worse and responders in-between.

Conclusions: The results are consistent with a hypothesis that poorer antidepressant response in later life depressive disorder may be linked to underlying vascular dysfunction and pathology. The study is small and the results require replication but if confirmed, trials with vasoprotective medication aimed at improving vascular
function in order to alter the prognosis of late-life depression would be a rational development.
6.2 Introduction:

Late life depressive disorder has a poor prognosis. Chronicity affects at least a third of patients and this can lead to wide range of negative outcomes including increased mortality (Cole et al., 1999). Several factors such as severity of symptoms, social isolation, late age at onset, family support, chronic physical illness, low socioeconomic status, living alone and non-availability of a confidant have been linked to poor prognosis (Baldwin et al., 2006).

Recent interest has examined the influence of brain white matter hyperintensities (WMH) on treatment response. Several studies have shown that WMH is associated with poor treatment response (Hickie et al., 1995; Simpson et al., 1998; Patankar et al., 2007; Alexopoulos et al., 2008) and that progression of WMH is likewise linked to poorer long term outcome (Taylor et al, 2003) but there are negative studies (Krishnan et al., 1998; Jansen et al., 2007). More recently, older depressed patients who failed to remit following treatment with antidepressant medication had significantly greater MRI signal hyperintensity burden than both patients who remitted and elderly comparison subjects (Gunning-Dixon et al., 2010), lending further support for a relationship between WMH and antidepressant treatment response.

Given that the long term prognosis of late-life depression is poor and has changed little over the years despite the availability of new antidepressants, the role of vascular function and disease in treatment response in depressive disorder is of both theoretical and practical relevance as WMH are most likely to due to vascular damage or ischaemia (Baldwin, 2005; Culang-Reinlieb et al., 2010). It may, for example, lead to new treatment paradigms such as deploying vasoprotective drugs (Alexopoulos, 2006; Taragano et al., 2005).
There is a range of measures to assess vascular function and pathology. This study focused on endothelial function and atherosclerosis as these are two potential mechanisms underlying WMH which in turn have been associated with treatment resistance in late life depression (Culang-Reinlieb et al., 2010). Furthermore, it has been suggested that endothelial dysfunction is a potential treatment target in late life depression (Isingrini et al., 2009). In addition, carotid Intima Media Thickness (IMT) is a validated measure of atherosclerosis which has also been specifically linked to the aetiology of late life depression (Tiemeier et al, 2004; Chen et al, 2006; Paranthaman et al, 2010).

Direct assessment of endothelial function of small resistance vessels within the brain is not feasible. However, conditions such as hypertension and diabetes which predispose to endothelial dysfunction usually have effects on multiple vascular beds (Di Napoli & Papa, 2005). Therefore, it is likely that endothelial function in one small vessel bed is mirrored in other areas. Accordingly, we have developed a technique which has been successfully applied to assess endothelial function in small groups of subjects (Malik et al 2005). This allows for the direct assessment of endothelial function following a biopsied sample of small arteries from the gluteal area. Following dissection, pharmacological challenge is applied in vitro (Aalkjaer et al., 1987; Greenstein et al., 2010). As far as we know, no studies as yet have examined the association between endothelial function, atherosclerosis and treatment response in late life depression. We hypothesised that poorer endothelial function as measured by in vitro analysis of small vessels acquired from gluteal fat biopsy and atherosclerosis as measured by carotid IMT would be associated with poor response to antidepressant monotherapy.
6.3 Methods:
As described elsewhere (Paranthaman et al., 2010), following ethical committee approval, 25 subjects with late life depression were recruited from case registers of two secondary care sites in greater Manchester. 21 Control subjects were recruited from spouses or partners of depressed subjects or via advert in local community centres. All of the participants gave informed consent. All studies were performed during a visit to the Manchester Wellcome Trust Clinical Research Facility.

6.3.1 Inclusion and Exclusion Criteria:
We recruited patients aged 60 and over who fulfilled criteria for past or present history of a depressive episode, moderate or severe, psychotic or nonpsychotic (World Health Organisation, ICD-10, 1992). All patients in the depressed group were under the care of secondary care psychiatric services. Patients with a history of stroke, space-occupying lesion, neurodegenerative disorders (including Parkinson’s disease), dementia, previous head injury with loss of consciousness, or a history of another psychiatric disorder besides depression were excluded, as were those with severe valvular heart disease or who were taking warfarin (because of contraindications to scan or biopsy). Control participants had no previous history of psychiatric disturbance and had stable medical health.

6.3.2 Definition of Treatment Response:
All subjects completed the Massachusetts General Hospital Antidepressant Treatment Response Questionnaire (MGH ATRQ) (Chandler et al., 2010), a valid and reliable self-rated scale, to gather data on dose and duration of antidepressant treatment. This data was supplemented by data from the treating psychiatrist and review of hospital psychiatric records. All the above treatment data was used to compute the degree of treatment resistance using two models of staging- Thane and Rush staging (TR-S)
method (Thase & Rush, 1995) and Massachusetts General Hospital staging (MGH-S) method (Fava, 2003). The TR-S method (Thase & Rush, 1995) uses a 5 stage categorical approach: 0 = response to monotherapy; 1 = nonresponse to antidepressant monotherapy; 2 = non-response to two trials of monotherapy with drugs of different classes; 3 = stage 2 plus failure to respond to one augmentation strategy; 4 = stage 3 plus failure of a second augmentation strategy; and 5 = stage 4 plus failure to respond to one course of electroconvulsive therapy (ECT). MGH-S generates a continuous variable which represents degree of treatment resistance taking into account adequacy, intensity and optimization of various treatments (Petersen et al., 2005). A score of 0 in the MGH-S indicates response to monotherapy and higher scores indicate greater resistance to treatment.

The vast majority of patients were allocated to stages 0 and 1 and only a very few to stages 2 and above in the TR-S (Table 1a). Similarly, the vast majority of patients scored 0 and 1 in the MGH-S and only a few scored between 1.5 and 5 (Table 1b). The mean TR-S score was 1.2 (±1.3) and the mean MGH-S score was 1.3(±1.6) and the two were highly correlated (Pearson’s r=0.971; p=<0.001). So the two measures were amalgamated to dichotomise the depressed subjects to two groups: responder to monotherapy versus non-responder as follows. Responders to monotherapy—Patients in stage 0 of TR-S and score 0 in the MGH-S; Non-Responders to monotherapy—Patients from levels 1–5 in the TR-S and scores 1 and above in the MGH-S.

6.3.3 Gluteal Fat Biopsy of Resistance Vessels:

A subset of 16 depressed subjects and 15 controls underwent gluteal fat biopsy to assess endothelial function. A single subcutaneous gluteal fat biopsy was obtained using 3 to 5 ml of 2% lignocaine, allowing tissue (2x1.5x1.5cm) to be harvested. Small arteries 200 to 250μm in diameter were dissected from the tissue and isolated.
vessels were then transferred to an arteriographic bath chamber and cannulated. Lumen diameter was recorded with the use of a Video Dimension Analyser (Living Systems Instrumentations). After viability assessment with potassium enriched physiological saline small arteries were preconstricted with $10^{-5}$ Norepinephrine. Endothelial function in the small arteries was then studied by constructing a cumulative dose response to Acetylcholine. The maximal vasodilation in response to a concentration of $10^{-5}$M of Acetylcholine was taken as a surrogate marker for endothelial function.

6.3.4 Intima Media Thickness (IMT):

All study subjects underwent an evaluation of their carotid arteries with high-resolution B-mode ultrasonography using a Philips/ATL HDI 5000 ultrasound system. IMT was measured by two-dimensional ultrasound at 3 sites (far wall) on both sides: (1) Common Carotid artery (CCA) 1cm proximal to the beginning of the carotid bulb. (2) Within the carotid bulb; (3) Internal Carotid artery (ICA) 0.5cm distal to the flow divider. The measurements were repeated thrice at each site and the average was calculated (32). An overall measure of IMT was calculated as the average of the CCA IMT, bifurcation IMT, and ICA IMT of both sides (Bots et al., 1993).

Both the gluteal fat biopsy and Carotid ultrasound scans were conducted by investigators who were blind to the subject’s group status.

6.4 Statistical analysis:

Statistical analysis was performed using Statistical Package for the Social Sciences database (version 16.0, www.spss.com ). One way Analysis of variance (ANOVA) was used to identify any significant differences in endothelial function between the 3 groups (Controls, responders to monotherapy and non responders to monotherapy) followed by a posteriori Bonferroni testing to allow identification of between group
differences. As carotid IMT data was non normally distributed, Kruskal–Wallis test was used followed by post hoc testing with Mann-Whitney test to identify between group differences. Multiple regression analysis with forward stepwise selection of variables was used to determine predictors of outcome with TR-S/MGH-S as dependant variables.

6.5 Results:

Nine subjects in the depressed group were responsive to antidepressant monotherapy (responders, 72.1±4.9 years) and 16 were not (non responders, 72.9±4.3 years) (Table 1a & 1b). There were no significant differences in demographics, range of vascular risk factors, serum glucose and lipids between the three groups-controls, responders to monotherapy and non responders to monotherapy (Table 2). The mean score on MADRS scale for the treatment groups was responders 8.3; non-responders 18.5 and for controls 2.6 which was the only (and expected) significant difference between groups on baseline variables. The MADRS score was not significantly between responders and non-responders after excluding five patients who had major depression at study entry (responders-8.4; non-responders -12.4; P=NS).

Three of the responders and 9 of the non responders were on selective serotonin re-uptake inhibitors (SSRIs). Four of responders and 5 of non responders were on noradrenergic and specific serotonin agonists (NaSSAs). Two of responders and none of the non responders were on selective noradrenaline reuptake inhibitors. None of the responders and 2 non-responders were prescribed tricyclics. One responder and 3 non responders were on antipsychotic medication. The single responder on an antipsychotic had a psychosis which was treated with an antipsychotic but depressive symptoms persisted, eventually resolving with an antidepressant. It was decided to
keep this participant in the responder group. Three subjects in the depressed group and all subjects in the control group were not taking any psychotropic medication. There were significant group differences in both Mean IMT and endothelial function (Table 3). There was a gradient in the scores (from best to worst) over the groups: control – responder – non-responder on both endothelial function and IMT. Mean IMT was significantly higher in nonresponders compared to controls (p=<.001) on a posteriori testing with Bonferroni correction. Similarly, non responders showed poorer endothelial function compared to controls (p=0.04) but there was no significant difference between responders and non-responders. In a multiple regression analysis with stepwise selection of variables to determine likely predictors of resistance (current age, gender, metabolic syndrome, hypertension, diabetes, mean IMT and endothelial function), mean IMT emerged as the only significant predictor of outcome (non response to mono therapy), explaining 10% of the variance (F= 4.41, P = .04).

6.6 Discussion:
We found that non-response to monotherapy treatment in late life depression is associated with atherosclerosis as assessed by IMT. There was also a significant difference between both treatment groups and controls on the measure of endothelial function but the same trend was seen as for IMT: worse function in the non-responders, best in the controls and responders in between. This suggests vascular dysfunction and pathology is linked to poorer antidepressant response at quite a low level of resistance. The interpretation is a cautious one given the small numbers but the differences could not be explained by group differences in physical health burden or a range of vascular risk factors including type 2 diabetes, metabolic syndrome, elevated blood pressure, and cholesterol and blood glucose. Moreover treatment with
agents which are known to influence small endothelial function (Malik et al, 2005) such as angiotensin converting enzyme inhibitors and statins were similar between the two groups of patients studied.

We have previously shown that late life depression is associated with endothelial dysfunction and atherosclerosis (Paranthaman et al., 2010; Greenstein et al., 2010). The present study adds to those findings by showing some differences in vascular function and pathology in those who do and do not show treatment response. These data are consistent with the vascular depression hypothesis which proposes that vascular disease or dysfunction contributes to brain matter lesions and with more recent refinements of the hypothesis which propose that in turn vascular disorder may be linked to antidepressant treatment resistance.

Vascular dysfunction and atherosclerosis can contribute to depression onset in several ways such as hypothalamic-pituitary-adrenal axis over-activity, increased platelet activation and hypercoagulability and altered inflammatory responses (Baldwin, 2005). Depression independently contributes to the cause and progression of vascular disease by various mechanisms such as raised cortisol levels and impaired endothelial function (Baldwin, 2005).

Although the interaction between depression and vascular disease may be bi-directional, the association of vascular impairment with treatment response in depression is of clinical importance. Statins can reduce progression of atherosclerosis and improve endothelial function (Furburg et al., 1994; Bonetti et al, 2003). Similarly it appears that vasoprotective drugs might improve the prognosis of late-life depression (Taragano et al., 2005).
6.6.1 Methodological considerations:

The study sample size was relatively small. Proportionally fewer subjects in the non-responders group had gluteal biopsy, thereby further reducing the chance of detecting group differences, but there were no differences in vascular or demographic factors between those who had the biopsy and those who had not. The cross-sectional nature of the study limits an understanding of causal relationships between vascular function and treatment response. Treatment outcome data was collected retrospectively and from hospital records and so is prone to error. The level of treatment resistance was low but is the same as two other studies showing neuropsychological differences likely to represent brain dysfunction between monotherapy responders and non-responders (Baldwin et al, 2004) and in brain Virchow robins spaces, representing atherosclerosis, also between monotherapy responders and non-responders (Pantankar et al, 2007). White matter hyperintensities (WMH) are associated with executive dysfunction (Aizenstein et al 2002; Culang-Reinlieb et al., 2010) which itself has been linked to poorer treatment response (Simpson et al., 1998; Baldwin et al., 2004; Sheline et al., 2010) but not all studies agree (Butters et al., 2004; Saghafi et al., 2007). It is likely though that in vascular depression, executive dysfunction and WMH have a common aetiology (Culang-Reinlieb et al, 2010). However, the latter authors have also highlighted perfusion deficits as a further potential factor in the formation WMH. This study has not assessed perfusion but it is likely that perfusion deficits linked to, for example, stiff arteries or autonomic problems have their aetiology in systemic atheromatous processes. The study population was predominantly white Caucasian, which may limit the generalizability of the findings. Treatment effects may have affected vascular function but recent studies have shown that SSRIs, the most commonly used antidepressants in this study, protect
or improve vascular endothelial function (Van Zyl et al., 2009; Serebruany et al., 2005) and repeating the analyses including antidepressant use as a covariate, did not alter the results. We did not have data on longitudinal treatment history of patients and this could be determinative as previous work has shown that outcome in one episode may not predict longer term outcome (Baldwin et al, 2006).

6.7 Conclusions:

With the caveats above, the study is in keeping in with other evidence that vascular abnormality is associated with poorer outcomes in late life depression. Endothelial dysfunction and atherosclerosis are two potential mechanistic explanations for this but there are others such as low perfusion states or cerebral auto regulatory failure (Naish et al, 2006). Further studies with larger samples are required to confirm the results. If confirmed further mechanistic research may lead to trials of treatments for vascular depression (and perhaps later life depression in general) involving vasoprotective agents alongside antidepressants.
References:


32. Taragano FE, Bagnatti P, Allegri RF. A double-blind, randomized clinical trial to assess the augmentation with nimodipine of antidepressant therapy in the treatment of "vascular depression". *Int Psychogeriatr.* Sep 2005;17(3):487-


Table 1a: Thase and Rush Treatment-Resistant Depression Staging:

<table>
<thead>
<tr>
<th>Thane and Rush Staging</th>
<th>Depressed (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 0</td>
<td>9</td>
</tr>
<tr>
<td>Stage 1</td>
<td>10</td>
</tr>
<tr>
<td>Stage 2</td>
<td>3</td>
</tr>
<tr>
<td>Stage 4</td>
<td>3</td>
</tr>
</tbody>
</table>

Stage 0: response to monotherapy
Stage 1: non response to antidepressant monotherapy
Stage 2 = non response to two trials of monotherapy with drugs of different classes
Stage 3 = stage 2 plus failure to respond to one augmentation strategy
Stage 4 = stage 3 plus failure of a second augmentation strategy
Stage 5 = stage 4 plus failure to respond to one course of electroconvulsive therapy (ECT).

Table 1b: Massachusetts General Hospital staging method (MGH-S)

<table>
<thead>
<tr>
<th>Score</th>
<th>Depressed (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>1.5</td>
<td>4</td>
</tr>
<tr>
<td>2.5</td>
<td>2</td>
</tr>
<tr>
<td>3.5</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

MGH-S method scoring:
(1) Non response to each adequate (at least 6 weeks of an adequate dose of antidepressant) trial of an antidepressant generates an overall score of resistance (1 point per trial)
(2) Optimization of dose, optimization of duration, and augmentation/comboination of each trial (based on the MGH or Antidepressant Treatment Response Questionnaire) increase the overall score (0.5 point per trial per optimization/strategy)
(3) ECT increases the overall score by 3 points
Table 2:

**Characteristics of study sample:** ANOVA for normally distributed data, Kruskal–Wallis (K–W) test for non normally distributed data with post hoc tests and Bonferroni correction for between-group differences and Chi-squared tests for categorical data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n=21)</th>
<th>Responders to monotherapy (n=9)</th>
<th>Non responders to monotherapy (n=16)</th>
<th>Between group statistic</th>
<th>Group statistic P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>72.2(6.5)</td>
<td>72.1(4.9)</td>
<td>72.9(4.3)</td>
<td>F=0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>13(62)</td>
<td>5(55.6)</td>
<td>12(75)</td>
<td>df=2, χ² =1.1</td>
<td>0.5</td>
</tr>
<tr>
<td>Ethnicity-European, n (%)</td>
<td>20(95.2)</td>
<td>8(88.9)</td>
<td>16(100)</td>
<td>df=2, χ² =1.7</td>
<td>0.4</td>
</tr>
<tr>
<td>MMSE†</td>
<td>29.1(1.1)</td>
<td>28.9(1.1)</td>
<td>29.3(1.1)</td>
<td>F=0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>MADRS</td>
<td>2.6(1.9)</td>
<td>8.3(5.0)</td>
<td>18.5(10.7)</td>
<td>F=24.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CIRS-G score†</td>
<td>6.0(2.6)</td>
<td>7.2(4.0)</td>
<td>7.3(3.5)</td>
<td>F=0.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Smoking status†, n (%)</td>
<td>2(9.5)</td>
<td>1(11.1)</td>
<td>3(18.6)</td>
<td>df=4, χ² =1.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Taking statin, n (%)</td>
<td>6(28.6)</td>
<td>4(44.4)</td>
<td>8(50)</td>
<td>df=2, χ² =1.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>16(76.1)</td>
<td>5(55.6)</td>
<td>10(62.5)</td>
<td>df=2, χ² =1.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>3(14.3)</td>
<td>1(11.1)</td>
<td>1(6.3)</td>
<td>df=2, χ² =0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>30.0(5.2)</td>
<td>28.9(3.9)</td>
<td>28.5(5.3)</td>
<td>F=0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Metabolic syndrome, n (%)</td>
<td>12(57.1)</td>
<td>6(66.7)</td>
<td>9(56.3)</td>
<td>df=2, χ² =0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Systolic Blood Pressure, mmHg</td>
<td>145.3(20.7)</td>
<td>141.2(18.6)</td>
<td>143.1(14.9)</td>
<td>F=0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Diastolic Blood Pressure, mmHg</td>
<td>76.5(9.6)</td>
<td>79.9(9.9)</td>
<td>75.6(12.4)</td>
<td>F=0.02</td>
<td>0.9</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.7(0.7)</td>
<td>5.1(0.9)</td>
<td>4.6(0.9)</td>
<td>F=1.0</td>
<td>0.4</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.9(1.3)</td>
<td>5.3(0.4)</td>
<td>5.5(1.8)</td>
<td>F=0.6</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Data are mean (Standard deviation) unless specified

1. CIRS-G, Cumulative Illness Rating Scale – Geriatrics
2. Current smokers; †Pack-years of smoking are for those who had ever smoked
3. Significance lies between responders vs. non responders and controls Vs non responders
**Table 3:**

**Vascular findings:** ANOVA for normally distributed data, Kruskal–Wallis (K–W) for non normally distributed data with post hoc tests and Bonferroni correction for between-group differences analyses

<table>
<thead>
<tr>
<th></th>
<th>Controls Mean (SD)</th>
<th>Responders a Mean (SD)</th>
<th>Non responders b Mean (SD)</th>
<th>Group statistic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean IMTc</td>
<td>0.09(0.01)</td>
<td>0.11(0.06)</td>
<td>0.12(0.03)</td>
<td>Kruskal Wallis test: df=2, $\chi^2=12.0$</td>
<td>P=0.002*</td>
</tr>
<tr>
<td>Endothelial functiond</td>
<td>96.2(5.7)</td>
<td>86.7(19.6)</td>
<td>82.9(13.1)</td>
<td>ANOVA: df=2, F=3.8</td>
<td>P=0.03**</td>
</tr>
</tbody>
</table>

a. Responders to monotherapy-Patients in Stage 0 of TR-S and score 0 in the MGH-S
b. Non-Responders to monotherapy-Patients from levels 1–5 in the TR-S and scores 1 and above in the MGH-S
c. IMT- results for 21 control subjects, 9 responders and 15 non responders
d. Endothelial function- results for 15 control, 9 responders and 7 non responders.

Higher scores mean better endothelial function

* Significance lies between non responders and controls
** Significance lies between non responders and controls and controls and responders
Chapter 7: Discussion

7.1 Vascular function in Older Adults with Depressive Disorder:

Late life depression is a heterogeneous entity with different aetiologies. Several lines of evidence support a link between vascular disease and late life depression. There is a bidirectional link between stroke and myocardial infarction and depression (Baldwin & O’Brien, 2002). White matter lesions in brain white matter and gray matter basal ganglia visualised on Magnetic Resonance Imaging (MRI) occur more often in depressed patients compared to non-depressed older people (Baldwin, 2005). WML are associated with ischaemic damage (Teper & O’Brien, 2008). This has led to the vascular depression hypothesis which proposes distinct clinical (Alexopoulos et al., 1997) and neuro radiological (Krishnan et al., 2004) appearances.

In this case control study, we compared vascular function and neuroimaging findings between 25 elderly depressed subjects and 21 healthy controls. Subjects underwent a variety of measures including Pulse Wave Analysis (PWA), Pulse Wave Velocity (PWV), carotid Intima Media Thickness (IMT) and MR brain scan. A subset (16 patients and 15 controls) also underwent assessment of biopsied pre-constricted small artery dilatation to acetylcholine.

The main finding was that patients with late-life depressive disorder had impaired vascular function compared to control subjects. This was seen most significantly seen for carotid intima media thickness and endothelial function. Pulse wave velocity and Augmentation index were higher in depressed subjects implying a higher arterial stiffness compared to controls but the differences were not statistically significant.

Regarding MRI changes, Virchow Robin spaces, thought to reflect cerebral microangiopathy, were significantly more frequent, notably in the basal ganglia. On the automated measure of white matter hyperintensity volume, the group difference
was not significant although the direction of the findings was as predicted.

Endothelial function and carotid IMT and both predicted about 75% of group membership. There were no baseline differences in vascular risk, inflammatory factors such as Serum C reactive protein, vitamin B12 and folate levels between the two groups.

The findings are consistent with research on major depression showing endothelial dysfunction and subclinical atherosclerosis in depressive disorder, both untreated (Rajagopalan et al., 2001) and treated (Broadley et al., 2002) and that this may represent trait rather than state as it may be independent of whether or not the person is in remission from depression (Rybakowski et al., 2006). The small sample size may account for the failure to find significant group differences in volumes of WML despite trends in the expected direction. Moreover, strategic location rather than counts of WML may be the more relevant in late-life depression (Sheline et al., 2008).

It is increasingly recognised that the relationship between depression and vascular disease is bi-directional. Vascular disease can contribute to depression by several mechanisms such as increased platelet activation, hypothalamic-pituitary-adrenal axis over-activity and abnormal folate or homocysteine-driven mechanisms (Baldwin, 2005). Depression independently contributes to vascular disease by several mechanisms such as platelet activation and raised cortisol levels (Thomas et al., 2004). Also, depressed patients often adopt unhealthy life styles and are known to comply poorly with medical treatment (Di Matteo et al., 2000).

As the study was cross sectional, it cannot address whether vascular dysfunction causes depression or depression directly interferes with vascular function. There were no differences between the groups across a range of vascular risk factors such as type 2 diabetes, hypertension, metabolic syndrome, serum lipids and blood glucose. Also
treatment with medication that are known to influence endothelial function (Malik et al., 2005) such as statins and angiotensin converting enzyme inhibitors were similar between the two groups of patients studied. So, it appears that depression itself may be causatively linked, either directly or via closely related factors such as lifestyle or inflammation. The dysfunction affected large, medium and small vessels suggesting that in late-life major depression there is a systemic disturbance in vascular function that is reflected in the brain as cerebral microangiopathy.

Endothelial function can improve with treatment with statins (Bonetti et al., 2003), Angiotensin Converting Enzyme (ACE) inhibitors and Angiotensin II Receptor Blockers (ARB) (Bertrand, 2004). The effects of vasoprotective drugs in depressive disorder have not yet been studied adequately. Further research is needed to see whether treatment of endothelial dysfunction can improve outcomes for major depression. This could lead to a dual approach to treatment based on both depression management and vasoprotective agents (Alexopoulos, 2006).

7.2 Small artery function and structure in late life depression:

Endothelial function has been shown to be significantly impaired in large arteries in major depression. This has been shown is both untreated (Rajagopalan et al., 2001) patients and those taking antidepressant therapy (Broadley et al., 2002). Chronic endothelial dysfunction plays a pivotal role in cerebral small vessel disease (Hassan et al., 2004; Lin et al., 2000). A direct assessment of cerebral vasculature in patients with depressive disorder would be ideal but, unlike some other end-organs, is clearly not feasible currently. However, risk factors for endothelial dysfunction are conditions with widespread effects such as hypertension, diabetes, smoking, exposure to high levels of low-density lipoprotein, homocysteine and fibrinogen (Di Napoli & Papa,
Therefore, it is likely that endothelial function in one small vessel bed is mirrored in other areas (Elesber et al., 2006).

In this study, small arteries were obtained from subcutaneous tissue by gluteal fat biopsy small artery function and structure was compared between 16 patients with depression and 15 control participants. There were no differences in contractility of small vessels to noradrenaline when patients with depression were compared with control participants. However, when endothelial function was assessed using by measuring the ability of Acetylcholine to dilate a preconstricted vessel, depressed patients showed significant abnormalities. Control participants showed normal relaxation to Acetylcholine whilst patients with depression showed significant impairment in function.

The structure of small arteries in 16 patients with depression and 14 control subjects were compared. Depressed patients showed significant increases in wall thickness and medial cross sectional area compared to the control subjects. There were no significant differences in wall to lumen ratio or distensibility or lumen diameter. In depressed patients, there was preservation of the lumen with an increase in wall thickness and wall cross-sectional area indicating hypertrophic growth of the arteries. This type of arterial remodelling is commonly seen in patients with type 2 diabetes mellitus (Schofield et al., 2002; Endemann et al., 2004; Rizzoni et al., 2005). The changes in peripheral small arteries were seen along with significantly higher changes in Virchow Robin Spaces in the basal ganglia and subinsular region in the MR brain.

In a logistic regression analysis endothelial function alone accurately predicted 76.7% of group membership.

The study findings could not be explained by group differences in vascular risk factors or socio demographic variables. Additionally, treatment with agents which are known
to influence small artery endothelial function (Malik et al., 2005) or wall remodeling (Thybo et al., 1995; Schiffrin et al., 2003) such as angiotensin converting enzyme inhibitors, angiotensin II receptor blockers or statins were similar between the two groups of patients studied. The main clinical difference between the two groups was depressive disorder itself suggesting that depression is linked to endothelial dysfunction.

As mentioned earlier, endothelial dysfunction can be reversed by treatment with angiotensin converting enzyme (ACE) inhibitors, statins and angiotensin II receptor blockers (Schofield et al., 2002; Bonetti et al., 2003; Bertrand, 2004). In addition, studies have shown that hypertrophic remodeling in Type 2 diabetes is reversible with ACE inhibitors and Angiotensin Receptor Blockers (Rizzoni et al., 2005 & 2006). Further research is needed to explain whether endothelial dysfunction associated with depressive disorder is reversible and whether vasoprotective drugs can alter the course or severity of major depression (Alexopoulos, 2006; Isingrini et al., 2009).

7.3 Age at onset and vascular function in late life depression:

There is substantial evidence to show that late onset depression (LOD) may be different from depression with an early age of onset depression (EOD). LOD patients are less likely to have family history of depression (Baldwin & Tomenson, 1995; Krishnan et al., 1995), but have greater cognitive impairment (Salloway et al., 1996), medical co morbidity (Lavretsky et al., 1998; Tupler et al., 2002) and vascular risk factors (Baldwin & Tomenson 1995; O’Brien et al., 1996). It has been suggested that early and late onset depression may have different aetiologies (Brodaty et al., 2001). Furthermore, the prognosis of depression in late life may be different for late as opposed to early-onset illness (Mitchell & Subramaniam, 2005).
This part of the study examined the relationship between age of onset of depression and vascular function between 11 subjects with EOD and 14 subjects with LOD. Early-onset illness was defined as having the first episode of major depression before age of 60 years and those who had their first episode at or above the age of 60 years were defined as having a late onset illness (Blazer, 2003). All subjects underwent a variety of vascular assessments including Pulse Wave Analysis (PWA), Pulse Wave Velocity (PWV), carotid Intima Media Thickness (IMT) and Magnetic Resonance Imaging (MRI).

There were no baseline differences in vascular risk or socio demographic variables between the two groups. The peripheral augmentation index as measured by pulse wave analysis was significantly higher in the late onset group than in the early onset group. Pulse Wave velocity was higher in the late onset group but not significantly so. The common carotid intima media thickness and total atheromatous plaque was significantly higher in late onset subjects compared to early onset subjects. Total WMH volumes and individual regional scores were uniformly higher in the late onset group but none was statistically significant. Age at onset moderately positively correlated with peripheral augmentation index, carotid IMT and plaque index. Total WMH scores positively correlated with common carotid IMT and plaque index suggesting a link between cerebral ischaemia and peripheral atherosclerosis.

The results suggest that vascular factors are of greater importance in late- than in early-onset depression. This is consistent with an earlier study by Smith et al., (2009), who showed age at onset of depression, was associated with a higher carotid IMT in a study of middle age and older adults with a mean age of 52. As far as I am aware this is the first study to show a relationship between age at onset and vascular function specifically in late life depression. As the study sample was small the findings are
preliminary. If replicated, it suggests that LOD subjects may be more responsive to new treatment approaches in the treatment of depression such as vasoprotective agents. It has been shown that statins and calcium channel blockers can reduce the progression of carotid IMT (Furberg et al., 1994; Wang et al., 2006) and also lower the incidence of cardiovascular events (Zanchetti et al., 1998).

### 7.4 Treatment response in late life depression and vascular function:

The long term prognosis of late life depression is poor (Mitchell & Subramaniam, 2005). A number of studies have shown that MRI signal hyperintensities which are markers of cerebrovascular damage predict poor antidepressant treatment response (Hickie et al., 1995; Simpson et al., 1998; Gunning-Dixon et al., 2010). Several mechanisms such as cerebrovascular microangiopathy (Patankar et al., 2007), endothelial function and atherosclerosis (Culang-Reinlieb et al., 2010) have been suggested as the underlying cause of cerebral WML have been suggested.

The aim of this study was to assess the relationship of endothelial function and atherosclerosis and treatment response in late life depressive disorder. Twenty four depressed subjects (9 who were responders to antidepressant monotherapy and 15 who were non responders) were recruited along with 21 Control subjects. All study subjects underwent direct assessment of small vessel endothelial function by gluteal fat biopsy (Greenstein et al., 2010) and Carotid Intima Media Thickness (IMT) measurement by ultrasound. There were no significant differences in age, gender, range of vascular risk factors, serum glucose and lipids between the two groups.

Both patient groups showed a trend towards greater vascular impairment compared to control subjects. Mean IMT was significantly higher in Nonresponders compared to controls. Similarly non responders showed poorer endothelial function compared to controls on a posterior testing with Bonferroni correction. This is the first study to
show a link between extra cerebral vascular function and treatment response in late life depression. If the study results are replicated, there may be a role for new treatment approaches as vasoprotective drugs in treatment resistant late life depressive disorder.

7.5 Limitations of the study:

In relation to the overarching aim of the research, the role of vascular function in late life depression, the study objectives were met. The data for the small artery biopsy are from a smaller number as not all participants had a gluteal biopsy. In some cases this was because of refusal but often it was because of contraindications and, overall, the participation rate was good. The main strength of this study is the intensive investigation of vascular function and structure using validated measures in a group of elderly depressed subjects and controls who were similar on most demographic and clinical measures, including of vascular risk.

Nevertheless, there were limitations. First is that the study is cross-sectional and so the observed relationships between arterial function and depression are associative rather than the causal. The absence of reliable data regarding duration of symptoms limits discussion of potential interactions between WML and chronicity of depression (Hickie et al., 1997). A type 2 error due to small numbers is possible and may explain an absence of statistical significance on some of the measures.

Previous research, albeit limited, suggests that the state of depression versus nondepression does not explain differences in vascular function (Rajagopalan et al., 2001; Broadley et al., 2002; Smith et al., 2009). Although not conclusive, it appears that vascular disease is likely to be a trait-like risk factor rather than an immediate precipitant of late-onset depression. Therefore, we recruited subjects to our depressed group who were a mixture of some who were currently depressed and others who
were in complete or partial remission. Also, we did not assess actual mood at the time of the vascular function evaluation. So, our study cannot sufficiently address whether vascular function is a state or trait factor in late life depression and it is possible but unlikely that some vascular measures might be state and not only trait-dependant. Treatment with antidepressants could be a potential confounder but studies have shown that SSRI, most commonly used antidepressant in this study’s protect and indeed improve vascular endothelial function in healthy volunteers and in those suffering from major depression (van Zyl et al., 2009; Lara et al., 2003; Serebruany et al., 2005) mainly through enhanced production of endothelium-derived nitric oxide (Chrapko et al., 2006). It is well known that tricyclic antidepressants have cardiac side effects, mainly anti arrhythmic (Sala et al., 2006). This is unlikely to account for our findings as only 2 of our subjects were on tricyclic antidepressants and statistically controlling for their use in the analyses did not change the results. There could be other potential confounders such as physical activity that were not measured in this study. As our study population was predominantly white Caucasian, the generalizability of our findings to other ethnic groups might be limited. Finally, the observed associations in the study should be interpreted with caution as a large number of statistical tests were performed in a small sample and needs to be confirmed in other studies.

7.6 Implications for future research:

Despite the limitations as above, the study data provide a basis for further mechanistic work to explore the underlying nature of the vascular disorder in late-life depression, whether it is one process or, as with the aetiology of late-life depression in general, there are a number of pathways including factors such as orthostatic blood pressure changes and cerebral autoregulation. Gluteal biopsy is not a routinely available
procedure and is labour intensive. Future work might focus on newer non-invasive techniques such as retinal artery analysis via photography, as mentioned in Chapter 1. Probably there is now enough data to propose an intervention study aimed at determining whether vasoprotective medication (ACE inhibitors, calcium channel agents) can lead to improved depression outcomes, as is suggested by the work of Tarangano et al., (2005) referred to earlier. Such a study could be combined with repeat vascular measures to see if any clinical change is accompanied by improvement in vascular function.
References:


## Appendix 1:

### Montgomery Asberg Depression Rating Scale (MADRS)

<table>
<thead>
<tr>
<th>1. Apparent sadness</th>
<th>2. Reported sadness</th>
</tr>
</thead>
<tbody>
<tr>
<td>no sadness</td>
<td>occasional appropriate sadness</td>
</tr>
<tr>
<td>looks dispirited but reacts</td>
<td>sad or low but can brighten</td>
</tr>
<tr>
<td>appears sad most of the time</td>
<td>pervasive sadness, still influenced</td>
</tr>
<tr>
<td>miserable all the time, despondent</td>
<td>varying sadness, despondent</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3. Inner tension</th>
<th>4. Reduced sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>placid, only fleeting tension</td>
<td>sleeps as usual</td>
</tr>
<tr>
<td>occasional edginess</td>
<td>sleep slightly reduced</td>
</tr>
<tr>
<td>continuous tension or intermittent panic</td>
<td>reduced by at least two hours</td>
</tr>
<tr>
<td>unrelenting dread, overwhelming panic</td>
<td>less than two hours sleep</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5. Reduced appetite</th>
<th>6. Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal or increased</td>
<td>no difficulty concentrating</td>
</tr>
<tr>
<td>slightly reduced</td>
<td>occasional difficulties</td>
</tr>
<tr>
<td>no appetite, food tasteless</td>
<td>difficulty reading or in conversation</td>
</tr>
<tr>
<td>needs persuasion to eat at all</td>
<td>unable to read or converse</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>7. Lassitude</th>
<th>8. Inability to feel</th>
</tr>
</thead>
<tbody>
<tr>
<td>no sluggishness</td>
<td>normal interests</td>
</tr>
<tr>
<td>difficulty getting started</td>
<td>reduced interest</td>
</tr>
<tr>
<td>simple routine an effort</td>
<td>loss of interest or feelings</td>
</tr>
<tr>
<td>needs help with anything</td>
<td>emotionally paralysed</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>8. Pessimistic thoughts</th>
<th>10. Suicidal thoughts</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>enjoys life</td>
</tr>
<tr>
<td>fluctuating failure or self reproach</td>
<td>weary, fleeting thoughts of suicide</td>
</tr>
<tr>
<td>self accusations, ideas of guilt</td>
<td>suicide an option but no plans</td>
</tr>
<tr>
<td>delusions of ruin, guilt or sin</td>
<td>explicit or active plans for suicide</td>
</tr>
</tbody>
</table>

Total Score ........
## Appendix 2: The Mini-Mental State Exam

<table>
<thead>
<tr>
<th>Score</th>
</tr>
</thead>
</table>

### Orientation
What is the (year) (season) (date) (day) (month)?
Where are we (state) (country) (town) (hospital) (floor)?

### Registration
Name 3 objects: 1 second to say each. Then ask the patient all 3 after you have said them.

### Attention and Calculation
Serial 7’s. 1 point for each correct answer. Stop after 5 answers.
Alternatively spell “world” backward.

### Recall
Ask for the 3 objects repeated above. Give 1 point for each correct answer.

### Language
Name a pencil and watch.
Repeat the following “No ifs, ands, or buts”

#### Follow a 3-stage command:
“Take a paper in your hand, fold it in half, and put it on the floor.”

Read and obey the following: CLOSE YOUR EYES
Write a sentence.
Copy the design shown.

### Total Score

CLOSE YOUR EYES
Appendix 3:

**Thase & Rush staging score for treatment of Depression**

0 - response to monotherapy

1 - Non-response to antidepressant monotherapy

2 - Non-response to two trials of monotherapy from drugs of different classes

3 - Stage 2 plus failure to respond to one augmentation strategy

4 - Stage 3 plus failure of a second augmentation strategy

5 - Stage 4 plus failure to respond to one course of ECT

**Score:**
Appendix 4:

**MGH Antidepressant Treatment Response Questionnaire**

**Instructions:** Please check the names of any medications that have been prescribed for at least 6 or 10 weeks since the beginning of THIS EPISODE or period of depression. Please also check if the daily dosage of the medication was equal to or greater than the minimum dose listed below. Finally, please check whether a drug (e.g., buspirone, lithium, atypical antipsychotics) was added to augment the antidepressant effect.

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Generic Name</th>
<th>At least 6 Weeks</th>
<th>At least 10 Weeks</th>
<th>Minimum Dose</th>
<th>Equal or greater</th>
<th>Maximum Dose</th>
<th>Equal or greater</th>
<th>Drug was added to augment or boost effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tricyclic Antidepressants</strong></td>
<td></td>
<td></td>
<td></td>
<td>--------------</td>
<td>-----------------</td>
<td>--------------</td>
<td>-----------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>Adapin</td>
<td>desipramine</td>
<td>---</td>
<td>---</td>
<td>150 mg/day</td>
<td>---</td>
<td>250 mg/day</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Anafranil</td>
<td>clomipramine</td>
<td>---</td>
<td>---</td>
<td>150 mg/day</td>
<td>---</td>
<td>250 mg/day</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Asendin</td>
<td>amoxapine</td>
<td>---</td>
<td>---</td>
<td>150 mg/day</td>
<td>---</td>
<td>250 mg/day</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Enaep/Elavil</td>
<td>amitriptyline</td>
<td>---</td>
<td>---</td>
<td>150 mg/day</td>
<td>---</td>
<td>250 mg/day</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Ludiomal</td>
<td>maprotiline</td>
<td>---</td>
<td>---</td>
<td>150 mg/day</td>
<td>---</td>
<td>250 mg/day</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Nortrofin</td>
<td>desipramine</td>
<td>---</td>
<td>---</td>
<td>150 mg/day</td>
<td>---</td>
<td>250 mg/day</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Pamelor</td>
<td>nortriptyline</td>
<td>---</td>
<td>---</td>
<td>75 mg/day</td>
<td>125 mg/day</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Sinequan</td>
<td>doxepin</td>
<td>---</td>
<td>---</td>
<td>150 mg/day</td>
<td>---</td>
<td>250 mg/day</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Sumomiptil</td>
<td>trimipramine</td>
<td>---</td>
<td>---</td>
<td>150 mg/day</td>
<td>---</td>
<td>250 mg/day</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Tofranil</td>
<td>imipramine</td>
<td>---</td>
<td>---</td>
<td>150 mg/day</td>
<td>---</td>
<td>250 mg/day</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Vivactil</td>
<td>protriptyline</td>
<td>---</td>
<td>---</td>
<td>30 mg/day</td>
<td>60 mg/day</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Monoamine Oxidase Inhibitors (MAOIs)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marplan</td>
<td>isocarboxazid</td>
<td>---</td>
<td>---</td>
<td>30 mg/day</td>
<td>60 mg/day</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Nardil</td>
<td>phenelzine</td>
<td>---</td>
<td>---</td>
<td>45 mg/day</td>
<td>90 mg/day</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Paroato</td>
<td>transcypramine</td>
<td>---</td>
<td>---</td>
<td>30 mg/day</td>
<td>60 mg/day</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Selective Serotonin Reuptake Inhibitors (SSRIS)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luvox</td>
<td>fluvoxamine</td>
<td>---</td>
<td>---</td>
<td>50 mg/day</td>
<td>150 mg/day</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Peril</td>
<td>paroxetine</td>
<td>---</td>
<td>---</td>
<td>18/20 mg/day</td>
<td>60 mg/day</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Prozac</td>
<td>fluoxetine</td>
<td>---</td>
<td>---</td>
<td>20 mg/day</td>
<td>60 mg/day</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Zoloft</td>
<td>sertraline</td>
<td>---</td>
<td>---</td>
<td>50 mg/day</td>
<td>150 mg/day</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Cipralex</td>
<td>citalopram</td>
<td>---</td>
<td>---</td>
<td>20 mg/day</td>
<td>60 mg/day</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Lexapro</td>
<td>escitalopram</td>
<td>---</td>
<td>---</td>
<td>10 mg/day</td>
<td>30 mg/day</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Serotonin-Norepinephrine Reuptake Inhibitors (SNRIS)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effexor</td>
<td>venlafaxine</td>
<td>---</td>
<td>---</td>
<td>125 mg/day</td>
<td>250 mg/day</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Cymbalta</td>
<td>duloxetine</td>
<td>---</td>
<td>---</td>
<td>60 mg/day</td>
<td>100 mg/day</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Other Antidepressants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desyrel</td>
<td>trazodone</td>
<td>---</td>
<td>---</td>
<td>300 mg/day</td>
<td>600 mg/day</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Serzone</td>
<td>nefazodone</td>
<td>---</td>
<td>---</td>
<td>300 mg/day</td>
<td>600 mg/day</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Wellbutrin</td>
<td>bupropion</td>
<td>---</td>
<td>---</td>
<td>300 mg/day</td>
<td>450 mg/day</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Remeron</td>
<td>mirtazapine</td>
<td>---</td>
<td>---</td>
<td>15 mg/day</td>
<td>45 mg/day</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

*Did you receive electro-convulsive treatment (ECT) during the current episode (please circle the correct answer): YES NO*
Massachusetts General Hospital (MGH) Staging

Method to Classify Treatment-Resistant Depression

(1) Nonresponse to each adequate (at least 6 weeks of an adequate dose of antidepressant) trial of a marketed antidepressant generates an overall score of resistance (1 point per trial)
(2) Optimization of dose, optimization of duration, and augmentation/com不远 combination of each trial (based on the MGH or Antidepressant Treatment Response Questionnaire) increase the overall score (.5 point per trial per optimization/strategy)
(3) ECT increases the overall score by 3 points

Total score:
Appendix 5:

Cumulative Illness Rating Scale for Geriatrics (CIRS-G)

<table>
<thead>
<tr>
<th>System</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td></td>
</tr>
<tr>
<td>Vascular</td>
<td></td>
</tr>
<tr>
<td>Haemotopetic</td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td></td>
</tr>
<tr>
<td>Eyes, ears, nose, throat and larynx</td>
<td></td>
</tr>
<tr>
<td>Upper gastrointestinal tract</td>
<td></td>
</tr>
<tr>
<td>Lower gastrointestinal tract</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>Renal</td>
<td></td>
</tr>
<tr>
<td>Genitourinary</td>
<td></td>
</tr>
<tr>
<td>Musculoskeletal/integument</td>
<td></td>
</tr>
<tr>
<td>Neurological</td>
<td></td>
</tr>
<tr>
<td>Endocrine/metabolic and breast</td>
<td></td>
</tr>
<tr>
<td>Psychiatric illness</td>
<td></td>
</tr>
</tbody>
</table>

1) Total score ......

2) Total number of categories endorsed ......

3) Severity index: (total score/total number of categories endorsed) ......

4) Number of categories at level-3 severity ......

5) Number of categories at level-4 severity ......

Rating strategy:
0 – No problem
1 – Current mild problem or past significant problem
2 – Moderate disability or morbidity requires “first line therapy”
3 – Severe/constant significant disability / “uncontrollable” clinical problems
4 – Extremely severe/immediate treatment required/end organ failure/severe impairment function
### Framingham Stroke Risk Scale

<table>
<thead>
<tr>
<th>Risk</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Age</td>
<td>54-56</td>
</tr>
<tr>
<td>Rx</td>
<td>No</td>
</tr>
<tr>
<td>DM</td>
<td>No</td>
</tr>
<tr>
<td>Cigs</td>
<td>No</td>
</tr>
<tr>
<td>CVD</td>
<td>No</td>
</tr>
<tr>
<td>AF</td>
<td>No</td>
</tr>
<tr>
<td>LVH</td>
<td>No</td>
</tr>
</tbody>
</table>
Appendix 7:  
Metabolic syndrome:  Yes/No  
Waist circumference—ethnicity specific (see table)  
Plus any two:  
Raised triglycerides  
>150 mg/dL (1.7 mmol/L)  
Specific treatment for this lipid abnormality  
Reduced HDL-cholesterol  
<40 mg/dL (1.03 mmol/L) in men  
<50 mg/dL (1.29 mmol/L) in women  
Specific treatment for this lipid abnormality  
Raised blood pressure  
Systolic ≥130 mm Hg  
Diastolic ≥85 mm Hg  
Treatment of previously diagnosed hypertension  
Raised fasting plasma glucose  
Fasting plasma glucose ≥100 mg/dL (5.6 mmol/L)  
Previously diagnosed type 2 diabetes  
If above 5.6 mmol/L or 100 mg/dL, oral glucose tolerance test is strongly recommended, but is not necessary to define presence of syndrome

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>Waist circumference (as measure of central obesity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europids*</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>≥94 cm</td>
</tr>
<tr>
<td>Women</td>
<td>≥80 cm</td>
</tr>
<tr>
<td>South Asians</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>≥90 cm</td>
</tr>
<tr>
<td>Women</td>
<td>≥80 cm</td>
</tr>
<tr>
<td>Chinese</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>≥90 cm</td>
</tr>
<tr>
<td>Women</td>
<td>≥80 cm</td>
</tr>
<tr>
<td>Japanese</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>≥85 cm</td>
</tr>
<tr>
<td>Women</td>
<td>≥90 cm</td>
</tr>
<tr>
<td>Ethnic south and central Americans</td>
<td>Use south Asian recommendations until more specific data are available</td>
</tr>
<tr>
<td>Sub-Saharan Africans</td>
<td>Use European data until more specific data are available</td>
</tr>
<tr>
<td>Eastern Mediterranean and middle east (Arab) populations</td>
<td>Use European data until more specific data are available</td>
</tr>
</tbody>
</table>