Ocular and systemic vascular alterations in overweight and obese individuals undergoing weight-loss interventions

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Thesis Summary

Obesity and its accompanying comorbidities play a crucial role in the pathogenesis of endothelial function which is a pre-cursor to atherosclerosis, subsequently leading to increases in cardiovascular risk. However, amelioration of these risk factors and improvements in endothelial function have never been fully explored in functional assessments of the retinal and peripheral microcirculations. The aim of this thesis was therefore to investigate the presence and impact of weight-loss interventions in overweight and obese individuals and also the relationships between functional measurements of different vascular beds.

The principle findings of this work were:

1. The relationship between retinal and peripheral vascular function in healthy individuals with low cardiovascular risk

- Participants with higher peripheral vascular reactivity indices had a higher amplitude change from maximum to minimum and also showed enhanced reaction times to flicker provocation, which correlated to the degree of peripheral vascular function.

2. The effects of physical training on retinal and systemic microvascular function

- Physical exercise positively influenced the retinal microcirculation through improvements in dilation and constriction reaction times to flicker provocation

3. The long-lasting effects of fasting during the month of Ramadan on retinal and peripheral vascular function

- Participants during fasting had a higher capacity to reach maximum dilation and also a greater percentage increase from baseline diameters. The retinal veins were also significantly less variable during baseline corrected measurements

4. The effect of bariatric surgery on retinal vessels structure and systemic microvascular function

- Increases were recorded for the diameter of retinal arteries but also for the veins. Peripheral vascular function was significantly improved and arterial stiffness was decreased. CVD risk was significantly decreased and also correlated with retinal vessel calibre measurements.

5. Is there an improvement in anterior ocular health after bariatric surgery?

- Anterior surface health doesn't necessarily cause ocular health problems in obese individuals nor can it be improved or ameliorated through bariatric surgery

Keywords: Overweight, Obesity, Weight-loss, Retinal vessel reactivity, Peripheral vascular reactivity, Cardiovascular disease, Endothelial function

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ABBREVIATIONS

Alx	Augmentation index
ANG	Angiotensin
ANGII	Angiotensin-2
aTR	Adjusted temperature rebound
AVR	Arteriolar-to-venular diameter ratio
AUCtr	Area under the curve temperature rebound
BCFR	Baseline corrected flicker response
BD	Baseline diameter
BDF	Baseline diameter fluctuation
BMI	Body mass index
BP	Blood pressure
BR	Bulbar redness
CCT	Central corneal thickness
COX	cyclooxygenase
CRAE	Central retinal artery equivalent
CRVE	Central retinal vein equivalent
CRP	C-reactive protein
CT	Constriction time
CVD	Cardiovascular disease
CHD	Coronary heart disease
CHOL	Total Cholesterol
DA	Dilation amplitude
DED	Dry eye disease
DBP	Diastolic blood pressure
DTM	Digital thermal monitoring
DVA	Dynamic retinal vessel analysis
EDRF	Endothelial derived relaxing factor
EDCF	Endothelial derived constricting factor
ET-1	Endothelin-1
FES	Floppy eyelid syndrome
FMD	Flow mediated dilation
HDL	High density lipoprotein- cholesterol
HIF	Hypoxia inducible factor
IGT	Impaired glucose tolerance
IOP	Intra ocular pressure
IL	Interleukin
LAGB	Laparoscopic adjustable gastric band
LASG	Laparoscopic sleeve gastrectomy
LDL	Low density lipoprotein- cholesterol
MC	Minimum constriction
MD	maximum dilation
MGD	Meibomian gland dysfunction
UCP-1	Mitochondrial uncoupling protein
MCP-1	Monocyte chemoattractant protein
NAFL	Sodium fluorescein
NC	Neck circumference

NIH	National institute of health
NIKBUT	Non-invasive keratography break up time
NO	Nitric oxide
eNOS	Nitric oxide synthase
OCT	Optical coherence Tomography
PWA	Pulse wave analysis
RAS	Renin angiotensin system
RNFL	Retinal nerve fibre layer
RT	Reaction time
SAS	Sleep apnea syndrome
SBP	Systolic blood pressure
TBUT	Tear break up time
TMH	Tear meniscus height
TNF-α	Tumor necrosis factor alpha
TR	Temperature rebound
WC	Waist circumference
WHO	World health organization
VGB	Vertical banded gastroplasty (Bypass)

1. Introduction

1.1. General background

Obesity has been recognised as a health issue for thousands of years. Early documents show that the privileged classes of roman society were affected by obesity[1]. The writer Gaius Plinius Secondus known as "the Elder", describes in his book[2] that the son of the consul Lucious Apronius complained that his excessive stature made him clumsy and heavy, and chose to have surgical removal of his skin and subcutaneous fat. The consul expressed the opinion that the operation would have physical and psychological health benefits for his son and would improve his ability to perform his professional duties[1 2]. Hippocrates also recognized that "sudden death is more common in those who are naturally larger than their leaner counterparts"[3].

The technological advances in farming during the 18th Century translated in a gradual increase of food supply and in a better quality and variety of food; this, in turn, led to an increased longevity and body size. Corpulence and increased "flesh" were a desirable attribute and a sign of beauty, as reflected in arts, literature, and medical opinions of those times[4].

In 1832, Adolphe Quetelet (1796-1874), a Belgian mathematician, astronomer, and statistician developed a formula for estimating body fat, called the *quetelet index*. His pioneering cross-sectional studies of human growth led him to conclude that 'weight increases as the square of the height'[5]. The need for a normative value index was also recognized in the early 19th century when actuaries noted an increased death claims among their obese policy holders[6]. As obesity gradually became a medical concern following the Second World War, the pursuit for a reliable and practical index for relative weight began[5]. One of the first studies to confirm the validity of the *quetelet index* was the Framingham study[5 7] and this index was renamed *the body mass index* (BMI)[8]. This index is still used today.

Even though the impact of obesity on health was being recorded from the late 18th century and recognized as a cause of ill health by the mid-19th century[4], its morbid complications and impact on increased mortality was documented only in mid-20th century[3 9-11]. Obesity has seen an exponential increase in the west most probably due to sedentary lifestyles, longer working office hours and the commodity of fast food.

Obesity has been described as a chronic disease in the same sense as hypertension and atherosclerosis[3]. In the past few decades it has reached epidemic proportions. Obesity has become one of the leading causes of disability and death, affecting not only adults but of varying ages, including children worldwide[12]. In 2014, more than 1.9 billion adults were overweight, 600 million of them were obese. Forty two million children under the age of 5 were overweight or obese in 2013[13]. Obesity has also been shown to decrease life expectancy by 7 years at the age of 40[14]. This increased risk of death declines progressively with each unit of BMI but remains substantial until about the age of 75[15 16]. The World Health Organization (WHO) has declared obesity as a global epidemic and world-wide public health crisis. WHO has characterized obesity as a medical condition in which excess amounts of body fat have accumulated, leading to health problems and reduced life expectancy[13].The WHO has declared obesity as the largest global chronic health problem in adults which is increasingly turning into a more serious problem than malnutrition[17]. The WHO world health statistics report in 2015 shows that in the European region the overall obesity rate among adults is 21.5% in males and 24.5% in females. The same report states that the prevalence for overweight among children under the age of 5 is 12.4%[18] (ref WHO 2015). It has been further projected that 3.3 billion people, could be overweight (2.2 billion) or obese (1.1 billion) by 2030 if these trends continue, equating to roughly 60% of the world's population[17 19].

Throughout the world, obesity is more common amongst women; they also have a mean BMI higher than men, possibly due to biological reasons, such as estrogen and insulin resistance[20]. The department of health (UK) estimates that men with a BMI of 25 kg/m2 have a reduced life

expectancy of 2 years, and given the progressive nature of obesity, life expectancy will be reduced by 5 years in 2050[16].

1.2. The role of adipose tissue

Historically adipose tissue has been has been viewed as one of the least complex organs in the body. The perspective was that the organ was simply for fat storing purposes in which excess calories were deposited after a meal and from which fuel would be released during episodes of increased energy demands, fasting and prolonged food deprivation[21]. Adipose tissue in normal weight persons can account for between 18% and 24% of total body weight and in contrast in obesity it can account for between 52% and 74%[22]. Adipocytes secrete cytokines, hormones and bioactive peptides that have a key impact on skeletal muscle and liver function to regulate energy, and maintain homeostasis as well as used in metabolism[23]. Adipose tissue has a dense network of micro-vessels ensuring sufficient exchange of nutrients and oxygen. The adipose tissue delivers lipids to their storage depot in the adipocytes and also exports nutrients in response to metabolic need. Adipokines and other vasoactive mediators are secreted from adipocytes and other cellular elements from the tissue, such as macrophages and via the tissue microvascular network are delivered into the blood stream to exert their remote effects. In obesity however, insufficient adipose tissue perfusion may result in local hypoxia which induces the levels of hypoxia inducible factor (HIF) in adipocytes and can lead to increased synthesis of various inflammatory adipokines that include tumor necrosis factor (TNF), Leptin, interleukin (IL)-6 and resistin[24-26].

Newer studies and further developments have demonstrated essential distinctions between the roles of white adipose tissue (WAT) and brown adipose tissue (BAT)[21]. BAT is specialized for the generation of heat, fuelled primarily by the oxidation of fatty acids through the presence of a specific mitochondrial uncoupling protein (UCP-1)[27 28]. This generation of heat is mainly responsible for the dissipation of excess calories as a mechanism for maintenance of energy balance and body weight[29 30]. In contrast WAT is responsible for fuels storage and provides a substrate to other tissues during

periods of high energy demands and fasting[21]. Further studies demonstrate that in some cases it appears to be that both types of adipose tissue can interact in a coordinated and compensatory manner[31] with little differentiation that makes this organ much more complex. Nonetheless, adipose tissue is a key endocrine organ with autocrine regulation[32]. Adipocytes are the signature cell of adipose tissue and are primarily the focus of cellular studies[21]. Indeed it has been realised that the cells (adipocytes) within adipose tissue become inflamed and this inflammatory state leads to the initiation and progression of the major obesity associated diseases[33-35] and has a central role in the development of complications associated with obesity that include dyslipidemia, insulin resistance, metabolic syndrome and low grade chronic inflammation that leads to an increased risk of cardiovascular disease[32 36].

Other adipose constituents include Adipokines such as adiponectin, leptin, resisten, vascular endothelial growth factor (VEGF)[23 37-39] and also proinflammatory cytokines such as TNF, IL-1, IL-6[40 41] and monocyte chemoattractant protein (MCP1)[42]. Adiponectin is thought to have a positive effect on vascular function[43]. In obesity the circulating levels of adiponectin are reduced[23] while the concentrations of leptin, TNF IL6, and MCP1[37 42 44] are increased. A study by Greenstein et al, has found that healthy adipose tissue around human small arteries secretes adiponectin that causes vasodilation by increasing nitric oxide (NO) bioavailability[45]. However, adiponectin from perivascular fat in obese subjects with metabolic syndrome lose its dilator effects[45]. Leptin is a hormone with appetite regulating properties which primarily acts on the hypothalamic neurons to activate catabolic and inhibit anabolic pathways resulting in weight reduction[46] and lower levels of leptin have been found to be associated with higher risk of weight gain in healthy young adults[47]. In many obese subjects, leptin secretion was found to be significantly higher than in lean subjects, indicating leptin resistance rather than insufficient leptin production[48], which can alter vasomotor function from the adverse effects of circulating levels of leptin[23]. In a study by Knudson et al, pathological concentrations of leptin attenuated dilation to acetylcholine (Ach) in coronary arteries, whereas physiological concentrations had no effect[49].

Resistin is another adipokine and is expressed almost exclusively in adipocytes and resistin levels elevate in obesity[50]. Elevated serum levels of resistin have been correlated with inflammatory markers including TNF and IL-6 along with increased coronary artery calcium score[51]. Verma et al, showed within endothelial cells, resistin increased the expression of endothelin-1 (ET-1) a potent vasoconstrictor[52]. Another study showed decreased eNOS levels in coronary endothelial cells which were incubated with resistin[53]. TNF is also secreted by adipocytes and its expression in adipose tissue is increased in obesity[54 55], which correlated with the severity of insulin resistance[56 57] It has an important role in the development of endothelial dysfunction in obesity[54 58 59]. Recently a study carried out found that obese individuals express almost 3 times higher TNF in adipose tissue relative to lean controls[35 56]. Recent reports provide evidence that TNF inhibits endothelium dependant NO mediated dilation of coronary arterioles[59 60]. In obesity, adipose tissue is infiltrated with a large number of macrophages[61], and can account for 40% of the cells within the adipose tissue when compared to normal adipose tissue[62]. Macrophages within adipose tissue were shown to contribute to the substantial release of TNF and Il6[63].

Apart from the cerebral artery and pulmonary vessels various quantities of perivascular adipose tissue (PVAT) surround blood vessels throughout the body[64]. PVAT provides mechanical protection for the blood vessels but also secretes vasoactive adipokines[65]. The adipokines secreted from PVAT can easily reach the adjacent blood vessels wall sicne there is no anatomical barrier between PVAT and adventitia[66]. They play an important role in the regulation of vascular tone and vessel wall remodelling via their paracrine or endocrine effects. Adipokines released from PVAT have been shown to be more inflammatory, proliferative and angiogenic compared to other adipose depot profiles such as visceral or subcutaneous adipose tissues in humans[67-70]. It has been shown in coronary artery disease (CAD) that inflammatory and proliferative adipokine expression or release such as IL6, il1, resistin, MCP1, Leptin and TNF are increased where as ant-inflammatory and anti-proliferative vasodilator such as adiponectin are decreased[71 72]. This imbalance between pro/anti-inflammatory adopikines can play a major role in cardiovascular diseases such as atherosclerosis and

hypertension by increasing vascular tone, inflammatory processes and vascular smooth muscle cell VSMC proliferation and migration[66]. Total quantity of PVAT around coronary arteries is strongly related to atherosclerotic plaque[73 74]. It has been demonstrated that adipose tissue quantity is negatively correlated with microvascular coronary vasodilation response and also coronary flow hyperaemia[75]. More recently, the role of TNF on the NO/ET-1 imbalance in PVAT has been investigated in small arteries of visceral abdominal fat from obese subjects. The results have suggested that PVAT can have a role in endothelial dysfunction[76].

Weight-loss procedures such as bariatric surgery have been shown to improve vascular response by restoration of the vaso-relaxant effect of PVAT and also reversal of endothelial dysfunction[77 78]. PVAT is associated with an increased vascular expression of ET-1 and endothelin receptors. In addition, the increase of TNF in obsese subjects proposed to be responsible for NOS uncoupling and decreased NO release due to NADPH oxidase activation and increased reactive oxygen species (ROS) generation[76]. Arteries surrounded by PVAT from obese subjects release less NO compared to PVAT from controls. Thus the vaso-relaxant effects of PVAT in physiological conditions transforms into an inflammatory pro-contractile state in obesity[54 76].

It is well known that proliferation and migration of vascular smooth mucle cells (VSMC) plays a pivotal role in vascular remodelling which is observed during pathogenesis of atherosclerosis and hypertension[66]. These proliferative factors of PVAT include the adipokines resistin and TNF[79 80] were found to be VSMC growth factors. Adipokines play a role not only in in VSMC proliferation but also endothelial cell proliferation. Studies have demonstrated that leptin and chemerin could promote endothelial cell proliferation and migration and mediate the formation of blood vessels to a similar extend as VEGF in human endothelial cells[75 81-83]. Similarly resistin and visfatin have induced angiogenesis by promoting VEGF via metalloproteinase[84 85] and increase endothelial cell proliferation, migration and expression of cell adhesion molecules in human endothelial cells[86 87]. In contrast adiponectin supresses human endothelial cell migration and proliferation[88 89]. This

imbalance of proliferative and anti-proliferative adipokines observed in obesity is proposed to lead to the development of cardiovascular diseases. In addition, most of the overweight/obese individuals have deviant lifestyle habits , including smoking , and these issues are discussed below.

1.3. Lifestyle and cardiovascular risk

The relationship between CVD and lifestyle habits have been explored with accumulating evidence that environmental factors, personal behaviour and genetic pre-disposition are indirect causes of CVD mortality. A review of studies on these inter-relationships are presented in Table 1.3

1.3.1. Sedentary lifestyle

Adults in the western population devote at least half their employed hours in *sedentary behaviours*[90]. The term *sedentary behaviour* refers to any waking behaviour characterised by low energy expenditure while in a sitting or reclining posture[91 92] and is completely separate from not participating in physical activity. After work, adults usually are continuing their *sedentary behaviour* at home watching television. Television itself has been shown to occupy a large amount of an adults leisure time[93], and is predominantly prevalent in older adults who watch up to 5 hrs a day[94]. This accumulative sedentary behaviour can eventually lead to increased health problems and television viewing has been associated with snacking on energy dense foods[95], poor self-rated health, low household income, and low education[91]. Excessive TV viewing has is also associated with an increased rates of cardiovascular disease and all-cause mortality[96-99], depression[100], type 2 diabetes[101], systemic hypertension[98], and a high level of total cholesterol (CHOL)[98]. Sedentary lifestyle first contributes to gaining weight, usually in the abdominal area. If continued, the individual will become overweight. Sedentary lifestyle is also associated with poor nutrition, excess alcohol consumption and smoking, all contributors to a high level of cardiovascular risk[102].

Office workers are also at higher risks of developing routines which could easily lead them to a sedentary lifestyle[103 104]. The only way around this problem is to incorporate physical activity before

or after work and in most cases there are only few which that can manage this due to feeling of exhaustion and fatigue mentally as well as physically. In particular, office workers are at higher risk of developing metabolic syndrome, which incorporates an abdominal obesity, systemic hypertension, impaired glucose tolerance, low HDL levels, all of which increase the risk of future cardiovascular events. Previous studies have well documented the risk for obesity, diabetes, and cardiovascular disease through sedentary and office style work environments[103-107].

Table 1.3 Overview of potential lifestyle and environmental relationships with cardiovascular disease

	Author	Year	Participants	Purpose	Main findings
Lifestyle	Stampfer[108]	2000	84,129	To assess the effect of a combination of lifestyle practices on the risk of coronary heart disease	82% of coronary events could be prevented by maintaining a healthy lifestyle. 41% of coronary events were attributed to smoking.
	Chiuve[109]	2008	43,685	To examine the impact of stroke risk that is attributable to unhealthy lifestyle choices	64% of all coronary events were avoidable if patients adhered to a low-risk lifestyle
	Akesson[110]	2007	24,444	To determine the benefit of combining healthy dietary and lifestyle behaviours in preventing MI in women	Low risk behaviour was associated with 92% risk reduction of Myocardial infarction
Circadian rhythm	Knutson[111]	2006	298	To determine the relationship between impaired sleep and glycaemic control	Sleep duration and quality were predictors of HbA1c. Data suggests that sleep duration should be examined as an intervention to improve glucose control.
	Kohatsu[112]	2006	990	To determine whether short sleep duration is related to BMI and obesity in a rural population	Sleep duration was negatively correlated with higher BMI after adjusting for cofounding variables.
	Gangwisch[113]	2006	4810	to analyse the relationship between sleep disorders and duration with the incidence of hypertension	Participants with sleep disorders that are >32 yrs and <59 yrs were at higher risk of developing hypertension even after correcting for obesity and diabetes
	Kawachi[114]	1995	79109	To examine the relationship between shift work (including nights) with CHD in women	Shift work for at least 6 years contributed to an increased risk of CHD in women
	Morris[115]	2016	14	to determine the impact of acute circadian misalignment on CVD risk	Shift workers are possibly more susceptible to hypertension, inflammation and CVD risk due to circadian misalignment increasing BP and inflammatory markers.
Altitude	Fujimoto[116]	1989	65	to Investigate risk factors for occlusive arterial disease in Tibetan highlanders	Tibetans showed lower serum lipids, phospholipids and BP. They also had lower apo-B levels when matched to controls without any CVD
	De mendoza[117]	1979	230	To assess the relationship between altitude and serum cholesterol levels	High altitudes of 1000m resulted in significantly lower total cholesterol levels
	Mohanna[118]	2006	82	To assess the relationship between altitude, lipid profile, WC and BMI	altitudes at 4100m in Peru Revealed that subjects had higher levels of cholesterol, WC and BMI particularly in women

Environment	Critchley[119]	2004	100,000	To assess the increase in CHD mortality with CVD risk factors in Beijing, China	CHD mortality rates increased by 50% in men and 27% in women. Majority can be explained by rise in total cholesterol through "western diet" influences.
	Pekka[120]	2002	180,000	To determine dietary and lifestyle changes on CVD risk	Environmental changes and education through local councils showed a decrease in ischaemic heart disease by 73%
	Zatonski[121]	2005	100,000	To determine the decline in mortality from CHD to changes in food consumption and economic policy.	Changes in dietary polyunsaturated to saturated fat resulted in a 24% drop in coronary mortality.
	Aspelund[122]	2010	100,000	To determine the decrease in mortality attributed to surgical, medical, and dietary changes	73% reductions in mortality were attributable to smoking, cholesterol, blood pressure and physical activity.
	Ford[123]	2007	100,000	To determine the factors that have contributed to the decline of CHD	There have been substantial decreases in CVD risk factors through patient education and a revolution in the treatments for vascular disease that are available
	patel[124]	2006	600	To determine whether migration effects CHD risk factors	Western diet was associated with disproportionate levels of dyslipidaemia and hypertension compared to the country they migrated from
	Hedlund[125]	2007	1534	To analyse the prevalence of CHD in migrants to Sweden compared with co-twins from Finland	Emigration to Sweden reduced the prevalence of CHD possibly due to physical activity, dietary habits and psychosocial factors.
Seasonal	Marti-Solar[126]	2014	237979	To assess the seasonality of CVD risk factors in a large set of population based studies	Seasonal patterns of CVD risk factors tend to be higher in the winter months with increased BP, HDL, LDL and HbA1c in patients
	Tung[127]	2009	4162	To examine whether seasonal variations affect acute coronary syndromes in patients receiving statin therapy	Authors suggested lipoprotein metabolism may be regulated depending on the season as a significant amount of participants were able to achieve their LDL target in the summer
	Spencer[128]	1999	259,981	To determine whether cases of acute myocardial infarction varied by season	53% more cases were reported during the winter months. This was the same for all sub groups and with different geographic areas.
	Ostro[129]	2010	21,900	To assess the association between temperature and hospital admissions and whether air condition (AC) usage attenuated this association.	10F difference in one day resulted in increased hospital admission in patients' with CVD, respiratory disease and diabetes. Ownership of AC's significantly reduced these effects

1.3.2. Smoking

The substances in cigarette smoke include: carcinogens, respiratory toxicants, cardiovascular toxicants, and reproductive or developmental toxicants[130]. All of the above play vital roles in the pathogenesis of many diseases. The high concentration of carcinogens in cigarette smoke leads to the release of free radicals and the formation of oxidants. This damages tissue and depletes the body's plasma and tissue antioxidant levels[131]. Nicotine, being the chief addictive constituent of cigarette smoke, is also notorious for depressing the body's immune system, increasing susceptibility to illness and disease, as well as making it more difficult to heal and repair wounded tissues[132-134]. Smokers are more susceptible to an increase in lipoprotein concentration and accumulation[135]. Lipoproteins are particles that transport triglycerides (TG) and CHOL in the blood stream. They are essential components to cell structure and metabolism that aren't soluble in aqueous solutions[136]. Low density lipoproteins (LDL) "bad fat" are by-products of VLDL metabolism and are the primary carriers of plasma cholesterol. High density lipoprotein (HDL) "good fat" particles are created by the liver and intestine and mature to become enhanced with other apolipoproteins and lipids[136]. LDL levels are higher in smokers and HDL levels are markedly reduced, adding to the negative effects that lead to CVD and an increase in atherosclerotic events[135]. Further impedance of blood distribution caused by smoking is due to the effects on NO mediated blood flow regulation. NO was first discovered to be an endothelium derived relaxing factor (EDRF) and named as such. It transmits inhibitory infomation from vascular endothelial cells to smooth muscle cells[137] (ref toda 2010) and is responsible for the maintenance of the vascular tone, blood flow, peripheral vascular resistance and systemic blood pressure[138] . The vasoactive substances released from the endothelium are described in detail in section 1.7.2. Briefly, endothelium nitric oxide (eNO) causes vasodilation, increased blood flow, lower blood pressure, platelet aggregation and adhesion with reduced smooth muscle proliferation, thus contributing to a reduction in athlerosclerotic events[139].

For the past decades it has been will established and documented that smoking cigarettes is a major modifiable risk factor for CVD's and the world health organization has attributed to 10% of all CVD

cases in the world[140 141]. The exact mechanisms of the relevant constituents in tobacco are still unclear as to how they initiate, progress and determine cardiovascular health through endothelial dysfunction. It is more likely that not a single compound of toxicants is responsible but rather a mixture of the elements that are responsible for cardiovascular outcome which are discussed below.

Since then many studies have analysed the effect of smoking on serum lipids. In 1989 Craig et al[142] showed a statistically significant correlation between smoking and increased total serum CHOL, very–low-density lipoprotein (VLDL), LDL, and TG concentrations[140]. Additionally, they found that HDL and apolipoprotein A1 levels to be decreased in smokers. Similar conclusions were described by subsequent clinical studies, all validating that smoking modifies serum lipid profiles in a pro-atherogenic manner[143 144]. Smoking was also found to cause changes within the biomolecules of lipids themselves. Free radicals and oxidants present in cigarette smoke as well endogenous produced oxidants and radicals (resulting from the smoke chemical induced alteration in the cellular redox system)[140] causes a pro-oxidative environment[145]. Findings of increased lipid peroxidation in the serum of smokers and oxidative modification of LDL (oxidative stress) has been reported by many studies suggesting an impairment of antioxidant systems within the body[146-149]. It is known that oxidative modified LDL are recognized by macrophage scavenger receptors, they are taken up and transformed into foam cells which are essential elements in lipid deposition and plaque formation[140], this results in the progression of atherosclerosis.

Smoking is also known to have influences on the immune system both locally and systemically through inflammation which is an essential element in atherogenesis[140 150 151]. Smokers have a significantly elevated white blood cell (WBC) count, which is positively correlated to the formation of atherosclerotic plaques[152]. This was more recently confirmed by Lavi et al[153] which reported smokers with increased levels neutrophils and lymphocytes when compared to controls. In addition, smokers were also found to have increased levels of pro-inflammatory cytokines, such as tumor necrosis

factor-@ and interleukin-1B[154]. Another marker of inflammation which is widely accepted is C-reactive protein (CRP), this was also found to be increased in smokers[155 156].

Systemic immunologic alterations were found on smokers and that they correlate with atherosclerotic plaque within the vessel wall, characterized by inflammation and increased expression of matrix metalloproteinases[157]. In addition, leukocyte recruitment facilitated by endothelial adhesion molecules is a key element in the initiation of atherosclerosis is amplified by smoking[140]. Cavusoglu et al[158] showed that smoking increases the plasma concentration of soluble vascular cell adhesion molecule-1 in patients with CVD.

Clinical data on smoking has clearly shown a pro-oxidative environment in which smoking increases serum levels of oxidative stress markers, oxidative modification of LDL, lipid peroxidation, and lipid deposition in the vessel wall leading to plaque development and proression[159]. Additionally, Orosz et al[160] showed in vivo a link between smoking-induced increase in reactive oxygen species and the expression of pro-inflammatory cytokines, interleukin-1b, 6 and TNF-α, in the vascular wall by activation of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH).

One of the first studies to document smoking induced vascular changes was in 1993 by Celemajer et al[161]. This study shows that continuous smoking decreased forearm flow mediated dilation (FMD) in a dose dependant manor. This was also confirmed in another study which showed the reduced dilative capacity of coronary arteries[162] in smokers. Leading to smoking as an early marker of atherogenesis and vascular dysfunction. Oxygen free radicals, particularly superoxide anion is known to reduce the bioavailability of NO by the formation of peroxynitrite[163]. This reduction of NO in the vascular wall in smokers results from an imbalance in oxididant-reducing and oxidant generating cellular systems[164 165]. Compounds within smoke have also been proven to decrease eNOS activity and increase eNOS acetylation[166-168]. As well as leading to adhesion molecue expression on the surface of endothelial cells and release of proatherogenic cytokines[165], which result in increased number of platelets and a prothrombotic and procoagulative state within the vascular wall of

smokers[169]. Several experimental studies also concluded that apart from causing endothelial dysfunction, smoking also causes physical damage to the vascular endothelium. Contraction of endothelial cells or endothelial cell death mediated by oxidation and collapse of the tubulin system[140], could be responsible for the reduction in FMD[165]. This reduction leads to reduced endothelial functions and can promote thrombogenic events and inflammation. It was presented that cigarette smoke and interleukin-1b in combination contributed to endothelial junction disassembly[154]. Several studies found that smoking also induces smooth muscle cell proliferation. Proliferation of smooth muscle cells are mediated through activation of the platelet derived growth factor protein Kinase C signalling cascade[170 171]. In addition, compounds within cigarette smoke, specifically polycyclic aromatic hydrocarbons were shown to activate aryal hydrocarbon receptor, which induces iNOS (responsible for NO production), leading to intimal thickening[172], as well as encouraging the transcription receptors for chemokines and adhesion molecules, thus intensifying the inflammatory signals to the vascular endothelium[173].

The key developments in smoking induced atherogenesis commencement are endothelial dysfunction and damage leading to reduced bioavailability of NO, increase in oxidation and of proatherogenic lipids, as well as decrease of HDL, stimulation of inflammation, and the alteration toward a pro-coagulant state in the circulation. Of important note is that these affects have not only been shown in active smokers but also patients exposed to second hand smoke[155]. Taken as a whole cigarette smoking still remains the most important modifiable risk factor for CVD outcome.

1.3.3. Hypertension

Obese individuals more often exhibit upper levels of office as well as ambulatory blood pressure (BP) from childhood to old age; moreover they exhibit higher BP levels than non-obese individuals even in the normotensive range[174]. This evidence indicates that excess adiposity and weight gain are major causes of human essential hypertension, possibly accounting for as much as 65–75% of the risk[175] for the development of hypertension. Despite this, the mechanism through which obesity

directly causes hypertension is still an area of research and is being intensively studied, even though the relationship between obesity and hypertension is well established in both adults and children[176] [177]. Nevertheless, one should not assume that every obese person is hypertensive but that weight gain increases the probability of becoming hypertensive, by shifting the distribution frequency of arterial pressure towards higher BP values[178-180]. Current research in experimental studies propose essential roles for reduced renal-pressure natriuresis (excessive sodium excretion) due to physical compression of the kidneys and activation of the Renin-Angiotensin-Aldosterone-System (RAAS) and SNS which are discussed below.

Increased visceral and retroperitoneal adiposity could increase BP by physical compression of the kidneys. Excess fat amassing in and around the kidneys is linked with impaired pressure natriuresis, amplified intrarenal pressures and hypertension[181]. In patients with visceral obesity, intra-abdominal pressure rises in proportion to sagittal abdominal diameter, reaching levels as high as 35–40 mmHg[175 182]. These elevated pressures compress the renal veins, renal parenchyma and lymph vessels. Moreover, retroperitoneal fat often encapsulates the kidney, adheres securely and occupies the renal capsule and sinuses, causing further compression and elevated intrarenal pressures[181]. During obesity, increases in retroperitoneal and renal sinus fat are associated with hypertension. The Dallas Heart Study that showed that retroperitoneal fat was uniquely correlated with incident hypertension[183]. In addition it was reported that renal sinus fat was associated with stage II hypertension[184]. Compression of the kidneys through retroperitoneal and renal sinus fat may cause inflammation and expansion of renal medullary extracellular matrix that could further impair renal function[175]. Increased sodium reabsorption initiated by renal compression could secondarily contribute to glomerular hyperfiltration, renal vasodilation, and augmented renin secretion in obese subjects[181]. Rapid weight gain can't account for the initial rise in BP due to renal compression, but it can explain why visceral obesity and increased renal fat are more closely associated with hypertension than subcutaneous fat[175]. As the metabolic and hemodynamic consequences of obesity are sustained over many years, renal damage progressively makes the hypertension more severe

and more resistant to therapy[175]. To date, the only established strategies to decrease visceral, retroperitoneal and their adverse effects on cardiovascular, metabolic and lifestyle modification are with dietary changes, increased physical activity and the latter stages of obesity through bariatric surgery.

In patients with obesity, activation of RAAS modulates insulin resistance, renal sodium handling, SNS activation, which jointly contribute to cardiovascular dysfunction[185-188]. Angiotensin II (ANG II) synthesis by intravascular and intrarenal RAAS can directly control vascular stiffness, endothelial and renal function[186 189]. Angiotensinogen expression by adipose tissue in obesity supports a role for local RAAS activation[190]. Moreover, it was shown that increased ANG II production by perivascular adipose tissue contributes to impaired vascular function[64 191]. Aldosterone has been implicated in the modulation of vascular remodelling through enhanced smooth muscle reactivity[186] and ED[192]. This result of increased plasma aldosterone levels in obesity are derived from adipose tissue that can stimulate adrenal aldosterone secretion[193 194] in turn which can activate NADPH that promotes oxidative stress and decreases NO bio-availability[195]. Aldosterone also increases endothelial rigidity by modifying epithelial sodium channel expression on the endothelial cell surface and NO release[195].

Structural and functional changes in the kidney that activate the intrarenal ANG II are also important in the development of obesity related hypertension[196]. It was previously shown that components between the RAAS, specifically ANG II and aldosterone can regulate vasoconstriction independently of renal control[185 197]. This can be the case in lean patients with hypertension that presented with an increase in peripheral vascular resistance, whereas obese hypertensive patients are mediated, in part, by sodium absorption in the kidney, increased intravascular volume and cardiac output[198].

Activation of the RAAS in obesity also contributes to glomerular injury and nephron loss not only by elevating BP but also through intrarenal effects through constriction of efferent arterioles by ANG II exacerbating the rise in glomerular hydrostatic pressure caused by arterial hypertension[181] to maintain sodium balance[176 199].

Obesity induced hypertension is also associated with activation of the SNS in diverse tissues which include the kidneys, heart, and skeletal muscles[200-202]. Individuals who are obese, irrespective of BP have increased renal SNS activity compared with healthy individuals, indicated by an elevation in renal norepinephrine levels[202]. Increases in both renal and cardiac SNS activity could possibly be one mechanism that leads to the development of hypertension in obesity. Remarkably, individuals who have obesity but are normotensive have suppressed cardiac SNS activity, however those who have obesity and hypertension have increased cardiac SNS activity[202]. There are studies that suggest SNS activation alone might not lead to the development of hypertension. It was speculated that a division between SNS activity and peripheral vascular tone can protect a small proportion of obese individuals from ever developing hypertension[203]. Increased alpha-adrenergic mediated vascular tone has been reported in overweight men with hypertension[204] however, rather than being overweight, it was more likely plausible that hypertension could account for the increase in SNS activity. Alternatively, In individuals who are normotensive and obese, sympathetic outflow to the forearm musculature did not lead to an increase in peripheral sympathetic vascular tone[203].

Increased leptin secretion from dysfunctional adipose tissue is also an important modulator of SNS activity[205]. Another factor by which Leptin is secreted by white adipose cells and its production correlates positively with the percentage of body fat[206]. In experimental studies it was shown that acute and chronic leptin infusion increased blood pressure and heart rate[207 208] despite decreases in food intake. However, leptin also has counterbalancing cardiovascular effects, the leptin receptor is expressed in endothelial cells[209] which induces the release of NO[210]. This influence of leptin on blood pressure homeostasis may be the result of a balance between the action through sympathetic activation and the endothelium dependant hypotensive effect of leptin[179]. Consequently, any factor that impairs endothelial function in obesity may dilute the vasodilative capacity of leptin. Advancement to a chronic hypertensive obese state can possibly be preceded by a loss of nocturnal BP dipping[211 212]. A non-dipping configuration of circadian BP increases the risk of CVD and Chronic kidney disease (CKD)[212 213]. The exact mechanisms for this non-dipping pattern of BP is unknown but

is possibly a combination of insulin resistance, autonomic nervous system (ANS) dysfunction, and increased SNS activity[212 214].

Endothelial dysfunction and arterial stiffness are thought to precede the development of hypertension which is the early manifestation of vascular dysfunction in obesity[215-218]. Vascular smooth muscle dysfunction[219] and changes in the extracellular matrix[220] contribute to arterial stiffness; there is also evidence suggesting that ED also contributes to vascular stiffness though the association with insulin resistance[215 221]. Impaired vascular reactivity to insulin in prehypertension can be seen in spontaneous hypertension[222] suggesting that insulin resistance is an early event in obesity-related hypertension development.

1.3.4. Metabolic syndrome

Obesity is a risk factor for the development of the metabolic syndrome which encompasses diabetes, hypertension, and dyslipidaemia that can occur simultaneously resulting in vascular complications[223]. However, epidemiological studies specify that the preferential accumulation of intra-abdominal (visceral) fat[224], neighbouring the gastrointestinal organs poses a superior cardiovascular risk than in other forms of obesity in which fat is amassed under the skin (subcutaneous) in the gluteal region[225].

To date the exact mechanism by which obesity results in diabetes and increased cardiovascular risk has yet to be elucidated; though a common denominator in this relationship is the link between excess adipose tissue and insulin resistance i.e. (the inability of insulin to exert its metabolic effects) and thus leads to increased glucose within the blood. A chronic low grade inflammation and an activation of the immune system are observed in abdominal obesity and may have a role in the pathogenesis of obesity related metabolic disorders[226-230]. Indeed, white blood cell counts and plasma levels of coagulation factors (fibrinogen and plasminogen activator inhibitor 1(PAI-1)), proteins like C-reactive protein (CRP);pro-inflammatory cytokines (tumor necrosis factor-alpha (TNF-α), interleukin (IL-1B) and (IL-6)) are elevated in obese and diabetic patients. When these patients

are engaged in rigorous activity and undergo lifestyle changes, these systemic markers of inflammation reduce[41 226 231-235].

Increased expression of TNF-@ in adipose tissue of obese individuals and its direct role in obesity induced insulin resistance was first described by Hotamisligil and colleagues[56]. Over time accumulating data has shown an over-production of inflammatory cytokines in enlarged adipose tissue[228] and was considered a crucial event leading to metabolic syndrome (MS), diabetes mellitus and atherosclerotic CVD's. More recently, adipose tissue has been associated with an accumulation of immune cells which causes an infiltration of macrophages in adipose tissue[63 227 236]. Indeed, their recruitment correlates with the degree of obesity and is linked to systemic inflammation, and insulin resistance[63]. Infiltration of macrophages have similarly been described within skeletal muscles, particularly in the inter-muscular adipose depots, and have been a major site of local insulin resistance in obesity and diabetes mellitus[226].

The development from obesity related insulin resistance to diabetes mellitus is a failure of the pancreatic b-cells in the islets of Langerhan to recompense effectively, leading to chronic hyperglycaemia. Inflammation was shown in the pancreas of patients with diabetes with presenting infiltration of macrophages, fibrosis and increased levels of pro-inflammatory cytokines[63]. This increase in inflammation can be seen before the onset of diabetes in obese individuals which can reduce insulin secretion and trigger b-cell death leading to a decrease in islet mass[226].

In the vasculature two components of insulin signalling exist: metabolic and growth factor signalling[237]. Metabolic signalling involves insulin receptor substrate-1 (IRS-1), phosphoinositide 3-kinase, protein kinase B (AKT), and endothelial nitric oxide synthase (NOS)[237]; growth factor signalling functions via the extracellular signal regulated kinases (ERK)1/2 and (ET-1) pathways[185 237-240].

In insulin resistant conditions, metabolism is compromised owing to serine phosphorylation of IRS-1, which leads to reduced NO bioavailability and impaired vascular dilatation[238]. Equally, in this state,

upregulation of the ET-1 pathway contributes to increased vascular contraction[238]. Coupled in the obese state, metabolic changes in adipose tissue can also lead to altered adipokines secretion and impaired adiponectin levels[241] within the blood plasma of obese individuals which promotes insulin resistance[187 188 242]. Together these factors contribute to the deleterious effects of metabolic syndrome which predispose the individual to hypertension, insulin resistance, atherosclerosis and ED which are all risk factors for the development of CVD.

1.3.5. Cardiovascular disease

Various studies have established the strong relationship of overweight and obesity with increased prevalence of most CVD's that include CHD, CAD and atrial fibrillation[243-246]. Though the relationship between obesity and cardiovascular disease is to some extent complicated; some investigators have suggested an indirect association due to the increased prevalence of diabetes, dyslipidemia and hypertension although others have demonstrated and independent association between obesity and cardiovascular disease[247-249].

Weight gain and obesity lead to various alterations in body's hemodynamics, including increased central blood volume, decreased systemic vascular resistance, a rise in cardiac output, and cardiac remodelling[250-253]. Obese individuals are more likely to have left ventricular (LV) remodelling, left atrial (LA) enlargement, and greater right ventricular mass and end-diastolic volume[254-257]. Interestingly, obesity's association with heart failure (HF) has been with a preserved ejection fraction of greater than 50%[258 259]. The area of the heart which is responsible for pumping blood through the aorta has a normal range of roughly 50%-70%[260]. Many studies have established a strong association amongst overweight/obesity and HF. In the Framingham Heart Study, every 1 kg/m^2 increment in BMI was found to increase the risk of HF by 5% in men and 7% in women[244 261]. In a separate analysis of the Framingham Heart Study it was revealed that obesity was associated with significant decreases in life expectancy and these results were similar to the control group which included smokers[14]. In addition, the risk of developing hypertension is 5x greater for obese individuals than

those of normal weight[262]. Other parameters in obesity, such as WC or waist-to-hip ratio, are useful for predicting individual risk. In a meta-analysis of 28 prospective studies, every 10 cm increase in waist circumference (WC) was associated with a 29% higher risk of HF[263]. In addition, a cross sectional study by Janssen et al[264] compared BMI and WC in a total 14925 participants from the national health and nutrition examination survey. The study revealed that those with high WC values were at greater risk to have hypertension, diabetes, dyslipidaemia and metabolic syndrome compared to controls. This was also significant in the case of comparing BMI values across groups even after adjusting for confounding variables. A cross sectional population survey by Allison et al[265] suggested that more than 80% of hypertension arises in individuals with a BMI of >25kg/m2 and up to 2 thirds of cases of hypertension are linked to excess weight[266]. Two separate meta-analyses also exhibited that overweight and obese individuals are at increased risk of CVD and mortality even in the absence of metabolic abnormalities[267 268]. A study by Sims et al[269] showed that in otherwise healthy young men without a family history *of* diabetes who were overfed for 6 months BMI increased to 28 kg/m^2 and there were reversible rises in concentrations of triglycerides, insulin glucose, and impaired glucose tolerance. The risk of developing diabetes greatly increased by early weight gain[270] especially in childhood and abdominal obesity. Furthermore, Stevens et al[271] showed that 90% of individuals who develop type 2 diabetes have a BMI higher than 23kg/m2.

Obesity and its accompanying comorbidities such as HTN, dyslipidemia, and diabetes play a crucial role in the pathogenesis of atherosclerosis, the hallmark finding of CHD[244 272]. It was shown that relatively young men, aged 15-35yr, who are obese have an association with accelerated coronary atherosclerosis[273], and that dyslipidemia progressively develops as BMI increases from 21 kg/m2 with a rise in small particle (LDL)[15]. This change increases the risk of coronary heart disease by 3-6 fold, resulting in Low concentration of HDL and high concentrations of triglycerides[270 274].

Overweight and obese individuals tend to have a higher prevalence of CHD compared to their lean controls[275]. The Framingham Heart Study demonstrated that roughly 23% of CHD in men and 15% of

CHD in women were attributable to excess visceral adiposity[246] even adjusting for covariates. In addition, sudden rupture of atherosclerotic plaque within the coronary arteries also known as Acute coronary syndromes (ACS) occur at earlier ages in overweight and obese individuals compared to those with a normal BMI[244]. A large retrospective cohort study showed that obese individuals with greater BMI than 30kg/m^2 had the prospect of suffering from ACS 7 years earlier as compared to normal weight individuals. This trend had a positive and linear relationship[276].

The obesity "paradox" is now a well-established phenomenon across different types of CVDs. It is derived from a complex interaction between several potential mechanisms but this remains poorly understood and can occur regardless of ethnicity and age and severity of CVD[252]. However, growing evidence suggests that muscle mass, strength training and cardio respiratory fitness (CRF) are the major determinants of the prognostic implications of obesity in CVD[277-279]. Increased CRF through physical activity and strength training has been associated with improvement in cardiometabolic risk factors, metabolic syndrome and can prevent/delay the development of hypertension[280-283] ameliorating the risk for CVD prognosis. Higher CRF has been associated with several changes in the cardiovascular structure and function, that include larger LV chamber size, improved diastolic function, and greater left atrial volume[284 285]. The FHS revealed that higher PA levels were associated with lower arterial stiffness[286]. In the Coronary Artery Risk Development in Young Adults (CARDIA) study higher baseline CRF even after 25 years was associated with better diastolic BP, and lower LV mass[287 288]. Broadly speaking, high CRF provides a cardio protective effect regardless of race, age and gender, and independent of other traditional risk factors, including smoking, DM, HTN, and BMI[102 244 289].

1.4. INTERVENTIONS TO REDUCE CVD RISK IN OVERWEIGHT AND OBESITY

Having a healthy BMI within the range of 20-25kg/m2 usually does not require any interventions. Once overweight BMI >25 one should consider necessary lifestyle changes, this usually includes physical exercise and eating a healthy diet. This can be taken further by restricting your daily calorie intake anywhere between 250-500 calories than you would normally consume. Fasting is also an option but usually only required under certain circumstances and/or under appropriate supervision. Pharmacotherapy has been used within the last 40 years[290 291] as a method of weight loss with promising results but is usually only prescribed once other methods have failed. Once the individuals BMI is >30kg/m^2 and they have not achieved any results your primary care physician will likely refer you onto a nutritionist/dietician. They will advise you of the appropriate management which will probably include all of the above with special emphasis on the diet including a breakdown of the macro-micro nutrient intake as well as a tailored exercise regimen. The last stage of management is bariatric surgery, usually only provided in certain circumstances which patients are suffering from morbid obesity BMI >40 kg/m2 or BMI >35 kg/m2 with $\geq$1 comorbidities.

1.4.1. Exercise and diet

Exercise as a method of weight loss is the best option. Firstly, if one exercises routinely they are at less risk of becoming overweight. It is known that regular exercise and healthy food choices reduce stress and puts the individual in a better mind set. If the individual has become overweight, this can be due to a number of factors such as a sedentary life style, high fat and condensed rich calorie meals, one has to start exercising because eventually this path will lead you to becoming obese. There are a number of studies which have looked at exercise training on overweight and obese individuals and the results show significant improvements in anthropometric measurements. Many studies have also shown that participants who exercise 3-4x a week without focusing on their diet make improve but individuals who are careful with their diet achieve better results and are more likely to maintain their habits. This can be done on their own or some type of counselling by a

nutritionist or physician. Fasting or a reduced calorie intake has shown to help individuals reduce their weight. Several studies have looked at reducing calorie intake roughly from 250-1000 a day. Other, studies have calculated the total calorie intake per person for the week and look at reducing it by roughly 5000 calories. Fasting is also a method of weight loss. Muslims fast during the month of Ramadan from sunrise to sunset and there are a few studies (table 1.4.1.1) which have looked at the benefits of this with promising results. Unfortunately it is very difficult for an individual to maintain this beyond Ramadan and eventually within a few weeks or so it has been shown that those individuals that have lost weight usually regain it back. There are also studies that looked at alternate day fasting. These studies usually looked at a complete fast for one day (table 1.4.1.1), with the exception of water, and alternate days of whatever the participants can fathom. It is important to note that the studies carried out saw reductions in weight and BMI but alternatively it is not something that most individuals can do on a regular basis due to the extreme will power one must contain within themselves. Tables 1.4.1 and 1.4.1.1 show the studies that have previously looked at physical exercise and reduced calorie intake for weight-loss management.

1.4.2 Pharmacotherapy for weight management

Pharmacological interventions have gained much attention even though the amount of weight-loss attributable is roughly 5kg a year[292 293] and no drug so far has the ability on its own to help patients lose weight. Patients that do require drugs to lose weight must also appreciate that a reduced calorie intake of 500-1000 calories will be necessary as well as lifestyle and behavioural changes with regards to the specific type of foods they eat. This brings the question into play as why is there a need for this since it has already been shown that maintaining a healthy diet, watching the type and quantity of calories you consume as well as regular exercise can help an individual shed the pounds while feeling better psychologically as well. The other aspect of using drugs to lose weight is that they have side effects, some of them which are life threatening, and there are numerous drugs in the past which have been banned for this reason. Nevertheless, they are being prescribed and newer

drugs are coming on the market every year but to this date no drug has been able to show weight loss without calorie restriction. Table 1.4.2 shows the studies which have investigated weight-loss interventions using pharmaceutical drugs.

1.4.3 Bariatric surgery

Bariatric surgery refers to the surgical techniques available for achieving weight-loss. The technique includes modification of the gastrointestinal tract to either reduce absorptive capacity or volume[294]. Currently there are a few techniques used worldwide which encompass Laporoscopic adjustable gastric band (LAGB), Laporoscopic sleeve gastrectomy (LSG), and vertical banded gastroplasty (VBG). LAGB is currently the most common surgical technique since 1996 and has now over taken the traditional stomach stapling procedure. The procedure is reversible and can be done in approximately 1 hour. The gastric band is an implantable device which consists of 2 parts, a round silicone band with an inflatable cuff that sits around the cardia and a reservoir that is placed over the fascia of the abdominal wall. The 2 parts are connected by silicon tubing. The gastric band is placed just below the gastrooesophageal junction (GOJ) around the gastric cardia, creating a 10–15 ml gastric pouch[294]. The band forms a aperture of about 10-12 mm in diameter so that food entering the stomach is slowed down. With stretching of the stomach wall leading to early satiety through signals transmitted via the nucleus tractus solitarius to the appetite centres in the hypothalamus thus reducing appetite[294].

The other techniques are not reversible and are permanent. LSG procedure restricts food intake by reducing the size of the stomach. The gut is also re-arranged which produces significant changes of several gastrointestinal hormones[294]. The hormone Ghrelin, which is secreted by the neuroendocrine cells and acts on the hypothalamus to regulate appetite, is suppressed after LSG. All other treatment options must be carried out first and, when unsuccessful, bariatric surgery is only indicated when the individuals BMI is >40 or >35 with comorbidities. Table 1.4.3 shows the studies that have been conducted on bariatric surgery for weight-loss management.

Table 1.4.1 Overview of weight loss management with exercise

Author	Year	Participants	Purpose/Summary	main findings	Disadvantages
Ross[295]	2000	52 obese men (11 controls)	To determine the effects of diet- or -exercise induced weight loss and exercise without weight-loss on abdominal/visceral fat, and insulin sensitivity in men	Weight loss induced by daily physical activity without calorie restriction significantly reduced obesity and insulin resistance. Exercise without weight-loss reduced abdominal fat and prevents further weight gain	Due to random group assignment there were more participants that dropped out then calculated, this let to approximately 10 people in each group. The calorie deficit among studies is usually 500, this study had a deficit of 700.
Ito[296]	2001	12 obese participants, longitudional study	to evaluate the effect of increased exercise and mild calorie restriction on heart rate vairability	After 3 months the patients had significant improvements in BMI, waste circumference, total cholesterol, triglycerides, and an improvement in parasympathetic modulation	As there were only 12 participants in the study they were not able to test the influences of systemic measures on their outcome measures due to a small sample size
Facchini[297]	2003	40 obese individuals (10 male)	To assess the impact of a 3 week integrated calorie restriction diet and high intensity exercise training in morbidly obese individuals	Resting SBP, DBP, BMI and total % body fat were all reduced following the exercise program. The reduction in Heart rate and increase in parasympathetic activity contribute to a reduced risk of cardiovascular morbidity.	The results were significant enough for a change in multiple parameters but no mention was made on the psychological aspect of the participants on a hypocaloric diet of 1200 calories and whether the participants can potentially to change after the study.
Trombetta[298]	2003	59 Obese women (24 diet alone) (25 diet + exercise) (10 controls)	To evaluate the effects of weight loss induced by moderate exercise and hypocaloric diet on neurovascular control	Diet and exercise significantly reduce BMI and %percent body fat, also increased resting forearm vascular conductance at rest and during exercise. Insulin sensitivity was also increased during the hypocaloric diet and even more so accompanied with exercise.	No measurements were reported on SBP and DBP only MBP and this did not change in any of the groups. Along with visits to the dietician, patients were instructed on diet and calorie break down but not everyone adhered to the advised protocol
Woo[299]	2004	82 overweight children, longitudinal study	To evaluate the reversibility of vascular dysfunction in children through exercise and diet	Diet alone improved arterial endothelial function but the combination of diet and exercise had significant improvements in body fat, endothelial function and lipid profile	The intensity of the exercise program was difficult to apply to obese children which possibly led to a selection bias of which group the patient should belong
Ross[300]	2004	54 obese women (10 controls)	To determine the effects of diet- or -exercise induced weight loss and exercise without weight-loss on abdominal/visceral fat, and insulin sensitivity in women	Daily exercise with no caloric restriction showed significant reductions in total fat, abdominal fat, and insulin resistance. Exercise without weight loss was also associated with reductions in total fat and abdominal obesity.	The study was underpowered due to dropouts not wanting to participate in a randomized group. If being part of a weight loss study requires one to do nothing (control) then it's difficult to have participants.
figueroa[301]	2007	28 obese women (10 with type 2 diabetes mellitus)	To evaluate the effects of moderate intensity endurance training in obese women with and without type 2 diabetes mellitus	After 16 weeks of speed walking training, resting SBP and DBP was significantly reduced in both groups. Heart rate was also reduced in both groups following recovery after 20 mins post exercise.	Heart rate did not differ when patients in both groups were tested at rest. There were no differences in BMI and Total body fat % after 16 weeks of training.
Goulopoulou[302]	2010	62 obese participants (26 type 2 diabetes mellitus)	To investigate the effect of aerobic exercise on cardiac function, body composition, central adiposity, insulin sensitivity, and lipid profile.	The group without diabetes had significantly reduced BMI, waste circumference, and diastolic blood pressure. The results were also significant in the non-diabetic group with reductions in fasting glucose, insulin sensitivity and reduced LDL cholesterol.	Unlike other studies that examined diabetics this study did not find anything significant after the intervention, Possibly due to their ingestion of a variety of oral glycaemic control medications and the intervention of 16 weeks. Diabetes effects endothelial function and other studies had only noticed improvements after 12 months
Blumenthal[303]	2010	144 overweight/obese individuals	To evaluate the effects of 4 months of dieting alone and dieting with exercise on participants with pre/stage 1 hypertension that are not on medication	The 2 different groups had significant improvements in SBP and DBP, reduced arterial stiffness and changes in overall aerobic fitness compared to controls. The results more significant if the participants included exercise. After the treatment period, the 2 groups were no longer classed as hypertensive	The group that was exercising was also being trained psychologically. Based on cognitive behavioural therapy, this teaches the participants to learn when and how to eat. This was not done on the diet alone participants even though it would have been appropriate

Table 1.4.1.1 Overview of fasting/reduced calorie intake studies for weight loss management

Author	Year	Participants	Purpose/Summary	Main findings	Disadvantages
Temizhan[304]	2000	52 (25 M) normal/overweight participants	To examine the effects of voluntarily fasting on blood lipid levels	There were significant decreases in total cholesterol, triglycerides, HDL, and LDL cholesterol. While there was an increase in glucose levels after 30 days.	The study showed that both groups had reductions in blood lipids though they reported that women had better results even though they did not lose any weight. That's slightly different in the table they reported showing a significant difference in weight after 30 days
Harvie[305]	2011	107 0verweight/obese women	To compare the feasibility and effectiveness of 25% energy restriction 2 days a week compared to 25% restriction 7 days a week on insulin sensitivity, weight loss, and metabolic disease risk factors	After 6 months both groups had Significant reductions in body weight, insulin sensitivity, serum leptin and increases in plasma adiponectin with no differences between groups.	Both groups had comparable reductions in total and LDL cholesterol, triglycerides, SBP and DBP but these weren't significant. As a cardiovascular disease risk factor a calculation on the long term risk might have provided added information to the study; such as the calculation of the framingham risk score before and after 6 months of calorie restriction
Teng[306]	2011	25 healthy overweight participants	to conduct a 3 month pilot study to investigate the effect of calorie restriction on quality of life, food intake and body composition	In the calorie restriction group compared to controls there were significant decreases in body weight, BMI, Body fat % and depression with participants in the CR group reporting increased energy levels. There were no differences in the levels of stress and sleep quality	The control group had also reduced their daily calorie intake even though they were advised not to make any changes. Which could have possibly narrowed the gap between measurements within the groups. The groups chosen didn't differ by age but the participants were above 50 as the experimenters believed they were more likely to follow fasting guidelines than younger counterparts
Varady[307]	2013	32 (16 control) overweight participants	To examine the effect of ADF on body weight and coronary heart disease risk in overweight participants	There were significant decreases after 12 weeks in terms of body weight, BMI, Body fat %, C-reactive protein (CRP), and leptin, while adiponectin increased compared to controls.	The fasting regimen included alternate days of calorie restriction and days of ad libitum intake but there were no documents on the quantity and quality of food participants were allowed to eat because they could have made up the excess in calories on the alternate day, which could represent why there were no differences if LDL and HDL cholesterol between the groups
Bhutani[308]	2013	64 obese participants	to investigate the effect of ADF with endurance exercise on endothelial function, relative to ADF end exercise alone	In the ADF group BMI decreased and in the combination group body weight decreased, waste circumference as well as plasma leptin levels. The increase in Endothelial function was only significant in the ADF group and not in the combination group.	Alternate days of fasting included ad libitum intake with no measures of calories consumed and the equality of food ingested. The baseline characteristics did not differentiate between ages as endothelial function is known to reduce with age.
Eshghinia[309]	2013	15 overweight participants	To examine the effects of modified ADF diet on BMI, waste circumference, SBP, DBP and cardiovascular risk factors	After 8 weeks significant decreases in total body weight, BMI, waste circumference, Fat mass %, SBP, and DBP were achieved. Total cholesterol and triglycerides were reduced but were not significant.	This study like others had no control group and included limited subjects with only 8 weeks duration. The study also found that men had significantly decreased LDL cholesterol while women has an increase but combined the results were not significant.
Norouzy[310]	2013	82 participants <35 yrs, 158 participants 36-70 yrs	to determine whether fasting during ramadan has any effect on body weight and composition relative to age and sex	Significant reductions in both groups in body weight and BMI but more so in males <35 yrs. Waist and hip circumference decreased in all except women >35 yrs. Percentage body fat only decreased in males. Dietary intake was similar but males reported decreased protein intake	Physical activity was not assessed so any changes observed couldn't be attributable to energy expenditure. There was only a sub group of participants who kept a food log and thus the analysis was only done on sex but not age. It is not known whether the changes persisted in individuals after the study as it is known for subjects reverting to their baseline values within a few weeks after ramadan
Varady[311]	2015	121 overweight/obese participants	To examine the characteristics, namely age, sex, baseline body weight/BMI that can predict weight loss success with alternate day fasting (ADF)	After 8 weeks of ADF; subjects aged 50-59 achieved the greatest weight loss. There were no differences between men and women but Caucasians achieved greater weight loss than other races. Baseline body weight and BMI did not predict outcome of success.	The study didn't perform a regression analysis to assess the independent contributions of age, sex and BMI on predicting weight loss even though that was their aim. The participants were allowed to eat whatever they chose on the alternate day but no record of what they ate nor was the amount of calories determined.
Antoni[312]	2016	10 (3F) overweight/obese participants	The aim of the study was to characterise the early metabolic response to varying degrees of energy restriction	Total energy restriction led to significant reductions in levels of fasting plasma glucose and triglycerides the following day. Partial energy restriction let to higher amounts of fat metabolism.	The women in the study showed an impairment of glucose tolerance but there were only three participants. Moreover, participants showed a prolonged period of decreased insulin sensitivity after the first day. 3 days is not long enough to determine the profile of participants. Ideally the study should have lasted for 3-4 weeks.

Table 1.4.2 Overview of weight loss management using pharmocotherapy

Author	Year	Participants	Purpose/Summary	main findings	Disadvantages
Munro[313]	1968	108 obese women, randomized placebo controlled, double blind study	To assess the effect of phenturmine on obese women after 36 weeks	The mean weight loss was 12.5 kg in patients taking the drug and 4.5 kg in the placebo group. The combination of calorie restriction and phenturmine produced promising results	The group which alternated the drug and placebo every 4 weeks had similar results to the group using the drug during the whole experiment. During the last 16 weeks of the trial there was no statistically significant change in weight loss between the three groups.
Alien[314]	1975	110 patients, randomized placebo controlled, double blind	To assess the use of Diethylpropion in conjunction with calorie restriction and exercise on obese patients	At 12 weeks there were significant reduction in weight compared to the placebo group, on average patient lost between 4-7Kg. Systolic and Diastolic BP were significantly lower as the 2 and 8 week follow up.	The drug group lost the most weight but the placebo group lost a significant amount as well. This study was only 12 weeks and possibly not long enough for obese patients to settle in a healthy weight range
Baird[315]	1977	38 patients, randomized placebo controlled, double blind	To assess the use of mazindol and calorie restriction on obese patients	At 12 weeks there were significant changes in body weight and waste circumference. Patients lost roughly 11.5 kg after treatment	The study did not find any significance between the 2 groups. This is probably because the 1000 calories a day would make anyone lose weight and be difficult to test the efficacy of the drug
Brun[316]	1988	44 patients, randomized placebo controlled, double blind study	To assess the effects of fenfluramine in hypertriglyceride obese subjects	Following 12 weeks follow up. Patients on the drug had decreased levels of LDL cholesterol and increased levels of HDL cholesterol, weight loss was roughly 3kg and was considered significant	Weight loss was only 3 Kg after 12 weeks, that's 0.25 Kilos a week which can be easily accomplished with diet and exercise. This drug has a potential to be used as a lipid lowering medication but not for weight loss.
Darga[317]	1991	45 obese patients, randomized placebo controlled, double blind study	To assess the combined effect of fluoxetine and diet after 52 weeks	The combined effect resulted in a mean loss of 12.5 kg after 26 weeks and 4.5kg for the placebo group	There was no information regarding the total caloric diet. The placebo group on the diet retained their weight loss from 26 to 52 weeks, while the drug group gained 5 Kg. Towards the end of the study. After 52 weeks there was no difference between the 2 groups
Van Gaal[318]	2005	1507 patients, randomized placebo controlled, double blind	To assess the effect of rimonabant on bodyweight and cardiovascular risk factors in overweight/obese patients after 52 weeks	Rimonabant 20mg was significantly associated with reduction in body weight, waste circumference and % BF. Patients lost a total of roughly 6 kg	Patients taking Rimonobant 5mg had some promising results but did not differ significantly from the placebo group. The 20mg dose reduced weight but all participants were put on 600 a day calorie deficit.
Hsieh[319]	2005	106 (55) controls	To assess the influence and effect of Orlistat on parameters of metabolic syndrome but without patients suffering from chronic diseases	There was a significant reduction in BMI, waste circumference and % BF in the drug group. At one year follow participants lost roughly 6 KG	Originally had 180 patients which only 106 completed the study. The participants were also given a specific diet to follow which included caloric restriction and daily exercise. This could possibly alter the effects of the drug in question
Fidler[291]	2012	4008 patients, randomized placebo controlled, double blind	To evaluate the effects of locraserin on body, weight cardiovascular risk factors and safety in obese/overweight patients.	There was a significant reduction in body weight, decreased body fat content and improved quality of life. At one year follow up participants lost roughly 4-6 KG	The placebo group that exercised and had a calorie restriction diet improved as well but the analysis only compared the drug groups to placebo. A within measures of the placebo group should have been carried out

Table 1.4.3 Overview of weight-loss management with bariatric surgery

Author	Year	Participants	Purpose/Summary	main findings	Disadvantages
Sjostrom[3][20]	2004	4047 obese participants	To assess the long term benefits of bariatric surgery. At 2 years and at 10 years.	After 2 years there was a 24% decrease in weight and 16% at 10 years. The participants which benefited the most kept physically active. All systemic plasma measure were reduced against the controls however there was no difference between the groups in total cholesterol.	The study could have calculated the framingham risk before and after surgery and at subsequent visits. Throughout the study obese controls were used for comparison. It would have been better to compare the surgery with age matched normal BMI controls to differentiate their actual risk.
Torre[321]	2008	45 Obese participants & 9 controls	To analyse the relationship between weight and circulating (VEGF-a) in morbidly obese subjects before and after bariatric surgery	After surgery there was a significant decrease in leptin and insulin concentrations. Adiponectin also showed an increase after surgery. Participants lost an average of25% of their body weight after 9 months.	The study carried out was only done on women and they can't make generalisations about the surgery without including men. There were no BP measurements and no anthropometric data
Mingrone[322]	2012	60 obese participants	Trial comparing the efficacy of bariatric surgery and conventional medical therapy in severely obese patients with type 2 diabetes.	Both groups had significant changes in BMI, WC, fasting glucose, total cholesterol but there were no differences in systemic BP. Those that had bariatric surgery had a 75% rate of remission of diabetes at 2 year follow up.	The study had 3 groups but the participants were not enough for the level of power. Sample size was small which led to differences in detecting important endpoints. Such as long term morbidity between the surgical procedures
Miras[323]	2012	84 Participants with type 2 diabetes	To assess whether bariatric surgery procedures are safe for retinal and renal complications of type 2 diabetes.	In the subgroup of participants with diabetic retinopathy 17.8% of patients improved and the mean retinoscopy scores decreased significantly. The stabilization of microvascular complications were observed in the context of a reduction in medication usage within all surgery groups.	There was only one grader for a total of 67 photos. Retinopathy was graded subjectively according to clinical protocol but no actual measurements were made of the photos and did not include the CRAE or CRVE or the AVR.
Lammert[3][24]	2012	30 obese participants	To assess the effect of bariatric surgery on retinal vessel as an early surrogate marker of atherosclerosis in morbidly obese participants.	After 9 months, there was significant reductions in total cholesterol, 53% of participants had complete remission of impaired glucose metabolism, mean weight loss was 37.5%. The AVR improved significantly the results indicated widening of the arterioles and narrowing of the venules.	The time to first postoperative visit was variable among the participants. There was also no mention of diet, eating behaviour and whether the participants were active in physical exercise.
Varadhan[3][25]	2012	23 obese participants with type 2 diabetes mellitus	Was only a pilot study and all grading was done subjectively. None of the photos had actual software or manual calculations of retinal parameters.	2 patients had new development of retinopathy, further two had progression of retinopathy and 2 patients had regression of retinopathy. Changes are unpredictable after surgery and patients should still be screened even though there is rapid improvement in glycaemic control	Even though patients improvement in glycaemic control was measured, they should be more frequently assessed for retinopathy.
Nerla[326]	2012	50 obese participants	To assess the effects of bariatric surgery on peripheral endothelial function and on coronary microvascular dilator function	After 3 months there was an improvement in flow mediated dilation (FMD), Coronary blood flow (CBF) and the cold presser test (CPT). 17 participants were no longer classed as hypertensive and 15 participants had quit smoking.	The study assessed short term effects at 3 months. It is important to note that participants are still in a liquid diet at that time. Yet, there was no mention of diet and the results of oral glucose tolerance test at the follow up visit.
Johnson[32][7]	2013	2580 obese participants and 13371 controls	To examine the long term effects of bariatric surgery and compare the macrovascular and microvascular complications to nonsurgical controls	Bariatric surgery was associated with a 5 year event free survival outcome. It is also associated with a 65% reduction in major macrovascular and microvascular events in obese patients with type 2 diabetes.	There are some limitations in administrative data when combining studies and have been well documented. There was a lack of data on body weight and markers of glycaemic control. There was also a lack of information on the duration and severity of diabetic or obesity status.
Aghamohammadzadeh[78]	2013	20 obese participants	To investigate the effects of bariatric surgery on small artery function and the underlying mechanisms	6 months after surgery there were significant improvements in insulin sensitivity, inflammatory cytokines, and SBP together with increased Perivascular adipose tissue (PVAT) anti-contractile function, adiponectin and Nitric oxide (NO) bio-availability	There was no monitoring of the patients exercise and dieting habits with regards to calorie restriction. The changes detected were only to subcutaneous gluteal PVAT after surgery and not necessarily represents all small functional arteries in different vascular beds

1.5. Obesity and anterior eye health

Not much is known about the impact of obesity on anterior eye health. Dry eye syndrome is a multifactorial disease of the ocular surface which results in symptoms of discomfort, visual disturbance and tear film instability[328]. It can be brought upon by certain medications, poor nutritional diets, contact lens wear and also laser refractive surgery, but the direct association has still yet to be elucidated. Floppy eye syndrome (FES) is a condition where the upper lids are extremely lax and when the patient falls asleep the eye lids turn inside out which results in conjunctivitis, keratitis, corneal ulceration, and palpebral inflammation. It was first described by Cumbertton & Olster[329] in 1981 and it was more prevalent in overweight/obese individuals. Since then there have been studies which have argued against the direct association but have now come to terms that being overweight/ obese is a risk factor for the development of FES. Xanthoma can be characterised as an ocular manifestation of hypercholesterolaemia. CHOL deposits can appear anywhere on the body, and when they appear around the eyelids, usually in the inner canthus, they are referred to as xanthelasma palpebrum. They are a result of high CHOL levels in the systemic system and present as deposits of fat that build up under the skin but are usually of no consequence and are not sight threatening. This is the same when certain patients have a ring around their cornea, known as Arcus Senilis or Juvenilis. The former of which is due to the accumulation of lipids over a prolonged period of time and could represent serum hyperlipidaemia. The latter happens much earlier on in younger individuals and this definitely represents that there is an underlying problem. These cases are also more frequent when the individual is overweight and more so obese. It is unequivocal that obesity has perilous effects on the body, and the eye. A study by Caffery et al[330] looked at the diet within the western world and its influence on tear function. In this review more than 25 years ago, it was noted that the maintenance of a healthy tear film requires sufficient amount of protein and vitamin A. Within the Western world protein intake is not the problem as there is an abundant supply but the real problem lies with the vitamins and minerals which are part of a balanced diet and this was hypothesized to be the problem of maintaining a healthy tear film.

More recently, elevated amounts of serum lipids was associated with dry eyes in the Beaver Dam eye study[331] and in the Taiwan nationwide population based survey[332]. The link between higher serum lipid levels and ocular deposition is well established for corneal arcus and xanthelasma[333 334]. However, the exact mechanism for the association between dry eye syndrome and obesity has yet to be elucidated. A study by Dao et al[335] identified high serum lipid levels as a risk factor for the development of Meibomian Gland Dysfunction (MGD). However this was identified as elevated HDL levels. Table 1.5 provides a summary of obesity related complications and their effect on anterior eye health.

The present research looks at both systemic and ocular circulation in relation to obesity and CVD risk. Therefore, an understanding of the cardiovascular anatomy and physiology is, in this context, necessary. This is offered below.

1.6. Anatomy of the cardiovascular system

The cardiovascular system is composed of the heart and circulatory blood vessels. It is a complex system with regulatory feedback mechanisms to ensure rapid alterations in blood flow which can be adjusted based on the metabolic needs of various tissues and the transport of materials to and from all regions of the body. On leaving the heart, the blood is distributed between two principle cardiovascular pathways, namely the pulmonary circuit and systemic circuit[336]. The systemic circulation refers to the flow of blood from the left ventricle of the heart to the tissues of the body and back to the right atrium. All of the arteries which make up the systemic circulation are branches of the aorta. The aorta branches further into the ascending aorta, the aortic arch and the descending aorta. The arteries that arise from the aortic arch (the brachiocephalic trunk, the subclavian artery, and the carotid artery) ultimately supply blood to the upper limbs, shoulders, neck, eye, and the head[336].

Table 1.5 Summary of studies of obesity related ocular health and systemic influences

Author	Year	Participants	Purpose/Summary	Main findings	Disadvantages
Caffery[330]	1991	Review Article	To review the dietary habits of north Americans and its effect on dry eye syndrome and the ocular tear film	Sufficient dietary intake of protein and vitamin A are essential. Fresh fruit and milk intake can be less than adequate in the western diet. Deficiencies in vitamin A, B6 and C, minerals, potassium, zinc, folate and linoleic acid have been identified in tear dysfunction.	An enormous amount of studies had been reviewed but the studies that were carried out were not really defined in terms of protocol and methodology. This review is also 25 yrs + outdated and since then many changes have to come light regarding dry eye disease.
Moss[331]	2000	3222 population based cohort study (beaver dam study)	The examine risk factors for the prevalence of dry eye syndrome	Diabetes, Elevated amounts of total and HDL cholesterol were associated with dry eye syndrome. Women report dry eye more than men but after 65 years of age there's no difference.	Dry eye was diagnosed by the patients self-report and no objective testing was conducted. This resulted in not differentiating between evaporative and aqueous deficiency. It is also known that patients can have dry eye but not suffer from any symptoms which probably led to an underestimation.
Mori[337]	2000	70139 (60% M) participants	To evaluate the association between IOP and obesity by cross sectional and longitudinal analysis in a Japanese population.	BMI significantly correlated with IOP after controlling for age, sex, and BP. There was also a significant association between longitudinal change in IOP and weight, this also remained after controlling for variables.	The variations were slight but in contrast all the values would still be considered normal and not pathological. Also at the last follow up visit there were only 2000 participants. That's almost a 96% drop out rate
Dao[335]	2010	46 (22 M) with meibomian gland dysfunction (MGD).	To determine whether MGD is associated with dyslipidemia	Patients with moderate to severe MGD have a higher incidence of dyslipidemia with regards to total and HDL cholesterol. No significant differences were noted for LDL cholesterol.	The study could not age match the patient population with controls. As a result the participants were older and could have possibly affected the results owing to the fact that cholesterol builds up as one ages.
Uchino[338]	2011	2644 (1221 M)	To investigate the prevalence and factors associated with dry eye disease in Japanese cohort	Low BMI and hypertension were risk factors for men having dry eye disease. Women were 2x more likely to have severe dry eye symptoms than men but were also more likely not to have dry eye disease if they were overweight.	There was no objective testing of dry eye disease done, rather the information was self-reported. In diabetics because of peripheral neuropathy this can undermine dry eye symptomology resulting in an underestimation.
Beis[339]	2012	135 (51 controls) obese/overweight participants	To investigate whether there is an association of floppy eye lid syndrome (FES) and BMI in Sleep apnea syndrome (SAS) patients and normal controls	The presence of hyper elasticity in obese SAS individuals was significant compared to controls. But there was no direct association between FES and Obesity	Other studies have found associations between BMI and FES and it was first noted in overweight individuals. This study probably didn't find an association due to a number of control participants who were overweight and 15 were obese.

Modulo[340]	2012	5 mice	To determine in transgenic mice models of dyslipidemia, whether there is an association with dry eye syndrome (DES)	Mice that were fed high lipid diets had reduced tear secretion but did not suffer from meibomian gland dysfunction.	Did not have the tests sensitive enough to analyse cholesterol esters within the tear film. Mice live in a different environment and there were only 5 that were analysed. It's very difficult to make generalisations.
Wang[341]	2012	12007 patients with dry eye disease	To investigate the comorbidities of dry eye disease in a nationwide population-based data in Taiwan.	Patients with dry eye syndrome compared to controls were more likely to suffer from hyperlipidemia, hypertension, diabetes, cardiac arrhythmias and peripheral vascular disorders. Almost all patients being treated for mental illness had dry eye disease as well.	The study had no medicinal information with regards to what patients were being treated for and if they were being treated for more than one condition. Such as diuretics, antihistamines, corticosteroids and antidepressants which are known to affect tear function.
Pinna[342]	2013	120 (63 control) patients with MGD	The aim was to investigate a possible correlation between MGD and hypercholesteremia in young and middle aged patients.	Hypercholesteremia was found in nearly half of participants. After adjusting for confounding variables MGD was significantly associated with higher total cholesterol levels, HDL, and LDL levels.	Only included patients of Italian ancestry and can't be used for the general population. They showed an association between MGD and hypercholesteremia but not necessarily proven. Larger trials with multi-ethnic populations should be carried out.
Wang[343]	2013	2355 (960 men) participants	To assess the association between longitudinal changes in IOP, blood pressure and BMI (the Beijing eye study)	A Higher IOP was significantly associated with a higher change in mean arterial pressure and a higher change in BMI.	From the original cohort there was many drop outs. 750 just did not want to attend and 158 died. Methods included a whole section on Visual acquity but did not report on it. Also would have been better to carry out Applanation tonometry as this is the gold standard.
Pihlblad[344]	2013	30 (15 control) obese Patients with keratoconus	To investigate the prevalence of obesity and obstructive sleep apnea (OSA) in keratoconic patients	The prevalence of OSA 24% and obesity 50% were higher in the keratoconic patients. Keratoconic patients also had increased lid laxity with a more rubbery tarsus further prolonging the symptoms of FES.	There were 15 controls and they were referring to them as the normal population rather than Group etc. The study size was small and in a case control there was only one examiner which could have possibly lead to bias results.
Dogan[345]	2016	96 (29 controls) morbidly obese participants	To assess the ocular factors using OCT in morbid obesity and compare them against matched controls	Intra ocular pressure (IOP) and central corneal thickness (CCT) were found to be significantly higher in obese subjects, while the retinal nerve fibre layer (RNFL) was found to be significantly thinner. There was a positive correlation between IOP and BMI.	There were twice as many morbidly obese patients as there were controls. Patients that attended also had systemic measurements taken but none of which was included. A regression analysis could have elucidated an influence on measurements and could have been corrected for.

1.6.1 The heart

The human heart consists of four chambers: the left and right atria and the left and right ventricle. The right atrium receives deoxygenated blood which is then pumped into the right ventricle and then into the pulmonary artery and the lungs. Oxygenated blood then returns to the heart, into the left atria from where is pumped into the left ventricle, aorta, and systemic circulation (Figure 1.6.1). Approximately 80 cm^3 of blood is pumped out of the heart in each of its beats, making a total of approximately 5.5 litres of blood every minute[346].

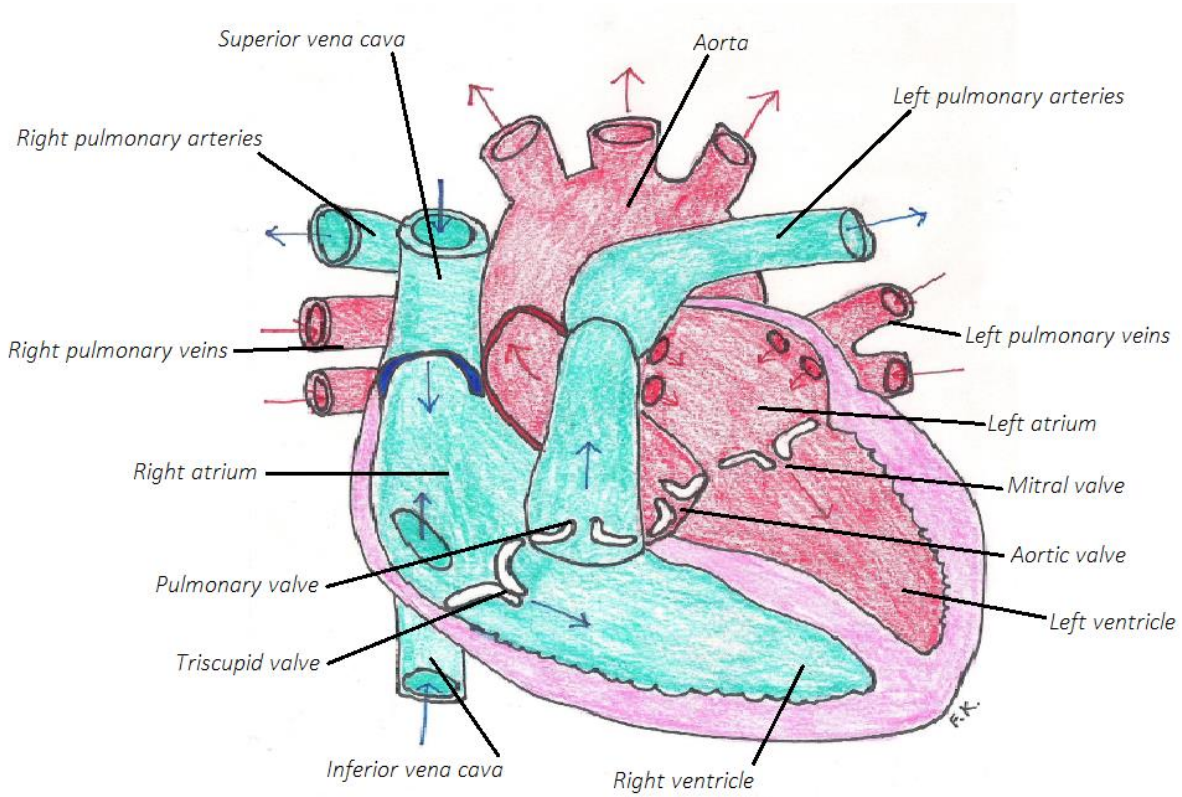

FIG 1.6.1. Illustration of the heart. The heart comprises of four chambers. It is divided into a right and left, each with an atrium and a ventricle. The atria act as reservoirs for venous blood. The ventricles are the major pumping chambers, delivering blood to the pulmonary (right ventricle) and systemic (left ventricle) circulations. The left ventricle has to generate greater pressures than the right ventricle, and has a thicker and more muscular wall. Four valves ensure that blood flows only one way, from atria to ventricle (tricuspid and mitral valves), and then to the arterial circulations (pulmonary and aortic valves)[346]. Illustration sketched by author based on Ganong's medical physiology[336].

The hearts' blood supply to the cardiac muscle is derived from branches of the aorta and the coronary circulation, mainly the right and left coronary arteries, which line of wall of the heart. The left coronary artery originates from the left side of the aorta and separates into three branches supplying the anterior wall of the heart and left ventricle. The right coronary artery originates on the right side of the aorta separating into two branches and supplies the right ventricle[347 348]. The coronary arterial blood supply to the cardiac muscle represents an "end circulation" since it is the only blood supply to the myocardium. As such any type of pathology (atherosclerosis) resulting in either blockage or dysfunction to the coronary arteries poses a significant threat that can lead to a heart attack or myocardial infarction[349].

1.6.2 The Carotid Arteries

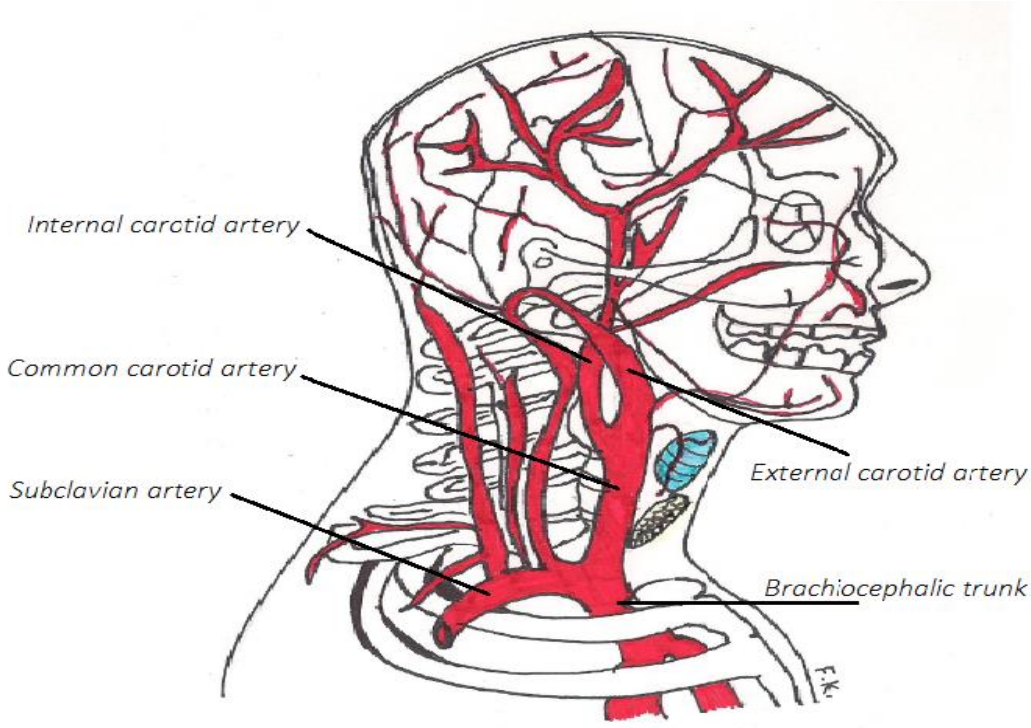

FIG 1.6.2 Illustration of the carotid artery and its branches. The brachiocephalic trunk arises from the aortic arch and divides into the right common carotid artery and subclavian artery. The left common carotid artery arises directly from the aortic arch. The internal carotid artery is a branch of the common carotid artery and is responsible for the main arterial blood supply to the eyes and brain while the external and its varying branches supply the head and face. Illustration sketched by author based Netter's medical illustrations[350].

The right common carotid artery (Figure 1.6.2) arises from the branch of the aortic arch via the brachiacephalic artery, whereas the left common carotid artery arises directly from the aortic arch

itself. Both right and left common carotid arteries extend superiorly within the corresponding parts of the neck before branching to form the right and left, internal and external carotid arteries[336]. The examination of the carotid artery can be undertaken, due to its close proximity to the skin, with ultrasonography and involves measurements of the thickness of the vessel. An increase of carotid intimal media thickness can be regarded as one of the first anatomical signs of atherosclerosis which is a precursor to atheroma formation[351].

1.6.3 Brachial Artery

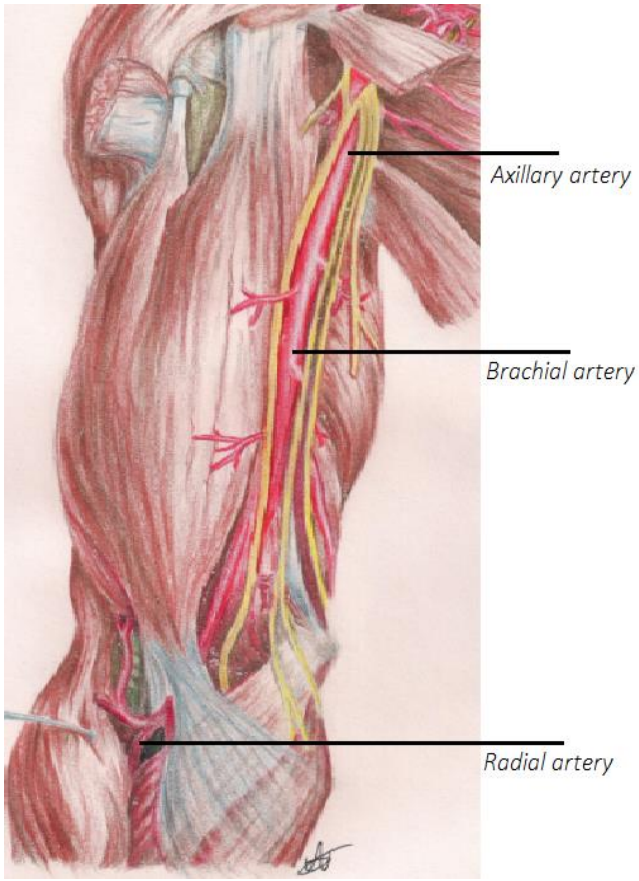

FIG 1.6.3. Illustration of the brachial artery. The brachial artery is the major blood vessel of the upper arm, especially the tricep muscles. It continues from of the axillary artery, which is a branch of the subclavian, travelling downwards to the surface of the arm until it reaches the cubital fossa at the elbow. Blood pressure cuffs are often used to measure the blood pressure at this point. The brachial artery then divides into the radial and ulnar arteries which run down the forearm. Illustration sketched by author based on Netter's medical illustrations[350].

The brachial artery, located in the upper arm, is formed from the subclavian artery which is a direct branch of the braciocephalic artery (Figure 1.6.3). Its location facilitates uniform compression and

the artery can be easily visualized with ultrasonography of reactive hyperaemic responses with the FMD technique or measurements of temperature rebound with the Endothelix. The brachial is in close proximity to the skin which allows for a non-invasive blood pressure assessment carried out routinely by a physician or using automated instruments that can allow for routine assessments in one's own home. Currently the gold standard of measuring reactive hyperaemia, is FMD, involves and induced increase in blood flow through the artery, based on the principal that shear stress exerted on the endothelium would stimulate the release of NO and cause vasodilation. An impaired vasodilative response is indicative of a decreased bioavailability of NO[352]. Although, given the subjective nature of the FMD assessment, it has been prone to large inter and intra-observer variability, making this technique difficult to master, and usually requires two operators simultaneously. The endothelix is a new instrument which also relies on the principle of reactive hyperaemia but the advantage of it being that it is fully operator independent.

1.6.4 Radial Artery

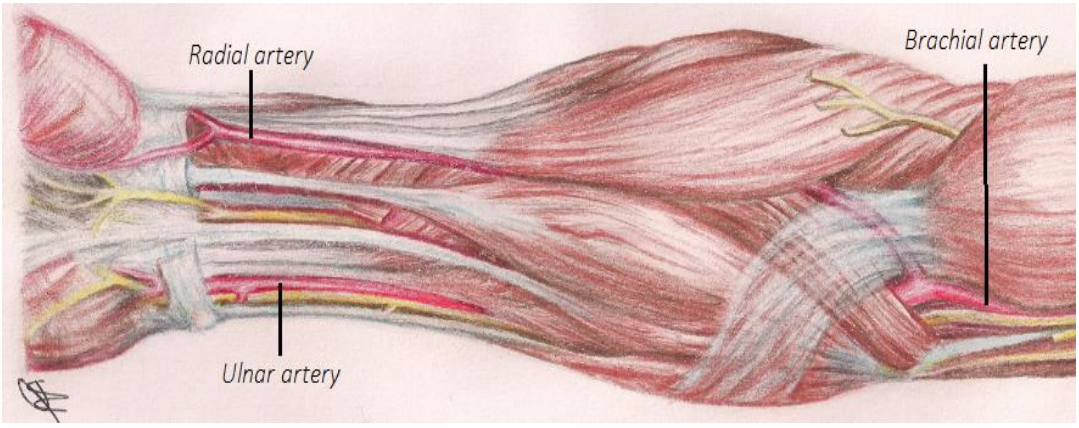

FIG 1.6.4. Illustration of the radial artery. The radial artery arises from the bifurcation of the brachial artery in the cubital fossa. It runs distally on the anterior part of the forearm travels down the radial side of the forearm towards the wrist. This is where pulse rate can be measured. Just beyond the wrist branches of the ulnar artery join and form a network of vessels which supply the hand and fingers. Illustration sketched by author based on Netter's medical illustrations[350].

As the brachial artery branches at the elbow it give rise to the radial artery that traverses along the radial side of the forearm to the wrist providing a site for pulse measurements as it approaches the skin surface[353]. Radial artery (Figure 1.6.4) elasticity is measured by pulse wave analysis. This

technique relies on pulse pressure waves that travel through the vasculature during cardiac contractions. However, it does not give an indication of how the artery interacts with the heart as part of an integrative system[353]. Typically, thicker arterial walls facilitate the dampening of pressure oscillations so that blood flows smoothly from the aorta to target tissues[354]. At inflection points in the vessel, pressure waves are reflected back to the heart to regulate the filling of coronary vessels during diastole. In individuals with impaired arterial elasticity, pressure wave forms return to the heart at a much quicker rate and this augments the pressure that the heart has to overcome in order to open the aortic valve. Stiffening of the arterial wall reduces elasticity[355], and has been attributed to decreased production of nitric oxide, and loss of smooth muscle tone[356].

1.6.5 Blood vessel structure and function

1.6.5.1 General considerations

Blood vessels carry blood to every part of the body. They decrease in size as they move away from the heart[357]. From major arteries to arterioles and finally capillaries which are then collected in venules to veins and back to the atrium via the superior and inferior vena cava[358]. Arteries are vessels that are robust and elastic which have to carry blood away from the heart at high pressures. These arteries branch off and subdivide into arterioles. The artery vessel wall can be divided into 3 distinct layers (Figure 1.6.5) which are separated by elastic connective tissue. The inner most layer known as the tunica intima is largely made up of simple squamous epithelium known as the endothelium. The role of the endothelium is to prevent coagulation and also regulate blood flow. The middle layer known, as the tunica media, makes up majority of the vessel wall and includes smooth muscle fibres and thick elastic tissue. The outer most layer known as the tunica adventitia is thinner than the middle layer and is mostly made up of connective tissue[358]. The walls of the aorta and other large diameter conducting arteries contain a large amount of elastic tissue within the laminas, either internally or externally. These walls are stretched during systole and recoil during diastole. Arterioles contain more smooth muscle and less elastic tissue. The smooth muscles

function as a constrictor through innervation by adrenergic nerve fibres and also dilate through innervation by cholinergic fibres[336]. Small changes within the lumen diameter of arterioles cause large changes in peripheral resistance; arterioles being the major site of blood flow resistance. Conducting arteries branch out further into smaller arteries such as the brachial and radial arteries which direct regional blood flow and divide into arterioles which are responsible for perfusion of the organ. Arterioles further divide into capillaries. Capillaries are semi-permeable and formed by a single layer of endothelial cells. Their main function is for nutrient, metabolite and gas exchange. The capillaries connect the arterial system to the venous system. The venules will branch together directing regional blood flow to the veins from which deoxygenated blood will travel through the peripheral veins and into the heart via the atrium and pulmonary circulation[336]. Veins can also be divided into 3 layers like their arterial counterparts but mainly have a larger lumen, smaller amount of smooth muscle cells and elastic tissue. Veins also contain valves which prevent back flow and are supported by skeletal muscle contractions to help return blood to the heart[221 336].

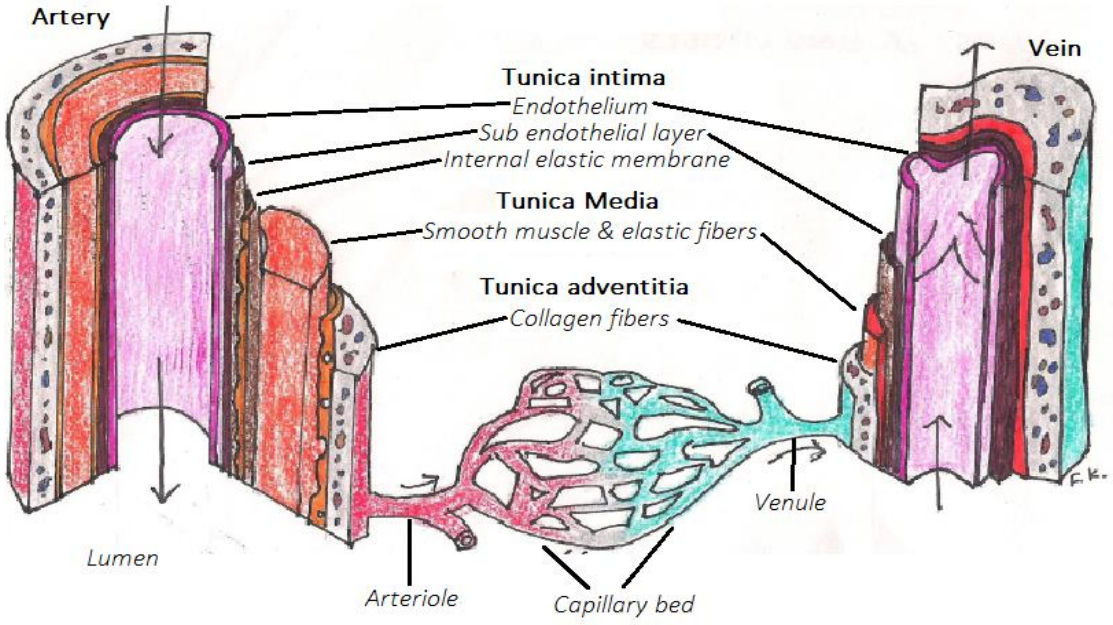

FIG 1.6.5. Illustration of the blood vessel types, structure and diameters. There are five classes of blood vessels within the circulatory system. Arteries, arterioles, capillaries, venules, and veins. Arteries deliver oxygenated blood to the organs through arteries and capillaries while de-oxygenated blood returns to the heart from the veins. Illustration sketched by author.

1.6.6 Anatomy and physiology of peripheral circulation

The vasculature of the superficial skin is organized into two horizontal plexi of arterioles and venules; one located just below the papillary dermis (1-1.5 mm below the surface) and one at the dermal-subcutaneous junction (3-5 mm below the surface). The upper (superficial) plexus supplies the individual dermal papillae with nutritive capillary loops and the lower (deep) plexus provides supply to the hair follicles and sweat glands (Figure 1.6.6). The many neural, environmental and hormonal signals involved in temperature regulation are integrated in the pre-optic anterior hypothalamus[359]. The hypothalamus has a thermos-neutral zone of accepted core body temperature, and deviation from this point by ±0.2°C initiates reflex activity by a variety of negative feedback-controlled methods[359]. Warm and cold responses are both mediated by the sympathetic nervous system. Blood flow to the cutaneous circulation has a remarkable range, from nearly zero in extreme cold to a total for the entire body surface of 7 l/min when body temperatures are high[360]. When expressed per unit volume of skin. Maximal skin blood flow can reach an estimated 300–400 ml/min per 100g skin[361-363].

During warm temperatures blood flow increases to the skin and can be as high as 8L/min. This is an effective manner of heat loss; though it can cause problems in subjects with some degree of cardiac abnormalities who can't increase their cardiac output sufficiently[359]. Cold temperatures result in reductions of blood flow to the skin. This response is mediated by aplha1-adrenoreceptors and peripheral alpha2-adrenoreceptors. These cause constriction of arteriovenous shunts in the skin (particularly fingers and toes), diverting blood away from the peripheries. This reduces the mass of tissue in the body that must be kept warm by reducing the size of the core. There can be a temperature difference of 6°C between the hands and core organs without damage to tissue or extra expenditure of energy[359].

Pathology-induced vascular dysfunction is evident in the cutaneous circulation[364-367] and may mirror generalized systemic vascular dysfunction in magnitude and underlying mechanisms[368-373]. Deficits in

cutaneous vaso-reactivity are measurable early in the progression of hypertensive vascular disease[366 367 374 375], and hypertrophy of the cutaneous resistance vasculature is predictive of future cardiovascular events[376].

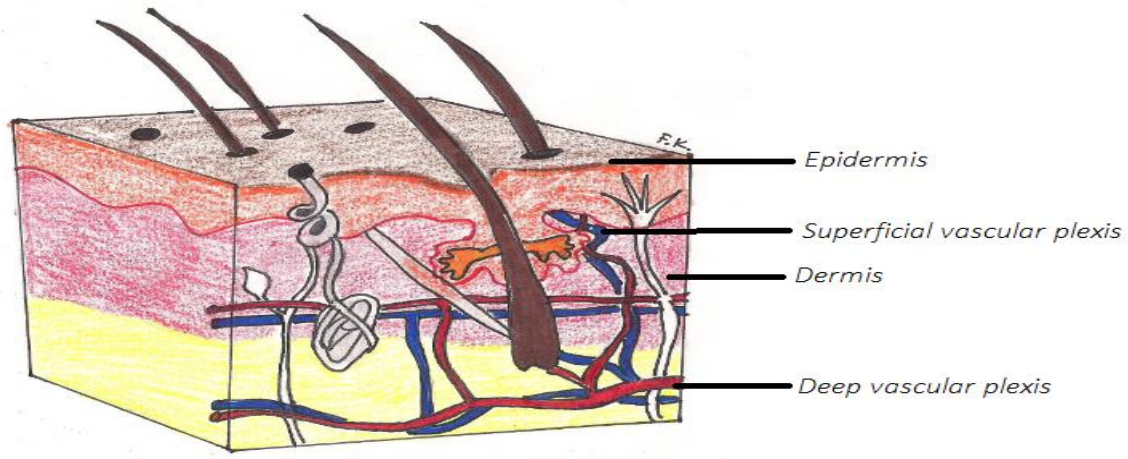

FIG 1.6.6. Illustration representative of cutaneous circulation. Skin blood flow has, as a prime function, the regulation of core body temperature. The superficial plexus acts as a large thermal radiator and has a blood-flow range of 1ml/min per 100g of tissue in the cold to 200 ml/min per 100 g tissue in the hot[346]. These extremes of flow are possible through the regulation of numerous arteriovenous communications by the ANS. The acral areas (hands and feet) in particular possess abundant direct connections between arterioles and venules; the vessels in these areas are controlled almost exclusively by sympathetic nerve fibers. Illustration sketched by author based on Netter's medical illustrations[350].

1.7. Physiology of the cardiovascular system

The principle function of the cardiovascular system is to maintain adequate blood flow to all tissues, ensuring their oxygen and nutrient demands are met and waste products removed. Blood flows through the cardiovascular system primarily as a result of the pressure produced by the contraction of the heart ventricles, referred to as the systemic BP[336]. Both systemic BP and heart rate (HR) are constantly regulated and controlled by the autonomic nervous system (ANS) in accordance with the hemodynamic needs of the body. The background and relevance of systemic BP and the ANS with regard to cardiovascular physiology is outlined in the following sections

1.7.1. Systemic blood pressure

Blood pressure defines the force exerted by the blood against the vessel wall and is determined by both the volume of blood in the vessel and the distensibility of the vessel wall. It is commonly described in terms of systolic (SBP) and diastolic (DBP) blood pressure, with systolic being the highest measured pressure, corresponding to ventricular contraction and diastolic being the lowest measured pressure, corresponding to ventricular relaxation and refilling. It is measured in milligrams of mercury (mmHg) from the brachial artery in the upper arm using a sphygmomanometer. The most recent World Health Organisation (WHO) and International Society of Hypertension guidelines, which are based on the 1999 publication for the classification of hypertension, are outlined in table 1.7.

Table 1.7 Classification of hypertension		
Category	**Systolic (mmHg)**	**Diastolic (mmHg)**
Optimal	<120	<80
Normal	120-129	80-84
High normal	130-139	85-89
Hypertension		
Grade 1- Mild	140-159	90-99
Grade 2- moderate	150-179	100-109
Grade 3-Severe	>180	>110
Isolated systolic hypertension	>140	<90

Based on WHO and international society of hypertension[377]

1.7.2. Endothelial function

The endothelium is the most inner portion of the vessel. It comprises of almost single layered cells and lines the entire vasculature throughout the body.Its structure and phenotype depends on the vessel size and location[378]. The cell structure is important for the maintenance of the vessel wall and circulatory function[379]. Endothelial cells have the ability to regulate a number of complex physiological functions depending on the location of the vascular bed. Its main role is regulation of vascular tone and blood flowto maintain homeostasis. It also regulates thrombosis, coagulation, platelet adherence and inflammation. The endothelium releases vasoactive substances which act on the diameter of the vessel. These are usually brought on but not limited to stress, mechanical

irritation or chemical provocations. These active substances are commonly known as endothelial derived constricting or relaxing factors which play an important part in the regulation of ocular blood flow[380]. In diseased, the endothelium undergoes structural and functional changes making it more prone to atherosclerosis and thus becoming more susceptible to develop vascular disease[381 382]. More commonly known as endothelial dysfunction.

The endothelium has a critical role as an active regulator of vascular tone[383 384]. Maintaning vascular homeostasis is brought upon by competing factors on the smooth muscle cell within the vessel wall from internal and external forces. These internal factors influence the smooth muscle cell within the vessel wall itself through the production of vasoactive substances such as NO and ET-1, which are the most potent vasodilating and vasoconstricting substances within our body[385 386]. External factors which act on the smooth muscle cell relate to innervation from the autonomic nervous system[138 387]. The endothelium also has a protective role within the vessel wall and regulates inflammation, inhibits the proliferation of smooth muscle cells, platelet aggregation, angiogenesis and thrombosis through its control on vascular permeability[382 388 389].

a. Endothelium derived relaxing factor

The endothelium plays a key role in maintaining basal vascular tone throughout the body. The endothelium derived relaxing factor (EDRF) was first described in 1980 by Furgatt and Zawadzki[139]. Their study had shown that Acetylcholine which acts on muscarinic receptors within the endothelial cells provided a substance which acted upon the relaxation of smooth muscle cells. They also noticed that Acetylcholine had no effect on relaxation when endothelial cells were not present or damaged. However this EDRF was not known at the time of their study. Soon after it was recognized that this EDRF was NO[385 390 391] and became an important research topic with regards to vascular physiology and has been investigated in depth.

NO is produced from the oxidization of the amino acid L-arginine to the L-citriline group by the enzyme Nitric oxide synthase NOS[385 390]. To date three isoforms have been identified, Neuronal

NOS1, inducible NOS2 and endothelial NOS3. NO synthesis has been mainly attributed by endothelial synthesis. When NOS1 and NOS3 are activated, NO is produced via the calcium/calmodulin complex. When NOS2 is activated NO production is independent of calcium[392]. Large amounts of NO, via NOS2, are produced and expressed only after immunological or inflammatory stimuli[392 393]. All the isoforms of NOS have been identified in the ocular circulation[394]. NO has a very short half-life of roughly 2-3 seconds. A single NO molecule can affect adjacent cells because of its ability to diffuse over large distances. NO has a key role in maintaining vascular tone in humans [138] and is a major regulator of systemic blood pressure[395 396]. Diffusion of NO into smooth muscle cells brings about vasodilation of the vessel through stimulation of the guanylate cyclise enzyme and increased production of cyclic guanine monophosphate (CGMP)[397 398].

NO plays an important role in the haemodynamics of the cardiovascular system, the regulation of physiological functions and the branches of the autonomic nervous system. In addition to its role as a relaxer it has many other functions including inhibition of thrombosis, cellular proliferation and inflammation. NO has also been identified at the ocular level as a mediator of vascular tone. NO is present in retinal vessels, in the endothelium of the optic nerve head and choroidal blood vessels[399-401]. Retinal arterioles and capillaries are in a constant state of vasodilation which is maintained by a continuous release of NO[138 402]. Any disturbances to homeostasis can have detrimental effects on the vasculature and it has been shown that NO deficiency, through an impaired endothelium, can result in excessive vasoconstriction, inflammation, leukocyte activation and infiltration[403]. As such, any disturbance to NO can have an impact on systemic and ocular haemodynamics, resulting in endothelial dysfunction ED, usually from impaired NO bioavailability which results in vasoconstriction from stimulation of the vasoconstrictor (ET-1). This shift from a normal vasodilative state can influence the vasculature permanently leading to a greater chance of vascular disease.

b. Endothelium derived constricting factor

Endothelium derived constricting factors (EDCF) were first described by Yanagisawa in 1988[404], having potent vasoconstrictor capabilities which are also produced in the endothelium alongside NO. Three distinct isoforms of endothelins exist and have been identified, ET-1, ET-2 and ET-3. The 21-amino acid peptide ET-1 is considered as the most potent isoform in controlling vascular tone[405]. Endothelins are formed via a 39-amino acid intermediate which is then processed by a family of endothelin converting enzymes[392 406]. Under physiological conditions, ET-1 is produced in very low concentrations within endothelial cells and there release is inhibited by the production and continual release of NO. However, under pathophysiological conditions, the production of endothelins is stimulated in a large number of different cell types such as smooth muscle cells, endothelial cells and inflammatory cells such as leukocytes and macrophages which can be measured in plasma[407].

There are two classes of endothelin receptors ET-a and ET-b, separated by pharmological receptor subtypes. The ET-a receptor is mainly located within smooth muscle cells and mediate the potent vasoconstriction affect. The ET-b receptor has been further divided into ET-b1 & ET-b2 based on their responses[408]. Stimulation of ET-b1 receptor cells induces vasodilation in generally low doses probably because they are formed within endothelial cells, but at higher concentrations of the peptide, vasoconstriction dominates. The ET-b2 receptor located within vascular smooth muscle cells when stimulated cause direct vasoconstriction. The total effect of endothelins may vary considerably from different vascular beds, which depends on the localization of the ratio of ET-a and ET-b receptors. This varies considerably within veins as they tend to have a lower receptor ratio compared to arteries[409]. In pathological states there is a predominantly ET-1 vasoconstrictive induced effect. This is because there is an upregulation of ET-b receptors on vascular smooth muscle cells and down regulation of ET-b receptors on endothelial cells[410].

Increased amounts of ET-1 due to a reduced bioavailability of NO have been implicated in a number of cardiovascular diseases including CAD, systemic hypertension and exasperation of

atherosclerosis[411 412]. Endothelin receptors ET-a are found at the ocular level within the retina and choroid and optic nerve head while Receptor ET-b sites are localized to the neural and glial substance of the retina[413 414]. Within the ocular circulation there have been numerous studies that have shown ET-1 being involved primarily in the regulation of vascular tone mainly exhibiting its vasoconstrictor effects on the microvessels[406 415 416]. The role of ET-1 in regulating ocular blood flow can be seen as it was shown that during exercise ET-1 is produced to counteract the effect of increases in ocular perfusion pressure[413 417]. But when the receptor ET-a antagonist BQ-123[404] was administered the vasoconstrictive role of ET-1 was ameliorated. Due to the role of ET-1 regulatory capacity of blood flow throughout the body it can be envisaged that any imbalance in vascular tone through cardiovascular disease, systemic or ocular, can result in retinopathy and future cardiovascular events.

1.7.3. The relationship between the Autonomic Nervous System and the endothelial function

In a normal state, the ANS and the endothelium work together to maintain vascular tone. There is a tonic balance between the release of vasodilating factors from the endothelium and vasoconstricting factors from the sympathetic nerve terminals[418]. The balance between these forces acts on the vascular smooth muscle cells to maintain the appropriate vessel tone[419]. Activation of b-adrenergic receptors on vascular smooth muscle cells contribute to vasodilation[420], whereas activation of a1-adrenergic and a2-adrenergic receptors on smooth muscle cells contributes to smooth cell contraction[402 420 421]. PSNS stimulation of muscarinic receptors on smooth muscle cells also results in contraction[138]. The release of NO from the endothelium stimulates smooth muscle cells which in turns causes dilation[422] (Figure 1.7.3). This endothelium-dependant vasodilatory effect of a2-adrenergic receptor activation counteracts the vasoconstricting effects of a1-adrenergic receptor stimulation of the vascular smooth muscle[421 423]. In addition, the SNS can stimulate the release of endothelium derived contracting factors, such as ET-1[419 424]. Endothelial cells also possess receptors

for the primary PSNS neurotransmitter acetylcholine and activation of these receptors produces endothelium-dependant relaxation of human coronary arteries [425]. Although endothelial cells in the microvasculature may receive ANS input, endothelial cells along the major conduit vessels do not receive direct neural innervation from the ANS[419]. Therefore the effects of neurotransmitters on endothelial function must be exerted by circulating levels or by diffusion through the smooth muscle cell layer without degradation[422] (figure 1.7.3).

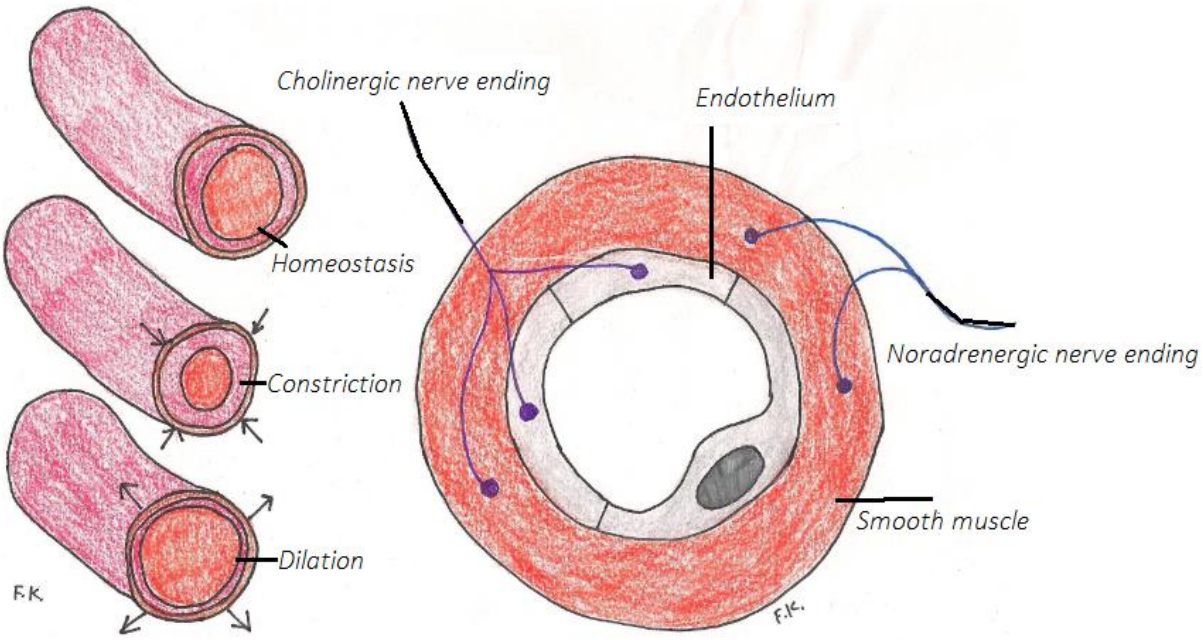

FIG 1.7.3. Illustration of ANS and endothelium and its effects on vascular diameters. There is a tonic balance between the release of vasodilating factors from the endothelium and vasoconstricting factors from sympathetic nerve terminals. The endothelium releases vasodilating factors, such as NO, which stimulates the smooth muscle cells causing vasodilation. The endothelium also releases contracting factors, such as ET-1 and ANGII, which stimulates vasoconstriction. Sympathetic activation of B-adrenergic receptors of smooth muscle cells contributes to vasodilation, whereas a-1adrenergic receptors contributes to vasoconstriction. Parasympathetic stimulation of muscarinic receptors on smooth muscle cells also results in vasoconstriction[138]. Illustration sketched by author.

Much of the evidence linking ANS activity and endothelial function comes from pathological states associated with impairments in both systems. Chronic diseases such as hypertension and diabetes have been independently associated with ANS and endothelial function[426-430]. It is unclear though, whether the ANS and endothelial systems are negatively affecting one another, or whether both systems undergo dysregulation as a consequence of the disease process[422]. An impairment in

endothelial function can cause irregularities within the ANS, through alterations in neurotransmitter release from the SNS may result from decreased NO release[431]. Conversely, an impairment in ANS function can cause irregularities in the endothelium. This can happen through an exaggerated SNS and can enhance endothelium mediated atherogenic processes[418] as well as it may also increase the uptake of low density lipoprotein by endothelial cells[432].

1.7.4. Vascular tone

In healthy individuals, the ANS works closely with the vascular endothelium to insure a stable vascular tone. In response to local factors, the vascular endothelium continuously releases vasodilating factors (e.g., NO, endothelium-derived hyperpolarizing factor, prostacycline, substance P, acetylcholine) which counteract the vasoconstrictor effect of the sympathetic activation occurring as a result of a more systemic requirement [418]. Although the vascular endothelial cells do not receive direct ANS innervation, it still can be influenced through the activation of its own alpha2 and beta-adrenoreceptors that results in NO release and vasodilation [420]. In addition, the parasympathetic nervous system can contribute to acetylcholine release and vasodilation [433], while the sympathetic nervous system can stimulate the release of endothelium-derived vasoconstrictors such as ET-1 [434]. More recently, Seddon et al. has demonstrated that neuronal NO synthase (nNOS) also contributes directly to the regulation of human vascular tone [435].

Blood flow is autoregulated in proportion to tissue demand for oxygen and other nutrients[436]. Therefore, during the night, the lack of physical activity together with a decreased demand for oxygen results in a low blood flow and an increased peripheral resistance [436 437]. In addition, the frequency of peripheral vasoconstriction is dependent on the stage of the sleep, being higher during REM than during non-REM sleep [438]. Endothelium-dependent vasodilation represents a protective mechanism against time-dependent changes in the body's haemodynamics. Any disturbances in this

mechanism could represent an important risk factor for circulatory ischaemic events [439]. Assessment of endothelial function, can therefore provide both a valuable insight into the pre-clinical phase of ischaemic diseases and an early marker for this type of circulatory disturbance. Moreover, due to a circadian variation in the occurrence of systemic and local circulatory ischaemic events, the rhythm of the endothelial function is equally important to assess. In healthy individuals, the vascular diameter is smaller in the morning. However, this status is accompanied by a normal dilation capacity should the body need it [440]. In addition, it has been demonstrated that the systemic secretion of NO has a peak early in the morning [441], possibly to buffer the concomitant increase in BP due to a high sympathetic tonus. Moreover, in normals, circadian rhythm of (ET-1), the most potent vasoconstrictor of the human body is characterized by two peaks (at 8h and 20h) and low levels during the night when the body should rest [442]. Therefore, maintaining a balance between all these factors is crucial for an appropriate perfusion with blood and any disturbance that occurs in either ANS or endothelial function is ultimately reflected in impairments that occur in both systems [418]. Indeed, disorders such as diabetes, cardiovascular disease, hypertension and congestive heart failure, have been associated with both abnormalities of ANS regulation [430 443 444] and abnormalities of endothelial function [445-447]. It is difficult to determine, however, whether a dysfunction in one system may have driven a dysfunction in the other and if so which occurred first, or whether both systems have developed a dysfunction independently as part of the disease process. In young patients with essential hypertension, both ET-1 levels and sympathetic activation were higher (ref kulinska 2010). Moreover, in patients with congestive heart failure, studies have found that ET-1 levels correlate negatively with some measures of HRV [448] and in patients with diabetes associations have been found between lower HRV and higher levels of von Willibrand factor (vWF), a marker of endothelial dysfunction [449]. Nevertheless, other external influences, such as the level of oxidative stress and the ageing effect on the vascular function, should also be considered when studying the complex interaction between ANS and endothelial function in health and disease. Indeed, an altered

vascular tone may result in higher vulnerability of various organs including the eye to damage from free radicals or inflammatory stress[450].

1.8. Anatomy and physiology of the retinal vessels and retinal circulation

The blood supply that nourishes the retina and ocular structures primarily arises from the internal carotid artery which is a direct branch of the aorta. The internal carotid artery (ICA) branches into the ophthalmic artery (OA), which further divides into the central retinal artery (CRA), and posterior ciliary arteries (PCAs). These vessels are known as retro bulbar vessels as they provide nourishment to the posterior segment of the eye.

1.8.1 The ophthalmic artery

The OA if the first major branch of the ICA arising just after the cavernous sinus. It travels anteriorly passing within the optic canal and piercing the dura meter infero-laterally reaching the optic nerve until it enters the orbit[451]. There are normal anatomical variations, such as OA piercing the orbital canal but not the protective dura sheeth, and other variations such as the OA entering the optic canal superiorly, medially, and nasally but in most cases it enters inferiorly[452 453] and is situated beneath the superior oblique muscle. As such, the anatomy will be discussed according to what is most frequently observed.

1.8.2 The central retinal artery

The CRA represents a direct branch of the ophthalmic artery. It commonly originates on the infero-medial side of the ophthalmic artery about 8.4mm distal to the orbital end of the optic canal[454]. It pierces through the optic nerve behind the globe. It then travels along the centre of the optic nerve before appearing at the surface of the disk through the lamina cribrosa. The central retinal artery further divides into 4 intra retinal arterioles; supplying each quadrant of the retina.

1.8.3. The ciliary arteries

The ciliary arteries can be divided into three groups; the short posterior, long posterior, and anterior ciliary arteries. Anatomical variations exist but there are roughly between 1-5 posterior ciliary arteries that arise from the ophthalmic artery. They branch from the ophthalmic artery, grouped into medial trunks situated on either side of the ophthalmic nerve and pierce the sclera laterally, medially (infrequently inferiorly, and superiorly) and serve as the main source of blood supply to the ocular structures and optic nerve head[455]. Short posterior ciliary arteries can range from 12-20 in number depending on the subdivision before reaching the sclera. They divide into the para-optic ciliary arteries which supply parts of the optic nerve head and distal ciliary arteries which supply the choroid. Long posterior ciliary arteries, run a course, and pierce the sclera medial and laterally forward to the iris. The anterior ciliary arteries leave the ophthalmic artery and run a course through the medial and lateral rectus muscles; then travel forward to the ciliary muscle and also to the major arterial circle of the iris, supplying the anterior uveal tract[456].

1.8.4 The retinal circulation

There are 2 main sources of blood for the retina. The CRA and its branches supplying the inner retina and the choroidal blood vessels which maintain and nourish the outer retina. Branches of the posterior ciliary arteries, are the main source of blood to the corresponding parts of the optic nerve head, peripapillary choroid, the circle of Zinn and Haller, and serve recurrent branches to the retrolaminar optic nerve head[455]. These short posterior ciliary arteries further divide into smaller segments and supply a corresponding segment of the choroid. Each terminal arteriole supplies a lobule of the choriocapillaries[455 457 458]. The choriocapillaries (a network of choroidal capillaries located beneath the retinal pigment epithelium) are extremely small vessels and are arranged

distinctly within the inner portion of the choroid. One of its major roles is to supply blood and metabolites to the retinal pigment epithelium and the outer neurosensory retina[459].

The retinal capillary network is organized into an inner and outer parapapillary radial network. The inner capillaries underlying the radial peripapillary network from the superficial layer and the outer capillaries form the deep plexus layer[460]. This capillary network extends towards the edges of the retina with the exception of a capillary free zone in the macular region[461]. Majority of blood that flows for the maintenance of the retina is through the choroid and its circulation and is approximately 40 times greater than that of the retinal vasculature[462]. Generally the retinal circulation is characterized by a low level of flow with a high level of oxygen extraction[463 464]. The circulation is autoregulated, allowing blood supply to be adjusted to metabolic demand. As sympathetic nerve terminals are localized in the smooth muscle cells of the choroidal vessels, retinal vessels do not receive autonomic innervation[465 466].

1.8.5. Venous drainage

The venous system consists of many orbital veins, of which the superior ophthalmic vein is the largest in diameter[467]. It is generally regarded as the main source of drainage within the orbit. It originates from two roots at the medial orbital rim. The superior root being the supraorbital vein, and the inferior route from the terminal part of the angular vein. The roots form posterior to the trochlea and travel posteriorly in the direction of the ophthalmic artery before leaving the orbit through the superior orbital fissure and draining into the cavernous sinus[468]. Venous drainage from the choroid is mainly by 4 vorticose veins representing the different quadrants of the eye which also drain corresponding quadrants of the Ciliary body and iris[469]. The inferior orbital venous system comprises of the inferior orbital venous plexus which is responsible for the drainage of the inferior lateral and medial vorticose veins. The superior medial and lateral vorticose veins ultimately join the superior ophthalmic vein and drain directly into the cavernous sinus[467 469]. The central retinal vein is responsible for venous drainage within the retina. It receives its de-oxygenated blood from its four

quadrants within the retinal branches. The central retinal vein courses centrally within the optic nerve but exits the nerve 1-2mm behind the entry point of CRA and then runs posteriorly below it. It can drain directly into the cavernous sinus but also drains into the superior ophthalmic vein or other intra orbital venous branches[470].

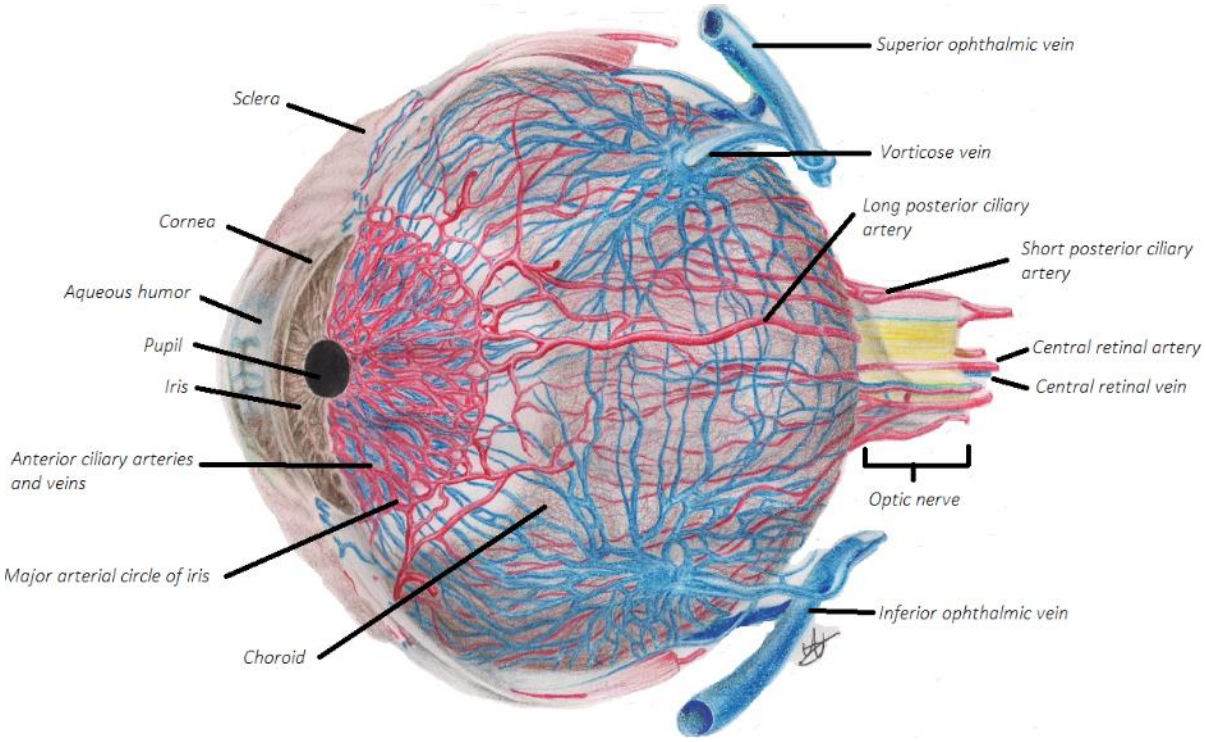

FIG 1.8. Illustration of the human eye. The outer protective layer of the eyeball, the sclera, extends anteriorly to form the cornea through which light rays enter the eye. Underneath the sclera is the choroid, a layer that is heavily vascularized and responsible for nourishment of the structures in the eye. Underneath the choroid is the retina, the neural tissue containing the photoreceptor cells which is responsible for transmitting the information to higher orders of the brain[336]. In front of the lens (not shown) is the pigmented and opaque iris, the colored portion of the eye. The iris contains circular muscle fibers that constrict and radial fibers that dilate the pupil. The space between the lens and the retina is filled primarily with a clear gelatinous material called the vitreous humor. Aqueous humor, a clear liquid that nourishes the cornea and lens, is produced in the ciliary body by diffusion and active transport from plasma. Illustration sketched by author based on Netter's medical illustrations[350].

1.9. Physiology of ocular blood flow

Blood flowing through the body is determined by the cardiac output, which depends on heart rate and stroke volume. An adequate supply is a basic requirement for all tissue beds and organs to remain healthy. The distribution of cardiac output between vascular beds varies according to the haemodynamic situation that arises, and can be influenced by systemic hormones and the autonomic nervous system[464]. Despite variations in systemic blood pressure, arteries can preserve

constant flow through tissues due to the existence of vascular tone mediated by smooth muscles cells and other contractile elements within the vessels wall[380], allowing blood supply to be adjusted to metabolic demand, this is autoregulation. Within each vascular bed, local factors regulate and supply the required blood. At the ocular level, this has been identified as a complex, multi-factorial process[471], dependant on both ocular perfusion pressure and vascular resistance.

Blood flow, inside a vessel is proportional to its perfusion pressure, and inversely proportional to vascular resistance[392]. This relationship is given by Q=ΔP/R, where Q is blood flow, ΔP is the difference in pressure between 2 ends of a vessel, and R which is due to the friction of blood moving through the vascular endothelium. The principles of blood flow in intraocular tissues are similar in that blood flow is dependent on arterial pressure, local venous pressure, and resistance to flow.

Based on the drop in blood pressure between the heart and OA, retinal arterial pressure can be estimated as 2/3 of the mean arterial pressure, (MAP=2/3DBP+1/3SBP) and venous pressure can be taken approximately equal to the level of IOP[464 472], except in instances where the IOP is very low[465].

1.9.1. Ocular perfusion pressure (OPP)

The IOP is determined as the force per unit area exerted on the ocular tissues within the anterior chamber by the fluids that contain them[473], mainly through the combination of aqueous humor production by the ciliary body and drainage through the trabecular meshwork and canal of schlemm. The normal range of IOP is defined as being between 10-21mmHg[474]. There is considerably a large amount of variability between people but it exists in a constant state and is known to slightly increase with age and can also measure slightly higher in the morning than later on in the day. It can vary with cardiac and respiratory cycles and can be influenced by external factors such as exercise, diet and posture[475]. OPP is influenced by pulsatile variations in IOP, these pulsatile variations are thought to be delivered during systole contraction while diastolic flow accounts for 2/3 of ocular flow[476]. Put in another way, the ocular circulation pressure gradient begins with the arterial pressure being supplied by the CRA and ends with the pressure exiting the CRV. Within the eye the

vasculature is exposed to a significant compressing force, the intraocular pressure; the pressure within the small arteries and arterioles are the primary site of resistance within the ocular circulation and the drop in pressure from the systemic circulation to the capillary level can indicate the force of blood[392]. Ocular perfusion pressure is the force blood flow through the intra ocular vessels and is the difference between venous and arterial pressure, this affective venous pressure is also known as the IOP. This force can't actually be measured directly in the retinal circulation and thus it can only be estimated using the following equation. OPP= 2/3MABP-IOP. The relationship between ocular perfusion pressure and blood flow is complex but a rise in systemic arterial pressure or a drop in intraocular pressure can potentially reduce OPP.

1.9.2. Autoregulation and local control mechanisms

Autoregulation represents the ability of organs and tissues to modify vascular resistance in order to allow constant and uninterrupted blood supply and its maintenance despite variations in perfusion pressure[477]. Autoregulation ensures tissues and organs receive adequate blood supply despite varying haemodynamic conditions and has been demonstrated within the retinal and optic nerve head circulation[478-480]. There are methods of evaluating the autoregulatory responses within the vasculature and they usually include a provocation method such as inducing hypoxia or hypercapnia, postural changes, exercise, hand grip testing, and stimulation of flickering light. These methods put the vascular system under stress and should evoke an autoregulatory response. A failure to respond results in dysregulation and is possibly indicative of altered regulatory capacity. The exact mechanisms which are involved in autoregulation are not quite clear as it is more probable to be a combination of factors that work to harmonize the relationship between responses in the systemic and ocular circulation. These local control mechanisms include, myogenic, metabolic, neurogenic, humoral as well as endothelial derived vasoactive agents[481]. In the absence of autonomic innervation, myogenic and metabolic stimuli are more involved within the retinal circulation. These adjustments of vascular tone are described in detail below.

a. Myogenic control

The myogenic mechanism responds to stretch and vascular wall tension which is characterised by a decrease in vessel diameter followed by an increase in transmural pressure[482], ultimately referring to the maintenance of constant blood flow despite variations in systemic blood pressure[477]. Myogenic autoregulation is thought to be independent from the endothelium and intrinsic to vascular smooth muscle cells. Whereby, stretching of the vessel wall is thought to lead to depolarization of the vascular smooth muscle cells membrane with an influx of calcium ion (CA^{++}) into the cytosol and inducing vascular constriction[483]. At the level of the systemic vasculature, myogenic autoregulation mainly facilitates capillary fluid exchange, but its role in the eye[484 485] is not clear. Although its auto regulatory capacity has been demonstrated within the retina and optic nerve head[485], it remains unclear whether this mechanism is involved within the choroidal blood flow[484].

b. Metabolic control

Metabolic control refers to the metabolites that regulate blood flow to maintain adequate nutrient delivery. A tight coupling mechanism between tissue metabolism and perfusion is thought to exist[477] with alterations in the concentrations of metabolites including oxygen, carbon dioxide[486 487], potassium, hydrogen[380], and adenosine[488]. Since the majority of tissues use aerobic metabolism, oxygen delivery to the tissue is of utmost importance and when there is a demand of oxygen, this mechanism releases a vasodilatory signal that increases blood flow and capillary perfusion. Oxygen is the most regulated variable. Partial oxygen pressure has been identified as one of the main factors that act upon metabolic autoregulation[489 490]. Within the ocular circulation under conditions of hyperoxia or hypoxia, the auto regulatory mechanism maintains the delivery of oxygen consistently to the retina and optic nerve head. Hyperoxic conditions reduce retinal blood flow and partial oxygen pressure, through causing the retinal arterioles to constrict[486 491]. While hypoxic conditions trigger retina arteriolar vasodilations, normalising partial oxygen pressure through increased retinal blood flow[492]. Other metabolic factors are also in play within the retinal circulation and include

endothelial cells, glial cells, and neurons that affect vascular tone, specifically NO, ET-1, prostacyclin, cyclooxygenase, and angiotensin 2.

c. Neurogenic control

The autonomic nervous system has a large network of vasomotor nerve fibres that are directed towards the uvea, posterior ciliary arteries, and the extraocular portion of the central retinal artery[462 493]. Vessels in the retina and pre-laminar portion of the optic nerve head, however, have no neural innervation[494]. Neuronal regulatory mechanisms play a key role within the regulation of choroidal blood flow but have little effect on retinal and optic nerve head blood flow[495], despite alpha and beta adrenergic receptors having been identified within the retinal vasculature[496]. Numerous agents have been implicated as mediators in neuronal regulation such as acetylcholine[497], vasoactive intestinal polypeptide[498], substance P[499] and NO[500]. However the exact role of these agents are still unknown. More recently, it was shown that even high levels of noradrenaline have little impact on retinal vascular tone[501]. It has been suggested however, that stimulation of the sympathetic nervous system regulates blood flow towards the eye, by regulating and preventing over perfusion during an increase in systemic arterial blood pressure, thereby assisting myogenic autoregulatory mechanisms[502].

d. Neurovascular coupling mechanism

Neurovascular coupling, also known as functional hyperaemia, refers to the relationship between neural activity and its subsequent effects in regulating blood flow in the cerebral vasculature and was first identified in the late 1800's[503]. The normal response at the cerebral level is characterized by an increase in dilation of arterioles and capillaries in a local brain region in response to amplified neural activity[504]. This results in rapid expansion increasing cerebral blood flow so the oxygen and glucose demands of the brain are met[504]. This is important because the brain, unlike the rest of the

body doesn't possess the ability to reserve energy and oxygen. Although the exact mechanism which drives the neurovascular response is still up for debate, it is more likely a complex combination of intercellular communications between neurones, astrocytes and microvessels, endothelial cells, pericytes and vascular smooth muscle cells[504 505], with NO having been identified as a key vasodilating contributor of the response[506 507] but not necessarily as the primary mediator. The key components of the neurovascular coupling response, are astrocytes, neurons, microvascular endothelium and the extracellular matrix, collectively are known as the "neurovascular unit". They are not only involved in the regulation of blood flow but are indispensable in preserving the health and function of the central nervous system[508]. The neurovascular unit is also responsible for maintaining homeostasis within the cerebral microenvironment through controlling blood brain barrier (BBB) metabolite exchange and contributing to immune surveillance[509]. The neurovascular coupling mechanism has also been detected at the retinal and optic nerve head circulations, where escalation in the retinal neuron activity increases blood flow to the excited regions[510 511]. This occurs through alterations in retinal vessel diameters, either through dilation or constriction of the retinal arterioles and capillaries[512 513].

1.10 Assessment of systemic vascular and endothelial function

The capability to detect and evaluate endothelial dysfunction (ED) is important with regard to the identification, understanding and treatment of overweight and obese patients at risk of developing CVD. The techniques for identifying endothelial function can be broadly separated into three categories. The first works by measuring soluble circulating endothelial markers such as NO, ET-1 and von Willibrand factor (vWF). The production of these markers is known to be increased in the presence of ED[514 515]. The second category of measurement is determination of functional endothelial dependent vascular tone at focal sites of circulation[516]. This gives information on how the calibre of the vessel in correlates with its function. The third category of technique used is the

identification of structural characteristics of the vascular wall[353 517] that can be altered in the presence of ED. A summary of these investigations are given in table 1.10.

The initial methods for assessing endothelial function were quite invasive and included such techniques as strain gauge plethysmography of the forearm[518], and quantitative coronary angiography using intracoronary infusion of acetylcholine (Ach)[519]. During both techniques, infusion of Ach would cause the vessel of choice to dilate. If there was a disrupted or dysfunctional endothelium then vessels would constrict due to direct activation of the muscarinic receptors on the vascular smooth muscle cells[516 519]. These tests however are only suited when clinically indicated and are not ideal for the assessment of the asymptomatic patient. The introduction of non-invasive techniques such as FMD have now superseded the aforementioned investigations. Currently systemic endothelial function as measured by FMD is the gold standard but can only be performed in highly specialized environments limiting its use within research settings. Indeed, it has previously been shown its application is technically challenging and requires extensive training[520-522]. Because of this, reproducibility has been hampered with inter-and intra-observer variations caused by a lack of a standardized protocol[523 524]. Although circulatory abnormalities have been known to occur in micro-vessels before they occur in larger vessels, there is a possibility that vascular changes in the retinal micro-circulation may represent an ocular manifestation of a generalized systemic disorder. A number of systemic endothelial assessments were therefore selected for the present study to provide a comprehensive view of an individual's vascular status. Namely, assessments of systemic endothelial function and arterial stiffness were achieved by way of digital thermal monitor (DTM), and PWA respectively.

DTM has been recently introduced as an alternative to FMD because of its similarity to FMD; occluding the forearm results in a temperature drop, when released reactive hyperaemia (RH) causes an increase in temperature rebound which is proportional to the RH response[525]. The advantage of DTM is its independence from the operator's skills and experience making its

repeatability enhanced, so that is has become a preferred method in research[526]. DTM was therefore the technique of choice for present study. Its principles are discussed in more detail in Section 3.3.2.

1.11. Assessment of retinal vessels

1.11.1 Static vessel analysis

The first published image of the retina by Von Tright in 1853 was the result of many years of research and improvements in the technique by Von Helmholtz 1851. The concept of fundus photography dates back to the days of the early 1900's when Gullstrand invented the fundus camera and won a Nobel prize for his achievement[527]. The photographic technique has not changed significantly per se but the availability of technological advancements in digital imaging has allowed the clinician to make adjustments and refine the images as necessary as well as allowing the operator to view the fundus as the image is being captured.

Since then fundus photography has facilitated the development of methods to quantify retinal vessel calibre and to yield specifications with regards to vascular characterization and risk stratification. It has been shown over time that the retinal vasculature can provide information regarding the future risk of systemic pathology[528-533]

Retinal vessel assessments can aid in the interpretation of pathology and risk factor exposure over the course of life. It has been used as a research tool in exploring cardiovascular risk; assessing microvascular changes associated with systemic pathology, and treatment of vascular therapies. There are numerous parameters used in quantifying fundus photographs. Among them, the use of retinal vessel diameters has been most often used for the assessment of disease. The vessel diameter measurements correlate well with systemic diseases such as obesity and metabolic syndrome[299 534 535], hypertension[536-538], diabetes[539-541], coronary heart disease[542 543], and endothelial dysfunction[543 544]. These alterations could suggest specific vascular signs that correlate with the

systemic abnormality. For example, venular widening could represent obesity and metabolic abnormalities while arteriolar narrowing could represent hypertensive profiles.

Even with the amount of information to date and the associations with retinal vessel calibre measurements it still only provides a snap shot of an individual's circulatory state and conveys more information on established structural abnormalities rather than developing functional abnormalities. Nevertheless, the retinal microcirculation provides a useful marker for the evaluation of physiological or pathophysiological deviations from the systemic circulation and can represent a microvascular phenotype of endothelial dysfunction.

Table 1.10 Summary of techniques used to assess endothelial function

Technique	Advantages	Disadvantages
Circulating markers		
Nitric oxide - urine cGMP	Elevated urine levels are in early indicator of endothelial dysfunction	Heavily affected by diet
Endothelin-1 (ET-1)	Can be detected through increased bioavailability or receptor blockade	Can only be determined in pathological states and not in controls
von Willibrand factor (vWF)	Production is known to be increased in the presence of damaged endothelial cells	Can be difficult to distinguish with an acute infection or injury
asymmetric dimethylarginine (ADMA)	Elevated levels are present in patients with atherosclerosis- precursor to ED	Can be affected by high salt and fat concentrations
Plasminogen activator inhibitor-1 (PAI-1)	increased levels correlate with the severity of atherosclerosis	Difficult to distinct between PAI-1 and the antigen PAI-A which is responsible for reduced fibrinolysis
Vascular cell adhesion molecules. E-selectin and P selectin	levels are increased with atherosclerosis and positively correlated with circulatory levels of inflammation	Can be difficult to distinguish between an acute infection or injury with atherosclerosis
Structural tests		
Pulse wave analysis	Easily detect arterial stiffness and is non- invasive	An indirect measure of endothelial function to be used in conjunction with other tests
Pulse contour analysis	Simple set-up and repeatable test for arterial stiffness	In patients with stiff vasculature SBP and DBP cannot be distinguished readily
Carotid wall distensibility co-eficient	Measures arterial wall ability to expand and contract with cardiac work. Useful index in late atherosclerosis	Only useful in adults. Uses ultrasonography but it's relationship with endothelial function is yet to be determined
Intima media thickness	Reliable non-invasive and reproducible measurement of arterial stiffness	Indirect measure of endothelial dysfunction and cannot be used for short term efficacy of therapy.
Functional tests		
Coronary angiograpy	Sensitive indicator of endothelial dysfunction	Invasive procedure and can only be used when indicated clinically
Coronary positron emission tomography	Non-invasive can also provide information on inflammatory cells within the vessel wall- is going to supersede coronary angiography	Relatively new technique but lacks specificity when used on its own to detect atherosclerosis. Can be used in combination with CT and MRI
Forearm venous occlusion plethysmography	Sensitive indicator of endothelial dysfunction	Invasive. Requires specialised training and insertion of a catheter
Brachial artery- Flow mediated dilation (FMD)	Non-invasive. Sensitive indicator of endothelial dysfunction	Requires specialised training to ensure repeatability and low variability. Protocols vary amongst studies

1.11.2 Retinal vascular function: Dynamic Vessel Analysis

Quantification of retinal vessel diameter was first derived from studies that observed the increase and decrease of oxygen on the retinal circulation[545], where by providing the individual with 100% oxygen caused a decrease in vessel diameter (vasoconstriction), and providing 10% oxygen resulted in vasodilation. This later resulted in dyes being imaged through the retinal circulation to look for microvascular abnormalities. Recent years have seen the advent of newer instruments that measure the haemodynamic parameters of the retina, such as, the laser Doppler flowmetry and the scanning laser ophthalmoscope[546]. Currently, the retinal vessel analyser (RVA) system (IMEDOS, GmbH, Jena, Germany) is the most widely used for assessing retinal microvascular reactivity in health and disease, and is considered to be a sensitive indicator of retinal microvascular function[547 548]. The dynamic nature of the instrument allows the retinal vessels to be assessed through a continuous online measurement of retinal vessel diameter changes in response to provocation[549 550]. This has led to the device being increasingly recognized as an alternate tool for the assessment of retinal vascular tone[551], and functional microvascular assessment of endothelial function at the retinal level[552].

Studies have examined the link between functional microvascular abnormalities and the risk of developing cardiovascular disease. On the other hand, there have been numerous studies that have shown functional deficits of microvascular function in persons with established disease[553] or as a consequence of ageing[554]. However, no study to date has looked at the effect on ocular health; specifically, retinal microvascular function responses during weight loss interventions. In order to enable a better appreciation of the vascular insufficiency and whether this can improve through advancements in weight reductions requires a review on the principles of blood vessels, and blood flow regulation within the systemic and retinal vasculature. (Refer to section 1.8 for further details). Table 1.11 shows the studies that have been used to describe the impact of obesity and higher BMI ranges on the microvasculature.

1.12. Assessment of the peripheral vascular function

The initial methods of assessing endothelial function was quantitative coronary angiography and was superseded by FMD due to the investigation being much less invasive. With this technique, currently regarded as the gold standard, the dilation response following temporary cuff occlusion of the forearm (ischaemia) or oral administration of nitroglycerin (potent NO vasodilating agent) is the amount of reactive hyperaemia observed and the amount of dilation measured. There have been different protocols among researchers especially since the technique is highly dependent on operator experience and expertise[520 555]. In most cases two operators are required for reliability and repeatability but the result itself can be variable since to date the normal reference range for a FMD response has yet to be characterized[516 556]. Other techniques have been used to study endothelial function through temperature changes within the microvasculature and cutaneous circulation.

The skin is affected in terms of structural and functional changes. Indeed, increased resistance due to microvascular dysfunction in hypertensive individuals is an important component on the maintenance of high blood pressure[364]. More specifically, spectral analysis of laser Doppler flowmetry (LDF) signals suggests that essential hypertension results in altered neurogenic and nitric NO synthase-dependent control of skin vasomotion[557]. Cutaneous vasodilation of vasoconstriction can be elicited through post-occlusive reactive hyperaemia[558]. When vascular function is compromised, the dilatation following ischaemia (hyperaemia) is markedly reduced. This reduction is attributed to endothelial dysfunction, a globalized systemic disease process consisting of attenuated endothelium-dependant vasodilatation, augmented vasoconstriction and microvessel structural remodelling[559]. A blunted post-ischemic increase in skin endothelial dependent vasomotion was observed in patients with chronic kidney disease (CKD)[560]. In addition, blunted skin endothelial-dependent vasomotion resulted from ACh iontophoresis in hypercholesterolemic patients without clinically manifest arterial disease[372 561] and a decreased skin endothelial- and sympathetic dependent vasomotion was observed in obese women under basal conditions[562]. All of these are

suggestive and consistent with microvascular endothelial dysfunction. It was also shown that peripheral endothelial function measured with peripheral arterial tonometry at the fingertip is correlated with coronary microvascular function in patients with early atherosclerosis [563]. Similarly, PORH assessed with LDF was found significantly reduced in untreated hypertensive subjects[564].

LDF is chosen as the standard to characterize microvascular function because 80% of blood in fingers is stored in the skin[565] and strongly affects skin temperature variation. Newer modalities that test skin reactive hyperaemia have been introduced. Digital thermal monitoring (DTM) works on the premise that the hyperaemic response following arterial occlusion can be characterized by recording and analysing temperature alterations at the fingertips. Reductions in the hyperaemic response, a hallmark feature of impaired vascular function, are observed as reductions in thermal monitoring. Previous studies have already indicated a high correlation between heat flux at the skin surface, skin temperature and blood perfusion[566 567]. Indeed, it was found that the fingertip temperature, as measured with DTM, follows the same response as that of perfusion or heat flux[361]. Recent studies have demonstrated the ability of the DTM test to discriminate individuals with established CVD or high risk of future CVD in normal and low-risk individuals[568-572].

More recently insights into the mechanism of cutaneous post-occlusive hyperaemia have shown that the inhibition of cyclooxygenase (COX) and NO do not alter skin PORH but that endothelial derived hyperpolarizing factor (EDHF) has been suggested as the major endothelial contributor to PORH[573]. Nevertheless, the cutaneous circulation is an accessible and representative vascular bed for the assessment of endothelial function and for mechanisms of underlying vascular disease. Table 1.12. shows the current studies which have used the DTM to assess peripheral microvascular function and their relationships with novel CVD risk markers. To provide a basis for the studies described in this thesis, it is important for an understanding of the current aetiological thinking for the development of anterior eye pathologies. An understanding of the anatomy and physiology of anterior ocular health and their regulatory mechanisms is required. A summary of these is presented below.

Table 1.11 Summary of studies on obesity and its effects on the microvasculature.

Author	Year	Participants	Purpose/summary	Main findings	Disadvantages
Leiden[574]	2002	626 participants	To study potential risk factors for retinopathy in diabetic and non-diabetic subjects	The prevalence of retinopathy was positively associated with elevated cholesterol, blood pressure and BMI. Elevated blood pressure and total cholesterol showed an association with retinal hard exudates	The level of retinopathy was graded subjectively and it was not known how many graders there were or how many photographs they each graded.
Wang[575]	2006	3654 (1460 M)	To investigate the associations among retinal vessel diameters, and BMI in an older population	Participants were 2x more likely to have wider venular calibres if they were obese even after adjusting for confounding variables.	There was a proportion of participants who did not attend the 5 year follow up which could have possibly led to a selection bias.
Wang[576]	2007	7494 participants	To examine whether smaller retinal arterioles or larger venules predicted coronary heart disease and stroke mortality	Participants that had smaller retinal arterioles and wider venular calibres were more likely to be older, have higher BMI and hypertension. This was significant in predicting higher risk of CHD mortality but was not significant in persons over age 70	Blood pressure values were relied upon patients self-reporting the diagnosis without any validation checks or measurements. They also did not account for the medications which some of the participants were taking.
Mitchell[577]	2007	768 school children	To examine the association of BMI and weight with retinal vascular calibre in children	After adjusting for covariates BMI was associated with wider venular calibre in healthy children. For each 3.1kg increase in BMI was associated with 2.55microns increase in venular diameter.	Photographs were only analysed from a subset of the original cohort and in this subset only half the participants underwent blood pressure measurements.
Kotliar[578]	2011	92 (46 controls) obese participants	To assess whether dynamic reaction to flicker stimulation is altered in obese subjects	Mean maximal diameter in response to flicker was reduced in the obese group and the reaction time to maximal dilation was also prolonged. Maximal venular dilation was reduced in obese subjects. There was a significant correlation between waist circumference and retinal arterioles after flicker provocation	They did not measure intra ocular pressure in obese subjects and this is not known whether it may affect retinal vessel response to flicker even though it is part of a normal protocol to measure IOP before mydriasis. The authors could have analysed at least 4 other parameters during DVA.
Hanssen[579]	2011	46 participants (14 obese, 15 lean, 17 elite)	To assess the effects of regular exercise training on AVR and to investigate changes in obesity impairment of the retinal microcirculation.	Results indicated at baseline that AVR ratio was significantly reduced compared to controls and that individual fitness levels positively correlated with AVR. The training program improved AVR improved in all groups and increased retinal arteriolar diameters in the obese subjects.	There were 14 dropouts due to musco-skeletal injuries and respiratory tract infections probably due to the intense exercise regimen which in turn decreased the sample of participants in each group. There was no mention of diet and how the groups differed in terms of that.
Hanssen[535]	2012	578 school children	To analyse the prevalence of obesity and the association between physical inactivity, metabolic risk factors and retinal vessel diameter	Overweight and obese children had significantly lower AVR compared controls. Wider venular diameter was independently associated with higher BMI, WC, and CRP. BP was associated with retinal vessel constriction.	Although there are associations between risk variables and vessel diameter, long term follow up still need to be carried out to prove the association.
Lammert[324]	2012	127 (87 W) obese participants	To assess cardiovascular risk factors and retinal vascular parameters in morbid obesity	WC was inversely proportional to AVR ratio. Impaired flicker reaction to retinal vessels was detected in 62% of the cohort, significantly reduced AVR in 56% and increased intima-media thickening in 26%.	There was no control group and the authors compared their results with already published normative data. The authors generalized their findings for the participants that underwent flicker analysis even though there were a lot of dropouts.
Boillot[534]	2013	44000 participants, meta-analysis	To determine the association between BMI and retinal vessel calibre	Obese participants had narrower arteriolar and wider venular calibres when compared controls independent of conventional cardiovascular risk factors. A 1kg increase in BMI was associated with a 0.07 micron decrease in arteriolar calibre and 0.22 micron increase in venular calibre.	There was a potential measurement bias due to different protocols, photographic procedures and the software being used to analyse the retinal vessel calibres resulting in either overestimation or underestimation of the true association between BMI and vessel calibres
Yau[580]	2014	90 obese (51 controls)	To assess retinal arteriolar diameter and retinal structure in obese adolescents with metabolic syndrome with changes in cerebral white matter microstructure	Obese adolescents with METs had significant reductions in arteriolar diameters. Waist circumference was the strongest component related to reduction in arteriolar diameter. White matter changes showed decreased microstructural integrity associated with narrower arteriolar calibre.	Out of the control participants 35% had insulin resistance and 29% were obese.

Table 1.12 Summary of studies utilizing digital thermal monitoring (DTM)

Author	Year	Participants	Purpose/Summary	Main Findings	Disadvantages
Dhindsa[581]	2008	40 healthy subjects	To assess the interrelationships between different non-invasive measures of macro- and microvascular reactivity.	No significant relationships were found due to the difference of physiological factors influencing the vascular bed being tested.	Did not examine any participants with or suspected subclinical atherosclerosis. A case control study with the different techniques could have shown a better correlation between micro and macrovascular function.
Ahmadi[568]	2008	233 asymptomatic	To assess the correlation between peripheral vascular dysfunction with the Framingham Risk Score (FRS) and extent of subclinical atherosclerosis measured by CAC.	DTM showed a negative correlation with FRS independent of cardiac risk factors was and superior to the FRS in predicting significant CAC	Study did not include controls or subjects with definitive diagnosis of atherosclerosis, rather asymptomatic patients were only included if they underwent CAC or increased FRS
McQuilken[525]	2009	N/A	To assess the relationship between two measures of peripheral vascular reactivity. DTM & LDF	Outcome measurements of peripheral vascular reactivity are strongly correlated. DTM sensors resemble the same frequency signals calculated by Doppler methods	The study did not mention how many subjects were used to derive their correlation or the statistical test used to come to their conclusions
Ahmadi[570]	2009	129	To determine the correlation between DTM and coronary artery disease (CAD) in symptomatic patients	The study indicated a strong correlation between low fingertip TR and the presence and extent of CAD measured by computed tomography.	The tests were conducted in accordance with a protocol that was not excluding subjects taking caffeine, alcohol or tobacco. Also, antihypertensive medication was allowed for certain subjects >65bpm
Gul[571]	2009	133 (19 with CAD)	To assess the clinical utility of using the DTM system on patients diagnosed with CHD	DTM of vascular dysfunction is associated with an increased FRS and CHD. Fingertip temperature rebound was lower in patients with CHD than without.	Protocol was different with regards to other studies that use a 5 min cuff-occlusion. Current study only used 2 minutes.
Ahmadi[569]	2009	233 (127MS/DM)	To assess peripheral vascular dysfunction in patients diagnosed with diabetes mellitus and metabolic syndrome (MS)	Temperature rebound was significantly lower in diabetes and metabolic syndrome compared to controls. Potential to detect asymptomatic patients with increased subclinical atherosclerosis	No mention was made on the extent and duration of DM or MS within these subjects and whether a relationship exists due to measurements of different vascular beds.
Akhtar[582]	2010	mechanical model	To assess the sensitivity of DTM parameters to reactive hyperaemia.	Temperature rebound was shown to correlate well with RH and had good sensitivity at the range of flow rates studied.	The results were only from a mechanical model which do not truly represent a clinical population with varying degrees physical parameters.
Ahmadi[526]	2011	18 healthy subjects	To evaluate the variability and reproducibility of DTM measurements.	DTM measures correlated well with Doppler ultrasound. In a controlled environment, DTM was reliable and reproducible from day to day clinical use.	This study had used a protocol consisting of a 2 minute cuff occlusive state, while previous studies have used a 5 min cuff-occlusive state.
Schier[583]	2013	60 Subjects	To evaluate the clinical utility of DTM as a surrogate for evaluating vascular function postoperatively after major thoracic surgery.	A significant difference was found for measurements taken between 24h and72h but not in between. Participants which were obese or had abdominal obesity displayed a higher TR after surgery.	Participants presented with multiple comorbidities during thoracic surgery and they had only one week follow up. A protocol of only 2 min cuff-occlusion was used.
Schier[584]	2013	30 subjects	To evaluate the increase in RH after acute exercise and whether a lack of RH increase would correlate with preoperative cardiovascular risk factors.	A single episode of anaerobic exercise enhanced RH. TR parameters increased significantly and all patients with preoperative cardiac risk factors had their values fall within the lower two thirds of the study population.	This study suggested that the increase in RH preoperatively may serve as a diagnostic tool for better surgical outcomes but no follow up was done on the patients to check whether the increase in RH would correlate with a better surgical outcome.
Zeb 2013[585]	2013	210 (105 controls)	To evaluate the association of vascular dysfunction and Coronary Artery Calcium (CAC) score in patients with chronic kidney disease.	Vascular reactivity is incrementally worse across CAC scores in patients with chronic kidney disease.	Vascular reactivity was reported with the area under the curve as being significant difference but no mention was made with regards to TR and other parameters measured by the DTM.

1.13. Anterior eye anatomy and physiology

The anterior portion of the eye consists of the eyelids, cornea, tear film, and the transparent conjunctiva that stretches from the superior and inferior fornix to the corneal limbus. The eye contains complex optical properties which allow for clear vision. In order to maintain clear vision at all times, the cornea, tear film, and the eyelids must act synergistically to maintain optical clarity and provide a smooth surface for light to travel through. It has antimicrobial properties within the tear film to protect itself against any foreign pathogens or objects and can also regenerate and washout debris simply by blinking. An overview with relevance to this thesis is presented below.

The part of the orbicularis oculi muscle within the eyelid brings about blinking and eyelid closure and is supplied by the facial nerve. The tarsal plate is internal to the orbicularis which provides rigidity to the eye lid and also contains the meibomian glands[586]. The meibomian gland openings are located at the eyelid margin between the palpebral conjunctiva and skin. There are about 35 meibomian glands in the upper lid and 25 in the lower lid, which extend to the full height of the tarsal plate[586]. The inner layer of the eyelid is covered by the palpebral conjunctiva. The eye lashes leave the distal part of the eyelid skin and curve away from the globe[586].

The conjunctiva is a very thin and translucent mucus membrane that lines the anterior portion of the sclera and covers the globe (bulbar conjunctiva) and inside the eye lids (tarsal conjunctiva). It reflects back in the conjunctival fornices and covers the globe as far as the corneal limbus[586]

1.13.1 The Cornea

The cornea is a transparent avascular connective tissue that acts as the primary infectious and structural barrier of the eye[587]. In conjunction with the tear film it provides the necessary conditions for light to refract and focus on the retina. The cornea relies on tiny blood vessels at the corneal

limbus, the tear film and aqueous humor for nutrients and oxygen. The shape of the cornea is flatter in the periphery and steeper centrally[587]. It is also well innervated with nerves which are derived from the nasociliary branch of the trigeminal nerve. The nerves enter the stroma radially in thick trunks which perforate Bowmans layer to provide a rich plexus beneath the epithelium[588]. The cornea is recognized as having 5 distinct layers which combine to give an overall thickness of 0.5mm centrally and increased towards the periphery. The distinct layers of the cornea from anterior to posterior are the Epithelium-Bowmans layer-Stroma- Descemets membrane- Endothelium. More recently, a 6th layer known as Dua's layer has been discovered anterior to Descemets membrane.

The epithelium is the outer most layer and is covered with the tear film. It is stratified with a non-keratinizing squamous layer[587]. It regenerates itself every 7 to 10 days[589]. The epithelium is attached to the underlying Bowmans layer by a basement membrane with a hemidesmosomal system. This is the sight of the strongest attachment to the underlying corneal layers. Bowmans' layer lies just anterior to the stroma and is actually a condensed part of the stroma but is also the junction at which the nerves enter the cornea. This has implications in diabetic patients due to the fact that they have reduced sensation to pain and are more susceptible to infections than non-diabetics. Bowmans' layer is also responsible for maintaining corneal integrity and shape. If damaged unlike, the epithelium, it cannot regenerate and scar tissue will form. The corneal stroma lies just anterior to the Descemets membrane and is the thickest of all the layers. It is comprised of collagen fibres which are arranged in parallel bundles. These collagen fibres are known to change direction as they approach the limbus[590]. This network of collagen fibres allows the reduction of forward light scatter by maintaining tight junctions which ultimately contribute to the mechanical strength and transparency of the cornea. Dua's layer lies posterior to the corneal stroma and anterior to Descemets membrane. It is acellular and contains only a few keratocytes. It is impervious to air[591]. Descemets membrane lies anterior to the corneal endothelium and is secreted by the endothelium. It has an amorphous structure and can increase in thickness with ageing. It is at this site along with

the stroma where lipid deposits can be seen on clinical examination and is known as corneal arcus. Lipid deposits are more densely compact towards the periphery of the cornea than centrally and is a sign of high CHOL[592]. The corneal endothelium is the most posterior portion of the cornea and is a mono-layer. The surface of the endothelium has many hemidesmosomes that promote adhesion to the Descemets membrane. Endothelial cells are constantly changing throughout life and decreasing in density as we age. The state of corneal deturgescence is mediated by a pump-leak process as fluid egresses from the stroma down the osmotic gradient from a relatively hypo-osmotic stroma towards a relatively hypertonic aqueous humor[587]. This process is facilitated by the transporting of ions to generate the osmotic gradient. The barrier portion of the endothelium is unique in that it is permeable to some degree, permitting the ion flux necessary to establish the osmotic gradient[593]. Transport of these important ions involves membrane bound Na+ and K+-ATPase sites and the intracellular carbonic anhydrase pathway[587]. The endothelium is unable to regenerate itself, and is susceptible to age, trauma and inflammation which result in polymegathism and pleomorphism.

1.13.2 Tear film function

The tear film has a unique tri-laminar composition that enables it to perform a number of functions. The normal corneal tear film is responsible for providing an optically smooth surface that allows clear vision. It protects the eyes from infections and environmental hazards, provides nourishment to the cornea, washes out cellular debris and foreign bodies and sustains comfort by ensuring a smooth movement of the eyelids over the globe with a healthy corneal epithelium[594]. Tears are contained within the conjunctival sac when the eyes are closed, and redistribution of these tears across the conjunctival and corneal surface happens when the eyes re-open[595]. The classic description by Wolff recognized the tear film as a three layer layered structure comprising of an outer lipid layer (approx. 0.1micron thick), intermediate aqueous layer (approx. 7 microns thick), and inner mucous layer (approx. 0.05 microns thick)[596]. This model is generally accepted though there is uncertainty about its true thickness. Other researchers have suggested that the actual thickness is

closer to 40 microns[597]. More recently, it has been proposed that the thickness is roughly 3 microns thick[598]. Although, the thickness is still being debated, it is generally accepted that the three layers work together to maintain integrity of the tear film and health of the cornea.

1.13.3 Lipid layer

This oily layer is produced by the Meibomian glands, which are modified sebaceous glands located in the lower and upper tarsal plates[599]. The lipid layers' main function is to reduce the evaporation of tears, provide a smooth optical surface, and to prevent contamination of the tear film debris[599]. Rapid and forceful blinking has also been shown to increase the thickness of the lipid layer[600]. The secretion of these glands are known as meibum. Meibum has a lower melting point then sebum, which allows it to remain a fluid when in the tear film[599]. It consists of both polar and non-polar lipids. The polar fraction of the Meibomian layer, acting like a surfactant, is comprised mostly of phospholipids and spreads over the aqueous layer of the tear film while the nonpolar fraction of the lipid layer lies more superficial[601 602], at the air-lipid interface[602]. The removal of the lipid layer results in increased hyper-osmolality, through the reduction of goblet cell density, and glycogen levels thus leading to inflammation[603].

1.13.4 Aqueous layer

The intermediate layer of the tear film is the aqueous layer and is responsible for lubrication, flushing away of desquamated epithelial cells and protection of the ocular surface. It washes away foreign materials introduced to the conjunctiva or cornea and contains antibacterial factors including immunoglobins[595]. Nutrients provided to the avascular cornea by the aqueous layer include glucose, oxygen, and protein[604]. The aqueous layer has antibacterial properties such as the effects of lysosome against gram +ve bacteria[605], effects of lactoferrin against –ve bacteria[606], as well as having anti-inflammatory properties[607]. It is produced mainly by the lacrimal and accessory lacrimal glands of Krause and Wolfring under the influence of autonomic innervation and various hormones[608]. The

aqueous layer also contains soluble mucin that decreases the surface tension, enhances the spread and coherence of the aqueous layer, and contributes the viscosity of the tear film[609].

1.13.5 Mucous layer

Ocular mucous is composed of mucin, immunoglobins, glucose, urea, salts, enzymes, leukocytes and cellular debris[610]. The mucous layer lubricates and protects the cornea, anchors the aqueous tear film to the epithelium protecting it from bacterial contamination, shear forces, and desiccation[611]. The mucus is responsible for creating a hydrophilic layer, which facilitates the spread of the aqueous layer on top of the hydrophobic corneal epithelium. The mucus layer is not tightly adhered to the epithelial layer, but rather attaches to the glycocalyx and undergoes free movement across the cornea[612]. This allows the mucus to spread evenly across the cells and prevents damage to the epithelium during blinking[613]. The majority of the mucus layer is secreted by conjunctival goblet cells which can be stimulated to secrete mucin by shear force, blinking or by histamines[614].

1.13.6 Tear film production

Tear production is a multifactorial process mediated by interconnected nervous, endocrine, vascular, muscular, and immune systems[615]. Tear secretion is a reflex response initiated by stimulation of the ocular surface or nasal mucosa[616 617]. Normal production of tears requires a healthy and normal functioning lacrimal unit. This functional unit is a subset of the ocular surface system[618], and consists of the eyelids, lacrimal glands, cornea, meibomian glands, and their interconnected nervous system. This unit is responsible for the tear secretion and blink reflex. The blink reflex is critical for tear function as it stimulates the release of lipids from the Meibomian glands and replenishes the tear film with tears from the inferior tear meniscus[600 619]. The lacrimal gland is an exocrine gland that lies within the lacrimal fossa of the bony orbit[620], and its secretory cells are the pyramidal acinar cells. The accessory lacrimal glands of Krause located in the stroma of the palpebral conjunctiva of the upper and lower fornix and Wolfring located in the superior tarsal plate, are thought to be similar to

the main lacrimal gland in both form and function. Previously it was though that the main accessory gland was responsible for reflex tearing, and the accessory lacrimal glands were responsible for basal tear secretion; however, it is now thought they contribute in unison, due to similarities in innervation, to both basal and reflex tear secretion[621]. The sensory nerve fibres in the cornea are primarily responsible for initiating tear secretion. These afferent sensory fibres relay signals to the spinal trigeminal nucleus where the information is relayed to the superior salivary nucleus of the pontine tegmentum[622 623]. Preganglionic parasympathetic neurons originating from the superior salivary nucleus project to the pterygopalatine ganglion[624 625]. Postganglionic parasympathetic neurons then project onto lacrimal glands, and also to the goblet cells and Meibomian glands[615], where they release neurotransmitters such as acetylcholine and vasoactive intestinal peptide that stimulate tear secretion[626-628].

1.14. Assessment of Anterior eye

Dry eye disease is an impairment of the ocular surface and tear ducts. It is multifactorial in nature and results in ocular discomfort and tear film instability[328]. Evaluation of anterior segment disorders has mainly relied on techniques such as the slit lamp bio-microscope. Traditionally tear break up time (TBUT) has been assessed with fluorescein, tear meniscus height has been measured with an attachment and Meibomian Gland Dysfunction has been judged on the blocked or atrophied meibomian glands on the lid margin. Bulbar and limbal redness are assessed by the naked eye or under the bio-microscope. It is important to note that all of these assessments are subjectively graded by the practitioner and that these measurements can thus vary amongst different examiners regardless of the addition of an anterior eye grading scale.

Computerized software can aid the clinician by allowing standardized measurements with higher repeatability. Indeed, The keratograph 5M (K5M, Oculus, Weltzar, Germany) has the capability of measuring these parameters within a single setting and does not require the use of staining agents, which alter tear volume and composition, for the determination of anterior segment disease.

Previous studies have reported that the K5M is a reliable method for scoring Bulbar redness (BR). Its reproducibility was the highest when compared to subjective examiner grading with 3 different scales[629]. The K5M also measures TBUT non-invasively as the first break up and as the average break up. A recent study compared these measurements in controls and in patients with DED. They showed the K5M was highly repeatable and reproducible and was comparable to the Schirmer test and fluorescein BUT[630]. In addition, the correlation of the *meiboscore* with functional dry eye parameters suggest that in patients with detectable meibomian gland atrophy as assessed with infrared meibography, there is also an impaired meibomian gland function[631]. However, the authors have stated that this assessment alone is not sufficient to diagnose MGD but should be used in conjunction with other measurements of DED for the overall picture. Therefore, the K5M was the technique of choice for the present study on anterior eye assessment. Its principles are discussed in more detail in section 3.3.5.

2.0 Research Rationale

Obesity is known to be a major risk factor for the development of several chronic diseases and the secondary implications associated with these risk factors have a direct impact on the quality of life and well-being for that individual. Together with an ever changing lifestyle directed towards longer working hours which leads to less time for an individual to revel in in extra-curricular activities has an important psychological affect; leading to laziness and an attitude that does not see the benefits of getting regular exercise and making healthier choices in grocery stores rather than eating the quickest meal possible that is loaded in saturated fats and refined sugars and salts.

Obesity has been associated with multiple chronic diseases that could lead to fatal events. One underlying common denominator is that endothelial dysfunction through atherosclerosis and constant inflammation is one of the main transgressors that leads to future vascular complications. Obese individuals, at least 50 % of the time, have elevated blood pressure, diabetes, and high cholesterol or a combination of these diseases. This in conjunction with the patient's age and whether they smoke, through a Framingham risk score calculation, can determine the risk that an individual can likely suffer from myocardial infarction or stroke. One of the main stays of treatment is through pharmacotherapy and studies have shown an increase in brachial artery function after treatment for hypertension and hypercholesterolemia. However, this considers macrovessel treatment and not the microvessels, and it is yet to be determined whether participants have improved in microvascular function alongside macrovascular function. It is understandable that, because of the nature of measuring different vascular beds, local control of vascular tone can be influential in revealing a possible relationship from vascular beds of different sizes and location throughout the body.

Another consideration is whether endothelial dysfunction can alter after weight-loss interventions. Obesity through inflammation and atherosclerosis can have a compounding effect on vascular

function through the body, specifically targeting the vascular endothelium. Once the endothelium has been damaged, the beginning stages of vascular complications usually follow and are unique to each individual. There has been some progress with weight management strategies Dieting has resulted in decreased blood pressure and bariatric surgery, which certain type 2 diabetic patients completed, has resulted in a 180 degree reversal. However, the relationship between weight-loss management and endothelial function have yet to be fully understood in terms of their role in returning microvascular function to normative values. Measurement of ocular circulation could significantly aid the assessment and diagnosis of vascular alterations in obesity and enhance our understanding and management of the disease.

Obesity can cause anterior eye pathologies but lacks concrete evidence. Neither have there been any studies on the impact of weight management strategies on anterior ocular health. Taking all of this into consideration, the principle purpose of this research was to investigate the presence of anterior eye pathologies and vascular alterations, at both the ocular and systemic level, in overweight and obese patients and to evaluate the potential impact of weight management strategies. In line with this, the overall aims of this research are as outlined in the following section.

2.1 Aims

- To investigate the relationship between retinal and peripheral microvascular function in healthy individuals
- To investigate the presence and impact of 16 weeks of physical exercise training in overweight individuals on retinal and systemic microvascular function.
- To investigate the impact of fasting during the month of Ramadan on retinal and systemic microvascular function.

- To investigate the impact of bariatric surgery on retinal and systemic micro and macrovascular function
- To compare anterior eye health in obese individuals 1 year before and after bariatric surgery.

3.0 Subjects and methods

This chapter outlines the recruitment procedure, inclusion and exclusion criteria for the obese, overweight and control participants involved in this research. It then goes on to outline the study protocol and investigative techniques used throughout this thesis for the assessment of anterior eye health, ocular and systemic vascular function.

3.1 Ethical approval

Prior to the study ethical approval was received from Coventry & Warwickshire West Midlands research ethics committee, Heart of England Foundations trust (HEFT) NHS Research Ethics Committees, Heartlands Hospital Research and Development (MIDRU) as well as the Aston University Life and Health Sciences Ethics Committee. Written informed consent was received from all subjects before entry into the study and all procedures were designed and conducted in accordance with the tenets of the Declaration of Helsinki.

3.2 Patient recruitment

3.2.2 Recruitment of obese participants

Successive diagnosed obese patients with BMI >40kg/m^2 were recruited from the Birmingham Heartlands, Heart of England Foundations Trust (HEFT, UK) weight management clinics by a specialist consultant. All obese participants recruited have never received weight reduction treatment. This ensured any vascular alterations identified were less likely to have occurred due to previous pharmacotherapy treatment and could be more reliably attributed to the weight loss itself.

3.2.2.1 Inclusion criteria

Only those patients diagnosed as obese and listed for surgery were considered for this study. Of these patients only those classified as having morbid obesity were included in the final study as per the WHO guidelines on obesity and based on their BMI value of greater than 40 kg/m^2.

3.2.3 Recruitment of overweight participants

Successive, participants that felt they were overweight with no previous health complications were recruited on campus from the Aston University's Sports Centre

3.2.3.1 Overweight participants' inclusion criteria

Only those patients that had a BMI greater than 25 kg/m² were enrolled in this study. They had to be relatively healthy with no known medical history or on any medications. Before inclusion, patients had agreed to involve themselves at least 4 times a week in programme of exercise over a period of 16 weeks.

3.2.4 Recruitment of fasting participants

Successive, patients that were willing to fast during the month of Ramadan were recruited for this study. Participants were recruited on campus at Aston University.

3.2.4.1 Fasting participants' inclusion criteria

Only those participants that were willing to fast for the whole month of Ramadan, from sunrise to sunset, were recruited. They had to be healthy with no medical history or taking any medications. Participants were recruited from volunteers at Aston University's Eye Clinic.

3.2.5 Recruitment of other healthy participants

Healthy participants not included in either of the above studies and of normal weight, were also recruited by inviting the participation of patients' spouses, same-generation relatives and friends, as well as through promotion of the study at the Aston University Health Clinics.

3.2.5.1 Healthy participants inclusion criteria

All healthy participants were screened for ocular disease with slit lamp biomicroscopy and volk assessment. Normal weight status was measured using a BMI calculation less than 25 kg/m² before inclusion in the study.

3.2.6 Exclusion criteria for all groups

Patients with closed iridocorneal angles, a history of intraocular surgery or any form of retinal or neuro-ophthalmological disease that could result in visual field defects were excluded from the study. Overweight and healthy participants were excluded if they had a positive diagnosis of cardio- or cerebro-vascular disease, (coronary artery disease - CAD, heart failure, arrhythmia, stroke, transient ischaemic attacks), peripheral vascular disease, severe dyslipidaemia (defined as plasma triglycerides>6.00mmol/L or cholesterol levels>7.00mmol/L), diabetes, and other metabolic disorders or chronic diseases that required treatment. Obese individuals were only excluded if they had a positive diagnosis of cardio- or cerebro-vascular disease and renal disease. Participants were screened for ocular disease and were excluded from the study if they had IOP>24 mmHg consistently, moderate to high cataracts or any other media opacity which did not allow proper fundus examination, a history of intraocular surgery or any form of retinal or neuro-ophthalmic disease affecting the ocular vascular system. Well-controlled systemic hypertension was neither an inclusion nor exclusion criteria; however any individuals taking medications which could potentially influence vascular function were excluded.

3.3 Methods

The investigative techniques used throughout this thesis were carefully selected to ensure the most accurate and reliable assessment of vascular function at both the ocular and systemic level that could be obtained. A summary of these techniques is given below in Table 3.3

Table 3.3 Summary of investigational parameters	
Technique	Purpose
Ocular level	
DVA	Assessment of ocular vascular function
Fundus photography	Asssessment of ocular vascular structure
Keratography	Assessment of anterior ocular structure/function
Systemic level	
DTM	Systemic endothelial function
PWA	Systemic arterial stiffness
Tanita body comp	Composition of total body fat and water
Blood analysis	
GlUC, CHOL, TG	Cardiovascular risk

Abbreviations: DVA, dynamic vessel analysis; DTM, digital thermal monitoring; PWA, pulse wave analysis; GLUC, fasting glucose; CHOL, total cholesterol, TG, triglycerides.

3.3.1 Experimental protocol

All measurements were performed between 8 am and 11 am following a 12 hour overnight fast, which included no alcohol or caffeine, water being the exception. All procedures were conducted in a consistent order outlined below and detailed in the following sections:

participant identified	•Approached and provided with the patient information leaflet
Explanation of study	•Procedures and risks explained, questions invited, concerns addressed
Consent	•Form read, understood, completed and signed by participant
Preliminary assessments	•Demographic questionaire •Lifestyle questionarire •Ocular assessment- slit lamp bio-microscopy/Indirect ophthalmoscopy •Height and weight measurement
Anterior eye	•Assessment of anterior ocular structures (Keratography, obese participants only)
IOP assessment	•O.4% Oxybuprocaine hydrochloride inserted in both eyes •Assessment of IOP with Perkins applanation tonometry
Dilation	•1% Tropicamide inserted into randomly selected eye
Endothelial function	•Assessment of systemic endothelial function (Endothelix, all participants)
Arterial stiffness	•Assessment of systemic arterial stiffness (PWA, obese participants only)
Body composition	•Assessment of body composition (Body Comp, exercise and healthy controls only)
Retinal vessel function	•Assessment of retinal endothelial function (DVA ,exercise, fasting, controls)
Fundus photography	•Final measurement ocular vascular structure (fundus photography, obese only)

3.3.2 Assessment of systemic parameters

3.3.2.1 Pulse wave analysis (PWA)

The method for assessing arterial stiffness in this thesis was pulse wave analysis (PWA) which was implemented using the SphygmoCor device (Atcor Medical, /PWV Medical Pty Ltd, Australia) in agreement with a well-established protocol[353 632 633]. This non invasive device has simple set up, and can be utilized for the assessment of arterial stiffness with high repeatability[353 634]. PWA represents an assessment of the arterial pulse contour and the relative degree of vessel stiffness alongside mathematical algorithms which can characterize the arterial pulse pressure waveform and generate indices of vascular interactions through identification of periods during systole and diastole[632]. Once the signal from the sphygmocor device is obtained, the algorithm converts the pulse pressure wave form into a central aortic pressure waveform which yields arterial stiffness parameters such as, the Augmentation index (Alx). The sphygomocor device also has quality control indices to ensure reliability of measurements. In this thesis, readings obtained with a reliability index of greater than 80 were used in the analysis.

3.3.2.1.1 Procedure

The procedure involves placement of the high fidelity pressure sensor (sphygmocor transducer) on the wrist underneath the thumb where the radial artery is located. The pressure sensor is then relatively flattened over the skin to generate a signal which represents the intravascular pulse of the radial artery. The profile generated by the system can provide important information of the stiffness of the systemic vasculature. This aortic pressure waveform is derived from two separate components; a forward pressure wave created by ventricular contraction (representing systole), and a reflected pressure wave created by the reflection of the incident wave back from bifurcations and peripheral vascular beds (representing diastole)[635].

In elastic vessels the reflected wave returns to the aortic root in diastole, supplementing coronary perfusion and creating the waveform profile (Figure 3.3.2). The speed at which the outgoing and reflected waveforms travel is dependent on the stiffness of the artery. This return of the waveform at a much quicker rate during systole, represents an augmentation of systolic pressure and decrease of diastolic pressure, along with decreased coronary perfusion.

The Augmentation index (Alx) value generated by the sphygmocor device is a widely used research parameter and is considered a sensitive value for assessing arterial stiffness[633]. In this thesis, Alx was the main parameter of interest. The Alx value is a percentage corrected for a heart rate of 75 bpm which indicates the amount the aortic pressure increase by the peripheral reflection of blood flow[636]. Influences of age, gender and ethnicity are known to exist, being greater in females and older persons[637 638] as well as being greater in Africans and Hispanics. A summary of the normal reference ranges matched with age are presented in table 3.3.2

Table 3.3.2 Normal reference values according to age for Alx (augmentation index)

Age	Mean	Lower 5% CI	Upper 5% CI
20	-4.67	-23.27	16.87
30	3.03	-15.57	24.57
40	10.73	-7.87	32.37
50	18.43	-0.17	39.97
60	26.13	7.53	47.67
70	33.83	15.23	55.37
80	41.53	22.93	63.07

PWA is a non invasive technique and poses no risk to the patient. One of the main weaknesses in the technique is that the estimation of the central pressure waveform may be inadequate to enumerate the magnitude of the pressure waveform reflections and therefore the true precision and reliability of any results through PWA may be limited[639]. However, studies have demonstrated that the technique is accurate with good reproducibility and repeatability with low inter-observer variation and is among the most widely used to assess arterial stiffness[640 641].

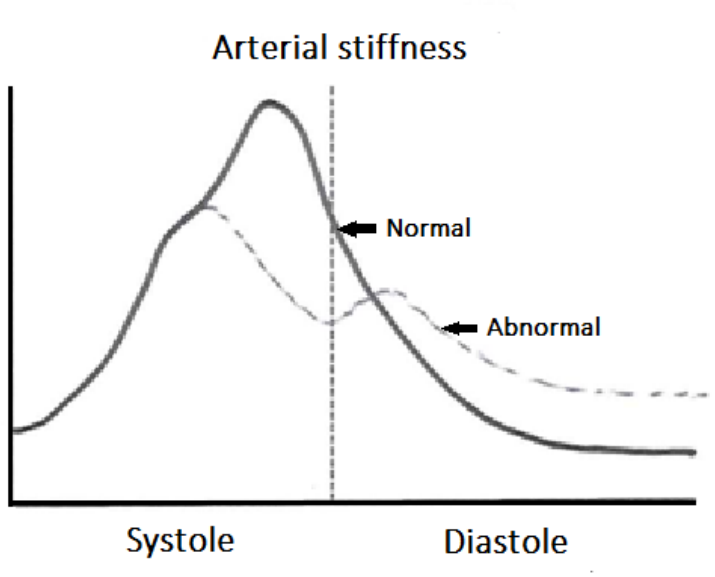

FIG 3.3.2. Representative example between normal and abnormal arterial stiffness

3.3.2.2 Digital thermal monitoring (DTM)

Peripheral microvascular reactivity at the level of the fingertips was assessed by way of DTM using VENDYS 5000BC Digital Thermal Monitoring (DTM) system (Endothelix, Inc., Houston, TX, USA). This FDA approved device consists of a computer-based thermometry system (0.006°C thermal resolution), with two special thermocouple fingertip probes designed to minimize the area of skin-probe contact and fingertip pressure. A standard sphygmomanometer cuff and a compressor unit to control cuff inflation and deflation is included to facilitate the occlusion-hyperaemia protocol[584].

3.3.2.2.1 Measurement Protocol

The test is conducted with the patient at rest for 30 minutes in the supine position, in a quiet, dimmed room with ambient temperature of 22°C to 26°C. VENDYS DTM probes are affixed to the index finger of each hand and after a period of stabilization of basal skin temperature (defined as stabilization within a 0.05°C threshold) the temperature is measured in the index fingers of both hands (of which the right arm only is subjected to occlusion-hyperaemia) with an automated, operator-independent protocol. The right upper arm cuff is rapidly inflated to ≥50 mmHg above systolic pressure for 5 minutes and then rapidly deflated to invoke reactive hyperaemia distally.

Thermal tracings are measured continuously and digitized automatically using a computer-based thermometry system with 0.006°C thermal resolution. Dual channel temperature data is simultaneously acquired at a 1 Hz sample rate.

(Figure 3.3.2.2) shows a representative example of a temperature-time trace and the primary DTM-derived measures, related to thermal debt and recovery, that were recorded and calculated. Measurements include (1) *Temperature rebound* (TR): maximum temperature- start temperature (just before cuff inflation), (2) *adjusted temperature rebound* (aTR): temperature rebound/ start temperature, (3) *area under the curve temperature rebound* (AUCTR): area under the curve between maximum temperature and minimum temperature. The post-occlusive adjusted temperature rebound aTR determined by the software algorithm is directly associated with the extent of the subjects vascular reactivity[582]. An aTR below 1 is considered to show poor cardiovascular reactivity, whereas an aTR of between 1 and 2 is considered intermediate, and an aTR of more than 2 is considered healthy.

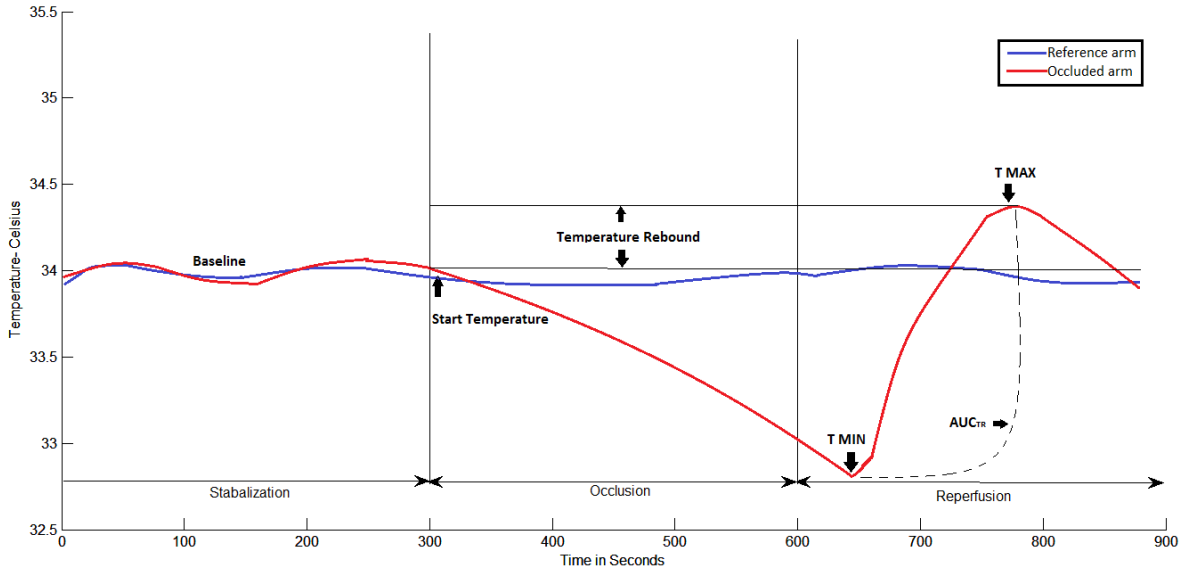

Figure 3.3.2.2: Graphical representation of the Digital Thermal Monitor software analysis; T MAX, maximum temperature; TMIN, minimum temperature; $AUC_{TR,}$ Area under the curve temperature rebound.

3.3.3 Assessment of ocular vascular structure

3.3.3.1 Quantification of retinal vessel calibre

Retinal vascular parameters can be extracted from the retinal image obtained from a specialized adapted camera. Currently, through advancements in technological capabilities, there are a wide variety of cameras that can take the necessary retinal images and software that can provide the user with the quantification of retinal images or provide the user with the basic data for manual calculations. These post processing techniques are used to aid the clinician in managing disease or progression. The quantitative values expressed and calculated are described below.

3.3.3.2 Central retinal artery equivalent

The central retinal artery equivalent is a measurement that incorporated all the retinal arterioles to give a single value that would represent the average width of a vessel within the retina. This method provides a quantifiable value that is far superior then objective grading of the vessel. In 1974, Parr and Spears[642 643] had developed a calculation to quantify retinal arteriole widths. First, retinal photographs were taken of the optic nerve head and arterioles were calculated out to 30 degrees. The rationale behind this measurement zone, according to Parr[643], was that pathological conditions affect the vessels as they branch out since the vessels are arterioles and venules respectively and they no longer have a continuous muscle layer and are void of an internal elastic lamina[644]. The following calculation incorporated the parent trunk vessel with its branches and is measured in micrometers.

$$Artery\ \ddot{W} = \sqrt{(0.87W_1^2 + 1.01W_2^2 - 0.22W_1W_2 - 10.76)}$$

Where W_1 (narrower), and W_2 (wider) are the paired retinal arteriole branches. $\ddot{W}$ Being the parent trunk arteriole diameter. All the arterioles were taken into account within the peripheral retina which yielded a single value known as the central retinal artery equivalent (CRAE).

3.3.3.3 Central retinal vein equivalent

Hubbard[645] expanded and devised a similar mathematical formula which is based on the above formula and experimental data that could be used to analyse the retinal veins. As the previous model Parr and Spears 1974 only takes into consideration the arterioles;

$$Vein\,\ddot{W} = \sqrt{(0.72W_1^2 + 0.91W_2^2 + 450.05)}$$

Where W_1 (narrower), and W_2 (wider) are the paired retinal venular branches. $\ddot{W}$ Being the parent trunk venular diameter. This calculation similar to the arterioles gives a single value which yields the central retinal vein equivalent (CRVE). Which can lead to the AVR ratio being calculated.

$$\text{AVR} = \frac{CRAE}{CRVE}$$

3.3.3.4 Artery Venous Ratio

The AVR has an advantage over generalised width measurements as the calculation does not require any modifications regarding refractive errors. Though absolute vessel diameters are important when correcting for refractive errors, this can be cancelled out when using equivalent measurements[646 647]. The resultant AVR can provide the clinician with a baseline value but changes in vascular morphology can result in simultaneous reductions or increases in the CRAE and CRVE possibly leading to the same AVR value as before. One must take into consideration the overall value of the equivalent diameter widths as well when contemplating disease progression or regression. Knudtson in 2003[648] revised the formula due to constant values within the PARR- HUBBARD equation which led to variability within the measurement calculation. The advantage of this being the revised formula does

not contain constant values and can be solved for different measuring units and is independent of image scale.

$$Arterioles\ \ddot{W} = 0.88 * \sqrt{(W_1^2 + W_2^2)}$$

$$Venules\ \ddot{W} = 0.95 * \sqrt{(W_1^2 + W_2^2)}$$

Where W_1 (narrower) and W_2 (wider) are the paired retinal vessel diameters. $\ddot{W}$ Being the parent trunk vessel. The branching co-efficient for the arterioles 0.88 and venules 0.95 were estimated based on the approximation of Hubbards experiment and as follows the equation.

$$Branching\ Co - efficient = \frac{(W_1^2 + W_2^2)}{W^2}$$

Where W_1, and W_2 are the widths of the narrower and wider branch and W is the parent trunk. After all the arterioles and venules are selected the six biggest of each group then go on to form the CRAE and CRVE which then can be used to quantify the AVR with the revised formula. The pairing process requires the biggest vessel to be matched with the smallest vessel and the next biggest with the next smallest, until all vessels are paired. The revised formula can begin with each iteration considered separately; in the case of an odd number vessel, this can be moved forward to the next iteration[648].

3.3.3.5 Measurement protocol

Mydriasis with 1% tropicamide (Chauvin pharmaceuticals, UK) was instilled 30 mins prior to fundus images being taken. The photograph taken 30^o centered on the optic nerve head. The ARIA[649] software was used. This is a Matlab (Matlab, Mathworks, VR2013a) plug in for the analysis of retinal images. The software protocol has been described elsewhere[649]. The measurement area is defined as a concentric ring centred on the optic nerve head at 1/2 to 1 disc diameter from the edge of the optic disc margin (Figure 3.3.3). Fundus photos are uploaded and the arteries and veins are chosen separately. Once all specified vessels are acquired they can be manually entered into the chosen formula, which in this thesis refers for Knudtson's 2003 revised formula.

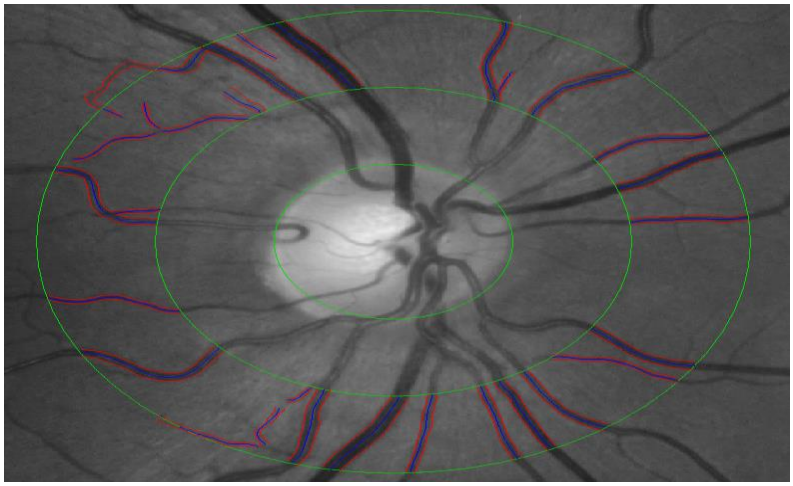

Figure 3.3.3. Aria measurement zone equivalent to 0.5 to 1DD from optic disc margin

3.3.4 Assessment of ocular vascular function

3.3.4.1 Dynamic retinal vessel analysis

This is a technique used to assess retinal microvascular function i.e. the behaviour of retinal arteries and veins in response to provocation. The RVA system (IMEDOS, GmbH, Jena ,Germany) enables the continuous recording of diameters of the retinal microvessels in real time. These diameter fluctuations occur physiologically owing to the pulsatile nature of blood flow and additionally can be altered through external provocation. Recordings can be stored on a video tape which is coupled to the CCD camera and can then be reviewed offline for further processing. Flicker light provocation was the method of choice in this thesis.

The RVA system (IMEDOS, GmbH, Jena ,Germany) consists of a fundus camera, (FF450, Zeiss Jena, Germany), a charged coupling device (CCD) camera, a high resolution video recorder, a real time monitor and a personal computer with analysis software.

In order to obtain a good quality illuminated fundus image with good contrast requires the use of dilating drops, usually 1% tropicamide (Chauvin, pharmaceuticals, UK). The camera itself is set at a 30^0 viewing angle to provide a proper field of view for examination with a temporal resolution of

$40_{ms.}$ This allows a sampling rate of 25 $H_{z,}$ such that 25 video frames are captured per second. The investigator will then choose a region of interest that incorporates appropriate vessel segments greater than 90 microns. These vessel segments are used in continuous diameter recordings over a specified time period. It encompasses a selection of an arteriole and venule between 0.5mm to 1mm in length, roughly 1 to 2 disc diameters away from the optic nerve head (figure 3.3.4).

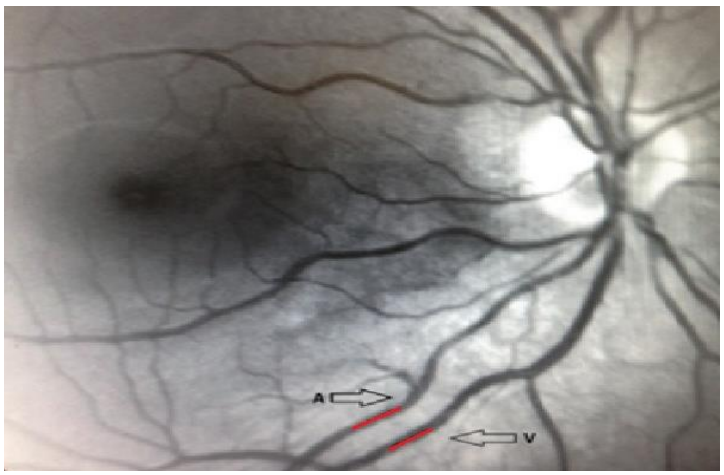

Figure 3.3.4. DVA analysis in real time

3.3.4.2 Measurement principle

The ability of the DVA to measure the diameter of vessels in real time depends on the brightness profile of the retinal vessels which is based on the absorption properties of erythrocytes. During retinal vessel analysis, maximum absorption of light occurs at 400-620nm, and by comparing the brightness profile of the red blood cell column within the vessel and the surrounding tissue, a continuous assessment can be made. Therefore, the DVA actually measures the width of the red blood cell column[549 650]. This is achieved as illumination from the fundus camera enters through a dilated pupil and is reflected by the different layers of the retina and is then delivered via the observation pathway to the CCD camera.

To ensure optimal patient alignment the operator can observe the fundus image on the display before the examination begins. A green filter is inserted into the fundus camera to ensure optimal contrast for visualizing the chosen segment. Furthermore, a series of adaptive algorithms compensate for any disruption to the brightness profile by shadowing structures from the background or reflections from the vessel surface. The software also has the capability to correct for slight eye movements and can continuously monitor image quality according to image contrast, and remove any inadequate measurements from the analysis[549 651].

However, it is important to note that the method for assessing retinal microvascular function does have its limitations which are summarized below along with its advantages in Table 3.3.4.

Table 3.3.4 Summary of DVA advantages and disadvantages

Advantages	**Disadvantages**
non-invasive measurement	Image quality is strongly dependant on an optically clear media
Vessel segments and different retinal vessels can be examined simultaneously	The patient requires good fixation for approximately 350 seconds
High reproducibility	Assumes the eye has no refractive error. Difficulty with high myopia or hypermetropia
Low variability	Dilation with the use of tropicamide 1% only (to avoid adverse vascular effects)
Potential to monitor drug effectiveness	Can not be performed on patients with epilepsy

In order to assess microvascular reactivity of the retinal vessels, the vessels need to be provoked. This can be done through a variety of provocation methods and have been used in other studies. Namely, through the use of suction cup IOP enhancement[652 653], pure oxygen breathing[654-656], inhalation of CO2[657], isometric exercise[658-661], infusion of vasoactive substances[662 663], and flicker light stimulation[664 665].

3.3.4.3 Flicker stimulation

The normal vascular response to flickering light has been widely studied[666 667]. Under normal circumstances the stimulation of flickering light should lead to an increase in vessel diameter, retinal blood flow and optic nerve head blood flow[668]. Flickering light can be considered the most natural provocation method for assessing the behaviour of the retinal vessels and has the advantage of only stimulating the retina and not another vascular bed. Throughout this thesis the DVA was equipped to generate flickering light at a sampling rate of 12.5H_z via an optoelectronic shutter placed in the optical path of the camera illumination system. The shutter generated flicker by interrupting the illumination to provide a bright to dark contrast ratio of at least 25:1. A sampling rate of 12.5 Hz lies within the optimum flicker frequency range and has been shown to provide appropriate retinal stimulation[667 669]. Flickering light increases the neural demand of the retina. An increase in the metabolic rate of the photoreceptors is thought to trigger the release of nitric oxide from the retinal endothelial cells causing an increase in vessel diameter followed by an increase in blood supply to meet the increased demand[670]. An altered vascular response could be indicative of impaired autoregulatory mechanisms or endothelial dysfunction in the form of increased levels of endothelin-1 or reduced bioavailability of nitric oxide. After the removal of the flicker stimulus, the vessel diameter normally resides to baseline values and is most accurate for the venous response; however, the artery has been shown to constrict below baseline immediately following stimulus cessation which is thought to be a regulatory mechanism of the retinal vascular endothelium as a result of an imbalance in nitric oxide and Endothelin-1[413].

3.3.4.4 Measurement Protocol

The measurement protocol in this thesis is in accordance with that introduced by Nagel et al[671]. The fundus camera is positioned to obtain a uniform illuminated fundus image through a dilated pupil. Adjustments of brightness and contrast to ensure a good quality image. The patient is directed to fixate on a needle so that the area of interest lies within the centre of the display. The user then

selects a rectangular area for the assessment. Within this region of the inferior temporal retina, an artery and vein is selected approximately 1 to 2 disc diameters from the optic nerve head for analysis. The measurement begins automatically and vessel diameters are continuously recorded over a period of 350 seconds. The baseline measurement consists of 50 seconds under still illumination (25 Hz), followed by 20 seconds of flicker stimulation (optoelectronically generated at 12.5Hz) and finally a recovery period of 80 seconds. This represents a single flicker cycle and under the examination it is repeated three times (figure 3.3.4.4).

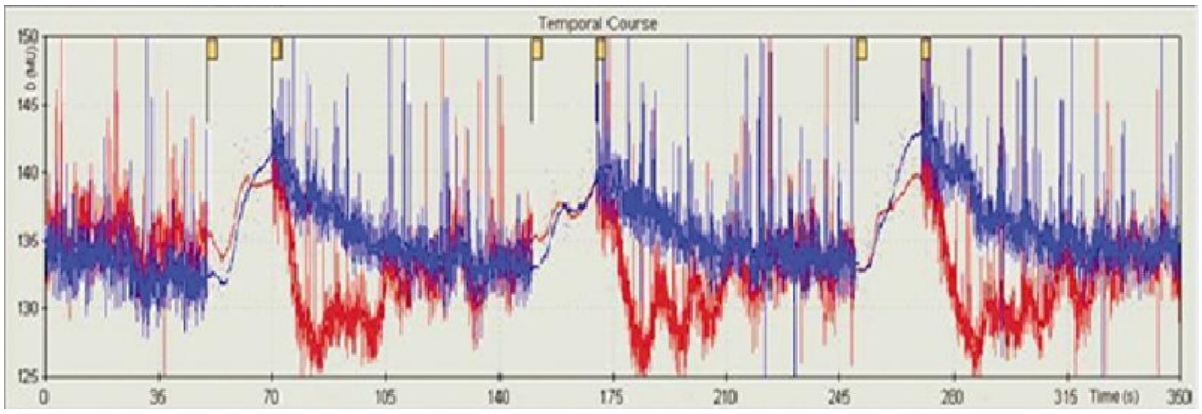

Figure 3.3.4.4. Example of DVA time course, showing 3 cycles of flicker provocation

Traditionally the inbuilt software of the DVA can provide an analysis of the retinal vascular response to flickering light. However, this has been identified to have a number of shortfalls and the need to evaluate the retinal response times in more detail has been increasingly recognised[651 672]. The algorithm used by the software calculates the maximum diameter response by averaging all three flicker cycles and then calculating the flicker response +/-3 seconds after clicker cessation[673 674]. This will actually cause an underestimation of arterial dilation times for those individuals that reach maximum dilation before 17 seconds or after 23 seconds. To overcome these shortfalls of the inbuilt software analysis, Heitmar et al[672] introduced the sequential and diameter response analysis SDRA. The SDRA has the advantage of utilizing the raw data generated by the DVA and allows each flicker cycle to be considered separately, enabling a more accurate assessment of the vessel response with additional parameters on top of the standard dilation and constriction response parameters. These

additional parameters include the baseline diameter fluctuation (BDF), baseline flicker response (BFR), dilation amplitude (DA), and the time taken for the vessel to reach maximum dilation or minimum constriction for the artery and vein for each individual flicker cycle. It is important to note that, analysis of individual flicker cycles relies on a full data set. If this cannot be obtained because of excessive patient movements, blinking or loss of concentration, then the average response times using SDRA are considered more reliable. The SDRA has been shown to be a sensitive measure of the vascular response to flicker light with good co-efficients of variation[672].

The principles of SDRA have been used in this thesis with the inclusion of additional parameters to evaluate the entire dynamic response profile. A statistical polynomial regression algorithm can be applied to the raw response data from the RVA software which can be implemented using the "polyfit" and "polyval" functions in Matlab (Mathworks, Inc., R2013a, USA) to create a visualization plot[675]. The statistical algorithm used for data visualization in this thesis was constructed and implemented in consultation with an experienced statistician at Aston University (Dr Aniko Ekart). These now follow.

Given the measurements y_i at times $t_i, i = 1,...,T$, it is possible to approximate $y = f(t)$ by a polynomial of degree n as:

$$p(t) = p_1 t^n + p_2 t^{n-1} + ... + p_n t + p_{n+1}$$

The polyfit function locates the coefficients $p_1, p_2, ..., p_n, p_{n+1}$ such that the error $\sum_{i=1}^{T} (y_i - p(t_i))^2$ is minimized.

This involves solving the system of equations

$$\begin{cases} p_1 t_1^n + ... + p_n t_1 + p_{n+1} = y_1 \\ \vdots \\ p_1 t_T^n + ... + p_n t_T + p_{n+1} = y_T \end{cases}$$

If $t_i^{n-j+1} = v_{ij}$ then $V = (v_{ij})$ is the Vandermonde matrix and the least squares problem to be solved can be written as $Vp = y$

with the vectors $p = \begin{pmatrix} p_1 \\ . \\ . \\ . \\ p_{n+1} \end{pmatrix}$ and $y = \begin{pmatrix} y_1 \\ . \\ . \\ . \\ y_T \end{pmatrix}$

The polyval function was used to calculate the fitted polynomials which provided curves that represent the dynamic vascular response. The degree of the polynomial, *n*, is an adjustable parameter. In this case, n = 20 was applied for consistency as this provided the closest fit polynomials on the data points. As well as the original parameters of the SDRA method the polynomial fitted curves allowed the nature of the dynamic response profile and the slope of the dilation and constriction responses to be calculated separately for the artery and vein. An example of the visualization plot (Figure 3.3.4.5) and a summary of the main parameters used in this thesis is provided below in table (3.3.4.6).

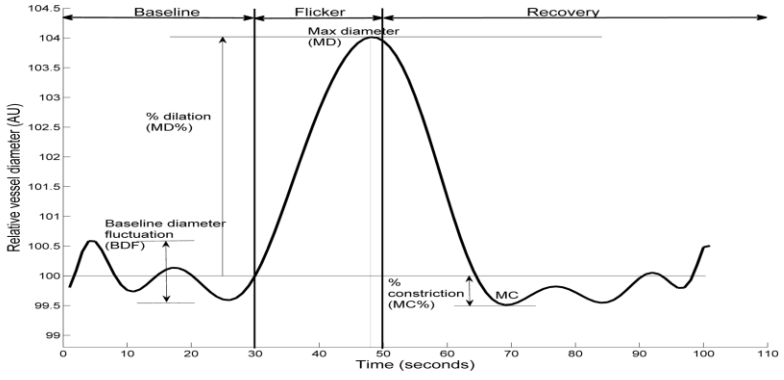

FIG 3.3.4.5. Representation of the SDRA response parameters
Abbreviations: BDF, baseline diameter fluctuation; MD,maximum diameter; MD%, percent dilation relative to average baseline diameter; MC, minimum constriction diameter; MC%, percent constriction relative to average baseline diameter.

Table 3.3.4.6 Summary of DVA parameters used for calculation of artery and venous profiles	
Parameter	**Calculation**
BD- Baseline diameter	Average diameter during baseline
BDF- Baseline diameter fluctuction	Maximal range of diameter measurements during baseline
MD- maximum dilation	Maximum diameter following flicker
% Dilation- expressed from baseline	((MD-BD)/BD)*100
MC- Maximum constriction	Minimum diameter after clicker cessation
% Constriction- expressed from baseline	((MC-BD)/BD)*100
RT- Reaction time	Time in seconds to reach MD
CT- Constriction time	Time in seconds to reach MC
DA- Dilation Amplitude	MD-MC
BCFR- Baseline corrected flicker resposne	(DA-BDF)
Slope D- Dilation slope	(MD-BD)RT
Slope C- Constriction slope	(MC-BD)/CT

3.3.5 Assessment of anterior ocular structure and function

3.3.5.1Keratography

The Keratograph (K5M, Optikgerate GmbH, Wetzlar, Germany), combines keratometry measuring processes with topographic mapping. It is an illumination system which has a special reflector illuminating a placido bowl which contains a series of 22 white concentric rings, and thus images obtained are reflected from this placido bowl from the patients' eye. Besides being an advanced corneal topographer it also contains additional imaging modalities to measure the anterior ocular surface. These include infra-red measurements of the meibomian glands at 840nm, evaluation of the tear break up time non-invasively, measuring the amount of bulbar and limbal hyperaemia, tear meniscus height, and a dynamic evaluation of the lipid layer. It also has a built in video recorder and software to analyse the data, and can be used for future reference when evaluating the ocular surface after treatment or post-surgical interventions.

3.3.5.2 Procedure

The test is conducted in a dim room to eliminate reflections from the surface of the keratograph. The patient is in a seated position with their chin placed on the chin rest and the outer canthus is aligned with the chin-forehead reference bar. The patient must focus on the red light located directly in the centre of the concentric rings. All subsequent measurements are taken from this reference point. The following procedures described below are in the order of the protocol used in this thesis.

3.3.5.3 Tear meniscus height

Images obtained of the tear meniscus height (TMH) from the keratograph are taken from the lower lid. The tear meniscus can be evaluated with different options to magnify the view. The height of the tear meniscus can give an indication of the tear volume i.e. quantity of tear reserve. TMH should be directly measured under the pupil centre at the lower lid. The classification of normal tear reserve is as follows: good >0.2mm; normal = 0.2mm; poor < 0.2mm[676]. The software allows the user to quantify the height of the tear meniscus with an integrated ruler. An example of the assessment is presented below in Figure 3.3.5.3.

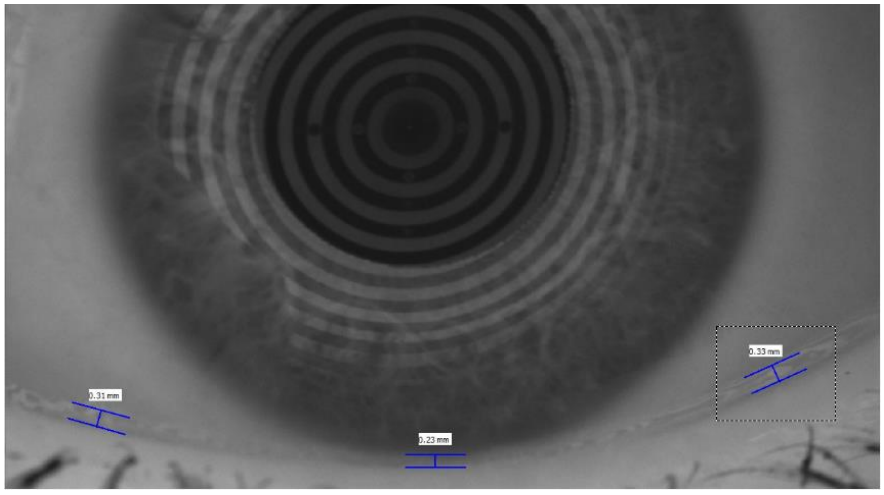

FIG 3.3.5.3. Example of TMH measurement

3.3.5.4 Non-invasive keratograph break up time NIKBUT

The non invasive break up time is evaluated and displayed in a color coded map of the cornea Figure 3.3.5.4. Once the patient is aligned, the clinician requires the patient to blink twice consecutively. This second blink triggers the program to initiate the recording. The recording of break up time ceases when one of two events occur; the patient blinks or there is a significant amount of distortion from the reflected image of the placido rings. The information is then encoded by the software and displayed to the clinician. The time it takes for the tears to break up can give an indication of the quality of the tear film. Normal tear break up time is > 10 seconds. Areas highlighted in red indicate an unstable tear film, and areas in green indicate a stable tear film.

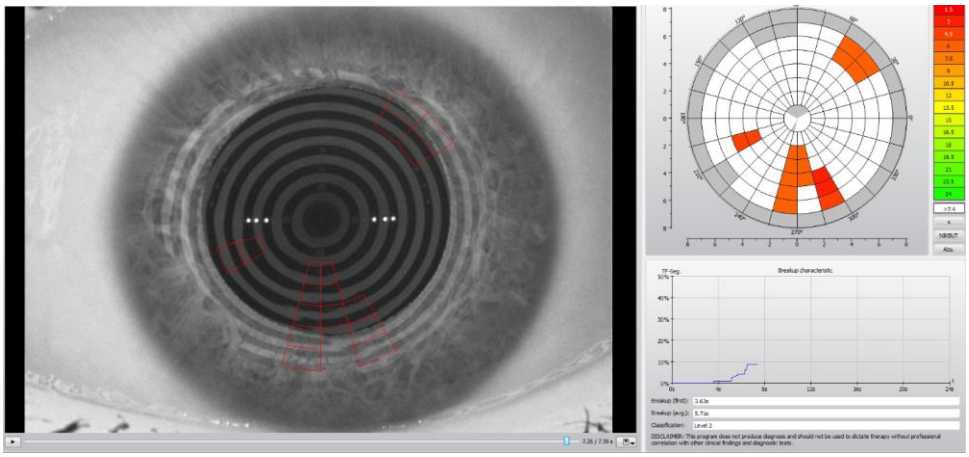

FIG 3.3.5.4. Example of NIKBUT measurement

3.3.5.5 Dynamic evaluation of the lipid layer

The evaluation of the lipid layer is performed with the patient looking directly at the centre of the placido disc Figure 3.3.5.5. Once aligned, the measurement commences and the clinician should advise the patient to blink normally to reveal the spread and formation of the lipid layer across the corneal surface. This measurement is usually recorded to assess the dynamic behaviour of the tear film, as well as giving an indication of the thickness of the lipid layer. If the interference pattern displays colours and structures it is regarded as normal; however, if no colours or structures are

visible it could be an indication of early tear film evaporation. Recordings can last from 5 to 10 seconds or longer if the clinician deems so. There is also the ability to capture images within the video recording for analysing images later on.

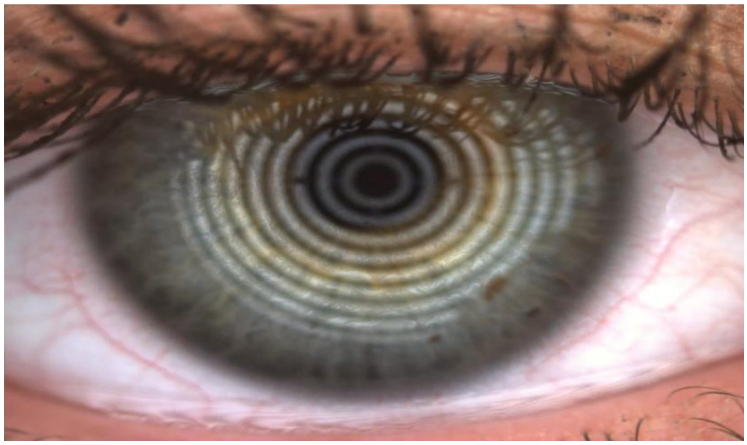

FIG 3.3.5.5 Example measurement of dynamic evaluation of lipid layer

3.3.5.6 Redness

The Keratograph is also able to evaluate the amount of limbal and bulbar hyperaemia. This feature can record and grade redness automatically and objectively with the inbuilt software that compares the amount of redness and analyses it according to the Efron grading scale. The area analysed is based on the area percentage between the vessels and the rest of the analysed area represented in figure 3.3.5.6.

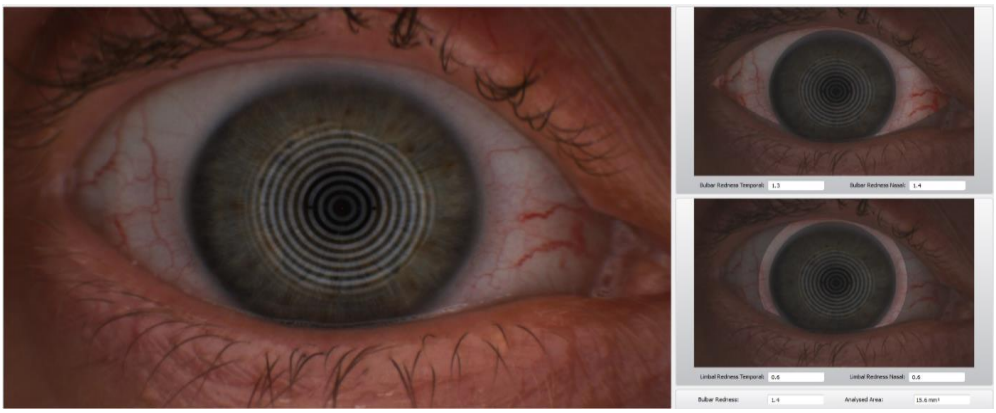

FIG 3.3.5.6. Measurement example of bulbar redness

3.3.5.7 Meibography

Meibomian gland structure can be imaged with infra-red light. The superior lid as well as the lower lid must be everted before capturing the image. The morphological changes in the glandular tissue can be made visible using a 3D meibo scan represented in figure 3.3.5.7. Once the image is captured the software makes a contrast enhanced image alongside a normal image which can be viewed by the clinician and is usually subjectively graded.

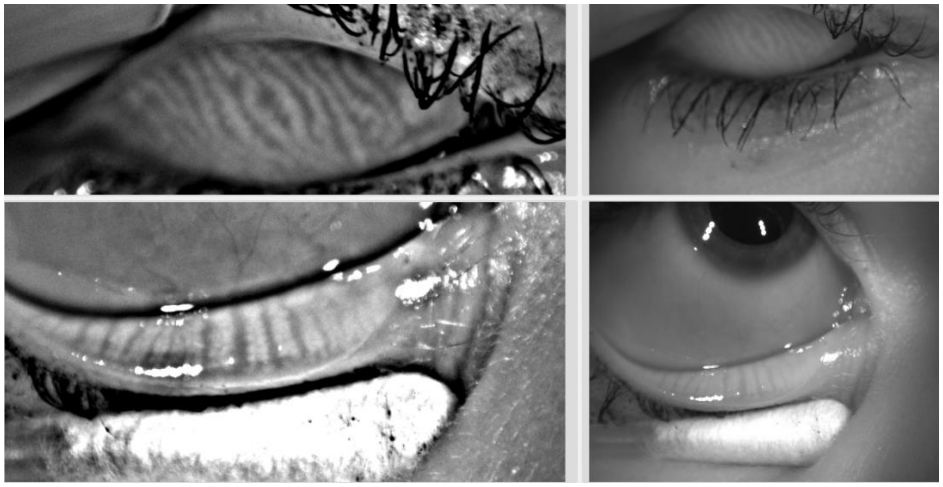

FIG 3.3.5.7. Example of infrared superior and inferior meibography

3.3.5.8 Data analysis

The in inbuilt software is capable of automated analysis. These analysed images are limited to the amount of hyperaemia, graded according to the EFRON grading scale, and the NIKBUT with the use of the placido discs. The algorithm shows good agreement with previous techniques and is highly repeatable and reproducible[629 630].

The quantification of tear meniscus height is made on the actual image with the use of an inbuilt ruler which measures to 1/10th of a mm and can be graded accordingly. The classification of lipid layer thickness can be made subjectively on the interference pattern observed during the video recording. An open/closed meshwork correlates to a thickness of 13-50nm, wave or flow pattern is

between 50-70nm, an amourphous pattern is between 80-90nm, colour fringes represent a thickness between from 90-180nm, and finally a globular network represents a thickness of >200nm.

The meibomian glands can be analysed according to a number of different protocols. Originally a 4 point scale was used to assess the level of meibomian gland drop out or a change in its morphology[677 678]. More recently a new scale was introduced by Pult et al[679] which incorporated a 5 point scale based on the level of Meibomian gland dropout. The infrared image is captured and then analysed with the imajeJ software V1.49 (Wayne Rasband, NIH, USA). The software allows the user to trace the whole area under analysis and then to trace the area where gland drop out occurs (figure 3.3.5.8). The percentage of gland drop is equal to (area of dropout/whole area)*100. In a further study, Pult et al[680] analysed the repeatability of objective grading and subjective grading using the meibomian gland loss ratio and found that intra-observer and inter-observer agreement was better in computerized grading than the subjective 5 point scale and 4 point scale conventionally used to diagnose meibomian gland dropout.

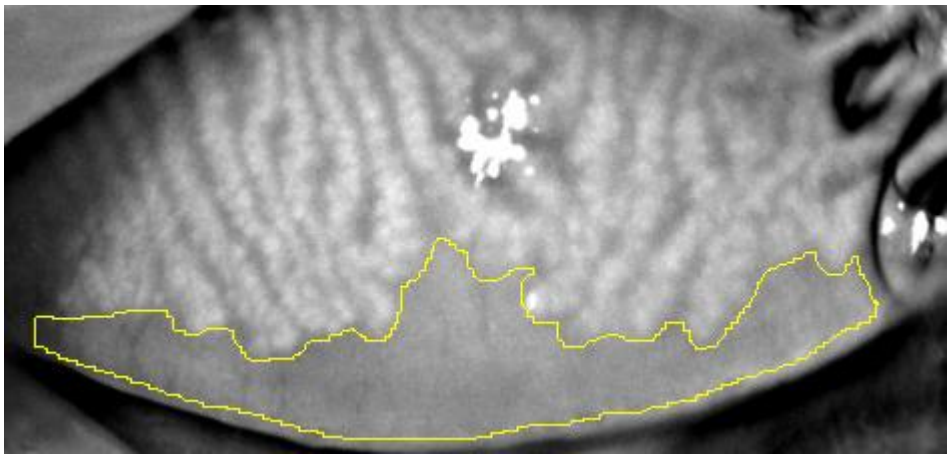

FIG 3.3.5.8. Measurement example with Image J representing Area of loss of the superior tarsus.

3.3.6 Assessment of cardiovascular risk

Determination of cardiovascular risk is often made based on health and lifestyle questionnaires which aim to determine the presence of obesity, smoking status, diabetes, hypercholesterolemia, and hypertension. A more accurate way of determining the risk specified for an individual is by calculating the Framingham risk score (FRS). The FRS provides an estimate of the 10 year absolute risk for developing coronary heart disease[681 682]. It is sex specific, taking into account age, total cholesterol, HDL cholesterol, smoking status, blood pressure, and diabetes and has been shown to be validated across different ethnic groups[681]. It has been shown that deviations in these risk variables normally account for the majority of cases that go on to develop cardiovascular disease[683]. In the present studies the FRS was calculated using the current version of the Framingham risk score[684]. The scoring algorithm is based on gender-specific points assigned for each risk factor variable that can be determined using FRS tables i.e. point scores by age group; age group and total CHOL; age group and smoking status; HDL-c level; SBP and treatment status. Ten-year risk percentage is then calculated by total points (1 point, 6%; 2 points, 8%; 3 points, 10%; 4 points, 12%; 5 points, 16%; 6 points, 20%; 7 points, 25%; 10 points or more, > 30%). Absolute CVD risk percentage over 10 years was classified as low risk (< 10%), intermediate risk (10-20%), and high risk (> 20%)[685]. Furthermore, fasting blood samples were obtained from the antecubital fossa vein of the participants and tested immediately for fasting triglycerides (TGs), glucose levels and total and HDL cholesterol, using a Reflotron Desktop Analyser (Roche Diagnostics, UK).

Recently our understanding of the pathogenesis of cardiovascular disease has progressed on a molecular level, being able to identify mechanisms that affect the vascular endothelium. Currently it is quite common to use quantitative values measuring blood pressure, cholesterol levels, glucose levels and more recently inflammatory markers. An alternative approach could be envisioned so that blood vessel health, loss of arterial elasticity, endothelial dysfunction, and or reduced reactive hyperaemia can provide additional prognostic value to better identify individuals with overt disease or those who are more at risk.

4.0 The relationship between retinal and peripheral vascular function in healthy individuals with low cardiovascular risk

4.1 Abstract

Purpose: To assess the relationship between retinal and systemic microvascular function in individuals with low cardiovascular risk.

Methods: Anthropometric measurements were collected on 136 healthy participants. Microvascular reactivity was measured using the dynamic vessel analyser (DVA, IMEDOS GmbH, Jena, Germany) and peripheral vascular reactivity was measured using the digital thermal monitor (DTM) VENDYS® system (Endothelix, Inc., Houston, TX). Additionally, blood analysis was assessed for the calculation of the Framingham risk score (FRS). Participants were categorised based on the value of their peripheral vascular reactivity indices.

Results: Participants with higher peripheral vascular reactivity indices showed a significant difference in arterial reaction time across groups ($p < 0.001$), dilation amplitude (p=0.006), and slopeAC (p=0.019). Digital thermal monitoring correlated positively with retinal arterial DA (r = 0.576, p = 0.002), BDF (r = 0.434, p =0.039) and negatively with mDRT (r = -0.643, $p < 0.001$) and slopeAC (r = -0.463, p = 0.027).

Conclusions: The findings of this study showed significant positive and negative associations between retinal and peripheral vascular measurements. As retinal microvascular reactivity reflected systemic reactivity then retinal measurements may serve as early warning of future CVD.

4.2 Introduction

In the primary prevention of cardiovascular disease, the use of predictive models such as the *Framingham Risk Score*[681 686 687](FRS), the Prospective Cardio- vascular Münster (PROCAM) score and the European Society of Cardiology Systematic Coronary Risk Evaluation (SCORE)[688-691] are useful preliminary steps for assessing each individuals' risk. However, such methods rely on measurements that are useful in predicting long-term risk assessment in populations and not in individuals with specific genetic and environmental influences[570 692-694]. As a direct result, such risk calculators either over- or underestimate risks in more than 50% of individuals[554 695-697]. It is now increasingly recognised that predictive, preventive and personalised approaches could better contribute to establishing integrative management tailored to the individual patient. Indeed, while there are various biomarkers offering a disease-specific individual biological profile, none of these techniques enable non-invasive assessments in primary care settings while being sufficiently sensitive to enable early detection and prevention.

The assessment of vascular and endothelial dysfunction (VED) is one very important marker for early CVD risk. It is well recognized that VED plays a role in the occurrence of hypertension, coronary heart disease (CHD), dyslipidemia, obesity, and types 1 and 2 diabetes[247 698-700]. Therefore, its quantification is very important when trying to establish an early risk and successfully implement preventive measures. The vascular function is usually assessed by employing techniques such as ultrasound flow-mediated dilation (FMD), pulse wave analysis (PWA), plethysmography and iontophoresis[701]. These tests can, however, be complex and time-consuming, and are performed only in highly specialized services. Among the more user-friendly methods developed to measure microvascular function, dynamic retinal vessel analysis (DVA) represents a non-invasive method that allows for continuous recordings of retinal arterial and venous diameter changes in response to flicker-light stimulation. The main advantage of the DVA assessment is that it provides integrated and dynamic data analysis that is specific to each individual. In addition, its output has proven to be modified not only by overt disease

but also in the presence of more subtle risk factors for CVD[578 702 703] including ageing[554], ethnicity [665] and impaired glucose tolerance [704]. Therefore, it is possible to use the assessment of retinal microvascular function as an early marker for VED.

Another method, called Digital Thermal Monitoring (DTM), has been developed to measures both cutaneous microvascular and vascular reactivity using reactive hyperaemia after increasing the blood pressure (BP) to suprasystolic values. This method was also found to detect increased risk of CVD and VED[568-570 584]. To date DTM has shown a strong relationship with FMD[581] , coronary calcium score, myocardial perfusion defects, and coronary angiographic findings[568-571 585 705-707] and has excellent reproducibility and low variability when used in controlled environments[526].

So there are at least a couple of candidates for non-invasive assessments of CVD risk in primary care settings. Nevertheless, the vascular beds assessed by the two machines have regional and physiological differences[525 708]. Moreover, the differences extend to the provocation stimulus used by each of the instruments to induce vascular dilation and constriction[569 581 671 708]. It would be useful to know, however, that regardless of the provocation stimulus and microvascular bed assessed, the existing methods are equally sensitive to signs of early CVD risk. Therefore, the aim of the present study was to investigate the relationship between retinal microvascular function as assessed by the DVA and peripheral vascular reactivity measured using the DTM in apparently healthy, normotensive individuals with no known history of cardiovascular disease.

4.3 Subjects and methods

Study participants were recruited through advertisements within Aston university (Birmingham, UK) campus, specifically the health clinics and the vascular research laboratory. The inclusion and exclusion criteria for the participants was detailed in section 3.2.5. Briefly, participants were healthy individuals that were currently not taking any medications and were willing to participate. The

investigative procedures performed in this study are outlined below and were conducted in accordance with the protocols set out in section 3.3.1.

1. Anthropometrics
2. IOP measurements
3. Blood pressure
4. Assessment of systemic vascular reactivity (DTM)
5. Assessment of retinal vascular reactivity (DVA)
6. Assessment of fasting blood samples (CVD risk)

4.4 Statistical analysis

All analyses were performed using Statistica® software (StatSoft Inc., Version 13, USA). Distributions of continuous variables were determined by the Shapiro-Wilks test. Group differences in clinical characteristics were assessed by one-way ANOVA. Univariate associations were determined using Pearson's (normally distributed) or Spearman's method (non-normally distributed), and multivariate analyses were performed to test the influence of clinical parameters, and circulating markers on the measured vascular reactivity variables. Differences between groups in retinal and peripheral vascular reactivity measures were computed by ANOVA or analysis of covariance (ANCOVA), where applicable, followed by a post hoc Bonferroni test. Statistical significance was defined at $p < 0.05$.

4.5 Power calculations

The sample size was calculated using the software G power[709] (University of Kiel, version 3.1.6, Germany) Based on previous studies, normal expected retinal arterial responses to flicker-light stimulation have been around 6.9 ± 2.8% which is a clinically significant amount of vessel diameter changes[710]. As the study design was multi-factorial in nature, it was anticipated that an analysis of variance ANOVA or covariance ANCOVA would be required in this study, therefore to provide a

statistical power of 80% and medium effect size of 0.5 with an alpha level of 0.05 it was estimated that a sample size of n= 138 participants was required.

4.6 Results

4.6.1 Clinical characteristics

A total of 150 participants were initially screened for study inclusion of which 14 individuals were excluded based on having a moderate to high FRS (>10%). The remaining 136 participants with low FRS (<10%) were included in the final analysis and classified into one of three groups based on the aTR vascular reactivity index cut-off represented in table 4.6.1.2: group 1 (high risk): aTR >0; group 2 (intermediate risk): aTR >1<2, and group 3 (low risk): aTR >=2. The number of participants in each group was: group 1: 45 (males=22, females=23); group 2: 46 (males=25, females=21); Group 3: 45 (males=22, females=23). There was no significant difference between the number of males and females in each of the study group (chi-square test p=0.715). The aTR values for each group are presented in table 4.6.1.1.

Table 4.6.1.1 Digital Thermal monitoring parameters

	Mean (SD)		
Parameter	**High risk**	**Intermediate risk**	**Low risk**
TR	-0.17 (0.60)	0.03 (0.54)	0.84 (1.26)
aTR	0.73 (0.35)	1.73(0.19)	2.62(0.78)
AUC_{TR}	121 (68.52)	267 (72.64)	420 (144.90)

Abbreviations: TR, temperature rebound; aTR, adjusted temperature rebound; AUC_{TR}, Area under the curve temperature rebound

Table 4.6.1.2 below provides a summary of the anthropometric and clinical data for the study groups. There were no significant differences in any of the measured clinical variables (ANOVA, all $p > 0.05$).

Table 4.6.1.2 Summary of clinical data				
		Mean (SD)		
Variable	**High risk**	**Intermediate risk**	**Low risk**	**P value**
N	45	46	45	
Age (years)	34 (11.51)	29 (9.56)	32 (10.57)	0.098
BMI (kg/m^2)	25(4.36)	24(4.50)	24(4.69)	0.428
SBP (mmHg)	122(11.98)	126(12.77)	122(11.22)	0.233
DBP (mmHg)	72(7.86)	73(9.01)	71(8.40)	0.462
HR (bpm)	64(11.45)	65(11.20)	67(9.60)	0.395
MAP	89(8.43)	91(9.19)	88(8.68)	0.306
IOP (mmHg)	14(2.25)	14(2.76)	13(2.85)	0.234
OPP	45(5.78)	46(7.03)	45(6.05)	0.740
CHOL (mmol/L)	4.38(0.82)	4.20(0.94)	4.76(1.09)	0.479
HDL-C (mmol/L)	1.48(0.56)	1.55(0.43)	1.67(0.59)	0.749
LDL-C (mmol/L)	2.48(0.90)	2.06(1.03)	2.46(1.07	0.673
CVD risk %	3.73(3.65)	2.17(1.82)	2.71(2.01)	0.593

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; IOP, intraocular pressure; OPP, ocular perfusion pressure; CHOL, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CVD risk%, cardiovascular disease risk percentage. * Significant *p*-values are indicated in bold where, ANOVA, $p < 0.05$ was considered significant.

4.6.2 Retinal vascular function measurements

The results of retinal vascular function assessments are displayed in Table 4.6.2 below. After controlling for all influential covariates identified in multivariate regression analysis, there was a clinically significant difference in the arterial mDRT between the 3 study groups. There was also a statistically significant difference in the DA & $slope_{AC}$ between group 3 and groups 1&2 Figure (4.6.2.) There were no other significant differences at the retinal microvascular level in either the measured arteries or veins (all p>0.05).

4.6.3 Correlations between retinal and peripheral vascular function measures

Retinal arterial DA, BDF correlated positively ($r = 0.576$, $p = 0.002$, $r = 0.434$, $p = 0.039$); mDRT and slopeAC negatively ($r = -0.643$, $p < 0.001$, $r = -0.463$, $p = 0.027$) with fingertip aTR which are represented in figure 4.6.3. No significant correlations between aTR and any of the other measured retinal arterial and venous reactivity parameters were identified (all p>0.05)

Table 4.6.2. Summary of retinal vascular function parameters					
			Mean (SD)		
Parameter	**High risk**	**Intermediate risk**	**Low risk**	**P value**	**Significance**
Arteries:					
Baseline	100(0.02)	100(0.03)	99.96(0.25)	0.398	-
BDF	5.43(2.42)	6.35(3.57)	6.24(2.66)	0.269	-
BCFR	3.02(2.52)	3.33(2.78)	2.86(2.63)	0.695	-
MD	105.05(2.75)	105.50(2.47)	104.95(1.96)	0.537	-
mDRT	25.27(7.79)	21.48(5.44)	17.28(4.17)	**<0.001***	**1>2>3**
Dilation %	5.05(2.74)	5.50(2.73)	4.98(1.96)	0.57	-
MC	96.59(1.80)	95.81(3.01)	95.83(2.39)	0.234	-
mCRT	29.07(9.09)	25.67(6.23)	26.42(6.57)	0.871	-
Constriction %	-3.41(1.78)	-4.20(2.98)	-4.13(2.43)	0.247	-
DA	23.80(10.76)	24.97(7.54)	29.54(7.80)	**0.006***	**3>2,1**
$Slope_{AD}$	0.31(0.23)	0.33(0.22)	0.38(0.23)	0.415	-
$Slope_{AC}$	-0.34(0.18)	-0.50(0.29)	-0.59(0.63)	**0.019***	**3>2,1**
Veins:					
Baseline	100(0.03)	99(0.03)	99(0.16)	0.254	-
BDF	4.69(2.82)	4.93(2.43)	5.27(2.74)	0.587	-
BCFR	3.12(2.13)	3.11(2.58)	3.35(3.28)	0.889	-
MD	106(3.51)	105(2.25)	106(3.43)	0.453	-
mDRT	21.42(4.87)	22.23(5.70)	22.19(6.44)	0.751	-
Dilation %	6.11(3.49)	5.81(2.25)	6.65(3.42)	0.427	-
MC	98.31(1.70)	97.75(2.03	98.01(1.55)	0.422	-
mCRT	33.78(6.41)	33.42(6.88)	33.25(7.92)	0.906	-
Constriction %	-1.83(1.93)	-2.26(1.99)	-1.98(1.55)	0.611	-
DA	31.50(7.08)	31.18(8.62)	31.05(8.71)	0.964	-
$Slope_{VD}$	0.31(0.16)	0.29(0.14)	0.37(0.21)	0.101	-
$Slope_{VC}$	-0.28(0.16)	-0.28(0.16)	-0.34(-0.24)	0.301	-

Abbreviations: Baseline diameter; BDF, baseline diameter fluctuation; BCFR, Baseline corrected flicker response; mDRT, reaction time to MD; MD (%), percent dilation; mCRT, reaction time to MC; MC (%), percent constriction; DA, dilation amplitude (difference between MD and MC during flicker) $Slope_{AD}$, slope of arterial/venous dilation; $Slope_{AC}$, slope of arterial/venous constriction. * Significant *p*-values are indicated in bold where $p < 0.05$ was considered significant. ANOVA, analysis of variance; ANCOVA, analysis of covariance. Multivariate regression analysis revealed that SBP, DBP, PR, CHOL were influential factors on some of the identified retinal measurements, which were then analysed using ANCOVA between groups. If there were no influential factors then analysis was carried out with ANOVA.

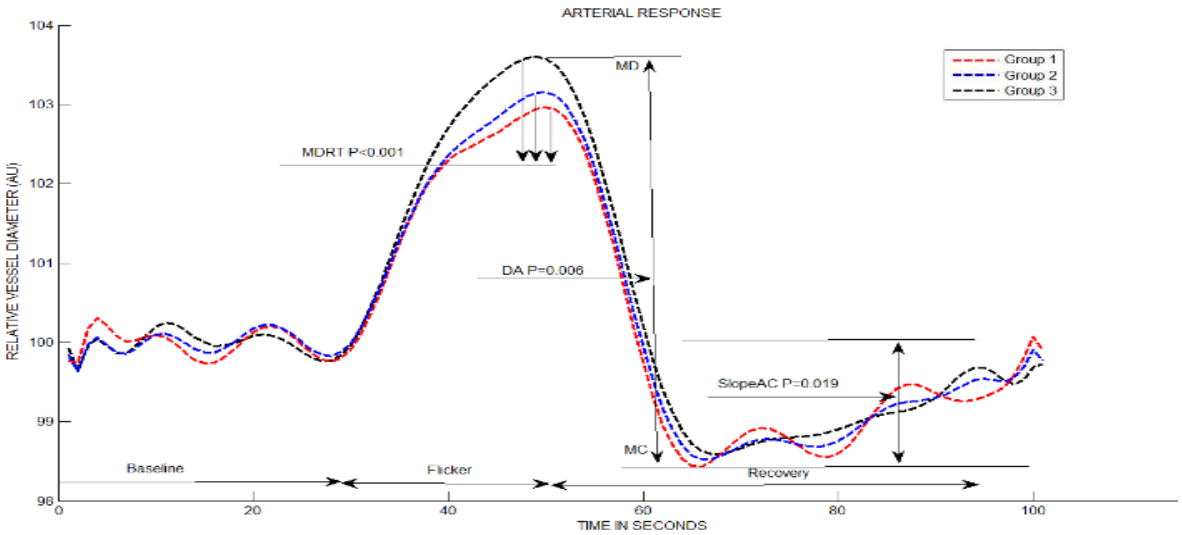

Figure 4.6.2: Comparison of retinal arterial response profile across groups based on the temperature rebound parameters. AU, arbitrary units; MD, maximum diameter during flicker; MC, maximum constriction post flicker; mDRT, reaction time in seconds to MD; DA, MD-MC; $Slope_{AC,}$,calculated as (MC-MD)/(mCRT).

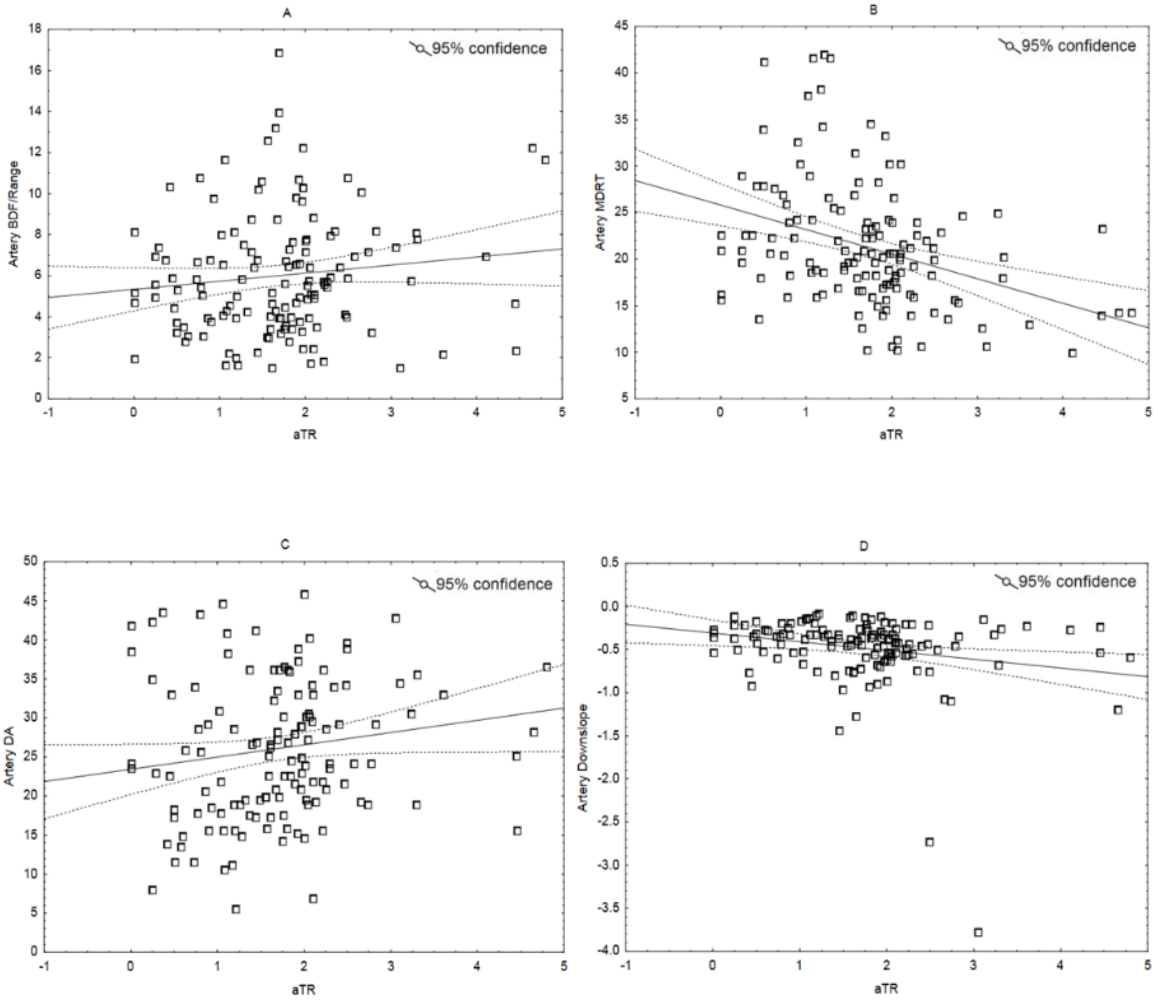

Figure 4.6.3: Correlations between retinal and peripheral vascular function measures, graphs A,B,C,D
BDF, Baseline diameter fluctuation; mDRT, reaction time in seconds; DA, Dilation Amplitude; Slope ac, Arterial constriction slope; aTR, adjusted temperature rebound.

4.7 Discussion

The results presented in this study show that otherwise healthy individuals with impaired peripheral microvascular reactivity (as detected by the DTM technique) also exhibit signs of impaired retinal vascular function demonstrated by an abnormal arterial mRDT, slopeac, and DA responses. This behaviour was not demonstrated in individuals without peripheral microvascular function abnormalities. Moreover, the dysfunction measured at the retinal microvascular level correlated with similar changes at the peripheral circulation, as measured by DTM.

Conventionally, endothelial dysfunction is assessed at the macro-circulatory level, using techniques such as brachial artery flow mediated dilation (FMD)[382] [711] [712] or venous occlusion forearm plethysmography[713]. Nevertheless, such techniques are challenging in more than one aspect, including availability, care setting and level of complexity. As endothelial dysfunction is known to have a profound and much earlier effect on the microvasculature[701] [714], efforts were made to develop methods that test vascular function at the microcirculatory level. Among these instruments, DVA was proven to be an invaluable asset for assessing risk for disease or for proving disease progression[664] [665] [704]. The reactive temperature rebound after sheer stress assessed by the DTM machine, also represents a candidate for microvascular function assessments in risk and disease progression demonstrations. Nevertheless, as the two tests investigate different vascular beds, with different structure and physiology, it would be important to show that there is an agreement between such methods and risk can be picked on regardless of the availability, choice or vascular bed assessed.The mechanism behind the retinal vascular response to flickering light includes nitric oxide (NO) release in response to increased metabolic demands. It also includes, however, neurovascular coupling driven response [708]. For the DTM, the mechanism involves a combined endothelial

NO synthase (eNOS) and cyclooxygenase (COX) inhibition which attenuates the acetylcholine (Ach)-induced, endothelium- dependent, cutaneous vasodilatation[715] but also a non-NO, non-prostanoid-dependent pathway, potentially attributable to endothelium-derived hyperpolarizing factor[519] (EDHF).

There are many conflicting views upon literature review in regards to endothelial function affecting vascular beds quite differently[522]. However, researchers are demonstrating correlations between retinal circulation and systemic circulation[716]. The retina provides a non-invasive assessment of an arterial bed that makes it possible to assess the structure and integrity of the vasculature and its function. The presence of retinal vasculature abnormalities such as arterio-venous nicking, focal and generalised narrowing, altered arterio-venous ratio, and micro-infarcts and haemorrhages can be indicative of a more widespread systemic dysfunction with blood supply[532 717-719]. A study by chapman et al[718] demonstrated the relationship between peripheral vascular disease and retinal arteriolar abnormalities that are also represented in the atherosclerosis risk in communities study (ARIC). The ARIC study further demonstrated that abnormalities in retinal arterioles (i.e. Hollenhorst plaque), are related to the presence of carotid artery plaque aggregation, serum markers of inflammation and endothelial dysfunction[717]. Park et al[720] also demonstrated that a significant correlation exists between endothelial function in the brachial artery as measured by FMD and in the subcutaneous small arteries where abnormally measured dilatory responses during FMD can predict the presence of endothelial dysfunction in resistance arteries.

Nguyen and colleagues in 2010 showed that increased venule calibres as an independent cardiovascular risk factor also had a reduced FMD response but showed no association with arteriorlar calibre[721]. The authors of the aforementioned study did not analyse the arterio-venous ratio in relation to FMD response. However, also in 2010, Katsi observed the relationship between alterations within the retina and arterial stiffness in untreated hypertensive patients[722]. Artery resistance plays a key role in the control of blood pressure due to the drop in hydrostatic pressure that occurs at these arteries[723 724]. To date, a great body of research data indicates that hypertensive patients show a greater tunica media thickness in resistance arteries, a reduced internal and external diameter with increased tunica media to lumen ratio[723]. Katsi et al[722], concluded that there was a progressive stiffening of the aorta that can be paralleled with signs of hypertensive retinopathy suggesting that a similar pathophysiological process of endothelial impairment or lumen wall remodelling occur in both

small and large blood vessels. Significant correlations between coronary flow reserve and subcutaneous small resistance artery remodelling was detectable in hypertension patients, which the study suggested that structural alterations in small resistance vessels may be simultaneously present in different vascular beds and that changes in morphology of the subcutaneous vasculature may even reflect alterations in the coronary vessels[375 723].

In addition to physiological factors responsible for vascular reactivity, measurement site and location of tissue used at which vascular reactivity was obtained is possibly responsible for the varying responses[581]. It was previously reported that measurements of endothelium dependant vasodilation that included FMD and intra-brachial artery infusion with AcH did not correlate with each other[725]. Gori et al,[726] also supported the finding of Eskurra et al[725]. This is likely due to the microvascular and macrovascular properties affecting each technique. FMD assess large conduit artery endothelial function in response to sheer stress and the latter from AcH infusion assess resistance vessel endothelium dependant vasodilation[581]. In the same study, where they measured microvascular reactivity by DTM and reactive hyperaemia index (RHI) with the ENDOPAT the authors found a surprising association with FMD as this is a measured of macrovascular reactivity, however, they note that further tests would be needed to confirm this finding.

It is of note to mention that all the current studies have made analysis of retinal vasculature with the use of static retinal vessel analysis that only provides a snap shot of the circulatory bed. Nevertheless, by being able to demonstrate that otherwise healthy individuals showing impaired peripheral microvascular reactivity also exhibit signs of impaired retinal vascular function, we indicate that endothelial dysfunction, when present, is a general process and is detectable with accuracy regardless of the method used or location of the vascular bed. As molecular and imaging biomarkers drive the shift towards personalized medicine, both retinal and peripheral vessel reactivity can be used for profiling individualized vascular risk by providing an integrated and dynamic analysis of vascular

function as a variable specific for each individual and, therefore, to be used in prediction, prevention and personalised intervention.

4.8 Conclusion

The findings of this study showed associations between retinal and peripheral vascular measurements. The identification of differences in vascular function in accessible peripheral arteries provides a means for the early detection in pre-symptomatic groups. The clinical information gathered with retinal and peripheral vascular function is essential given that they can provide prognostic information to previously un-identified ‘at-risk’ individuals.

5.0 The effects of physical training on the retinal microvascular function in healthy individuals.

5.1 Abstract

Purpose: To assess the effect of regular physical exercise on retinal and systemic microvascular function in overweight individuals.

Methods: Retinal vascular function was assessed in 20 healthy overweight volunteers by way of DVA and systemic vascular function with the DTM. General anthropometric measurements were also collected. Participants who were enrolled did a minimum of 45 mins exercise which included 30mins of weight lifting 4 times per week for 16 weeks. Nutritional intake was on ad libitum basis. They were followed up in 16 weeks for a repeat of their measurements.

Results: In comparison to baseline measurements, subjects had an increased arterial reaction time to dilation (MDRT) for the first flicker cycle (p=0.001) and through 3 cycles (p=0.024) as well as increased reaction time to constriction (MCRT) for the second cycle (p=0.046) and through 3 cycles (p=0.044). Although arterial minimum constriction MC was decreased (p=0.002), overall arterial % constriction from baseline was increased (p=0.002) following flicker. On the venous side, MCRT and DA were decreased at follow up (p=0.010, and p=0.029, respectively).

Conclusions: Retinal microvascular function was positively influenced by regular physical exercise.

5.2 Introduction

More individuals now have an increased propensity of being classified as overweight mainly due to poor dietary habits and sedentary lifestyles. Excess adipose tissue induces several metabolic changes, including dyslipidaemia, elevated blood pressure (BP), oxidative stress, and increased inflammation. In so doing these contribute towards atherosclerosis, increased arterial stiffness, and macro- and micro-vascular endothelial dysfunctions[727-729]. These changes in obesity are associated with higher levels of adiposity and indicate increased risk of CVD compared to controls by 60%[730]. It has been demonstrated that sedentary lifestyles consisting of very low energy expenditure, such as

watching TV or the use of computers, is a risk factor for increased weight gain[290 731 732]. More recently, researchers were able to predict midlife endothelial dysfunction based on factors such as, BMI, total cholesterol and fitness level in children, noting that these predisposing factors in childhood lead to adult obesity and in turn risk factors for CVD[733]. In addition, overweight individuals exhibit signs of microvascular dysfunction compared to their leaner counterparts[727]. A normal functional endothelium is critical to maintaining a normal vascular tone. When endothelial cells lose their ability to release NO, the amount of the constricting factors, such as ET-1, are increased and endothelial dysfunction ensues[138]. This latter step, is actually the initial step in a cascade of events that leads to atherosclerosis. It is known that endothelial dysfunction affects the microcirculation before the macrocirculation[734] in the course of disease development. With an increase in mortality rates due to being overweight or obese, it is proposed that those who maintain a healthy lifestyle with physical activity are at less risk of developing CVD. Physical exercise has been recognized as an important modifiable lifestyle intervention, mainly due to its effects on energy expenditure, reduction of adiposity[579 735 736], and increasing NO bioavailability[737]. Indeed, studies that utilized the FMD technique have reported increases in endothelial function[738-740] with physical exercise. Physical exercise also improved in the elderly. However this was only during intense bouts[741] and not with moderate exercise. More recently, it was shown in obese women that a single session of weight lifting can actually decrease vasodilator function[742]. Hanssen et al[579] measured static retinal photographs to determine arterio-venous ratios (AVR) after an exercise program. The results indicated an increase in AVR that is associated with dilatation of the arterioles, which indicates that individuals who exercise are at less risk of microvascular events.

However, despite all this information pertaining to structural retinal assessments and the risk of future CVD events, there have only been measures made via static retinal photography which only provide a snapshot of an individual's circulatory state. Limitations in these earlier studies can be

overcome by use of the dynamic retinal vessel analyser (DVA) that measures retinal diameter changes in real time in response to flickering light. Therefore the aim of the study was to determine the effects of physical training on systemic vascular function as measured by cuff-occlusive reactive hyperaemia (DTM) retinal microvascular function by way of DVA.

5.3 Subjects and methods.

Study participants were recruited through advertisements within Aston University (Birmingham, UK) campus, specifically the Health Clinics and Sports Centre. The inclusion and exclusion criteria for the participants was detailed in section 3.2.3.1. Briefly, participants that enrolled had to work out at-least 4 times a week for 45 mins. Feeding was on an ad libitum basis. Follow up appointments were conducted 16 weeks after baseline measurements were performed. The investigative procedures performed in this study are outlined below and were conducted in accordance with the protocols set out in section 3.3.1.

1. Anthropometrics
2. IOP measurements
3. Blood pressure
4. Assessment of systemic vascular reactivity (DTM)
5. Assessment of retinal vascular reactivity (DVA)

5.4 Statistical analysis

All analyses were performed using Statistica® software (StatSoft Inc., Version 13, USA). Distributions of continuous variables were determined by the Shapiro-Wilks test. Baseline and follow up differences in clinical characteristics were assessed by a paired t-test. Multivariate analyses were performed to test the influence of clinical parameters such as age, SBP, BMI, HR on the measured vascular reactivity variables. Differences between baseline and follow up in retinal and systemic

vascular reactivity measures were computed by t-test or analysis of covariance (ANCOVA) where applicable, follow by a post hoc Bonferroni test. Statistical significance was defined at $p < 0.05$.

5.5 Power calculations

The sample size was calculated using the software G power[709] (University of Kiel, version 3.1.6, Germany), Based on previous studies, normal expected retinal arterial responses to flicker-light stimulation have been around 6.9 ± 2.8% which is a clinically significant amount of vessel diameter changes[651 667 710]. It was anticipated that a paired t-test or ANCOVA would be required in this study for the baseline and follow up measurements. Therefore to provide a statistical power of 80% and medium effect size of 0.5 with an alpha level of 0.05, it was estimated that a sample size of n=19 paired observations was required.

5.6 Results

5.6.1 Clinical characteristics

A total of 26 participants were initially screened for study inclusion of which 6 individuals were lost to follow up. The remaining 20 healthy participants were included in the final analysis and repeated measurements were performed 16 weeks from baseline. The clinical characteristics of the study groups are presented in table 5.6.1. During the follow up measurements. Participants presented with lower SBP (p=0.006), MAP (p=0.024), and IOP (p<0.001). There were no significant differences in any of the other measured clinical variables. Systemic vascular assessments did not reveal any differences between baseline and follow up all p>0.05.

5.6.2 Retinal vascular function measurements

The results of the retinal vascular function parameters are presented in table 5.6.2 and 5.6.2.1. All values reported are based on each individual flicker cycle and averaged cycles with the artery and vein considered separately using traditional SDRA analysis. The dynamic retinal response profiles were generated using the polynomial computational analysis (Matlab; Mathworks inc, Vr2013a,

MA). After controlling for all influential covariates identified in multiple regression analysis, the follow up parameters showed a clinically significant arterial MDRT for the first flicker cycle and when averaged (p=0.001, p=0.024), MC had also reduced for the first cycle and when averaged (p=0.030, p=0.002), % constriction from baseline had increased for the first flicker cycle and when averaged (p=0.027, p=0.002), Finally MCRT had also reduced for the second flicker cycle and when averaged (p=0.046, p=0.044 respectively) (Figure 5.6.2a). On the venous side, MCRT had reduced for the third flicker cycle (p=0.010), and DA had reduced for the third flicker cycle and when averaged (p=0.013, 0.029) (figure 5.6.2b).

Table 5.6.1 Summary of clinical data

	Mean (SD)			
variable	Baseline	Follow up	P value	Significance
BMI (kg/m2)	27.385 (4.75)	27.355 (4.50)	0.916	-
SBP (mmHg)	133.600 (12.75)	127.550 (10.12)	**0.006***	1>2
DBP (mmHg)	74.900 (7.79)	72.450 (6.22)	0.144	-
HR (bpm)	66.750 (13.04)	65.550 (11.75)	0.555	-
MAP (mmHg)	94.467 (8.20)	90.816 (6.53)	**0.024***	1>2
IOP (mmHg)	14.925 (2.54)	12.25 (2.15)	**<0.001***	1>2
OPP	48.052 (5.85)	48.289 (3.74)	0.826	-

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; IOP, intra ocular pressure; OPP, ocular perfusion pressure. * Significant p-values are indicated in bold were p< 0.05 was considered significant.

Table 5.6.2 Summary of retinal vascular function parameters (arteries)									
Arteries		(SD)				(SD)			
Baseline	Baseline (1)	Follow up (2)	P value	Significance	MC	Baseline (1)	Follow up (2)	P value	Significance
F1	99.98 (0.01)	100.01 (0.01)	0.15	-	F1	96.61 (0.71)	94.71 (0.60)	**0.030***	**1>2**
F2	100.00 (0.01)	100.00 (0.01)	0.24	-	F2	95.78 (0.62)	95.38 (0.54)	0.61	-
F3	100.00 (0.01)	100.02 (0.01)	0.38	-	F3	96.65 (0.92)	95.42 (0.60)	0.17	-
AVG	99.99 (0.008)	100.02 (0.009)	0.07	-	AVG	96.35 (0.46)	95.17 (0.40)	**0.002***	**1>2**
BDF					% constriction				
F1	5.83 (0.74)	6.40 (0.59)	0.55	-	F1	-3.36 (0.71)	-5.30 (0.60)	**0.027***	**1<2**
F2	6.53 (0.79)	6.01 (0.50)	0.53	-	F2	-4.21 (0.62)	-4.63 (0.54)	0.59	-
F3	6.17 (1.04)	4.85 (0.56)	0.13	-	F3	-3.35 (0.93)	-4.60 (0.60)	0.16	-
AVG	6.36 (0.78)	5.75 (0.46)	0.48	-	AVG	-3.64 (0.46)	-4.84 (0.40)	**0.002***	**1<2**
BCFR					RTMC				
F1	4.69 (0.89)	4.27 (0.64)	0.69	-	F1	28.95 (2.91)	24.6 (3.08)	0.32	-
F2	2.92 (0.93)	3.52 (0.67)	0.61	-	F2	29.40 (3.45)	22.65 (2.58)	**0.046***	**1>2**
F3	2.29 (1.27)	5.13 (0.99)	0.09	-	F3	27.30 (3.03)	24.15 (2.96)	0.34	-
AVG	3.30 (0.61)	4.31 (0.51)	0.17	-	AVG	28.55 (2.49)	23.80 (1.97)	**0.044***	**1>2**
MD					DA				
F1	107.15 (0.94)	105.39 (0.59)	0.14	-	F1	25.5 (2.94)	30.55 (3.12)	0.24	-
F2	105.24 (0.56)	104.92 (0.78)	0.67	-	F2	26.55 (3.69)	22.1 (3.56)	0.27	-
F3	105.65 (0.68)	105.40 (1.02)	0.83	-	F3	28.1 (2.32)	27.2 (3.10)	0.68	-
AVG	106.01 (0.51)	105.24 (0.63)	0.30	-	AVG	26.71 (2.47)	26.7 (1.92)	0.98	-
RTMD					Slope AD				
F1	23.45 (2.35)	14.05 (1.37)	**0.001***	**1>2**	F1	0.34 (0.04)	0.48 (0.08)	0.08	-
F2	22.85 (2.83)	20.55 (1.74)	0.45	-	F2	0.25 (0.02)	0.29 (0.07)	0.63	-
F3	19.20 (2.70)	16.45 (2.02)	0.34	-	F3	0.37 (0.06)	0.54 (0.14)	0.14	-
AVG	21.83 (1.94)	17.01 (0.86	**0.024***	**1>2**	AVG	0.32 (0.03)	0.44 (0.07)	0.07	-
% Dilation					Slope AC				
F1	7.17(0.93)	5.37 (0.58)	0.13	-	F1	-0.43 (0.04)	-0.61 (0.11)	0.15	-
F2	5.24 (0.55)	4.89 (0.76)	0.64	-	F2	-0.43 (0.09)	-0.49 (0.05)	0.92	-
F3	5.64(0.68)	5.38 (1.02)	0.81	-	F3	-0.43 (0.06)	-0.50 (0.05)	0.27	-
AVG	6.02 (0.50	5.21 (0.62)	0.29	-	AVG	-0.45 (0.05)	-0.53 (0.04)	0.16	-

Abbreviations: BDF, baseline diameter fluctuation; BCFR, baseline corrected flicker response;MD, maximum diameter; RTMD, reaction time to MD; % Dilation, percent dilation during flicker; MC, minimum constriction; % constriction, percent constriction post flicker; RTMC, reaction to MC; DA, dilation amplitude; SLopeAD, slope of arterial dilation; SLopeAC, slope of arterial constriction. *significant p-values are indicated in bold where p<0.05 was considered significant.

Table 5.6.2.1 Summary of retinal vascular function parameters (veins)									
Veins	(SD)					(SD)			
Baseline	Baseline	Follow up	P value	Significance	MC	Baseline	Follow up	P value	Significance
F1	100.00 (0.01)	99.98 (0.01)	0.181	-	F1	97.84 (0.72)	97.02 (0.83)	0.516	-
F2	99.99 (0.01)	100.03 (0.01)	0.21	-	F2	97.12 (0.76)	97.51 (0.53)	0.666	-
F3	100.00 (0.01)	99.99 (0.01)	0.598	-	F3	98.45 (0.57)	98.63 (0.56)	0.806	-
AVG	100.00 (0.008)	100.00 (0.007)	0.976	-	AVG	97.80 (0.45)	97.72 (0.55)	0.905	-
BDF					% constriction				
F1	4.17 (0.44)	4.02 (0.57)	0.728	-	F1	-2.16 (0.73)	-2.96 (0.83)	0.525	-
F2	5.10 (0.76)	4.32 (0.67)	0.42	-	F2	-2.87 (0.76)	-2.51 (0.54)	0.693	-
F3	4.85 (0.79)	4.09 (0.54)	0.438	-	F3	-1.55 (0.56)	-1.35 (0.56)	0.787	-
AVG	4.70 (0.58)	4.14 (0.49)	0.35	-	AVG	-2.19 (0.46)	-2.28 (0.55)	0.906	-
BCFR					RTMC				
F1	4.50 (1.03)	3.88 (0.81)	0.59	-	F1	35.10 (2.99)	30.65 (2.84)	0.244	-
F2	3.31 (0.74)	3.79 90.63)	0.547	-	F2	29.65 (2.78)	31.35 (2.25)	0.396	-
F3	3.54 (1.06)	2.84 (0.92)	0.658	-	F3	32.40 (2.76)	23.6 (2.89)	**0.010***	**1>2**
AVG	3.78 (0.53)	3.50 (0.59)	0.746	-	AVG	32.38 (2.01)	28.53 (1.82)	0.142	-
MD					DA				
F1	106.51 (0.88)	104.93 (0.56)	0.109	-	F1	33.60 (3.52)	20.05 (2.70)	0.412	-
F2	105.53 (0.49)	105.63 (0.67)	0.859	-	F2	28.9 (2.90)	29.65 (2.97)	0.775	-
F3	106.85 (1.56)	105.57 (0.95)	0.538	-	F3	33.65 (2.61)	22.70 (3.34)	**0.013***	**1>2**
AVG	106.30 (0.77)	105.38 (0.56)	0.32	-	AVG	32.05 (1.86)	27.46 (1.69)	**0.029***	**1>2**
RTMD					Slope VD				
F1	21.50 (2.17)	20.60 (1.64)	0.789	-	F1	0.35 (0.05)	0.25 (0.02)	0.068	-
F2	20.75 (1.77)	21.70 (2.07)	0.735	-	F2	0.41 (0.14)	0.34 90.08)	0.607	-
F3	18.75 (1.03)	20.9 (2.29)	0.402	-	F3	0.33 (0.06)	0.47 (0.15)	0.459	-
AVG	20.33 (1.01)	21.06 (1.25)	0.695	-	AVG	0.37 (0.06)	0.35 (0.06)	0.9	-
% Dilation					Slope VC				
F1	6.50 (0.88)	4.94 90.560	0.112	-	F1	-0.30 (0.04)	-0.33 (0.06)	0.727	-
F2	5.53 (0.49)	5.60 (0.65)	0.907	-	F2	-0.35 (0.04)	-0.34 (0.09)	0.951	-
F3	6.84 (1.54)	5.58 (0.94)	0.54	-	F3	-0.34 (0.08)	-0.86 (0.51)	0.311	-
AVG	6.29 (0.77)	5.37 (0.56)	0.316	-	AVG	-0.33 (0.04)	-0.51 (0.17)	0.34	-

Abbreviations: BDF, baseline diameter fluctuation; BCFR, baseline corrected flicker response;MD, maximum diameter; RTMD, reaction time to MD; % Dilation, percent dilation during flicker; MC, minimum constriction; % constriction, percent constriction post flicker; RTMC, reaction to MC; DA, dilation amplitude; SLopeAD, slope of arterial dilation; SLopeAC, slope of arterial constriction. *significant p-values are indicated in bold where p<0.05 was considered significant.

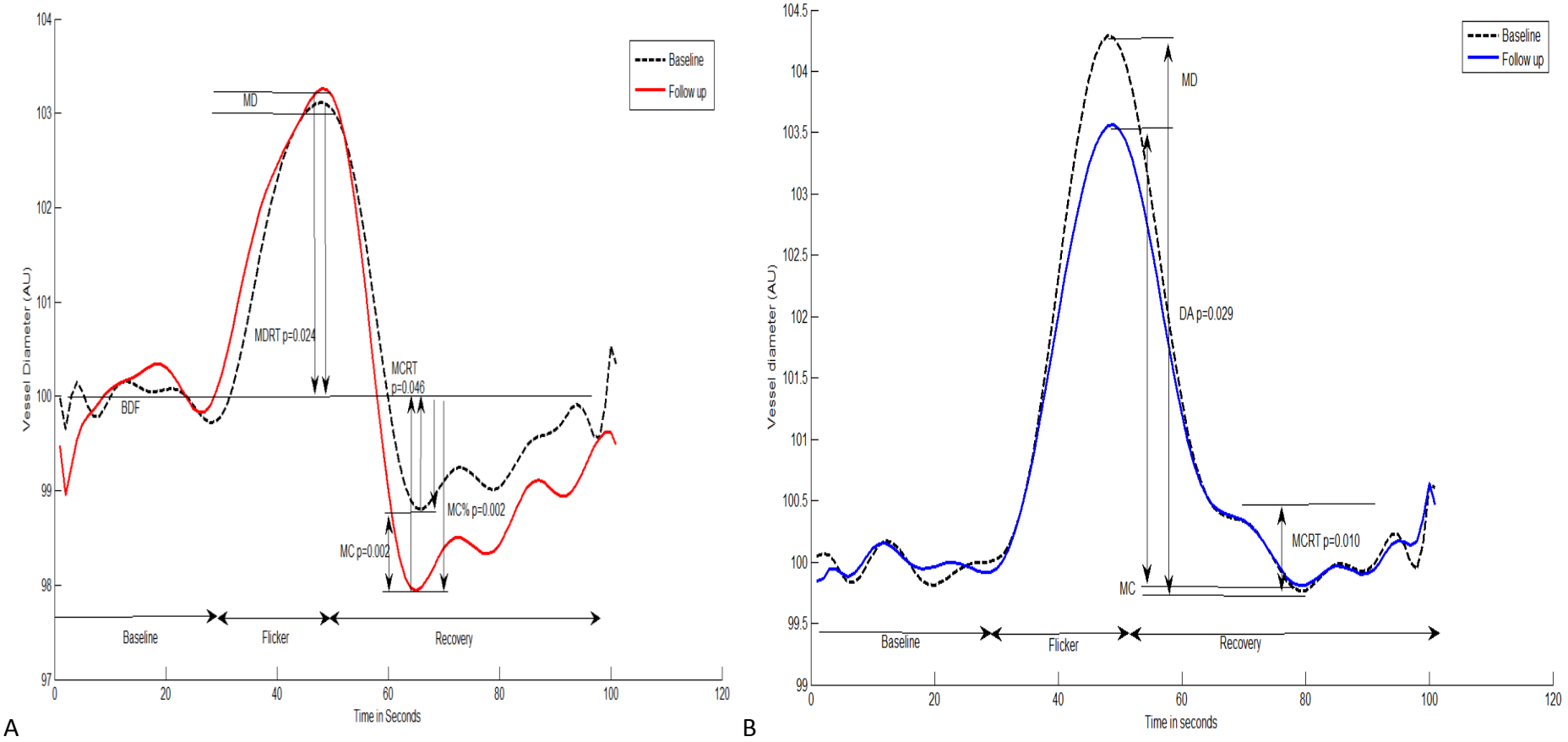

Figure 5.6.2. Retinal arterial and venous response profiles.

(A) Arterial response, (B) Venous response.

Abbreviations: BDF, baseline diameter fluctuation; MD, maximum diameter; MDRT, maximum diameter reaction time; MC, minimum diameter; MCRT, minimum constriction reaction time; MC%, percent constriction; DA, dilation amplitude, difference between MD&MC.

5.7 Discussion

The present study demonstrated, for the first time, that participants engaged in physical exercise had a lower arterial RTMD for the first flicker provocation and overall average throughout the three flicker cycles compared to baseline. It was noted that the MC was lower on the first flicker cycle and on average while MC% was markedly increased for the first flicker cycle and on average. RTMC was also reduced which results in a shorter period of time required for the vasculature to return to baseline values. Our results show that physical exercise has positively modified retinal microcirculation's function and moreover, for the first time, we were able to show that the retinal microvascular reactivity can be augmented through exercise.

Physical inactivity has been associated with reduced endothelial function which is a notable early marker of atherosclerosis propensity and in turn, CVD[743 744]. Thus, the need for understanding how exercise and microvasculature function was researched. Adipose tissue is a metabolically active endocrine organ and also a major source of pro-inflammatory molecules and cytokines. Current findings suggest that excess adipose tissue, especially in the visceral region, propagates an inflammatory response characterized by leukocyte infiltration[745] and increased secretion of adipose tissue- derived pro-inflammatory adipokines (i.e. leptin and resistin)[746] and well-established inflammatory mediators (i.e. tumor necrosis factor-alpha and interleukin-6)[747] In addition, production of anti-inflammatory adipokines and cytokines (i.e. adiponectin and interleukin-10) is reduced in the presence of excess adipose tissue which exacerbates the inflammatory response[748]. The pro-inflammatory environment associated with excess adipose tissue can directly impact vascular endothelial function[745 749]. During obesity, inflammatory cytokines such as TNF-α and IL-6, act on leukocytes and vascular endothelial cells producing Reactive oxygen species. Excess production of ROS can, in turn, induce oxidative stress and endothelial dysfunction[750].

Leptin is an adipokine that is increased during obesity[751] and contributes to ROS mediated endothelial dysfunction in human endothelial cells during weight gain[752]. Previous studies have demonstrated that circulating leptin is reduced in adults performing resistance training and

associated with improved conduit artery endothelial function during weight loss[753 754]. Impaired endothelial function represents a pathophysiologic mechanism linking sedentary lifestyle to CVD risk. Vascular endothelial dysfunction due to increased BMI represents an early marker of atherosclerosis and[755] and can affect the microcirculation much earlier than the macrocirculation[734] which can precede the clinical manifestations of CVD. In patients with a higher than average BMI, normal or mildly diseased coronary arteries was independently associated with reduced coronary dilation to intra-arterial infusions of ACh compared to normal patients with normal BMIs[756]. In addition, from early in life, increases in BMI have been associated with low levels of NO[757] and elevated adult BMI has also been associated with increased carotid intima media thickness[727] and lower retinal microvascular dilation[727] as well as narrower retinal arteries and wider venular calibres[534]. Physical activity has been shown to provide a protective effect against health risks associated with increased BMI, conferring health benefits independent of changes in body weight or composition[758 759] which suggests that overweight and obese individuals can obtain the same benefits of physical activity as normal weight individuals.

Regular exercise training has been recommended as a non-pharmacologic therapeutic strategy for the prevention and treatment of increased adipose leading to hypertension and CVD[760]. It is well recognized that aerobic training has BP lowering effects consistent with our findings. It was shown that 3 months of moderate intensity exercise training reduced systolic BP and diastolic BP in normotensive and previously sedentary older adults[761]. An important finding of the present study was the detection of an improvement in retinal vessel function in response to flickering stimulus (a decreased RTMD and decreased RTMC and an increased % constriction) in the follow up group. It has previosuly been reported that decreases in BMI have been associated with increases in retinal vessel calibre[324 579] and that exercise training has been associated with increases in microvascular reactivity as measured with laser doppler flowmetry[762 763]. Dietary factors (such as quantity and quality of food) have not been assessed as well as circulating markers of endothelial function in the

present study and this could therefore be perceived as a limitation. Other possible limitations of the study was that participants were relied upon actually attending and performing the exercises them selves without any proper supervision, nor was there a control group. It can be invisaged that not all participants were able to attend the gym four times a week and also possible some could have suffered injuries and made up the time at a later date.

Nevertheless, this is the first report showing functional retinal vascular changes in participants undergoing physical exercise, opening a new opportunity for early detection and prevention of future cardiovascular complications. This study did not show any signs of macrovascular changes. This is possibly due to the macrovessels being of larger diameters and less susceptible to slight changes that can effect their reactivity.

5.8 Conclusions

The results indicate that exercise training is an important non-pharmocologic intervention that can improve microvascuar endothelial function in asymptomatic overweight individuals. It is important for maintaining physical fitness and is widely accepted that regular exercise provides enormous health benefits that can promote and sustain physiological welfare.

6.0 The long-lasting effects of fasting during the month of Ramadan on retinal and peripheral vascular function.

6.1 Abstract

Purpose: To assess the effect of fasting during the month of Ramadan on retinal and peripheral microvascular function.

Methods: Retinal vascular function was assessed in 18 healthy, normal weight (BMI>18kg/m^2<25kg/m^2) volunteers by way of DVA and systemic vascular function by DTM. General anthropometric measurements were also collected. Parameters were recorded during the 3rd week of fasting. Non-fasting values were established by measures performed 7-8 weeks after Ramadan.

Results: Compared to the non-fasting status, during fasting subjects had an increase in arterial MD for the first flicker cycle (p= 0.043) and on average when flicker cycles were combined (p= 0.022). Additionally, there was also an increase in % dilation for the first flicker cycle (p=0.046) and when averaged through 3 cycles (p=0.022). On the venous side, Baseline diameter was increased on the second flicker cycle (p=0.048) but decreased when taking into account the baseline corrected flicker response (BCFR) for the first, third and averaged flicker cycles (p=0.041, p=0.012, p=0.007 respectively).

Conclusions: These findings suggest that fasting alone is an effective way of increasing the elasticity of retinal arterial diameters to provocation and ameliorating the venous baseline response possibly through an increase in endothelial function.

6.2 Introduction

Fasting during the month of Ramadan is one of the five fundamental pillars of Islam during which capable adults must fast from sunrise to sunset[764 765]. This type of fasting is defined as periodic food and water deprivation during daylight hours or the duration of the month[765]. Fasting itself causes a reduced caloric intake in which people usually resort to two meals a day rather than the typical 3

meals along with snacks. This reduced caloric intake has been measured to approximately 1200 calories a day and those who partake can lose up to 2-4kg in body weight[766]. Studies have also demonstrated that individuals can experience decreases in body mass index (BMI) and waist circumference (WC) in both males and females[766 767]. More recently, studies have shown to be in agreement with these previous findings and have confirmed that BMI and WC do decrease[768 769]. In contrast there have also been studies which have shown no significant differences[770 771].

It has been indicated that participants who are under 36 years of age will benefit greatly during the period of abstinence with reductions in BMI and WC compared to older participants. A reduction in blood pressure also occurs[310 768]. Sayeeda et al[768] also demonstrated that high density lipoprotein (HDL-c) increased while low density lipoprotein (LDL-c) decreased despite unchanged total cholesterol (CHOL). A similar increase in HDL-cholesterol occurs in participants on reduced calories diet and physical exercise for weight loss[772]. All the above findings would suggest that fasting during Ramadan should have a beneficial effect on cardiovascular risks, where these exist. Therefore, measuring endothelial function could provide important information on the benefits of fasting and elucidate whether fasting can be used as an intervention for the improvement in vascular function.

The assessment of vascular and endothelial dysfunction (VED) is one very important marker for early CVD risk. Yousefi et al[773] examined endothelial function during Ramadan fasting but mainly using biochemical markers rather than clinical functional measurements. While there are various blood biomarkers offering a disease-specific individual biological profile, none of these techniques enable non-invasive assessments in primary care settings while being sufficiently sensitive to enable early detection of endothelial dysfunction. The main advantage of the DVA assessment is that it provides integrated and dynamic data analysis that is specific to each individual. In addition, its output alters not only by overt disease but also in the presence of more subtle risk factors for CVD[578 702 703] including ageing[554], ethnicity[665] and impaired glucose tolerance[704]. Therefore, it is possible to use the assessment of retinal microvascular function as an early marker for VED. There is one other study to

date that recorded retinal microvascular function by way of DVA in a single case report. The authors stated that retinal vessel reactivity was blunted during a single day 20hr fast compared to eating a meal right after[774].

In addition peripheral microvascular reactivity was measured by DTM. This method was found to detect increased risk of atherosclerotic cardiovascular disease[568 569 571], as well as improvements in reactive hyperaemia during acute exercise[584], & surgery[583]. This technique has the advantage of being operator independent without the use of expensive ultrasound equipment allowing for use in community clinical settings. Thus, the aim of our study was to determine the long-lasting effects of fasting during the month of Ramadan on the retinal and peripheral vascular endothelial function. As we are studying the long term effect of fasting, the study was designed to analyse whether the observed "beneficial" changes in micro-macrovascular function can be sustained beyond the end of Ramadan.

6.3 Subjects and methods.

Study participants were recruited through advertisements within Aston University (Birmingham, UK). The inclusion and exclusion criteria for the participants was detailed in section 3.2.4. Briefly, Initial fasting measurements were performed mid-day during the 3rd and 4th week of Ramadan 2016, while the participants were within their fasting hours. The latter weeks in Ramadan were chosen because the body has had time to adapt to reduced calorie intake and energy demand. Non-fasting baseline measurements were conducted 7-8 weeks after the last day of fasting, at the same time of the day when the initial measurements were performed in each of the participants. Feeding was on an ad libitum basis when participants break their fast at sundown. The investigative procedures performed in this study are outlined below and were conducted in accordance with the protocols set out in section 3.3.1.

1. Anthropometrics
2. Body composition

3. IOP measurements
4. Blood pressure
5. Assessment of systemic vascular reactivity (DTM)
6. Assessment of retinal vascular reactivity (DVA)

6.4 Statistical analysis

All analyses were performed using Statistica® software (StatSoft Inc., Version 13, USA). Distributions of continuous variables were determined by the Shapiro-Wilks test. Group differences in clinical characteristics were assessed by a paired t-test. Multivariate analyses were performed to test the influence of clinical parameters such as age, SBP, BMI, HR on the measured vascular reactivity variables. Differences between fasting and non-fasting measurements in retinal vascular reactivity measures and systemic microvascular function were computed by t-test or analysis of covariance (ANCOVA) where applicable, followed by a post hoc Bonferroni test. Statistical significance was defined at $p < 0.05$.

6.5 Power calculations

The sample size was calculated using the software G power[709] (University of Kiel, version 3.1.6, Germany). Based on previous studies, normal expected retinal arterial responses to flicker-light stimulation have been around 6.9 ± 2.8% which is a clinically significant amount of vessel diameter changes[667 710]. It was anticipated that a paired t-test or ANCOVA would be required in this study, therefore to provide a statistical power of 80% and medium effect size 0f 0.5 with an alpha level of 0.05 it was estimated that a sample size of n=19 paired observations were required.

6.6 Results

6.6.1 Clinical characteristics

A total of 23 participants were initially screened for study inclusion and had completed all the baseline measurements of which 5 participants were lost to follow up. The remaining 18 healthy participants were included in the final analysis and repeated measurements were performed 7-8 weeks from fasting. The clinical characteristics of the study groups are presented in table 6.6.1. There were no significant differences in any of the measured clinical variables between the 2 periods (all $p > 0.05$).

Table 6.6.1. Summary of clinical data

	Mean (SD)		
Variable	Baseline	Follow up	P value
N	18 (5F)	-	-
BMI (kg/m2)	24.8 (5.61)	25.3 (5.58)	0.774
BF %	25.5(9.19)	25.09(8.56)	0.891
MM Kg	51.31 (13.08)	52.7(13.25)	0.93
BW %	53.94(6.72)	54.24(6.43)	0.892
SBP (mmHg)	124.83(11.22)	125.77(13.05)	0.817
DBP (mmHg)	74.44(6.95)	70.11 (9.02)	0.115
HR (bpm)	65.44 (10.15)	71.6(11.70)	0.096
MAP (mmHg)	75.00 (6.93)	70.71(8.99)	0.118
IOP (mmHg)	12.74 (2.00)	14.07(3.06)	0.131
OPP	48.08 (5.82)	45.03 (7.40)	0.178

Abbreviations: : BMI, body mass index; BF%, body fat percentage; MM%, muscle mass percentage; BW%, body water percentage; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; IOP, intraocular pressure; OPP, ocular perfusion pressure. * Significant *p*-values are indicated in bold where $p < 0.05$ was considered
significant.

6.6.2 Systemic vascular function

Table 6.6.2 provides a summary of the parameters using DTM that were assessed during fasting and non-fasting periods and follow up. There were no significant differences in any of the measured clinical variables (all $p > 0.05$).

Table 6.6.2 Summary of DTM measurements

	Mean (SD)		
Parameter	Baseline	Follow up	P value
TR	0.68(1.51)	0.08(0.64)	0.105
aTR	2.30(1.18)	2.15(0.69)	0.63
AUCtr	408(256.25)	329(151.38)	0.23

Abbreviations: TR, temperature rebound; aTR, adjusted temperature rebound; AUCtr, area under the curve temperature rebound. * Significant *p*-values are indicated in bold where $p < 0.05$ was considered significant.

6.6.3 Retinal vascular function measurements

The results of the retinal vascular function parameters are presented in table 6.6.3 and 6.6.3.1. All values reported are based on each individual flicker cycle and averaged cycles with the artery and vein considered separately using traditional SDRA analysis. The dynamic retinal response profiles were generated using the polynomial computational analysis (Matlab; Mathworks inc, Vr2013a, MA). After controlling for influential covariates identified by multiple regression analysis, there were no significant differences between baseline and follow up measurements on each flicker cycle or their averages for the following parameters; baseline, BDF, MDRT, MC, % constriction, DA, BCFR, MCRT, Slope AD, and Slope AC (all ANCOVA P >0.05). Nevertheless, during baseline measurements there was a significant difference in MD for the first flicker cycle (p= 0.043) and on average when flicker cycles were combined (p= 0.022). Additionally, there was also a significant increase in % dilation for the first flicker cycle (p=0.046) and on average when the flicker cycles were combined (p=0.022) (figure 6.6.3). On the venous side, there was a significant difference in the baseline parameter for the second flicker cycle (p=0.048), BCFR for the first flicker cycle (p=0.041), third flicker cycle (p=0.012), and combined averages of flicker cycles (p=0.007) (figure 6.6.3). There were no significant differences between baseline and follow up measurements on each flicker cycle or there averages for the following parameters; BDF, MD, MDRT,% Dilation, MC, MCRT, % constriction, SlopeVD, and slopeVC (all ANCOVA P >0.05,).

6.7 Discussion

The present study demonstrated, for the first time that during the period of fasting individuals had a higher arterial MD for the first flicker provocation and overall average throughout the three flicker cycles comparing to their non-fasting status. Similar results were achieved in the amount of dilation %, which shows that retinal arteries on average were able to dilate from baseline to MD significantly

better during fasting than during non-fasting period. For the retinal veins it was noted that the BCFR was notably stable during the fasting period as compared to the follow up which showed great

Table 6.6.3 Summary of retinal vascular function parameters (arteries)							
Arteries	Mean (SD)				Mean (SD)		
Baseline	Baseline	Follow up	P Value	MC	Baseline	Follow up	P value
F1	100.03 (0.09)	100.00 (0.09)	0.326	F1	94.46 (3.25)	92.87 (4.30)	0.172
F2	100.02 (0.54)	100.03 (0.58)	0.799	F2	94.88 (3.99)	95.92 (3.85)	0.899
F3	100.01 (0.08)	100.00 (0.74)	0.783	F3	95.66 (3.39)	94.05 (5.37)	0.288
AVG	100.02 (0.03)	100.01 (0.05)	0.529	AVG	95.01 (2.87)	94.28 (3.21)	0.463
BDF				MCRT			
F1	8.59 (4.71)	7.82 (3.81)	0.417	F1	29.66 (11.12)	29.44 (11.95)	0.961
F2	7.75 (3.96)	6.66 (3.03)	0.193	F2	23.61 (10.84)	25.88 (11.30)	0.547
F3	8.02 (4.06)	7.22 (4.46)	0.603	F3	24.83 (10.48)	23.16 (12.05)	0.614
AVG	8.14 (2.97)	7.23 (3.31)	0.271	AVG	26.03 (6.70)	26.16 (6.47)	0.953
BCFR				% constriction			
F1	4.51 (3.32)	4.67 (3.07)	0.894	F1	-5.56 (3.24)	-7.12 (4.28)	0.174
F2	4.40 (4.80)	3.66 (4.44)	0.645	F2	-5.13 (4.00)	-4.10 (3.84)	0.902
F3	4.00 (4.03)	4.67 (4.56)	0.641	F3	-4.35 (3.37)	-5.95 (5.37)	0.292
AVG	4.30 (2.00)	4.33 (2.56)	0.97	AVG	-5.01 (2.89)	-5.73 (3.21)	0.467
MD				DA			
F1	107.58 (4.22)	105.37 (3.62)	**0.043***	F1	30.11 (9.59)	29.44 (16.57)	0.896
F2	107.04 (2.18)	106.25 (3.45)	0.252	F2	27.44 (11.53)	24.33 (11.50)	0.316
F3	107.74 (3.19)	105.95 (3.04)	0.156	F3	25.66 (10.89)	23.83 (12.93)	0.656
AVG	107.45 (2.17)	105.85 (2.54)	**0.022***	AVG	27.72 (6.09)	25.87 (8.66)	0.391
MDRT				SlopeAD			
F1	19.55 (7.14)	20.00 (9.18)	0.867	F1	0.422 (0.27)	0.302 (0.32)	0.231
F2	16.16 (6.75)	21.55 (13.77)	0.081	F2	0.539 (0.35)	0.488 (0.54)	0.743
F3	19.22 (9.47)	19.33 (6.68)	0.962	F3	0.527 (0.47)	0.452 (0.60)	0.701
AVG	18.31 (5.79)	20.29 (5.99)	0.128	AVG	0.496 (0.23)	0.414 (0.30)	0.344
% Dilation				SLopeAC			
F1	7.54 (4.15)	5.36 (3.58)	**0.046***	F1	-0.509 (0.32)	-0.51 (0.29)	0.965
F2	7.02 (2.17)	6.22 (3.43)	0.145	F2	-0.686 (0.50)	-0.471 (0.23)	0.121
F3	7.72 (3.15)	5.94 (3.00)	0.152	F3	-0.609 (0.41)	-0.608 (0.32)	0.996
AVG	7.43 (2.16)	5.84 (2.52)	**0.022***	AVG	-0.601 (0.32)	-0.531 (0.19)	0.441

Abbreviations: BDF, baseline diameter fluctuation; BCFR, Baseline corrected flicker response; MDRT, reaction time to MD; % dilation, percent dilation; MCRT, reaction time to MC; % constriction, percent constriction; DA, dilation amplitude; SlopeAD, slope of arterial dilation; SlopeAC, slope of arterial constriction. * Significant *p*-values are indicated in bold where $p < 0.05$ was considered significant

Table 6.6.3.1 Summary of retinal vascular function (veins)							
Veins	Mean (SD)				Mean (SD)		
Baseline	Baseline (1)	Follow up (2)	P Value	MC	baseline	follow up	P value
F1	99.98 (0.06)	99.98 (0.07)	0.937	F1	97.54 (3.87)	96.12 (3.92)	0.359
F2	100.04 (0.09)	99.99 (0.05)	**0.048***	F2	97.80 (1.70)	97.78 (7.02)	0.988
F3	100.02 (0.07)	100.01 (0.07)	0.616	F3	96.93 (2.96)	96.04 (4.97)	0.54
AVG	100.01 (0.05)	99.99 (0.04)	0.258	AVG	97.42 (2.11)	96.65 (2.10)	0.243
BDF				MCRT			
F1	6.13 (2.77)	5.34 (2.66)	0.377	F1	32.55 (13.59)	32.11 (8.53)	0.919
F2	6.91 (3.09)	6.07 (2.85)	0.416	F2	35.44 (13.15)	31.33 (9.13)	0.298
F3	6.92 (3.60)	5.64 (3.53)	0.321	F3	32.5 (12.00)	33.61 (11.96)	0.804
AVG	6.65 (2.28)	5.68 (2.26)	0.069	AVG	33.5 (8.26)	32.35 (5.21)	0.682
BCFR				% constriction			
F1	2.50 (4.70)	6.17 (5.31)	**0.041***	F1	-2.44 (3.87)	-3.85 (3.90)	0.357
F2	2.33 (3.62)	3.85 (3.99)	0.362	F2	-2.23 (1.68)	-2.20 (7.02)	0.988
F3	2.27 (3.32)	5.63 (4.31)	**0.012***	F3	-3.08 (2.94)	-3.97 (4.93)	0.542
AVG	2.37 (2.05)	5.22 (2.96)	**0.007***	AVG	-2.58 (2.09)	-3.34 (2.11)	0.255
MD				DA			
F1	106.17 (4.08)	107.65 (5.06)	0.374	F1	30.27 (12.57)	28.38 (11.95)	0.685
F2	107.06 (4.08)	107.70 (7.83)	0.787	F2	33.00 (14.52)	25.50 (17.76)	0.145
F3	106.12 (3.31)	107.31 (3.86)	0.359	F3	34.77 (12.14)	32.11 (12.93)	0.601
AVG	106.45 (2.71)	107.55 (3.68)	0.381	AVG	32.68 (7.76)	28.66 (8.21)	0.153
MDRT				Slope VD			
F1	22.27 (8.54)	23.72 (9.34)	0.632	F1	0.33 (0.26)	0.37 (0.32)	0.66
F2	22.44 (10.60)	25.83 (14.30)	0.205	F2	0.36 (0.23)	0.49 (0.67)	0.399
F3	17.72 (8.28)	21.5 (8.94)	0.287	F3	0.53 (0.59)	0.42 (0.31)	0.559
AVG	20.81 (6.63)	23.68 (7.23)	0.143	AVG	0.40 (0.28)	043 (0.26)	0.811
% Dilation				Slope VC			
F1	6.18 (4.05)	7.66 (5.04)	0.369	F1	-0.44 (0.69)	-0.39 (0.25)	0.768
F2	7.01 (4.05)	7.71(7.83)	0.771	F2	-0.33 (0.22)	-0.33 (0.20)	0.917
F3	6.10 (3.25)	7.30 (3.84)	0.351	F3	-0.33 (0.23)	-0.46 (0.50)	0.281
AVG	6.43 (2.67)	7.56 (3.70)	0.369	AVG	-0.37 (0.24)	-0.39 (0.24)	0.779

Abbreviations: BDF, baseline diameter fluctuation; BCFR, Baseline corrected flicker response; MDRT, reaction time to MD; % dilation, percent dilation; MCRT, reaction time to MC; % constriction, percent constriction; DA, dilation amplitude; SlopeVD, slope of venous dilation; SlopeVC, slope of venous constriction. * Significant *p*-values are indicated in bold where $p < 0.05$ was considered significant.

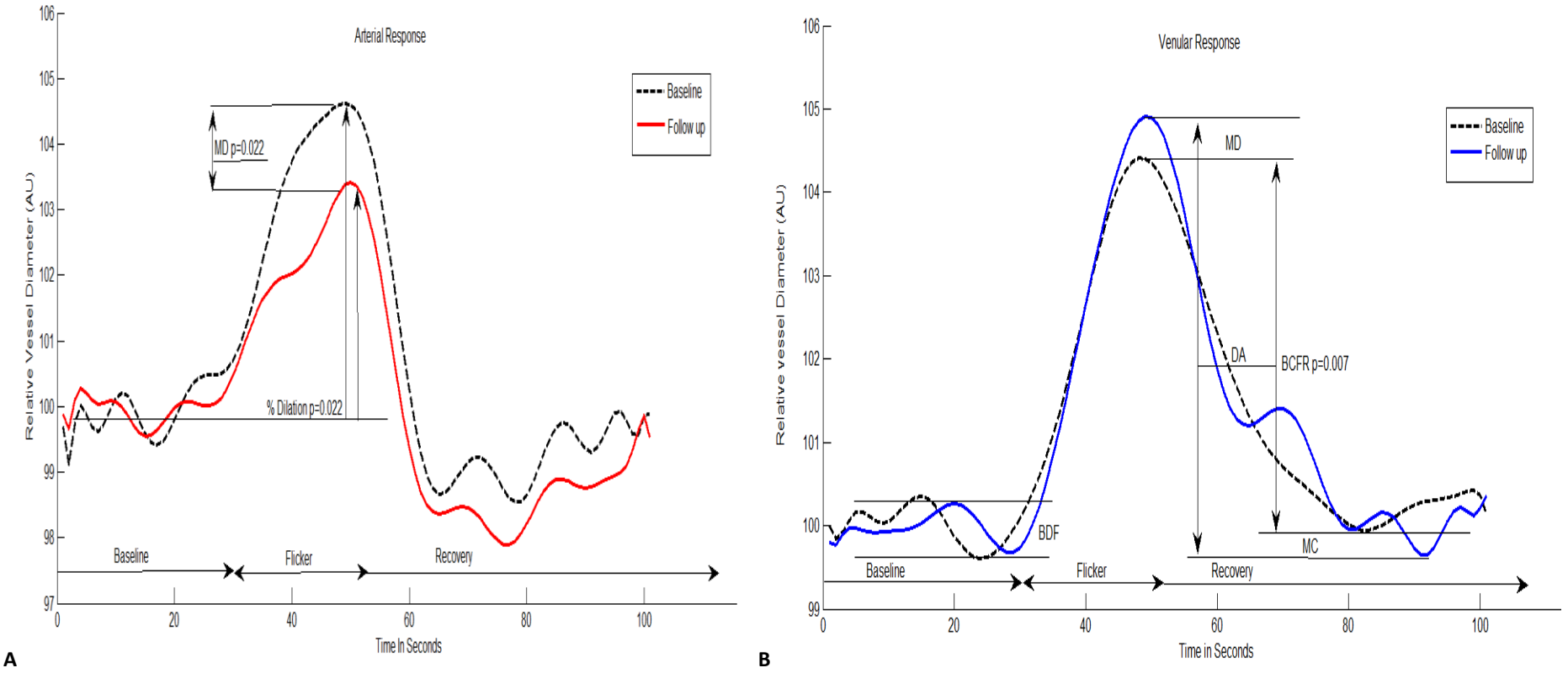

Figure 6.6.3. Retinal arterial and venous response profiles.

(A) Arterial response, (B) Venous response.

Abbreviations: AU, arbitrary units; BDF, baseline diameter fluctuation; BCFR, baseline corrected flicker response,DA (MD-MC)-(BDF); MD, maximum diameter; & Dilation, percent dialtion; DA, dilation amplitude.

variations in assessment. The BCFR was first described by Nagel et al[775] and is the difference between the peak of dilation and constriction of the vessels accounting for the fluctuation during baseline resting condition and has been successively reported in other studies when comparing parameters of healthy individuals to that of pathology[675 704]. It has also been shown in the presence of vascular disturbances to vary significantly in smokers and vasospastic subjects[672 776 777]. Accordingly, this study, findings would indicate that the increased BCFR of retinal micro vascular function after fasting is a result most likely related to increased vascular tone. These results are in contrast to a previous study which showed a blunted dilation response after fasting[774]. This is most probably due to our participants having already been adapted to the fasting cycle rather than a single case study.

The participants of the present study did not show any differences in anthropometrics in contrast to other studies[778]. however one study compared fasting participants to controls and did not record any significant changes[779]. Reductions in IOP due to fasting are still debatable as some studies have shown a reduction in IOP[780-782], while others have not[783 784]. The participants of the present study at follow up did have lower IOP readings but were not significant. It may be that any benefit in IOP reduction that is maintained during the fasting state is absent when individuals begin their normal feeding routine. This however, needs further confirmation.

In the present study a specific computational model was used to evaluate the dynamic response profile of the retinal micro vessels before and after fasting. The results show that participants had differing dilatory and constricting responses to flickering stimulation in retinal arteries and veins. This was the first study of its kind to determine the profile of retinal veins and arteries during fasting. The retinal vascular response to flickering light occurs to an increase in retinal metabolic demand and is predominantly a neurovascular coupling driven response[670 777]. It could be hypothesized that the altered retinal vessel activity demonstrated could be a consequence of endothelial dysfunction or a reduced bioavailability of NO. The endothelium plays an integral role in the regulation of vascular tone, platelet activity, inflammation, leukocyte adhesion and thrombosis and is ultimately involved in

the development of atherosclerosis[714]. It exerts its effects on the surrounding vascular smooth muscle cells by way of vasodilation, vasoconstriction and also has pro-inflammatory and anti-inflammatory properties[785] to maintain vascular homeostasis[786]. Notably, the process of atherosclerosis begins early in life, and endothelial dysfunction contributes to atherogenesis and precedes the development of morphological vascular changes[787 788]. It is known that the microcirculation represents the first vascular area to be affected by pre-clinical signs of endothelial dysfunction[734 789] and early vascular disturbances could be anticipated to occur before they can be detected in the macro vessels.

There has been only one study that has shown that CVD risk is reduced during fasting by assessments of the Framingham risk score but it was noted that participants were physically active during the month although the amount of activity was not recorded[790]. Another study by Yousefi et al[773] looked at the amount of Nitric oxide bioavailability after fasting had increased compared to baseline values. There have been other studies that looked at individual biochemical markers such as triglycerides and cholesterol but have revealed conflicting results[791-793]. This is possibly due to the fact that it has been revealed that individuals that come from a well-established economic country gain weight after fasting[794 795], while losing weight is associated from being in a deprived economic country[796 797].

No functional vascular changes identified were detected during systemic endothelial assessments. Similar to FMD, the DTM uses ischaemia-induced reactive hyperaemia as a stimulus to induce changes in vascular reactivity and increased blood flow[584] (schier 2013). This technique has been shown to strongly correlate with the Framingham risk score and coronary artery calcium[568]. In the present study trends were observed that showed individuals on average had better vascular reactivity during fasting but that these measured parameters were not significant after our follow up measurements (all $P > 0.05$).

There have not been any studies to date that have examined systemic endothelial function during fasting. Studies that have examined endothelial function as a consequence of weight loss have mainly focused on the effects of exercise which have shown promising results[798-800]. To date majority of the

studies have looked at individuals before fasting and during the last week of fasting. Most studies have noted a difference in biomarkers, clinical, and anthropometric measurements. What would be useful to know is whether these improvements can be sustained beyond the end of fasting and if there is truly a reduction of CVD risk?

Limitations of the present study are now considered. The amount of calories consumed was not recorded. This could have differed greatly between individuals. Physical exercise was not recorded and some of the participants were in relatively good physical shape compared to others. It would have been useful to know if they were still exercising. The power calculation was for 19 participants but due to the loss of follow up for some participants the study was slightly underpowered. Further research in this field should include the constituents of the diet, physical activity and at least 3 measurements that should encompass before fast, during, and sometime after wards to determine if any value gained or lost is sustainable.

6.8 Conclusion

In conclusion, this study demonstrated a higher MD and % dilation for retinal arteries during the fasting period and a decreased BCFR for retinal veins during the follow up period. This can be important for individuals during phases of long term fasting for weight loss and significant health benefits (not only to individuals but community health practices) would arise with early detection and appropriate preventative management. Retinal vasculature function of this type is non-invasive and gives real time indications of function or dysfunctions so that either holistic or pharmaceutical interventions may be applied before individuals develop cardio-vascular diseases.

7.0 The effect of bariatric surgery on retinal vessels structure and systemic microvascular function

7.1 Abstract

Purpose: To assess the effect of bariatric surgery on cardiovascular risk, retinal vessel calibre and endothelial function.

Methods: A total of 29 obese participants were measured at baseline and after one year from bariatric surgery. General anthropometric data was collected including waist/neck circumference as well as circulating markers for calculation of Framingham risk score. Retinal vessel calibre measurements were done on a randomly selected eye. Arterial stiffness was measured by pulse wave analysis (PWA), and microvascular reactivity by way of DTM.

Results: General anthropometric data was significantly decreased as expected. Additionally intra ocular pressure (IOP) was also reduced ($p<0.001$). CVD risk was significantly diminished ($p<0.001$). Central retinal artery equivalent trunk (CRAET) ($p=0.003$) and central retinal vein equivalent CRVE ($p=0.007$) had increased in calibre although the artery/venous ratio for the branches had decreased ($p=0.010$). Arterial stiffness had declined and peripheral microvascular reactivity improved ($p<0.001$, $p=0.008$ respectively).

Conclusions: These findings suggest that bariatric surgery alone is an effective way of reducing CVD risk through metabolic factors as well as improving retinal vessel diameters. Augmented vascular reserve through improvements in endothelial function are proposed for the enhancements in retinal vessel calibres, peripheral microvascular reactivity and arterial stiffness.

7.2 Introduction

Maintaining an appropriate weight and active lifestyle has been encouraged globally as these lead to many health benefits. Studies have demonstrated the positive correlations to individual health when individuals are within normative values pertaining to body weight. Obesity increases the risk of

cardiovascular disease and is targeted when treating patients who have progressed to a diseased state[801]. Obesity has become an important health issue. The WHO has described it as a growing global epidemic and has characterised it as a medical condition in which excess amount of body fat has accumulated which can lead to increased health problems and reduced life expectancy[13]. The prevalence of individuals who are overweight or obese is increasing rapidly in the developed world. The percentage of overweight or obese adults in the United Kingdom has risen sharply, increasing the prevalence rates as much as three to four times since the 1980's. There has also been an increase of 500% in obesity from 1972 to 2002 for boys and girls aged 10 years old[802], though there have been some signs of it slowing down. It has been projected that 60% of the United Kingdoms population could be obese by the year 2050 if these current trends continue[803]. These trends appear to be related to the typical Western diet high that is high in trans fats[804-806] and decreased physical activity[804]. Although the prevalence of people who are overweight and obese has increased for both sexes in all age and racial/ethnic groups, certain disparities may exist. Obesity in childhood, which is dramatically increasing, is a strong predictor of obesity in adulthood and studies have demonstrated that being overweight in early adult years is associated with a substantial incidence of obesity by middle age[807]. The causes of obesity are complex though a global consensus is that people become obese due to a combination of predisposed, socio-economic factors and behavioural factors including inherited genes that confer susceptibility, a lifestyle consisting of low levels of physical activity and consumption of excess calories (especially of poor nutritional value)[247]. Cardiovascular disease is an important health epidemic growing in prevalence as a result of greater childhood obesity rates and currently, worldwide, 10% of all children are classed as being obese or overweight[15 808].

Due to the relation between abdominal obesity and cardiovascular disease, it is useful to determine the waist circumference in addition to BMI. For individuals with a BMI of 25.0 to 34.9, a waist circumference >40 inches for men and >35 inches for women is associated with an increased risk of obesity-related cardiovascular disease[18]. Obesity plays a key role in endothelial dysfunction and involves either an increase (or a decrease) in any of the endothelial-related chemical messengers

and/or by alteration in function. The most commonly accepted endothelial dysfunction alteration pertains to abnormalities in the regulation of the lumen of vessels where endothelial cells within the lining prevent adhesion to the vessel wall in order to maintain morphology and function. However, any damage to the vessel wall, subsequently decreases blood vessel function[809]. Some examples of endothelial dysfunction include an increased permeation of macromolecules[810], increased or decreased production of vasoactive factors producing abnormal vasoconstriction/vasodilation, and increased pro-thrombotic and pro-coagulant activity[140]. A critical balance between endothelium-derived relaxing and contracting factors maintains vascular homeostasis. When this balance is disrupted, it predisposes the vasculature to vasoconstriction, leukocyte adherence, platelet activation, mitogenesis, pro-oxidation, thrombosis, impaired coagulation, vascular inflammation, and atherosclerosis[714]. Endothelial dysfunction has been described in many cardiovascular and metabolic disorders such as hypertension, coronary heart disease, dyslipidemia, and types 1 and 2 diabetes[77 698 699]. Endothelial dysfunction appears to precede the clinical manifestations of many of these cardiovascular disorders, hypertension for example, and also atherosclerosis, where abnormal vasoconstriction can be observed at the future site of plaque development[544].

An indicator that has also been used for the evaluation of endothelial dysfunction is the assessment of the arteriole-to-venue ratio of retinal vessels. It has been shown that the narrowing of arterial vessels is a marker for elevated blood pressure[324 575 811 812] and the increasing diameters of retinal veins have been linked to obesity, metabolic syndrome and, dyslipidemia[542 813 814]. Thus, endothelial dysfunction is one of the earliest hallmarks of vascular abnormality and is measurement of is proposed to help detect early stage increased risk for CVD. The AVR of retinal vessels is the most important static parameter of endothelial function associated with CVD and is independent of classic CVD risk factors[815 816].

It is known that reversible changes can be made with the loss of weight and decreasing central abdominal obesity and BMI. Bariatric surgery is the most effective treatment that reduces obesity and

in turn reduces an individual's risk of CVD[324 817]; and is a reliable pathway to sustained and substantial weight-loss in morbidly obese patients[818 819]. Although benefits of surgery have been associated with a reduction in abdominal obesity and certain macrovascular complications, few studies have assessed the effects of bariatric surgery on the peripheral microcirculation. Therefore, the purpose of this study was to determine the reduction in CVD risk as measured by the Framingham risk score (FRS) and to determine a relationship between the amount of weight-loss and the caliber of retinal vessels.

7.3 Methods and subjects

Successive diagnosed obese patients with a BMI > 40 kg/m^2 and listed for surgery were recruited from the Birmingham Heartlands Hospital, Heart of England Foundations Trust (HEFT, UK) weight management clinics by a specialist consultant. The inclusion and exclusion criteria for the participants was detailed in section 3.2.2. Initial baseline measurements were performed 1 month prior to the participant undergoing surgery. Additional circulatory markers were also included for the calculation of the Framingham risk score (section 3.3.6). Follow-up measurements were conducted 12 months after, at the same time of day when the initial measurements were performed in each of the participants. The investigative procedures performed in this study are outlined below and were conducted in accordance with the protocols set out in section 3.3.1.

1. Anthropometrics
2. IOP measurement
3. Blood pressure
4. Assessment of systemic vascular reactivity (DTM)
5. Assessment peripheral arterial stiffness (PWA)
6. Assessment of retinal vessel calibre (DVA)

7.4 Statistical analysis

All analyses were performed using Statistica® software (StatSoft Inc., Version 13, USA). Distributions of continuous variables were determined by the Shapiro-Wilks test. Group differences in clinical characteristics were assessed by a paired t-test. Multivariate analyses were performed to test the influence of clinical parameters such as age, SBP, BMI, HR and circulating markers such as CHOL, HDL, LDL, and HbA1c on the measured vascular reactivity variables. Differences between groups in retinal calibre and systemic vascular reactivity measures were computed by t-test or analysis of covariance (ANCOVA) where applicable. Statistical significance was defined at $p < 0.05$.

7.5 Power calculations

The sample size was calculated using the software G power[709] (University of Kiel, version 3.1.6, Germany). Based on previous studies, retinal vessel calibres in healthy individuals have a mean diameter of 202.3_{um} that vary $10\text{-}15_{um}$ but can change up to 30% with pathology[324 820 821]. Pulse wave analysis is also known to change by 4.8% for every 10bpm in heart rate[353]. Additionally, DTM measurements in healthy individuals have been known to change in temperature between 2.4 +/- $1.6^{0}c$[361]. It was anticipated that a paired t-test or ANCOVA would be required in this study, therefore to provide a statistical power of 95% and large effect size 0.7 with an alpha level of 0.05 it was estimated that a sample size of n=23 participants were required.

7.6 Results

7.6 1 Clinical characteristics

A total of 40 participants were initially screened for study inclusion and had completed all the baseline measurements of which 11 participants were lost to follow up. The remaining 29 obese participants were included in the final analysis and repeated measurements were performed 12 months from baseline. The clinical characteristics of the study groups are presented in table 7.6.1.

Bariatric surgery resulted in a significant mean reduction in BMI (10.82 kg/m2; ⩗22%; p<0.001), as well as, reductions in WC (25.13cm; ⩗18%; p<0.001), and NC (4.55cm; ⩗10%; p<0.001). Reductions in SBP (15.49mmHg; ⩗11%; p<0.001), DBP (5.62 mmHg; ⩗7%; p=0.039), HR (4.93bpm; ⩗7%; p=0.046), and interestingly IOP reduced (2.42 mmHG; ⩗16%; p<0.001) were also observed.

Bariatric surgery also resulted in significant improvements in mean fasting glucose GLUC (0.38mol/L; ⩗6%; p=0.004). Lipid profile also showed improvements with reduction in total cholesterol, CHOL (0.37 mmol/L; ⩗8%), LDL-C (0.48mmol/L; ⩗16%; p<0.001), TG (0.31mmol/L; ⩗21%; p=0.002), and increases in HDL-C (0.27mmol/L; ⩗21%; p<0.001).

Amelioration of CVD risk as was observed with a 42% reduction in FRS (p<0.001).

7.6.2 Systemic vascular function

Table 7.6.2 provides a summary of the systemic investigations using DTM and PWA that were assessed at baseline and follow up. Bariatric surgery resulted in an amelioration of endothelial dysfunction through mean increases in DTM parameters, specifically aTR (0.68; ⩗39%; p=0.08), and AUCtr (96.45; ⩗36%; p=0.025). Additionally arterial stiffness, as measured with PWA, also decreased (5.69; ⩗22%; p<0.001 respectively).

7.6.3 Retinal microcirculation

Bariatric surgery resulted in an improvement in CRAE T (5.61µm; ⩗4%; p=0.003), and conversely a deterioration in CRVE (8.52µm; ⩗4%; p=0.007), and AVR B (2.58µm; ⩗4%; p=0.010 respectively). Table 7.6.3 provides a summary of the retinal vessel calibres that were assessed at baseline and follow up.

7.6.4 Correlations between retinal vessel calibres and systemic parameters.

In the follow up group, decreases in SBP and DBP were associated with increases in CRAE T (r= -0.62, p<0.001, r= -0.70, p<0.001), whereas increases in HDL-C were associated with increases in CRAE T (r=

0.46, p=0.011 respectively) (figure 7.6.4). However, a similar trend was not observed in the baseline group. No other within group correlations were identified all (P >0.05).

Table 7.6.1. Summary of clinical data				
	Mean (SD)			
Variable	Baseline	Follow up	P-value	Significance
BMI (kg/m2)	49.2 (7.69)	38.38 (7.84)	**<0.001***	1>2
WC cm	137.17 (20.42)	112.04 (24.21)	**<0.001***	1>2
NC cm	42.58 (4.74)	38.03 (4.18)	**<0.001***	1>2
SBP (mmHg)	144.24 (14.35)	128.75 (13.23)	**<0.001***	1>2
DBP (mmHg)	78.96 (11.35)	73.34 (10.84)	**0.039***	1>2
MAP (mmHg)	100.72 (10.58)	92.48 (10.94)	**<0.001***	1>2
HR (bpm)	74.10 (13.45)	69.17 (9.88)	**0.046***	1>2
IOP (mmHg)	15 (2.40)	12.58 (1.95)	**<0.001***	1>2
OPP	52.14 (8.01)	49.06 (8.11)	**0.026***	1>2
CHOL (mmol/L)	4.90 (1.21)	4.53 (0.97)	**0.003***	1>2
HDL-C (mmol/L)	1.24 (0.35)	1.51 (0.40)	**<0.001***	1>2
LDL-C (mmol/L)	2.97 (1.01)	2.49 (0.81)	**<0.001***	1>2
TG (mmol/L)	1.44 (0.72)	1.13 (0.50)	**0.002***	1>2
GLUC (mmol/L)	5.75 (0.75)	5.37 (0.42)	**0.004***	1>2
FRS %	12.00 (7.73)	6.51 (5.17)	**<0.001***	1>2

Abbreviations: : BMI, body mass index; WC, waist circumference; NC, neck circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; IOP, intraocular pressure; OPP, ocular perfusion pressure; CHOL, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglycerides; GLUC, glucose. * Significant *p*-values are indicated in bold where $p < 0.05$ was considered significant

Table 7.6.2. Summary of systemic vascular function				
	Mean (SD)			
Parameter	Baseline	Follow up	P- Value	Significance
TR	0.05 (1.05)	0.38 (1.42)	0.283	-
aTR	1.71 (1.02)	2.39 (1.04)	**0.008***	2>1
AUCtr	264.51 (159.18)	360.96 (181.40)	**0.025***	2>1
AIx	25.79 (8.85)	20.10 (10.45)	**<0.001***	1>2

Abbreviations: TR, temperature rebound; aTR, adjusted temperature rebound; AUCtr, area under the curve temperature rebound; AIx, augmentation index. * Significant *p*-values are indicated in bold where $p < 0.05$ was considered significant.

table 7.6.3. Summary of retinal vessel calibres				
	Mean (SD)			
Parameter	baseline	follow up	P -value	Significance
CRAE T µm	143.70 (13.97)	149.31 (12.05)	**0.003***	2>1
CRAE B µm	136.95 (15.67)	137.02 (13.42)	0.968	-
CRVE µm	204.72 (23.26)	213.24 (20.75)	**0.007***	2>1
AVR T µm	70.60 (6.61)	70.33 (5.95)	0.802	-
AVR B µm	67.31 (7.62)	64.73 (7.89)	**0.01***	1>2

Abbreviations: CRAE T, central retinal artery equivalent-Trunk; CRAE B, central retinal equivalent- Branch; CRVE, central retinal vein equivalent; AVR T, arteriolar to venular diameter ratio-Trunk; AVR B, arteriolar to venular diameter ratio-Branch. * Significant *p*-values are indicated in bold where $p < 0.05$ was considered significant.

7.6.5 Results of differences between surgical procedures

Although there was a significant difference in the amount of participants between the Bypass group compared to the Sleeve and the Band p=0.002, the surgical procedures as described in section (1.4.3) did not reveal much differentiation between the measured anthropometric, systemic and retinal measurements. However, participants that underwent the sleeve procedure had a more favourable outcome of reducing total CHOL p=0.028 and reducing LDL-C compared to the other groups. However, this latter finding was borderline and not significant Table (7.6.5).

Table 7.6.5. Summary of different surgical procedures					
	Mean (SD)				
Procedure	**Bypass**	**Sleeve**	**Band**	**P value**	**Significance**
N	17	5	7	0.002	-
BMI (kg/m2)	-11.57 (3.66)	-7.79 (2.43)	-10.28 (4.76)	0.162	-
WC cm	-26.91 (23.22)	-21.64 (16.10)	-23.28 (9.72)	0.839	-
NC cm	-4.88 (2.28)	-3.00 (2.12)	-4.64 (1.02)	0.207	-
SBP (mmHg)	-14.11 (14.32)	-21.00 (15.00)	-14.85 (6.76)	0.587	-
DBP (mmHg)	-5.88 (12.51)	1.4 (14.44)	-5.87 (5.04)	0.454	-
MAP (mmHg)	-8.62 (11.51)	-6.06 (12.51)	-8.85 (5.35)	0.880	-
HR (bpm)	-9.00 (13.64)	5.40 (9.12)	2.42 (7.56))	0.064	-
IOP (mmHg)	-2.52 (2.06)	-1.80 (2.68)	-2.57 (2.93)	0.820	-
OPP	-3.22 (7.66)	-2.24 (8.21)	-3.33 (5.34)	0.961	-
CHOL (mmol/L)	-0.12 (0.49)	-0.86 (0.76)	-0.57 (0.50)	**0.028***	2>1
HDL-C (mmol/L)	0.31 (0.25)	0.28 (0.34)	0.16 (0.14)	0.402	-
LDL-C (mmol/L)	-0.27 (0.55)	-1.02 (0.56)	-0.58 (0.63)	0.050	-
TG (mmol/L)	-0.37 (0.55)	-0.28 (0.17)	-0.15 (0.46)	0.609	-
GLUC (mmol/L)	-0.24 (0.66)	-0.58 (0.64)	-0.55 (0.57)	0.419	-
FRS %	-5.22 (4.05)	-8.36 (3.16)	-4.07 (2.08)	0.127	-
TR	0.56 (1.43)	0.85 (1.48)	-0.66 (1.77)	0.158	-
aTR	0.87 (1.09)	1.05 (1.74)	-0.05 (1.29)	0.220	-
AUCtr	114.64 (180.92)	195.60 (315.21)	-18.57 (219.44)	0.224	-
Alx	-6.64 (7.88)	-4.80 (5.89)	-4.00 (5.65)	0.683	-
CRAE T µm	6.45 (11.28)	0.82 (8.86)	6.98 (3.64)	0.482	-
CRAE B µm	-2.53 (10.26)	0.40 (7.24)	6.17 (9.92)	0.159	-
CRVE µm	8.01 (17.99)	7.52 (12.88)	10.46 (13.92)	0.936	-
AVR T µm	0.53 (6.33)	-2.51 (5.10)	-0.58 (3.87)	0.572	-
AVR B µm	-3.39 (5.94)	-2.50 (3.28)	-0.63 (3.41)	0.494	-

Abbreviations: BMI, body mass index; WC, waist circumference; NC, neck circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; IOP, intraocular pressure; OPP, ocular perfusion pressure; CHOL, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglycerides; GLUC, glucose; TR, temperature rebound; aTR, adjusted temperature rebound; AUCtr, area under the curve temperature rebound; Alx, augmentation index; CRAE T, central retinal artery equivalent-Trunk; CRAE B, central retinal equivalent- Branch; CRVE, central retinal vein equivalent; AVR T, arteriolar to venular diameter ratio-Trunk; AVR B, arteriolar to venular diameter ratio- Branch. * Significant *p*-values are indicated in bold where $p < 0.05$ was considered significant. Differences are calculated for baseline and follow up between the 3 different surgical procedures.

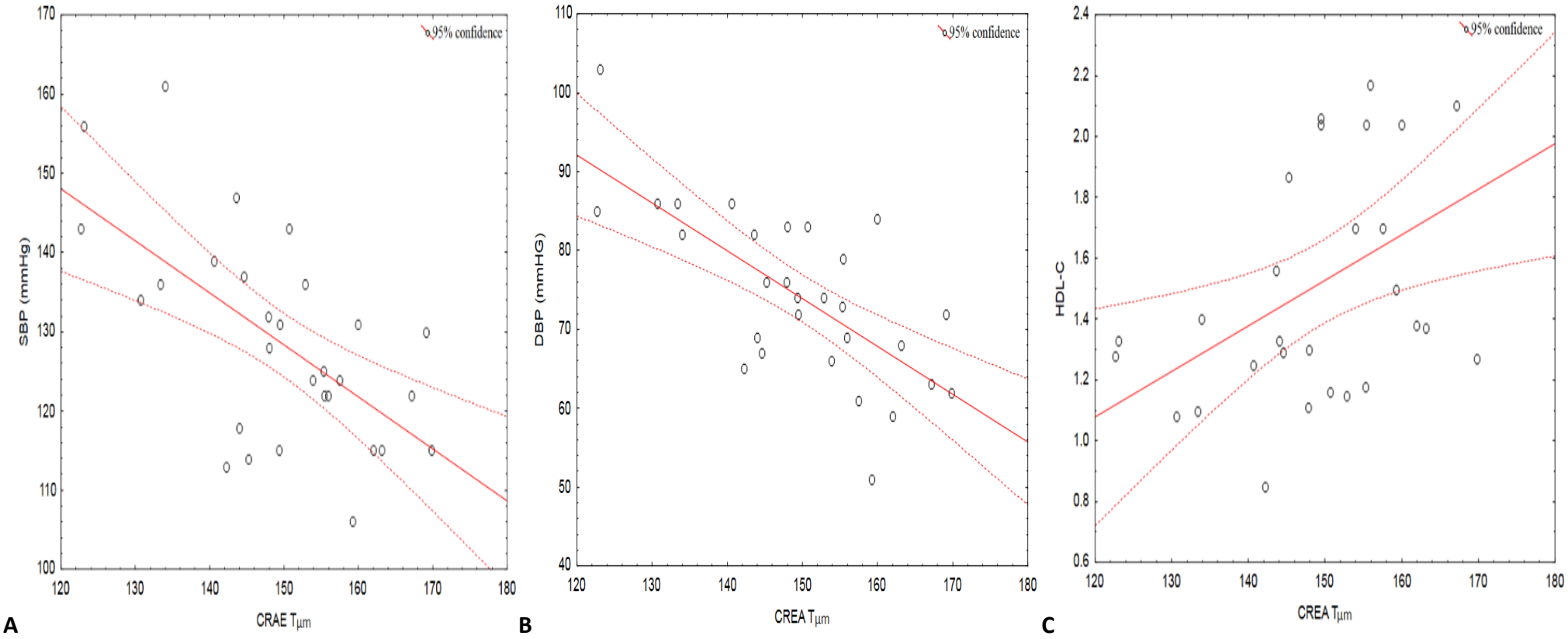

Figure 7.6.4. Correlation between SBP, DBP, HDL-C and CRAE T in the follow up subjects.
Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high Density lipoprotein cholesterol; CRAE T, central retinal artery equivalent-Trunk.

7.7 Discussion

The results show that bariatric surgery has made improvements in the retinal microcirculation through an improvement in the CRAE T, which are consistent with other studies[324 579]. This confirms that there is a marked reduction in peripheral arterial stiffness as measured with PWA. Bariatric participants, as expected, also benefited from a significant reduction in anthropometrics and shown for the first time, that post occlusive reactive hyperaemia was improved with the novel DTM.

Classically, increasing diameters of retinal venules have been linked to obesity, metabolic syndrome, systemic inflammatory markers, and dyslipidemia[575 813 814]. Large observational studies have established an association of arterio-venous ratio AVR with cardio-vascular disease (CVD), in part independent of classical cardiovascular risk factors[815 816]. A clinically assessable indicator of vascular disease is the AVR of retinal vessels. Narrowing of retinal arteries is a marker of chronic damage from elevated blood pressure[811 822 823] as in the results also showed the positive relationship between SBP and CRAE T, and DBP and CRAE T. Interestingly, an increase in venular diameter (CRVE) was found after bariatric surgery while other studies have reported the decrease in diameter after interventions[324 579]. Additionally the overall ratio for the trunk arterioles did not change. This was due to the fact that our venular diameters had increased and consequently had also affected the ratio for the branches (AVR B) resulting in a decrease after bariatric surgery. Arterial stiffness, which is characterized by the loss of elasticity and increased rigidity owing to the formation of atherosclerotic lesions, was also suggested as a biomarker for primary and secondary CVD prevention[824]. AIx has been a recognized marker of CVD risk and an indicator of arterial stiffness[825]. Increased arterial stiffness was identified as a risk factor for all-cause mortality and cardiovascular disease in recently published papers referring to the different risk factor groups and examined populations such as like obese children[826-828] and individuals with metabolic syndrome and its components[829 830]. Furthermore, the principal atherogenic lipoprotein in the blood is the LDL, and

has been implicated in obesity in many studies[272 831 832]. Increased levels of LDL promote cholesterol accumulation and an inflammatory response in the artery wall, which drives the process of atherosclerosis and in turn increases arterial stiffness by promoting the cellular outflow of cholesterol. Conversly, HDL opposes this process and reduces inflammation[833 834]. The results show that obese individuals benefited from decreases in arterial stiffness and LDL cholesterol with gains in HDL cholesterol.

Clinical studies have shown that endothelial dysfunction is manifested by impaired vascular reactivity, which can be measured by assessing flow mediated dilation (FMD) using non-invasive techniques such as brachial artery reactivity testing[835 836]. While FMD is a non-invasive measurement of vascular reactivity and endothelial-dependent vasomotion[382], this technique is dependent on technical expertise and expensive ultrasound equipment, thereby restricting it to a vascular research laboratory. Previous studies by Dhindsa et al[581] found that DTM parameters were significantly associated with FMD suggesting that DTM may be used as an alternative approach to FMD. Indeed, DTM has previously been positively correlated with the FRS, CAD[568] and, Doppler flow velocity[525]. DTM responds to both the macro- and microvascular reactivity. The macrovascular reactivity component is attributed to the dilation of the macrovessels induced by the vasodilatory response to increased shear stress levels caused by surge in flow rates (RH) postocclusion which is primarily NO mediated[581 837]. RH plays a pivotal role in delivering oxygen to the ischemic tissues in order to maintain the homeostatic balance of the vasculature[838]. The microvascular reactivity is induced by a macrovascular response to brachial occlusion and is primarily responsible for the amount of peripheral resistance[582].

The current study demonstrated that fingertip TR, as measured by DTM, increased after bariatric surgery, possibly though an increase in NO bioavailability. Indeed, reactive hyperaemia after a period of ischemia is a physiologic response of the vasculature and endothelial system that results in rapid increases in local blood flow and temperature[352 571 839]. This is strongly dependent on the degree of

endothelium-derived nitric oxide release following local ischemic stress[571 837]. However, an increase in AUCtr, which corresponds to faster temperature rebound response after induced ischemia, is a marker of improved endothelial function because blood traveling through more elastic vessels takes a longer time[840].

The increase in CRAE with the additional benefits of improved endothelial function and decreases in arterial stiffness as well as, reduction in the FRS point towards a favourable outcome for obese individuals, and this reduction in risk (≠40%) was similar in other studies assessing CVD risk after bariatric surgery[324 841]. It should be noted that the participants during the follow up period were still registered as obese due to BMI not falling below 30kg/m^2. Participants are at decreased risk compared to baseline values but are perhaps more at risk when compared to people of normal weight. This could also be perceived as a limitation to this study in that controls with less BMI were not measured. Other limitations that could have modified our results were exercise habits of the individuals and the precise calorie intake, both of which were not recorded. Additionally, there were unequal numbers of participants in the surgery groups, any findings would need to be confirmed in a larger study.

7.8 Conclusion

In conclusion, our study shows that bariatric surgery can significantly decrease morbid obesity and reduce CVD risk through improvements in retinal arteriolar calibre and systemic endothelial function. Further enhancements to participants' habitual diet and exercise as well as advice on the psychology of sedentary behaviour must be reinforced to the patient.

8.0. Is there an improvement in anterior ocular health after bariatric surgery?

8.1 Abstract

Purpose: To assess the effect of bariatric surgery on the anterior ocular surface.

Methods: A total of 29 obese participants were measured at baseline and followed up one year after bariatric surgery. General anthropometric data were collected including serum lipid markers of cholesterol. Anterior eye measurements were taken with the keratograph (K5M, Optikgerate GmbH, Wetzlar, Germany) instrument and included tear meniscus height (TMH), tear break up time (TBUT), bulbar/limbal redness and infrared meibography. Staining was evaluated using fluorescein and lissamine green.

Results: There were no differences between any of the measured clinical variables measured at the 5% significance level. There were tendancies for a reduced TMH and a decrease in Meibomian gland drop out after bariatric surgery, but these were insignificant ($p>0.05$).

Conclusions: Weight reduction through bariatric surgery did not have an effect on ocular health. Further study is warranted to determine if Meibomian gland loss can be reduced.

8.2 Introduction

Obesity has been gaining much attention over the past few decades and it has been reaching epidemic proportions within the western world. Obesity is an established risk factor for the development of cardiovascular diseases such as hypertension, diabetes mellitus, coronary heart disease[18]. It is typically associated with the western diet which is low in nutritional value but excessively high in saturated fats. The World Health Organization (WHO) has defined obesity as BMI greater than 30 kg/M^2 and the current international obesity task force has estimated that worldwide there are around 325 milliion people who meet this criterion[18]. The impact of obesity on health and its deleterious effects on the body are well known[842]. There have been studies which have tried to

establish associations between obesity and ocular pathologies such as glaucoma, through increased IOP[843 844], cataracts, by way of increased BMI[845 846], and diabetic retinopathy[574 847]. The associations found, however, have mainly been due to obesity related risks of systemic diseases that manifest themselves through retinopathy. For example, diabetes is a risk factor for the development of cataracts earlier on in life. Less, however, is known about the impact of obesity on the tear film and ocular surface health. Dry eye syndrome is a multifactorial disease of the ocular surface which results in symptoms of discomfort, visual disturbance and tear film instability[328]. It can be brought upon by certain medications, poor nutritional diets[333], contact lens wear and also laser refractive surgery[848]. More recently, Floppy Eyelid Syndrome (FES) has been associated with ocular surface disease resulting in dry eyes and a number of studies have attributed this to the severity of disease progression[849 850]. This is important because the number one risk factor for the development of FES is obesity.

A study by Caffery et al[330] looked at the diet within the western world and its influence on tear function. In this review more than 25 years ago, it was noted that the maintenance of a healthy tear film requires sufficient amount of protein and vitamin A. Within the western world protein intake is not the problem as there is an abundant supply. The real problem lies with the vitamins and minerals which are part of a balanced diet and this was hypothesized to be the problem of maintaining a healthy tear film. More recently, elevated amounts of serum lipids were associated with dry eyes in the Beaver Dam eye study[331] and in the Taiwan nationwide population based survey[332]. However, there was no link with obesity or BMI. The link between higher serum lipid levels and ocular deposition is well established for manifestations such as corneal arcus and xanthelasma[333 334]. However, the exact mechanism for the association between dry eye syndrome and obesity has yet to be elucidated. A study by Dao et al[335] identified high serum lipid levels as a risk factor for the development of Meibomian gland dysfunction. However, this was identified as elevated HDL levels. Along this line, another study by Uchino et al[338] found the prevalence of dry eye syndrome was higher in men with a low BMI than women. A further study by modulo et al[340],

conducted on mice, found no association between serum cholesterol levels and dry eye syndrome but noticed that males who were fed a higher fat diet secreted less tears, though the tests that were involved were probably not sensitive enough to detect changes in serum cholesterol levels.

The tear film plays a vital role in the maintenance of ocular health by keeping the cornea lubricated. The meibomain glands are responsible for the secretion of the lipid layer, which helps the aqueous from evaporating too quickly. The tear films stability can be assessed through different methods including tear break up time (TBUT), its volume through the schirmer test or measurement of the meniscus height. Staining is also a reliable indicator of an irregular tear film through drying of the corneal and conjunctival surface. A comprehensive review of these methods has already been published in the DEWS report 2007[328].

Among the various methods developed to measure dry eyes, the keratograph features as a non invasive method that allows for simultaneous evaluation of the corneal tear film, bulbar and palpebral conjunctival surfaces as well as an infrared sensor to measure meibography. It has in-built software that analyses hyperaemia, based on the Efron grading scale. It has been used within the last few years within specialist ophthalmic departments for the evaluation of corneal topography before and after surgery, specialist contact lens fitting and dry eye disease management. The main advantage of the keratograph is that it provides integrated and dynamic data analysis that is specific to each individual and can be used to show patients' progress during any regiments or to help in the understanding of certain ocular conditions. Therefore, the aim of this study was to use the keratograph for ocular health assessment of obese individuals before and after bariatric surgery.

8.3 Methods and Subjects

Successive diagnosed obese patients with a BMI > 40 kg/m^2 and listed for surgery were recruited from the Birmingham Heartlands hospital, Heart of England Foundations Trust (HEFT, UK) weight management clinics by a specialist consultant. The inclusion and exclusion criteria for the participants was detailed in section 3.2.2. Briefly, initial baseline measurements were performed 1 month prior to the participant undergoing surgery. Measurements of circulatory markers were also included to determine their relationship with anterior eye health. Follow-up measurements were conducted 12 months after, at the same time of day when the initial measurements were performed in each of the participants. The investigative procedures performed in this study are outlined below and were conducted in accordance with the protocols set out in section 3.3.1.

1. Anthropometrics

2. Assessment of anterior eye health (keratograph)

8.4 Statistical analysis

All analyses were performed using Statistica® software (StatSoft Inc., Version 13, USA). Distributions of continuous variables were determined by the Shapiro-Wilks test. Group differences in clinical characteristics were assessed by a paired t-test. Multivariate analyses were performed to test the influence of clinical parameters such as age, SBP, BMI, HR and circulating markers such as CHOL, HDL, and LDL on the anterior eye variables. Differences between groups in ocular health were computed by t-test or analysis of covariance (ANCOVA) where applicable. Statistical significance was defined at $p < 0.05$.

8.5 Power calculations

The sample size was calculated using the software G power[709] (University of Kiel, version 3.1.6, Germany). As this is the first study of its kind, power calculations were based on similar studies that used the Keratograph system for the assessment for dry eye disease and infrared meiobography[629]

[680 851 852]. Nevertheless, It was anticipated that a paired t-test or ANCOVA would be required in this study, therefore to provide a statistical power of 80% and medium size of 0.5 with an alpha level of 0.05 it was estimated that a sample size of n=27 participants were required. Therefore, the recruited participants from the previous study would suffice for the sample size.

8.6 Results

8.6.1 Clinical characteristics

A total of 40 participants were initially screened for study inclusion and had completed all the baseline measurements of which 11 participants were lost to follow up. The remaining 29 obese participants were included in the final analysis and repeated measurements were performed 12 months from baseline. The clinical characteristics of the study groups are presented in table 8.6.1 and have previously been described in the previous chapter (section 7.6.1).

8.6.2 Anterior ocular health

Bariatric surgery did not improve any of the anterior ocular health measurements. However, there was a tendancies for TMH to be reduced at the follow up which would point towards a reduced TBUT but no changes in TBUT were recorded. Additionally, there was also a trend for the amelioration of meibomian gland drop out but this was also non-significant (table 8.6.2).

No correlations were identified in within group analysis all $p>0.05$.

8.7 Discussion

This study was the first to look at anterior eye health changes in obese subjects undergoing bariatric surgery. No identifiable changes were measured at the ocular level at 1-year follow up post reduction in obesity.

Table 8.6.1. Summary of clinical data				
	Mean (SD)			
variable	Baseline	Follow up	P-value	Significance
BMI (kg/m2)	49.2 (7.69)	38.38 (7.84)	**<0.001***	1>2
SBP (mmHg)	144.24 (14.35)	128.75 (13.23)	**<0.001***	1>2
DBP (mmHg)	78.96 (11.35)	73.34 (10.84)	**0.039***	1>2
MAP (mmHg)	100.72 (10.58)	92.48 (10.94)	**<0.001***	1>2
HR (bpm)	74.10 (13.45)	69.17 (9.88)	**0.046***	1>2
CHOL (mmol/L)	4.90 (1.21)	4.53 (0.97)	**0.003***	1>2
HDL-C (mmol/L)	1.24 (0.35)	1.51 (0.40)	**<0.001***	1>2
LDL-C (mmol/L)	2.97 (1.01)	2.49 (0.81)	**<0.001***	1>2
TG (mmol/L)	1.44 (0.72)	1.13 (0.50)	**0.002***	1>2

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; CHOL, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglycerides. * Significant *p*-values are indicated in bold where $p < 0.05$ was considered significant.

Table 8.6.2. Results of anterior eye measures			
	Mean (SD)		
Parameter	Baseline	follow up	P-value
TMH (mm)	0.30 (0.10)	0.27 (0.08)	0.074
NIKBUT			
First (s)	10.08 (7.08)	10.29 (6.72)	0.875
Average (s)	11.92 (6.71)	12.14 (6.53)	0.849
REDNESS			
Bulbar temp.	0.86 (9.36)	0.86 (0.44)	0.952
bulbar nasal	0.99 (0.47)	0.93 (0.41)	0.451
Limbal temp.	0.57 (0.32)	0.57 (0.39)	0.948
limbal nasal	0.71 (0.33)	0.62 (0.32)	0.082
MGD			
Superior loss %	22.12 (11.73)	18.64 (10.88)	0.062
Inferior loss %	16.99 (9.94)	14.39 (11.06)	0.181
NAFL staining %	1.76 (2.46)	2.73 (4.53)	0.333
Lissamine staining %	12.10 (9.32)	11.03 (4.72)	0.372

Abbreviations: TMH, tear meniscus height; NIKBUT, non-invasive keratography break up time; MGD, Meibomian gland dyfunction; NAFL, sodium fluorescein. * Significant *p*-values are indicated in bold where $p < 0.05$ was considered

The tear film is essential for maintaining the health of the ocular surface and is an important optical element which ensures a smooth refracting surface[853]. It forms a complex and stable hydration and nutrient delivery system for the ocular surface. As a result, the instability of a disrupted tear film may compromise ocular health and lead to dry eye syndrome[630].

Hypercholesterolemia is considered to be related to MGD[854]. One study reported that patients with moderate to severe MGD had significantly higher total cholesterol than the controls[854] It was suggested that the increased concentration of cholesterol increased viscosity and induced meibomian plugging, aggravating MGD[599 848]. It is well known that severe MGD can induce dry eye symptoms[328]. In addition Hypercholesterolemia was associated with dry eye disease in the Beaver Dam Eye Study[331] and in the Taiwan nationwide population- based survey[569]. Further population-based studies have found that dry eye syndrome was more prevalent with a decreased BMI[338].

There have been a few studies that looked at diet intervention on ocular surface disease but human studies are scarce. There was a single case study that reported gradual improvement in tear volume in a subject on a calorie restricted diet for approximately eight years[855] but this is not really feasible and cannot translate into today's world. Whole-body hydration may be an important consideration for the tear film. One study concluded that religious fasting has no effect on tear stability and volume[856], while others showed an increased tear secretion rate following the pre-dawn meal during Ramadan[857]. Experimental rodents fed on calorie-restricted diets for 6 months showed increased tear volume and lacrimal gland acinar density as well as decreased ocular surface staining and lacrimal gland inflammatory cell density[858].

8.8 Conclusion

In conclusion, the results showed no effect of bariatric surgery on anterior eye and ocular surface health. This is perhaps due to the cohort having relatively good tear secretion as measured by TBUTs greater than 10 seconds and a TMH greater than 0.20mm. The meibomian glands lipid secretion allows the tears to stay on the ocular surface and prevent evaporation and is the reason why our participants' had a longer TBUT. The staining of fluorescein and lissamine green was minimal to begin with and no change was noticed after the intervention. Although obesity is a possible risk factor for FES, this would have been indicated through increased bulbar redness and staining. Neither of which were present in the cohort.

9. Summary and conclusions

Evaluating the importance of retinal and systemic vascular risk factors in terms of the aetiology stemming from overweight and obese individuals has been a research area of interest for some time. The scientific literature relating to this has been reviewed in Chapter 1 of this thesis. However, it is still unclear how disturbances within the vasculature, affecting multiple vascular beds, may interrelate to increase the risk from overweight to obese persons, and whether weight management interventions can reduce the risk through augmented vascular reserve or improve endothelial function. Further questions arise when considering how valid the assessment of vascular function at the ocular level may be as an indicator of endothelial dysfunction systemically.

Revealing or confirming these relationships could not only enhance aetiological understanding of early CVD risk but also provides insight into the intervention outcomes. It could also potentially open up new diagnostic or therapeutic avenues for overweight and obese individuals. Therefore, this study was concerned with investigating the presence, impact, and interactions of ocular and systemic vascular alterations in healthy, overweight, and obese individuals to ascertain how they may relate to increases in CVD risk and whether weight-loss interventions can ameliorate a person's risk profile.

The findings of this work were:

9.1 The relationship between retinal and peripheral vascular function in healthy individuals with low cardiovascular risk.

The uses of predictive models are indicated as preliminary steps for assessing each individual's CVD risk. However, such methods rely on measurements that are useful in predicting long-term risk assessment in populations and not in individuals with specific genetic and environmental influences. As a direct result, such risk calculators either over- or underestimate risks in more than 50% of individuals. Indeed, while there are various biomarkers offering a disease-specific individual biological

profile, none of these techniques enable non-invasive assessments in primary care settings while being sufficiently sensitive to enable early detection and prevention. It would be useful to know, however, that regardless of the microvascular bed assessed, that existing methods are equally sensitive to detecting signs of early CVD risk. Therefore, the aim of the present study was to investigate the relationship between retinal microvascular function and peripheral vascular reactivity in apparently healthy, normotensive, individuals with no known history of cardiovascular disease.

Post dynamic retinal vascular analysis, impairments in peripheral microvascular reactivity also exhibit signs of impaired retinal vascular function demonstrated by an abnormal arterial MDRT, slopeAC, and DA responses. This behaviour was not demonstrated in individuals without peripheral microvascular function abnormalities. Furthermore, the dysfunction measured at the retinal microvascular level correlated with similar changes in the peripheral circulation. Causes of the retinal vascular alterations identified in this study can only suggest that ED, when present, is a general process and is detectable with accuracy regardless of the method used or location of the vascular bed. Identification of retinal vascular dysfunction in patients with peripheral microvascular abnormalities showed, for the first time that functional impairments within the ocular circulation as assessed by DVA were correlated. Correlating these two different vascular beds indicates that dynamic analysis of vascular function is a variable specific for each individual that could be used to predict, prevent and monitor cardiovascular health in individuals.

9.2 The effects of physical training on retinal and systemic microvascular function.

It has been demonstrated that a sedentary lifestyle is a risk factor for increased weight gain. Excess adipose tissue induces several metabolic changes, including dyslipidaemia, elevated BP, oxidative stress, increased inflammation, and in so doing, contributes towards atherosclerosis, increased arterial stiffness, and macro- and micro-vascular endothelial dysfunction. More recently researchers were able to predict midlife endothelial dysfunction based on factors such as, BMI and total

cholesterol and fitness level in children; noting that these predisposing factors in childhood lead to adult obesity and in turn risk factors for CVD development. Physical exercise has been recognized as an important modifiable lifestyle intervention, mainly due to its effects on energy expenditure, reduction of adiposity and increasing NO bioavailability. As such the aim of this study was to investigate the presence of microvascular dysfunction in overweight individuals and to assess the effects the physical exercise on these measured clinical variables. It was hypothesised that, based on recent evidence highlighting the improvements in macrovascular function measured by FMD through exercise, that overweight patients would demonstrate improvements in retinal vascular reactivity to flicker light in conjunction with improvements in systemic vascular function post weight-loss through exercise.

The findings showed that after the exercise regimen, participants had lower arterial reaction times for maximum dilation for the first provocation and overall throughout the three flicker cycles compared to baseline. The minimum diameter was also lower on the first flicker cycle and was maintained throughout. The percent constriction from baseline was markedly increased for the first flicker cycle and throughout. Lower reaction times to constriction were also reduced which results in a shorter period of time required for the vasculature to return to baseline values. However, no differences were found for the peripheral vasculature. Correlations were nevertheles found between the retinal and peripheral vascular parameters explored in this study.

Physical exercise has positively modified the retinal microcirculation function and, moreover, for the first time, results showed that the retinal microvascular reactivity can be augmented through exercise. These findings indicate that subclinical signs of retinal vascular dysfunction appear to be present in overweight individuals and that these measured clinical variables can be improved with physical exercise. The importance of considering retinal vascular function in the prevention and management of overweight patients has been highlighted and, furthermore, through the utilisation of this technique vascular function that contributes to increases of endothelial function.

9.3 The long-lasting effects of fasting during the month of Ramadan on retinal and peripheral vascular function.

Having established that the manifestation of retinal vascular dysfunction can be improved through physical exercise in overweight individuals, an exploration into whether this could be accomplished through fasting was the next logical step. Ramadan fasting is defined as periodic food and water deprivation during daylight hours and itself can cause a reduced caloric intake in which people usually resort to two instead of three meals a day. It has been indicated that participants who are under 36 years of age will benefit greatly during the period of abstinence with reductions in BMI, WC and BP. Furthermore, studies have also demonstrated favourable lipid profile enhancements which can suggest that decreased calorie intake through fasting can improve individual CVD risk profile. Endothelial dysfunction is first pre-clinically noted in microvasculature circulation. Thus interventions to target detection at the micro-vascular level in conjunction with macro vascular level could provide early anticipation of endothelial dysfunction that leads to a negative impact on an individual's CVD risk profile. Therefore, the aim of our study was to measure, for the first time, endothelial function at the microvasculature level that can be observed non-invasively. Retinal and peripheral level of endothelial functions were examined to determine important information on the benefits of fasting. The hope was that this would clarify whether fasting can be used as an intervention for the improvement in micro-vascular function.

The findings did not indicate any measureable changes in anthropometrics but higher arterial maximal diameters for the first flicker provocation were found and overall throughout the three flicker cycles compared to their non-fasting status were found. Similar results were found in the amount of dilation %, which shows that retinal arteries on average were able to dilate from baseline to MD significantly better during fasting than during non-fasting period. For retinal, BCFR was notably stable during the fasting period compared to the non-fasting period that showed great variations in assessment. The study showed tendencies in individuals on average had better

peripheral vascular function during fasting but that these measured parameters were not significant during the non-fasting period. These findings provide support for the concept that dietary restriction through fasting is another method that can improve endothelial function through enhancements of the microcirculation. Interestingly, the nature of the retinal vascular alterations identified in the fasting participants appear to differ from those of the previous chapter. Fasting individuals retinal vessels have a better capacity to maximally dilate while in individuals who exercised, it was distinguished that the retinal vessels were much faster at responding to provocations. This is a novel finding which would benefit from further investigation to clarify its significance and determine the fasting versus exercise approach of weight-loss and their effects on the retinal vessel profile.

9.4 The effect of bariatric surgery on retinal vessels structure and systemic microvascular function

The prevalence of individuals who are overweight or obese is increasing rapidly in the developed world. Although this has increased for both sexes and all age and racial/ethnic groups, certain disparities may exist. Obesity in childhood, which is dramatically increasing, is a strong predictor of obesity in adulthood and studies have demonstrated that being overweight in early adult years is associated with a substantial incidence of obesity by middle age. Obesity increases the risk of cardiovascular disease and is targeted when treating patients who have progressed to diseased state. Indeed, obesity plays a key role in endothelial dysfunction and involves either an increase (or a decrease) in any of the endothelial-related chemical messenger and/or by alteration in any of the structural or functional vascular changes. Thus, endothelial dysfunction is one of the earliest hallmarks of vascular abnormality and measurement of endothelial dysfunction is proposed to be an early stage increased risk for CVD and may help in early detection and management of individuals at risk of developing CVD. As such the aim of this study was to assess the effects bariatric surgery on ocular and systemic vascular alterations in previously untreated morbidly obese patients. It was hypothesised that, based on recent evidence and from the previous findings, weight-loss

interventions should improve ocular and systemic vascular dysfunction and reduce overall cardiovascular risk.

Findings showed that participants had greatly reduced anthropometric measurements including a favourable drop in blood pressure. Interestingly, IOP was also reduced one year after surgery. Furthermore, changes in lipid profiles were prominent that also resulted in a decreased CVD risk as calculated by FRS. However, it should be noted that these decreases were substantially lower than initial baseline measurements but our cohort it still categorized as obese due to their BMI >30kg/m^2. Arterial stiffness was reduced, and microvascular function improved possibly through an increase in NO bioavailability. Furthermore, increases in the area under the curve, is a marker of improved endothelial function because blood traveling through more elastic vessels takes a longer time. This is the first time DTM has been used for comparisons after bariatric surgery in morbidly obese individuals. The inter-relationships identified between the investigated ocular and systemic parameters provide support for the decreases in CVD risk and the improvements in endothelial function.

Retinal vessel structural alterations in the arterioles had improved and were negatively correlated with the degree of systemic BP. Additionally favourable lipid profile such as increases in HDL-c were also positively correlated with increases in arteriolar diameter. Interestingly the findings showed, for the first time increases in venule diameter, which is classically associated with obesity, after an intervention while other studies have reported decreases. Although these findings suggest favourable CVD risk profile outcomes after bariatric surgery, supplementary enhancements to participants' habitual diet and exercise as well as advice on the psychology of sedentary behaviour must be reinforced to the patient.

9.5 Anterior eye health after weight reduction through bariatric surgery. Is there an improvement in anterior ocular health?

The impact of obesity on health and its harmful effects on the body are well known. There have been studies which have tried to establish associations between obesity and ocular pathologies, such as glaucoma, cataracts, and diabetic retinopathy but these are mainly due to obesity related risks of systemic diseases that can manifest themselves through visual disorders of the posterior eye. However, less is known about the impact of obesity on the tear film and whether there are any association's related to ocular surface health. Dry eye syndrome is a multifactorial disease of the ocular surface that can result in tear film instability. It can be brought upon by certain medications, and poor nutritional diets. More recently, Floppy Eyelid Syndrome (FES) has been associated with ocular surface disease resulting in dry eyes, this is important because the number one risk factor for the development of floppy eyelid syndrome is obesity. Additionally, elevated amounts of serum lipids was associated with dry eyes in the Beaver Dam eye study and in the Taiwan nationwide population based survey. Conversely, there have been studies that have also shown no relationship between serum lipid markers and ocular surface health.

The exact mechanism for the association between dry eye syndrome and obesity has yet to be explained. Therefore, the aim of this study was to evaluate ocular surface health in obese individuals before and after bariatric surgery. Findings showed that bariatric surgery did not improve any of the measured clinical variables. However, there were tendencies for the reduction in the tear meniscus height which would suggest a negative finding, as well as, a trend for meibomian gland drop out to be reduced which suggests a positive finding but again, these were not significant. There were also no correlations between serum lipid markers and any of the measured ocular surface parameters even though previous studies had noted these findings. The participants were asymptomatic and the results show that obesity does not necessarily cause ocular health problems nor can it be ameliorated or improved through bariatric surgery.

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