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Factors and markers predicting subclinical atherosclerosis in type 2 diabetes

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III. Kärberg K, Forbes A, Lember M. Visfatin and Subclinical Atherosclerosis in Type 2 Diabetes: Impact of Cardiovascular Drugs. Medicina (Kaunas). 2023;59(7):1324.

IV. Kärberg K, Forbes A, Lember M. Unlocking the Dietary Puzzle: How Macronutrient Intake Shapes the Relationship between Visfatin and Atherosclerosis in Type 2 Diabetes. Medicina (Kaunas). 2024;60(3):438.

Personal contribution:
Kati Kärberg was involved in study planning, protocol conception, and subject recruitment. She obtained the questionnaire data, performed ultrasound examinations, conducted statistical analyses of study materials, and was the primary author writing papers.

2. ABBREVIATIONS

24HR	24-hour recall
ABI	ankle-brachial index
ACEI	angiotensin-converting enzyme inhibitor
ARB	angiotensin II receptor blocker
ARIC	Atherosclerosis Risk In Communities
BB	beta-blocker
BMI	body mass index
BMRest	estimated basal metabolic rate
CAD	coronary atherosclerotic disease
CCA	common carotid artery
CCB	calcium channel blocker
CIMT	carotid intima-medial thickness
Cu	copper
CV	cardiovascular
CVD	cardiovascular disease
DLW	doubly labelled water
DM	diabetes mellitus
EASD	European Association for the Study of Diabetes
EIrep	reported energy intake
EIrep:BMRest	ratio of reported energy intake and estimated basal metabolic rate
FFQ	food frequency questionnaire
HbA1c	haemoglobin A1c
HDL-C	high-density lipoprotein cholesterol
hsCRP	high-sensitive C-reactive protein
ICA	internal carotid artery
IMT	intima-media thickness
IPAQ	International Physical Activity Questionnaires
LDL-C	low-density lipoprotein cholesterol
MESA	Multi-Ethnic Study of Atherosclerosis
MUFA	monounsaturated fatty acid
PAD	peripheral artery disease
PAL	physical activity level
PUFA	polyunsaturated fatty acid
RDA	recommended dietary allowances
SD	standard deviation
SE	standard error
SFA	saturated fatty acid
Zn	zinc
Zn:Cu	zinc to copper ratio
T2DM	type 2 diabetes mellitus
VAI	visceral adiposity index
WHO	World Health Organization

3. INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2DM) and its vascular complications is steadily increasing worldwide. In Estonia, the Health Development Institute reported 5397 newly diagnosed cases of T2DM in 2022, with a slightly higher prevalence among women than men. The number of newly diagnosed cases has remained similar each year since 2016 (Health Statistics and Health Research Database 2024). In 2016, the same database also reported 8953 new cases of ischemic heart disease, 5659 new cases of cerebrovascular diseases, and 465 new cases of lower limb peripheral artery diseases (PAD) (Health Statistics and Health Research Database 2024).

Diseases attributable to atherosclerosis remain notably underdiagnosed, particularly those pertaining to the lower limb peripheral arteries (Konya et al. 2024). Studies like PARTNERS (US) and PANDORA (Europe) highlight the high prevalence of asymptomatic PAD, with 29% and 18% of participants, respectively, displaying abnormal ABI despite many showing no symptoms (Hirsch et al. 2001, Cimminello et al. 2011). This highlights potential missed opportunities for identifying PAD in primary care due to insufficient recognition of cardiovascular (CV) risk markers and associated symptoms.

Subclinical atherosclerosis, a precursor to clinically significant CV events, often remains undetected until it has progressed to more overt forms of cardiovascular disease (CVD). The Hamburg City Health Study of 10,000 participants found that in the general population, the prevalence of lower limb PAD (ABI $\leq$0.9) was 23.6%, and the prevalence of carotid artery disease (IMT $\geq$1 mm) was 30% (Behrendt et al. 2023). In the context of patients with T2DM, the presence of CVD assumes an even greater significance, as diabetes not only increases the risk of atherosclerosis but also complicates its detection and management.

Unlike its symptomatic counterpart, subclinical atherosclerosis manifests as early stages of arterial wall thickening, plaque formation, and endothelial dysfunction without overt clinical manifestations such as myocardial infarction, stroke or acute limb ischaemia. This insidious progression underscores the importance of reliable markers for its early detection and intervention, especially in high-risk populations like those with T2DM. Identifying the internal and external factors of the human body that can be used to detect subclinical atherosclerosis or to slow its progression has a great potential for use in clinical practice.

One of the major players in the development of atherosclerosis is obesity, a chronic, complex, and multifactorial disease that frequently coexists with T2DM (Anitschkow et al. 1914; Siiteri 1987). Adipose tissue serves as an active endocrine organ, secreting various adipokines that participate in multiple processes, including the development of atherosclerosis (Siiteri 1987; Liu et al. 2022). These adipokines influence the progression of atherosclerosis through their protective or aggravating effects (Liu et al. 2022). Investigating the relationship between circulating adipokines and subclinical atherosclerosis could potentially uncover additional early risk markers for atherosclerosis.

Dietary advice has been one of the cornerstones of diabetes management (Wood et al. 1986; DNSG 2023). Studies indicate that implementing energy-restricted healthy diets moderate in fat, low in saturated fat and rich in fibre, whole grains, fruit, vegetables, and legumes, along with increased physical activity, significantly supports weight management and diminishes the likelihood of type 2 diabetes onset and associated complications (Uusitupa et al. 2019). Considering the important role of diet in T2DM management, assessing the dietary patterns and adherence to nutritional recommendations of people with T2DM and exploring their correlation with both subclinical atherosclerosis and adipokines has the potential to help identify dietary factors associated with early atherosclerosis.

Numerous studies have investigated the associations between advanced atherosclerosis and the risk factors contributing to its progression, particularly in the context of atherosclerosis-related complications. However, research focusing on the early stages of atherosclerosis is much less abundant. This thesis aims to identify simple markers for detecting subclinical atherosclerosis in a general physician's office, which, in turn, can help motivate patients to adopt lifestyle modifications or seek appropriate treatment.

4. REVIEW OF THE LITERATURE

4.1 Diabetes and atherosclerosis

Diabetes is rapidly becoming a major global health challenge, with its prevalence nearing 10% and continuing to rise, provoking major CVD morbidity and mortality (IDF diabetes atlas 2021). In the absence of symptoms, the diagnostic criteria of T2DM involve fasting glucose levels ≥7.0 mmol/L, confirmed by two tests, or a 2-hour postprandial glucose level ≥11.1 mmol/L; 2-hour postprandial glucose level of 7.8–11.0 mmol/L suggests impaired glucose tolerance (WHO/IDF 2006; ADA 2022). For symptomatic diagnosis, a single random glucose level reading of ≥11.1 mmol/L suffices; without symptoms, two readings are needed (WHO/IDF 2006; ADA 2022). Pre-diabetes is defined by the World Health Organization (WHO) as fasting glucose of 6.1–6.9 mmol/L (WHO/IDF 2006) and by the American Diabetes Association (ADA) as 5.6-6.9 mmol/L (ADA 2022). Glucose level readings below these levels are considered normal glucose metabolism. Diabetes diagnosis also includes HbA1c ≥48 mmol/mol (≥6.5%) (WHO 2011), with WHO setting pre-diabetes at 42–47 mmol/mol (6.0–6.4%) and ADA at 39–47 mmol/mol (5.7–6.4%) (WHO 2011; ADA 2022). It is widely acknowledged that atherosclerosis progresses more swiftly in the presence of diabetes. However, the potential link between subclinical atherosclerosis and both emerging and traditional risk factors of CVD remains poorly documented.

Atherosclerosis is a progressive asymmetrical systemic disease that may be asymptomatic before its complications emerge. It refers to intimal calcification with focal plaques that narrow the lumen. The initial event in atherosclerosis pathogenesis is the accumulation of lipids, which triggers the initiation of an inflammatory cascade (Sakakura et al. 2013; Stary et al. 1992, 1995). Lipid deposition prompts the migration of immune cells, notably macrophages, into the arterial intima, where they undertake phagocytosis of lipids, which, in turn, results in the formation of foam cells (Sakakura et al. 2013; Stary et al. 1992, 1995). Concurrently, there is a proliferation of connective tissue and smooth muscle cells, culminating in the formation of a fibrous plaque (Sakakura et al. 2013; Stary et al. 1992, 1995). As the plaque matures, it may undergo calcification, characterised by the deposition of calcium within its structure. This calcification imparts rigidity to the plaque, enhancing its stability but compromising its flexibility (Sakakura et al. 2013; Stary et al. 1992, 1995). Consequently, the risk of rupture increases, potentially leading to life-threatening CVD events (Creager et al. 2002). The American Heart Association (AHA) has developed criteria to assess the stages of atherosclerosis (Table 1 and Figure 1).

Subclinical atherosclerosis refers to the presence of atheromatous disease in one or more arterial territories before there are any signs, symptoms, or events attributable to clinically manifest atherosclerotic disease in that territory (Simon et al. 1993). This definition relies solely on symptoms or complications rather than the histological morphology of the condition. Based on the definition, subclinical atherosclerosis includes non-ruptured plaques with mild stenosis (<50%),

which can vary in morphological types, such as pathological intimal thickening and fibroatheromas (Insull et al. 2009). It also encompasses thrombus formation following plaque rupture, plaque erosion, or calcified nodules that do not cause ischemic symptoms but result in luminal narrowing of less than 50% (Insull et al. 2009; Kawai et al. 2024). Additionally, the definition of subclinical atherosclerosis covers lesions with 50% to 99% stenosis and chronic total occlusion that is caused by progressive atherosclerosis that presents without clinical symptoms (Blaha et al. 2023; Kawai et al. 2024). When interpreting study findings, it is essential to consider the definition being used.

T2DM significantly increases the risk of atherosclerosis due to a combination of factors, such as chronic hyperglycaemia, insulin resistance, dyslipidaemia, oxidative stress, and chronic inflammation (Creager et al. 2002). These conditions contribute to endothelial dysfunction, altered lipid profiles, increased oxidative damage, and heightened inflammatory responses, all of which promote the formation and progression of atherosclerotic plaques (Creager et al. 2002). Additionally, common comorbidities like hypertension and obesity further exacerbate this risk, making CV events more likely in individuals with T2DM (Zimmet et al 1986). This multifactorial interplay significantly increases the risk of CV events in individuals with T2DM (Einarson et al. 2018; Zhou et al. 2024). However, early detection of atherosclerosis motivates patients to take proactive steps for timely interventions that have the potential to prevent or delay the progression to clinical CV events such as heart attacks, and strokes or lower limb events such as gangrene or acute limb ischaemia.

Table 1. Criteria for American Heart Association lesion classification system and correspondence with classification of gross arterial specimens (Stary et al. 1995).

AHA Grade	Criteria	Comments and Corresponding Gross Classification
0	Normal artery with or without adaptive intimal thickening; no lipid	Normal tissue
1	Isolated MFCs containing lipid; no extracellular lipid; variable adaptive intimal thickening	Initial atherosclerotic lesion, sometimes visible grossly with lipid staining
2	Numerous MFCs, often in layers, with fine particles of extracellular lipid; no distinct pools of extracellular lipid; variable adaptive intimal thickening	Fatty streak, visible grossly with lipid staining
3	Numerous MFCs with ≥1 pools of extracellular lipid; no well-defined core of extracellular lipid	Fatty plaque, raised fatty streak, intermediate lesion, or transitional lesion
4	Numerous MFCs plus well-defined core of extracellular lipid, but with luminal surface covered by relatively normal intima	Atheroma, fibrous plaque, or raised lesion

AHA Grade	Criteria	Comments and Corresponding Gross Classification
5	Numerous MFCs, well-defined core or multiple cores of extracellular lipid, plus reactive fibrotic cap, vascularization, or calcium	Fibroatheroma, fibrous plaque, or raised lesion
6	All of the above plus surface defect, hematoma, haemorrhage, or thrombosis	Complicated lesion

Abbreviations: MFC, macrophage foam cell

Note: Adapted from Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W Jr, Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation. 1995, 92(5), 1355−74.

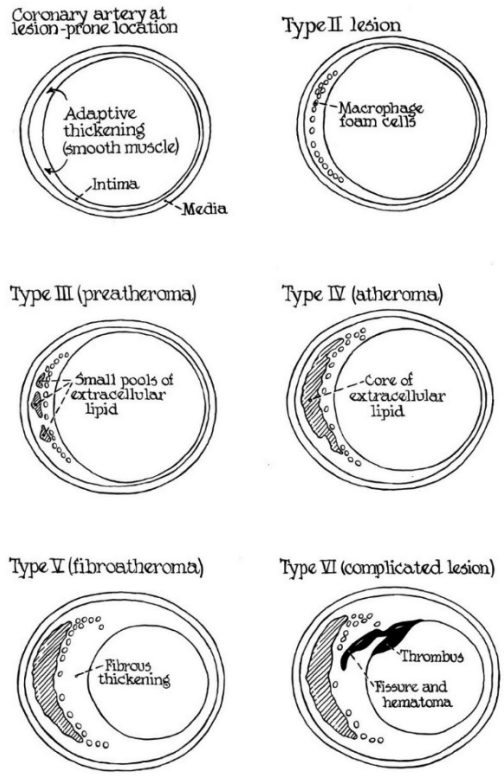

Figure 1. Intima morphology from adaptive thickening to advanced type VI lesions in atherosclerosis (Stary et al. 1995).

Note: Adapted from Stary HC., Chandler A., Dinsmore RE, Fuster V, Glagov S, Insull WJ, et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation. 1995, 92(5), 1355–1374.

4.2 Markers of subclinical atherosclerosis

Subclinical atherosclerosis precedes clinical symptoms and can be detected through functional or structural changes in arterial walls. Factors like age, inflammation, dyslipidemia, and hypertension disrupt the balance between collagen and elastin, leading to increased collagen deposition and reduced vessel elasticity (Swislocki et al. 1989; Li et al. 2023; Kawai et al. 2024). Prolonged hyperglycemia triggers harmful changes in vascular tissue by inappropriate renin-angiotensin-aldosterone system activation, nonenzymatic glycosylation of proteins and lipids, mitochondrial dysfunction, excessive oxidative stress, protein kinase C activation, platelet hyperactivity, coagulant disorders and hypofibrinolysis (Tribe et al. 1996; Aronson et al. 2002; Ageno et al 2008). All these mechanisms promote diabetic vasculopathy.

Arterial stiffness indicates functional damage and can be assessed through various methods. Pulse wave velocity measures the speed of pressure waves along arteries, while the augmentation index evaluates arterial pressure changes during systole (Townsend et al. 2015). The cardio-ankle vascular index measures arterial stiffness from the aorta to the ankle arteries with minimal dependence on blood pressure fluctuations (Shirai et al 2006). Assessment of flow-mediated dilation describes endothelial function (Raitakari et al. 2000; Saarikoski et al. 2010), while carotid intima-media thickness (CIMT) (Salonen et al. 1991) and grey-scale median of the intima-media complex are associated with atherosclerotic vessel wall structural changes (Karim et al. 2023).

Studies predominantly focus on CIMT to track disease progression. In contrast, the potential of carotid artery ultrasonography, which can measure plaque that correlates directly with CVD and stroke risk, often gets overlooked (Spence 2006). Carotid artery ultrasonography is a non-invasive yet observer-dependent method. Some studies have focused on detecting the presence of plaque, quantifying plaque size and evaluating plaque morphology (Inaba et al. 2012; Powell et al. 2024; Shore et al. 2015). These studies have linked carotid artery plaque to coronary heart disease and stroke (Bots et al. 1997; Inaba et al. 2012).

The Ankle-Brachial Index (ABI) offers a portable method for detecting lower limb arterial malperfusion due to vessel calcification, stenosis or occlusion (Winsor 1950; Aboyans et al. 2012), which, in turn, strongly predict future CVD mortality (Vogt et al. 1993). Computed tomography is used for assessing coronary and abdominal aortic calcification. Although coronary artery calcium scoring correlates with heart disease, it involves radiation exposure and specialised clinic visits (Spence 2006).

Overall, to alleviate both the clinical and economic impact of CVD in the long term, there is a need to identify safe, effective, and cost-effective approaches that can detect early atherosclerosis.

4.2.1 Ankle-brachial index

The ABI, calculated as the systolic blood pressure in the posterior tibial artery divided by that in the brachial artery (Figure 2), is a simple, non-invasive method for diagnosing PAD and a marker of lower-extremity peripheral artery atherosclerosis.

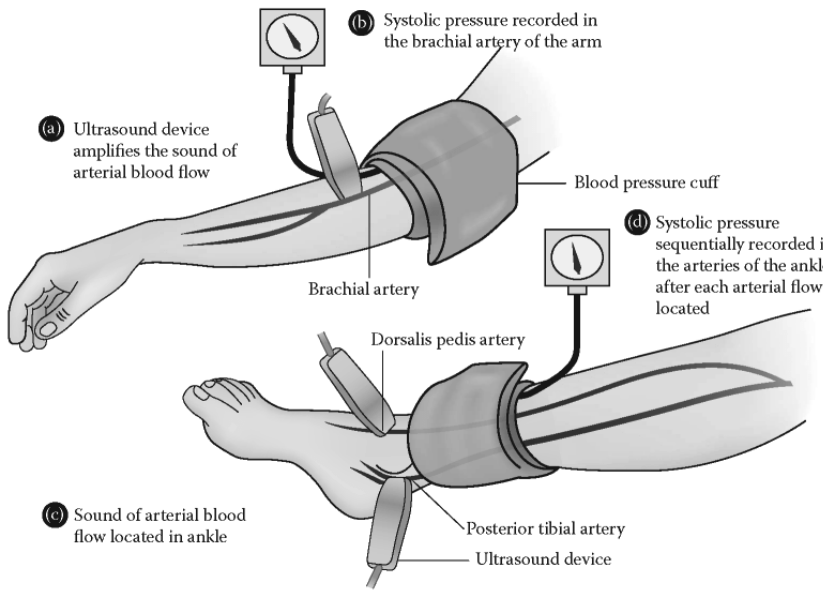

Figure 2. Measurement of ankle-brachial index.
Note: Adapted from Gilbert J, Hutt L, Ikram R, Jones P, Kyle C, Lack L et al. The ankle-brachial pressure index: An under-used tool in primary care? Best Practice Journal, 2014, 60, 21.

Measurements are performed for both the posterior tibial and dorsalis pedis arteries and depending on the purpose of the examination, the lowest (screening for lower limb PAD in asymptomatic high CVD risk populations) or the highest (to determine the severity of PAD, for pre- and post-operative assessments in the context of lower limb revascularisation and longitudinal surveillance) recorded ankle pressure is used in the calculations (Nordanstig et al. 2024). The systolic blood pressure in both arms is then measured, and the ABI is calculated by dividing the recorded ankle pressure by the highest recorded arm pressure (Nordanstig et al. 2024). Despite its potential, ABI is undervalued and underutilised in clinical practice.

Diseases attributable to atherosclerosis remain notably underdiagnosed, particularly those pertaining to the lower limb peripheral arteries, as only cases diagnosed within the confines of clinical consultations get recorded in the health

information system and health statistics and health research database. An online survey done in 2024 outlined current practices among general practitioners in England and the Republic of Ireland for diagnosing suspected PAD. The survey revealed that ABI measurement is unavailable in 25% of the practices in England and 55% of the practices in the Republic of Ireland (Konya et al. 2024). In the PARTNERS study of 6,979 participants attending primary care practices across the United States and undergoing ABI screening for PAD, 29% displayed an ABI ≤0.90, indicating PAD. Of those newly identified with PAD, 48% were asymptomatic (Hirsch et al. 2001). In the PANDORA study, which included 9,816 European participants at 'non-high' CV risk (patients with diabetes were excluded from the study), the prevalence of asymptomatic PAD (ABI ≤0.9) was 17.8% (Cimminiello et al. 2011). This suggests that a significant number of referrals rely solely on medical history and physical exams and lack formal diagnostic support. Additionally, the findings highlight missed opportunities for PAD identification in primary care due to insufficient recognition of CV risk markers and associated symptoms.

Identifying individuals at higher risk of early atherosclerosis is important for implementing more aggressive preventive treatments, potentially delaying or even preventing the onset of complications. The ABI is a valuable tool in this regard, yet its application has limitations and gaps that warrant further exploration.

The variability in ABI measurements, caused by factors such as measurement sequence, difficulty in locating the dorsalis pedis artery, and variable physical activity levels, complicates the establishment of a reliable threshold (Hiatt et al. 1995). Additionally, individual factors like body height influence ABI values, adding another layer of complexity to its interpretation (Hiatt et al. 1995). To address these challenges, further research is needed to assess the relationship between ABI values and early atherosclerosis.

The relationship between ABI and measures of subclinical atherosclerosis remains unclear. ABI values of 1.1–1.3 are considered normal, ABI values between 0.9 and 1.1 are regarded as the "grey area", and low ABI (≤0.9) values are well-established as indicators of advanced arterial occlusive disease and increased CV risk (Zheng et al. 1997). The precise ABI value that indicates the onset of early atherosclerosis is still unknown. The two-vessel criterion that incorporates measurements from both the posterior tibial and dorsalis pedis arteries has shown an increased risk of PAD with advancing age, T2DM, higher body mass index (BMI), smoking, hypertension, left ventricular dysfunction, carotid stenosis, and elevated cholesterol and creatinine levels (Hiatt et al. 1995; Newman et al. 1993). A low ABI (≤0.9) is a strong indicator of arterial occlusive disease and CVD mortality (Vogt et al. 1993). The sensitivity and specificity of diagnosing PAD with an ABI <0.9 at rest have been reported to be between 69–89% and 69–99%, respectively (Nordanstig et al. 2024). Adults without PAD typically have ABIs >1.0, while those with intermittent claudication have a mean ABI of 0.8 (Newman et al. 1993). An ABI <0.8 significantly increases the likelihood of myocardial infarction, angina, stroke or transient ischemic attack, and

congestive heart failure (Newman et al. 1993). In those aged 65 and older, lower ABIs are progressively linked to higher risks of myocardial infarction, angina (<1.0), and congestive heart failure and stroke (<0.9) (Newman et al. 1993). Some studies have proposed an ABI threshold of 0.9–1.0 (Hayashi et al. 2004) or 1.0–1.1 (Clairotte et al. 2009) as a potential marker of systemic atherosclerosis in asymptomatic patients in clinical practice. Nevertheless, the specific ABI threshold that definitively indicates early atherosclerosis remains undetermined.

ABIs exceeding 1.3–1.5 are considered abnormal, as they indicate arterial rigidity, which results in falsely high readings. Arterial medial calcification, detectable at ABI >1.30, indicates vessel wall calcification distinct from atherosclerosis and is non-occlusive. It is associated with increased pulse pressure and arterial stiffness and is common in the elderly, especially those with diabetes and end-stage renal failure (Nordanstig et al. 2024).

4.2.2 Carotid artery intima-media thickness

The arterial intima, spanning from the endothelial surface at the lumen to the luminal margin of the media, is traditionally delineated by the internal elastic lamina, the border between the intima and the media (Figure 3). However, its clarity may vary in regions of arterial geometric transitions. This variability poses challenges in demarcating the intima and the media. The intima comprises two layers, often discernible in segments with thickening (Figure 3).

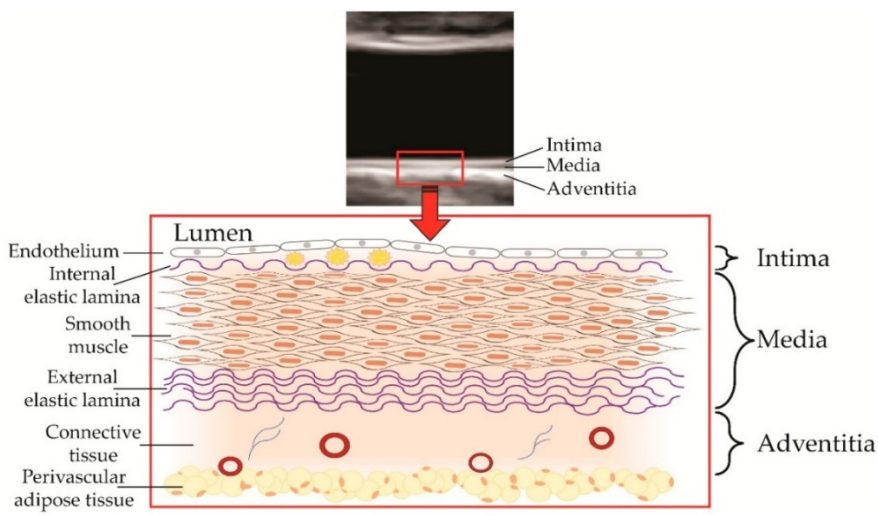

Figure 3. Components of the arterial wall (Skilton et al. 2019).
Note: Adapted from Skilton MR, Celermajer DS, Cosmi E, Crispi F, Gidding SS, Raitakari OT, Urbina EM. Natural History of Atherosclerosis and Abdominal Aortic Intima-Media Thickness: Rationale, Evidence, and Best Practice for Detection of Atherosclerosis in the Young. J Clin Med. 2019, 8(8), 1201.

The inner layer, termed the proteoglycan layer and located adjacent to the lumen, is characterised by its rich connective tissue composition and sparse elastic fibres (Stary et al. 1992). Beneath lies the musculoelastic layer where smooth muscle cells occur as widely spaced single cells rather than in layers. It is also abundant in elastic fibres and collagen (Stary et al. 1992). The tunica media comprises smooth muscle cells arranged in a circular manner, enveloped by the basal lamina and elastic fibres. Surrounding the tunica media is the external elastic membrane that has a structure akin to the internal elastic membrane.

Intima media thickening is not considered synonymous with atherosclerosis, as it can result from non-atherosclerotic processes like smooth muscle cell hyperplasia and fibrocellular hypertrophy, leading to medial hypertrophy and compensatory arterial remodelling (Spence 2006). As atherosclerosis progresses, advanced lesions initially form in select regions exhibiting adaptive thickening of the intima (Figure 4.). These regions are atherosclerosis-prone areas of arteries such as the carotid, coronary, abdominal and descending aorta, and the iliac artery (Nakashima et al. 2002).

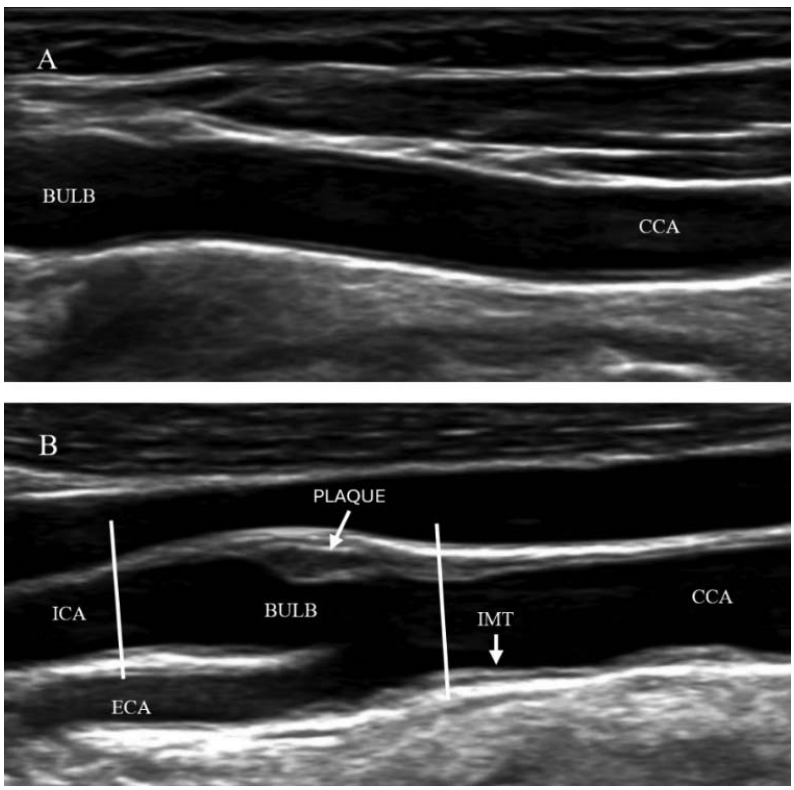

Figure 4. Carotid artery ultrasound with IMT measurement.
A – Carotid artery without atherosclerosis; B- Carotid artery with increased IMT and plaque.

A normal value of IMT is controversial, as it is sex- and age-dependent; it increases by 0.010 mm/y in CCA and ICA and by 0.020 mm/y in bifurcation (Howard et al. 1993). The European Society of Cardiology/European Society of Hypertension 2018 guideline has set 0.9 mm as the threshold of an abnormal value (Williams et al. 2018). In the scientific literature, subclinical atherosclerosis is usually defined as IMT values greater than or equal to the 75th percentile (≥0.9 or 1 mm) (Stein et al. 2008); the highest age-adjusted (97.5th) percentile of IMT is associated with a markedly increased risk of stroke and myocardial infarction (Shore et al. 2015).

Most studies utilise CCA IMT measurements due to the ease of access and the simplicity and speed of visualisation (Figure 5). However, measuring IMT in the carotid bulb has been shown to provide better discrimination of CVD presence in T2D subjects than measuring IMT in the CCA (Shore et al. 2015).

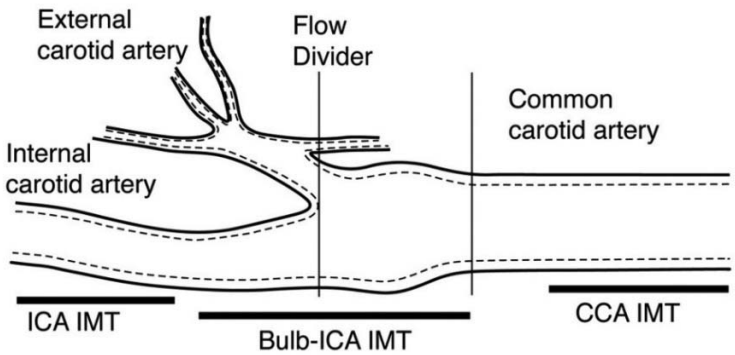

Figure 5. Localisation of measurements of carotid artery intima-media thickness (IMT). Note: Adapted from Polak JF, Person SD, Wei GS, Godreau A, Jacobs DR Jr, Harrington A, Sidney S, O'Leary DH. Segment-specific associations of carotid intima-media thickness with cardiovascular risk factors: the Coronary Artery Risk Development in Young Adults (CARDIA) study. Stroke. 2010 Jan;41(1):9–15.

4.2.3 Carotid artery plaque

Atherosclerotic plaque is defined by focal wall thickening exceeding 0.5 mm or it being 50% greater than the adjacent vessel wall, or by an IMT surpassing 1.5 mm (Catalan et al. 2015; Feng et al. 2015; Li et al. 2012; Stein et al. 2008). However, some studies have classified plaque with IMT measurements of >0.8 mm (Shore et al. 2018), >1.2 mm (Kadoglou et al. 2014) or >1.3 mm (Pitoulias et al. 2017). Notably, carotid plaque has emerged as a superior diagnostic indicator compared to CIMT in predicting future myocardial infarction events (Inaba et al. 2012). Incorporating CIMT and plaque information in intermediate-risk patients resulted in a clinical net reclassification of approximately 9.9% of the patients to a higher risk category (Nambi et al. 2010). This distinction underscores the importance of precise plaque characterisation in assessing CV risk and guiding therapeutic interventions.

4.3 Obesity and atherosclerosis

Obesity, resulting from excess nutrients and a positive energy balance, is characterised by an accumulation of adipose tissue. Obesity leads to various metabolic complications and is a significant risk factor for CVD, including atherosclerosis (Hubert et al. 1983). The transformation of white adipose tissue into a state marked by inflamed and dysfunctional adipocytes and immune cell infiltration is central to the pathology of obesity (Kawai et al. 2021). Visceral fat is more metabolically active and more prone to inflammation than subcutaneous fat, and exacerbates systemic metabolic disturbances (Harman-Boehm et al. 2007).

Obesity is closely linked to atherosclerosis, primarily through systemic inflammation and metabolic disturbances (Dandona et al. 1998). Inflamed adipose tissue, particularly visceral fat, releases pro-inflammatory cytokines and adipokines which contribute to the development of systemic inflammation. These inflammatory markers directly affect endothelial function, arterial smooth muscle cells, and macrophages, promoting atherogenesis. Obesity-induced inflammation impairs insulin signalling, leading to insulin resistance, which, together with elevated blood glucose and lipid levels, contributes to endothelial dysfunction and plaque formation (Henning 2021). Adipokines and free fatty acids from visceral fat induce oxidative stress and endothelial damage, increasing arterial stiffness, a hallmark of early atherosclerotic changes (Henning 2021). Furthermore, obesity is associated with increased sympathetic nerve activity and the activation of the renin-angiotensin-aldosterone system, promoting vasoconstriction, inflammation, and thrombosis, exacerbating atherosclerosis (Tribe et al. 1996; Aronson et al. 2002; Ageno et al 2008; Henning 2021).

Measuring obesity accurately, using clinical or imaging-based methods to differentiate between visceral and subcutaneous adipose tissue, is essential for assessing health risks and customising interventions. While BMI is used widely, it does not distinguish between fat, muscle, and bone mass, and this can lead to overestimating obesity in muscular individuals. Waist circumference and waist-to-hip ratio measurements assess central adiposity, a better indicator of visceral fat than BMI. However, neither of these measurements differentiates between subcutaneous and visceral fat. Some advanced imaging techniques, such as dual-energy X-ray absorptiometry (DEXA) which provides precise measurements of fat mass, lean mass, and bone mineral content, have low cost and low radiation compared to other imaging techniques. Multidetector computerised axial tomography (CAT) and magnetic resonance imaging provide detailed body composition and fat distribution analysis but are more costly. Some of them, including CAT, also involve higher radiation exposure.

Visceral fat tissue, the fat stored around internal organs, is associated with a higher risk of metabolic disorders and CVD (Hubert et al. 1983; Rexrode et al. 1998). Markers of visceral fat dysfunction are indicators that help identify the metabolic and inflammatory state of visceral adipose tissue, which is linked to various health risks, including atherosclerosis and metabolic disorders.

Various biochemical markers are used to assess visceral fat tissue dysfunction (Amato et al. 2014). These include adipokines, inflammatory cytokines (such as interleukin-6, tumour necrosis factor-alpha, c-reactive protein etc.), chemokines (monocyte chemoattractant protein-1), free fatty acids, lipid metabolism markers (triglycerides, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) etc.), oxidative stress markers (such as malondialdehyde, oxidised LDL etc), insulin resistance markers (homeostatic model assessment of insulin resistance, fasting insulin levels etc.) and endothelial dysfunction markers (endothelin-1, nitric oxide level etc.).

Practical strategies to reduce visceral fat and inflammation are essential for managing obesity-related atherosclerosis. It is also important to identify an easy-to-use clinical marker that can effectively assess the location and function of adipose tissue.

4.3.1 Markers of visceral fat tissue dysfunction

4.3.1.1 Visceral adiposity index

Introduced in 2010, the Visceral Adiposity Index (VAI) is a sex-specific measure that estimates visceral adiposity dysfunction by combining waist circumference, BMI, triglycerides, and HDL cholesterol into one metric. The formula for calculating VAI differs between men and women, accounting for physiological differences in fat distribution and metabolism.

A VAI of 1 is typical in healthy, non-obese individuals with normal adipose distribution, triglyceride levels, and HDL-C levels (Amato et al. 2010). In contrast, a VAI of 2.00 has been identified as the optimal threshold for detecting adipose tissue dysfunction, with a sensitivity of 65% and a specificity of 80% (Shen et al. 2024). Notably, a 1-unit increase in VAI is associated with a 44% higher risk of developing T2DM. Additionally, VAI predicts CVD incidence and mortality, with threshold values of 1.89 and 2.83, respectively (Fakhrolmobasheri et al. 2023). The index is also recommended for identifying early hypertension risk in adults (Zhou et al. 2024).

In patients with diabetes, VAI demonstrates significant correlations with various adipocytokines. It positively correlates with leptin, visfatin, and resistin while inversely correlating with adiponectin (Amato et al. 2014). These relationships underscore VAI's utility in reflecting underlying adipose tissue dysfunction and its broader metabolic implications.

A large prospective population-based cohort study highlighted a gender discrepancy in the effectiveness of VAI. The index proved to be a stronger risk marker for T2DM in women compared to traditional anthropometric and laboratory measures and was comparable to DEXA measures (Brahimaj et al. 2019). However, this correlation was not observed in men, suggesting that BMI remains a simple and useful tool for diabetes risk screening in the general male population (Brahimaj et al. 2019).

Figure 6 illustrates the comparative predictive ability of various anthropometric indices, highlighting VAI's superior ability to identify individuals with the worst adipocytokine profiles. This reinforces the index's role as a valuable tool in assessing visceral adiposity dysfunction, as it reflects nonclassical risk factors like altered adipocytokine production, increased lipolysis, and elevated plasma free fatty acids (Amato et al. 2010, 2014).

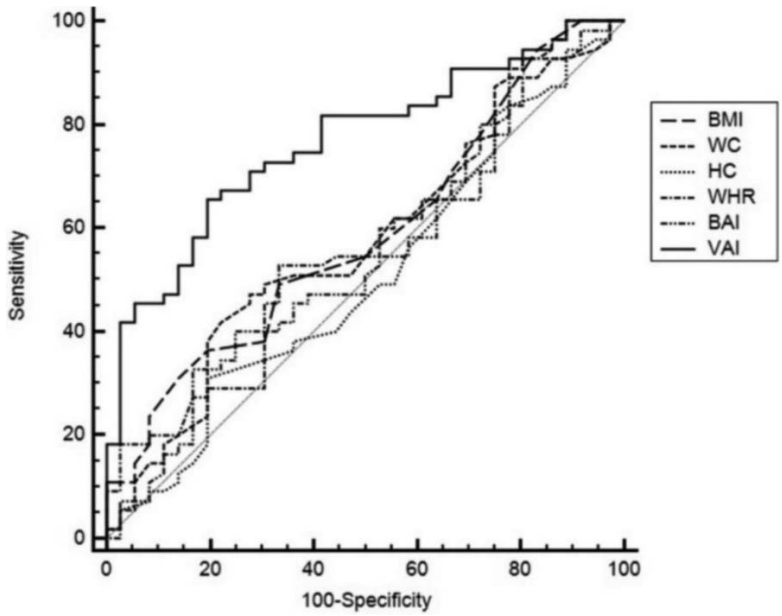

Figure 6. ROC curves of the various anthropometric indices for "hypothetical adipose tissue dysfunction" in DM (Amato et al. 2014).
BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-hip ratio; BAI, body adiposity index; VAI, visceral adiposity index
Note: Adapted from Amato MC, Pizzolanti G, Torregrossa V, Misiano G, Milano S, Giordano C. Visceral adiposity index (VAI) is predictive of an altered adipokine profile in patients with type 2 diabetes. PLoS One. 2019, (3), 91969.

4.3.1.2 Android to gynoid fat ratio

BMI has long been a standard measure for obesity, yet it lacks specificity in assessing central obesity. Advanced imaging techniques like magnetic resonance imaging and computed tomography are precise but costly (Wang et al. 2010). DEXA offers a cost-effective alternative for distinguishing between visceral and subcutaneous fat and is frequently employed in studies (Messina et al. 2020). Male (android) and female (gynoid) obesity were first differentiated by Vague in 1947 (Wang et al. 2010). Men typically store fat centrally, while women accumulate it in the gluteal and femoral regions. A higher android-to-gynoid (A/G)

fat ratio is linked to systemic inflammation (Bogl et al. 2016; Zhang 2024), prediabetes, T2DM (Manigrasso et al. 2005; Sun et al. 2022), metabolic disorders, and CV risks (Arnberg et. 2012; Ding et al. 2021). Research shows the A/G ratio correlates with visceral fat area, CCA IMT, and fatty liver disease in T2DM patients (Bouchi et al. 2016). Measuring body fat dysfunction is challenging, and no simple method exists for routine clinical use. Thus, while BMI is commonly used, more specific measures and imaging techniques offer better insights into obesity-related health risks.

4.3.2 Adipokines and atherosclerosis

Adipocyte dysfunction results in an imbalanced production of pro- and anti-inflammatory adipokines. While adiponectin and leptin are well-studied, the roles of newer adipokines such as resistin, retinol-binding protein (RBP) 4 and visfatin in the development of subclinical atherosclerosis in T2DM are not extensively researched. Few studies have explored the relationship between adipokines and subclinical atherosclerosis, dietary patterns, or adherence to nutritional recommendations, particularly regarding the inflammatory effects of food.

4.3.2.1 Adiponectin

Adiponectin, initially recognised as an insulin-sensitising adipokine, also plays a vital role in cardiovascular health due to its anti-inflammatory, anti-atherosclerotic, and cardioprotective effects (Cavusoglu et al. 2006; Gardener et al. 2012; Lawlor et al. 2005; Pischon et al. 2004; Saarikoski et al. 2010). Though primarily secreted by adipocytes, it is also produced by bone marrow, osteoblasts, fetal tissue, myocytes, and cardiomyocytes (Zhao et al. 2021). Before entering circulation, adiponectin undergoes modification into different multimers; its high molecular weight form exhibits potent biological activity (Zhao et al. 2021).

Research has shown that adiponectin is inversely associated with IMT in patients with cerebral infarction, particularly those with diabetes (Gardener et al. 2012). High plasma adiponectin levels correlate with a reduced risk of myocardial infarction in men without prior CVD, independent of traditional risk factors (Pischon et al. 2004). Conversely, lower serum adiponectin levels are linked to low ABI and PAD in haemodialysis patients, suggesting its predictive value in high-risk populations with inflammatory states (Lai et al. 2019).

However, contrasting findings reveal that while elevated adiponectin levels inversely correlate with diabetes, hypertension, and other metabolic markers in patients with stable angina, troponin-negative unstable angina, and non-ST-elevation myocardial infarction (Cavusoglu et al. 2006), they unexpectedly predict increased mortality and myocardial infarction risk over shorter follow-up periods (Cavusoglu et al. 2006). This paradoxical finding indicates that hyperadiponectinemia may not always be protective. In women without coronary heart disease, adiponectin correlated with favourable metabolic markers but did not predict coronary heart disease (Lawlor et al. 2005). Variations between studies include

the gender and race of the patients, co-existing conditions, and the timing of adiponectin assessment relative to the onset of atherosclerotic complications.

Limited research exists on adiponectin in early-stage atherosclerosis. In young, healthy adults, low adiponectin levels are linked to increased carotid IMT (Saarikoski et al. 2010). In individuals with T2DM, lower adiponectin predicts higher IMT, especially in men (Saarikoski et al. 2010). Dietary interventions, such as Mediterranean diets, have been shown to improve adiponectin levels and lipid profiles, suggesting their benefits for managing inflammatory states (Daidone et al. 2024). These findings suggest potential benefits of managing inflammatory states with dietary counselling.

Even less research has focused on the effects of CV drugs on adiponectin levels. However, existing studies indicate that atorvastatin, pravastatin, fenofibrate, niacin, ACE inhibitors, and ARBs increase adiponectin levels, whereas simvastatin and beta-blockers have been found to decrease them (Tian et al. 2010; Wanders et al. 2010; Otsuka et 2009).

Adiponectin's multifaceted role in CV health highlights its complex interplay with metabolic pathways and disease outcomes. While high adiponectin levels initially appear protective, further research is crucial to unravel its nuanced associations across diverse patient populations and disease states. Understanding these complexities is essential for developing targeted therapeutic strategies for mitigating CV risk in high-risk individuals.

4.3.2.2 Leptin

Leptin, the first hormone identified as being produced by adipocytes, regulates food intake and energy expenditure by acting on the hypothalamus (Wang et al. 2010). Synthesised mainly by the subcutaneous white adipose tissue (Van Harmelen et al. 1998), leptin levels in adipose tissue and plasma reflect the mass and size of adipocytes (Suárez-Cuenca et al. 2021; Yannakoulia et al. 2003). During fasting, decreased leptin levels trigger increased appetite and reduced energy expenditure (Mattu et al. 2013), and in individuals with obesity, a relative leptin deficiency after weight loss can lead to weight regain (Sadaf Farooqi et al. 2007). Leptin also affects brain regions that control the cognitive and emotional aspects of eating behaviour (Sadaf Farooqi et al. 2007).

Individuals with obesity often exhibit high circulating leptin levels due to central leptin resistance, contributing to obesity (Lee et al. 2014). The mechanisms of hyperleptinaemia in obesity are complex and involve changes in leptin expression, mutations, and impaired transport across the blood-brain barrier (Obradovic et al. 2021; Vilariño-García et al. 2024). High leptin levels are linked to adverse CV outcomes (Puurunen et al. 2017), including low ABI in hypertensive and dialysis patients (Huang et al. 2017), and predict major cardiac events in coronary artery disease patients (Puurunen et al. 2017). However, studies on leptin and CV outcomes often lack detailed body composition data and gender-specific analyses.

The relationship between leptin and early atherosclerosis is inconsistent. In healthy postmenopausal women, leptin levels did not correlate with CIMT (Karim et al. 2023), but in overweight and obese individuals, leptin positively correlated with IMT (Chen et al. 2017). This relationship has been further supported by studies indicating that plasma leptin concentrations are independently associated with CCA IMT in healthy subjects (Ciccone et al. 2001). The discrepancies between the studies stem from the methods used to measure CCA IMT and the atherosclerosis status of the participants (Karim et al. 2023).

In obese patients with T2DM, post-bariatric surgery reductions in IMT were associated with decreases in visceral fat and leptin levels, indicating leptin's role in vascular health improvement (Csongrádi et al. 2017). Leptin correlated with markers of platelet hyperactivity, hypercoagulability, and IMT, regardless of the presence of atherosclerosis (Csongrádi et al. 2017). A multicentre randomised controlled trial demonstrated that an intensive weight loss intervention, including the Mediterranean diet and physical activity, significantly reduced leptin levels compared to the control group (Salas-Salvadó et al. 2019).

Fenofibrate and niacin therapy decrease leptin levels, whereas carvedilol increase leptin concentrations. In contrast, bisoprolol and ACEI did not affect leptin levels (Kovacić et al. 2008; Wanders et al. 2010; Eriksson et al. 2020). These findings emphasise the importance of personalised approaches, including sex, metabolic health heterogeneities, medication use, in managing metabolic and CV disorders related to leptin and obesity.

4.3.2.3 Resistin

Resistin is primarily produced by visceral white adipose tissue and inflammatory cells such as macrophages and monocytes and it functions as a pro-inflammatory adipokine (Niaz et al. 2019). Like other adipokines, resistin levels have sex-associated differences, with plasma resistin levels being higher in women than men (Reilly et al. 2005; Yannakoulia et al. 2003). Resistin plays a significant role in the pathogenesis of obesity-related insulin resistance, as increased resistin levels induce central leptin resistance, worsening disruptions in glucose and lipid metabolism (Asterholm et al. 2014; Gerber et al. 2005; Gharibeh et al. 2010). Accordingly, plasma resistin concentrations are higher in obese than in non-obese patients with type 2 diabetes (Gharibeh et al. 2010).

Studies have demonstrated a progressive increase in serum resistin levels in patients with hypertension and coronary artery disease (Niaz et al. 2022). In healthy nondiabetic subjects with a family history of premature coronary atherosclerotic disease (CAD), resistin correlated with inflammatory markers, coronary atherosclerosis, and metabolic syndrome, but not insulin resistance (Reilly et al. 2005). Higher serum resistin levels are linked to greater severity of coronary heart disease, with elevated levels observed in patients with unstable angina or acute myocardial infarction compared to those with stable angina (Zhang et al. 2017). Elevated serum resistin levels independently predict lower limb PAD in hypertensive patients and chronic kidney disease (Hsu et al. 2017; Ng et al. 2021).

Research results vary due to differences in the characteristics of studied populations, such as weight, BMI, race, age, gender distribution, sample size, comorbidities, and medication usage. While variations in CV medication use may influence research outcomes, antihypertensive treatments such as amlodipine, bisoprolol, and indapamide demonstrate a capacity to lower resistin levels (Skoczylas et al. 2016). In contrast, statins appear to have no significant impact on resistin levels, highlighting a distinction in how these medications affect metabolic markers (Kadoglou et al. 2021).

There is limited research on the early phase of atherosclerosis. In a Finnish middle-aged population-based cohort, resistin was associated with proatherogenic inflammatory markers but did not have an independent association with early atherosclerosis (Kunnari et al. 2006). In psoriatic patients, resistin serum levels were associated with diabetes and metabolic syndrome and correlated with total cholesterol and triglycerides but not with CIMT (Schlenker et al. 2023). In children with idiopathic growth hormone deficiency, no correlation was found between CIMT, body composition parameters, and resistin (Saygılı et al. 2023). Additionally, no significant correlations were observed between serum resistin concentrations and total energy or macronutrient intake (Yannakoulia et al. 2003). These findings highlight the complex role of resistin in metabolic and cardiovascular health, emphasising the need for further research to understand its mechanisms and potential therapeutic targets fully.

4.3.2.4 Retinol binding protein 4

Retinol-binding protein 4 (RBP4) traditionally serves as a transporter of retinol from the liver to the peripheral tissues (Fan et al. 2024; Stefan et al. 2007). While primarily secreted by the liver, RBP4 is also produced by adipocytes, classifying it as an adipokine (Newcomer et al. 2000). Its role in obesity has yielded mixed results. For example, a study of Chinese subjects found a positive correlation between serum RBP4 levels and visceral adipose tissue area (Jia et al. 2007), while a German study reported no significant association with total body fat or visceral fat (Stefan et al. 2007). These discrepancies might be attributed to differences in measurement methods, sample sizes, and ethnic backgrounds.

The association between RBP4 levels and T2DM is contentious. In morbidly obese patients, those with metabolic syndrome or T2DM had higher RBP4 levels compared to euglycaemic subjects; RBP4 levels correlated with triglycerides but not insulin resistance (Rocha et al. 2013). RBP4 levels increase with the duration of T2DM (Jia et al. 2007; Tan et al. 2024). RBP4 is implicated in β-cell dysfunction and insulin resistance, showing an inverse correlation with insulin sensitivity (Broch et al. 2007; Stefan et al. 2007). A 2024 meta-analysis confirmed that higher RBP4 levels are associated with an increased risk of T2DM (Broch et al. 2007; Stefan et al. 2007).

RBP4 has emerged as a potential predictor of CVD. Higher serum RBP4 levels are found in patients with CAD than in those without it (Lambadiari et al.

2014). High RBP4 levels are independently associated with symptomatic PAD requiring revascularisation and established carotid atherosclerosis, correlating with the severity of the condition (Kadoglou et al. 2014, 2021). In T2DM patients, higher RBP4 levels were observed in those with increased CIMT and carotid artery plaques (Feng et al. 2015). In postmenopausal women, both low and high RBP4 levels were linked to coronary artery calcification; however, no correlation with CIMT was found (Huang et al. 2012). Research on the effects of cardiovascular medications on RBP4 is minimal, as only lipid-lowering drugs reduce RBP4 serum levels (Wanders et al. 2010).

Gender differences in RBP4 levels further complicate its role in metabolic and CVDs, with men generally exhibiting higher levels than women (Jia et al. 2007; Ribel-Madsen et al. 2009). The conflicting study results highlight the need for standardised measurement techniques and consideration of population-specific factors. Despite the inconsistencies in the results of existing studies, emerging evidence suggests that RBP4 is a significant marker in metabolic and CV health, warranting further research to elucidate its biological functions and therapeutic potential.

4.3.2.5 Visfatin

Visfatin, also known as pre-B-cell colony-enhancing factor or nicotinamide phosphoribosyltransferase (NAMPT), is an adipokine with multifaceted roles in metabolic regulation and CV health (Chen et al. 2006). Visfatin is predominantly secreted by macrophages in visceral white adipose tissue and is also expressed by foam cells and macrophages in unstable atherosclerotic lesions (Curat et al. 2006; Dahl et al. 2007).

Elevated levels of vistatin are observed in individuals with obesity, metabolic syndrome, insulin resistance, and T2DM (Abu-Farha et al. 2014; Chen et al. 2006). However, the relationship between visfatin and insulin resistance is complex; some studies have not confirmed their direct association (Pagano et al. 2006; Zhong et al. 2008). This variability suggests that further research is needed to elucidate the precise role of visfatin in insulin resistance.

Visfatin is associated with inflammation, endothelial dysfunction, and increased CIMT in patients with diabetes, linking it to atherosclerosis (Vanhoutte 2009; Kadoglou et al. 2010). It contributes to the development and instability of atherosclerotic plaques (Dahl et al. 2007), with elevated levels correlating with myocardial damage (Lu et al. 2012) and plaque instability in metabolic syndrome patients (Zhong et al. 2008). Visfatin is a novel biomarker for atherosclerosis. Understanding the factors that affect visfatin levels is essential for its use as a diagnostic marker.

Some studies hint at variable drug-specific effects on visfatin levels. Simvastatin and rosuvastatin reduce visfatin levels, while atorvastatin does not (Friebe et al. 2011; Krysiak et al. 2014; Kostapanos et al. 2008). Metformin shows limited effects (Kong et al. 2015). Antihypertensives like losartan increase visfatin levels, whereas ramipril does not (Derosa et al. 2011). These intra- and inter-drug group

differences highlight the need for further investigation into how commonly used drugs modulate visfatin levels.

Diet can influence inflammatory activity and the levels of adipokines. Dietary interventions such as Mediterranean and vegetarian diets significantly reduce visfatin levels, demonstrating their beneficial impact on metabolic health (Daidone et al. 2024). The composition and timing of meals also affect visfatin levels. Diets high in fat or carbohydrates modulate daily profiles of visfatin secretion. Specifically, eating a high-fat meal as the first meal of the day and a high-carbohydrate meal later in the day has been shown to reduce visfatin levels, as opposed to eating a high-carbohydrate meal first and a high-fat meal later. This indicates the importance of dietary composition and timing in visfatin regulation (Kessler et al. 2018). Diets rich in saturated fatty acids (SFAs) increase visfatin levels, while diets rich in monounsaturated fatty acids (MUFAs) decrease them (Haghighatdoost et al. 2012). Although the statistical significance of these findings has not been achieved due to small sample sizes, these studies suggest that fatty acid composition in the diet plays a role in visfatin regulation.

4.4 Diet and atherosclerosis

4.4.1 Dietary guidelines for managing type 2 diabetes

The European Association for the Study of Diabetes (EASD) recommends a variety of foods and dietary patterns for effective diabetes management, aligning closely with general population guidelines (Aas et al. 2023). Patients with diabetes should receive nutrition counselling to achieve personalised goals, such as optimising glycaemic control, managing weight, and improving cardiovascular health (Evert et al. 2019). Dietary approaches like Mediterranean or plant-based diets, rich in unsaturated fats, are recommended to reduce CV risk (Aas et al. 2023; Marx et al. 2023; Salas-Salvadó et al. 2019). The Mediterranean diet, supplemented with olive oil or nuts, has been shown to reduce atherosclerotic CV risk significantly (Estruch et al. 2018). Transitioning to a plant-based diet also lowers atherosclerotic CV risk (Qian et al. 2019).

Meta-analyses indicate that weight-loss diets, such as low-carbohydrate, high-protein, and vegetarian diets, effectively manage weight in T2DM if they meet nutritional guidelines (Churuangsuk et al. 2021). Adhering to specific dietary recommendations is important for improving metabolic control. A range of carbohydrate intakes is acceptable if fibre, sugar, saturated fat, and protein guidelines are met, but very low carbohydrate diets, like ketogenic diets, are not recommended (Aas et al. 2023; Korsmo-Haugen et al. 2019). Fibre intake should be at least 35 grams per day, as this enhances glycaemic control and reduces cardiovascular mortality (Evert et al. 2019; Reynolds et al. 2020).

Dietary fats should come mainly from plant-based sources, with saturated fats making up less than 10% and trans-fatty acids less than 1% of total energy intake (Aas et al. 2023). Replacing saturated fats with monounsaturated or

polyunsaturated fats leads to favourable lipid profile changes (Mensink 2016). Higher intakes of saturated and trans fats are associated with an increased risk of coronary heart disease, T2DM, and mortality (Reynolds et al. 2022). Protein intake recommendations vary by age, body type, and kidney function (Aas et al. 2023). High-protein diets effectively reduce cardiovascular risk factors in overweight and obese individuals. The source of protein matters, as plant protein has been shown to improve glycaemic control (Evangelista et al. 2021; Viguiliouk et al. 2015).

To lower blood pressure and reduce CV risk, the WHO advises limiting sodium intake to less than 2 grams per day (WHO 2018). EASD and ESC guidelines support this recommendation (Aas et al. 2023; Marx et al. 2023; Gornik et al. 2024). Customising diets to include Mediterranean or plant-based approaches, optimising protein intake, and controlling sodium levels are important strategies for enhancing metabolic health and reducing CV complications.

Scientific literature has paid little attention to nutritional guidelines among people with T2DM. A study in Poland found that patients with T2DM frequently exceed recommended levels of saturated fatty acids, dietary fibre, and sodium (Gętek-Paszek et al. 2020). Similarly, a Malaysian study showed inadequate protein intake among patients, around 30% exceeding the recommended levels. Ideally, dietary practices for individuals with T2DM should mirror those of healthy individuals, but a significant percentage of T2DM patients exceed recommended protein and fat intake limits.

Scientific literature often overlooks how well people with T2DM follow nutritional guidelines. Studies in Poland and Malaysia found that patients often exceed the recommended levels of saturated fats, fibre, sodium, protein, and fat (Chieng Tan et al. 2015; Gętek-Paszek et al. 2020). Ideally, their diets should mirror those of healthy individuals, but many exceed these limits.

Assessing micronutrient status and their role in diabetes and its complications remains uncertain. The European Food Safety Authority has set dietary reference values to indicate the nutrient intake needed for health in otherwise healthy individuals or populations (EFSA 2017). However, despite evidence of increased oxidative stress in diabetes (Tribe et al. 1996), research has not demonstrated clear benefits from antioxidant vitamins, leaving healthcare providers unsure about recommending micronutrient supplements for diabetic patients (Hasanain et al 2002; Mooradian et al 2006).

4.4.2 Dietary assessment methods

Accurately evaluating dietary intake is crucial in metabolic research, yet obtaining precise dietary information from subjects is challenging. The three most commonly used methods are dietary records (Ferrari et al. 2002; Kessler et al. 2018; de Luis et al. 2008; Öztürk et al. 2023; Yannakoulia et al. 2003), 24-hour recall (24HR) (Thompson et al. 1994), and food frequency questionnaires (FFQs) (Gętek-Paszek et al. 2020; Salas-Salvadó et al. 2019).

In dietary record methodology, participants fill in a document with a detailed overview of their food and beverage consumption (Thompson et al. 1994). Quantification may involve using scales, household measuring utensils, or estimations based on portion-size guidelines. Trained personnel provide detailed instructions, and the collected food intake data will be analysed. Advantages of the dietary record method include accurate intake quantification, elimination of recall bias, facilitation of self-monitoring to affect behavioural changes, and provision of data on typical meal and food patterns (Thompson et al. 1994). However, it also presents disadvantages, including high subject burden, elevated staff costs and workload, potential alteration of eating behaviours, literacy requirements, and the need for multiple records over several months to capture habitual intake (Thompson et al. 1994).

For the 24HR method, subjects report all foods and drinks consumed in the past 24 hours via telephone or face-to-face interview, with trained staff prompting for details such as cooking methods and portion sizes (Tooze et al. 2012). Benefits include quantified intake, lower subject burden and no impact on eating behaviours. Downsides include high staff costs, reliance on subject memory, and the need for multiple recalls over months to capture habitual intake (Tooze et al. 2012).

The food frequency questionnaire assesses food and beverage consumption over a specific period of time and takes portion sizes and cooking practices into account (Thompson et al.1994). Nutrient intake is calculated using reported frequencies, typically over a period of 6 to 12 months. Advantages of the FFQ include a low burden on participants and staff, a lack of influence on eating behaviours, and the ability to capture habitual intake. Disadvantages include reliance on memory, approximate quantification, lack of meal pattern details, and unsuitability for short-term assessments (Thompson et al. 1994).

Despite the potential reporting bias that comes with the nutritional intake assessment approaches, only a minority of studies have implemented strategies for excluding under- or over-reporters from their study cohorts (Johnson et al. 1994). However, some studies have implemented relevant strategies to identify these individuals and ensure data accuracy (Ejima et al. 2019; Johnson et al. 1994). Energy misreporting is more prevalent among overweight or obese individuals compared to those of normal weight, and diabetes patients tend to underreport even more than individuals with obesity (Burger et al. 2012). In contrast to men, underreporting of energy intake in women is negatively correlated with body fat percentage (Johnson et al. 1994).

Common methods for excluding under- and over-reporters from data analysis include the doubly labelled water (DLW) method (Ferrari et al. 2002) and the Goldberg method, which uses the ratio of reported energy intake and total energy expenditure (Black 2000; Burger et al. 2012). The DLW method measures average energy expenditure over 3–21 days without restricting movement, offering a more accurate estimate of habitual energy expenditure. However, the DLW method is costly and lacks detail on activity patterns. The Goldberg method

identifies under-reporters as those below the 95% confidence interval of the total energy intake and total energy expenditure ratio.

A large DLW study showed that the Goldberg method might misclassify the reporting status of some individuals (Tooze et al. 2012). The Goldberg method had a sensitivity of 92% and a specificity of 88% for the FFQ, and a sensitivity of 50% and a specificity of 99% for 24HR. The positive and negative predictive values were similar for both FFQ and 24HR (Tooze et al. 2012). Applying the Goldberg cutoffs for self-reported energy intake estimates in nutrition studies has been shown to decrease the bias to a greater extent than weight and waist circumference, and the method appears suitable for studying patients with T2DM (Ejima et al. 2019).

5. STUDY RATIONALE

Atherosclerosis progresses faster in patients with diabetes and is the leading cause of morbidity and mortality in this patient group. However, early detection and aggressive treatment of atherosclerosis in these high-risk patients have the potential to improve health outcomes, prevent premature mortality, and reduce the economic burden on the medical system.

Although carotid artery ultrasound is reliable for early identification of arterial wall changes, it requires specialised equipment and expertise, making it less accessible for routine use in primary healthcare settings. Therefore, there is a need to identify simpler and more accessible methods for assessing the risk of atherosclerosis.

The ABI has emerged as a practical alternative for assessing systemic advanced atherosclerosis. Few studies have assessed ABI for detecting subclinical atherosclerosis. Some individual studies have suggested potential thresholds for abnormal ABI but research in T2DM patients has presented varied and wide-ranging values.

Overweight and obesity are major risk factors for the development of T2DM and atherosclerosis. The increasing prevalence of overweight and obesity correlates strongly with the incidence of T2DM. Adipose tissue acts as an endocrine organ, producing adipokines that influence atherosclerosis. Adiponectin exhibits anti-atherogenic properties by enhancing lipid metabolism and insulin sensitivity. Leptin regulates body weight, and leptin resistance in T2DM correlates with inflammation and atherosclerosis. Resistin promotes vascular inflammation and plaque destabilisation and correlates with cardiovascular disorders. RBP-4 indicates endothelial dysfunction and correlates with carotid atherosclerosis in newly diagnosed T2DM patients. Visfatin, though less studied, has been observed at higher levels in T2DM, obesity, and atherosclerosis. However, the results of some studies on visfatin are conflicting. Some have found no association between visfatin levels and CIMT in T2DM patients, while others have described lower visfatin levels in obese patients without T2DM than in obese patients with T2DM. The latter suggests that visfatin may be more closely linked to diabetes than to obesity.

The search for effective biomarkers of early CVD has been challenging. Adipokines have been explored as potential suitable biomarkers. There is a lack of research examining changes in adipokine concentrations and their predictive value in identifying subclinical atherosclerotic disease in T2DM patients without previously diagnosed atherosclerotic changes. Furthermore, most of the existing research has not considered drug use.

There is a lack of studies supporting the routine measurement of adipokines for screening early diabetic complications in patients with T2DM who are already receiving CV-oriented combination therapies. Moreover, the impact of medication on circulating adipokine levels has mostly been investigated in isolation for specific drug groups.

Associations between adipokines and atherosclerosis have primarily been studied in advanced stages of atherosclerosis. Identifying a blood-based marker that correlates with early-stage atherosclerosis is important for implementing preventive strategies and initiating timely treatment.

Since determining adipokine concentration is currently not used in daily clinical work, one of the goals was to find factors that should be considered when using new adipokines for diagnostic purposes. Previous studies have shown the effect of statins and some antihypertensive drugs on visfatin concentration. Variations have been noted both between and within drug classes, and it is likely that these variations can be attributed to differences in pharmacokinetics. Some studies have offered thresholds for adipokines for acute myocardial infarction and unstable angina, but no thresholds have been suggested for subclinical atherosclerosis. Additionally, it is important to identify factors influencing the association between circulating adipokines and subclinical atherosclerosis.

There is a recognised link between diet and low-grade inflammation. Lifestyle factors such as a healthy, balanced diet have been shown to reduce inflammation. Especially the Mediterranean diet has demonstrated anti-inflammatory effects. Since diet forms the cornerstone of diabetes management, it is important to understand the dietary patterns of individuals with diabetes and the association of these patterns with early signs of atherosclerosis.

Optimal nutrition for T2DM aims to enhance glycaemic control through weight management. The ideal carbohydrate intake for maintaining health in T2DM remains uncertain due to varying study approaches and recommended percentage ranges. Dietary fats, particularly essential fatty acids, are contentious in CV risk. Fat is important for energy production, transport of lipid-soluble vitamins, and overall physiological function. Higher total fat intake has been linked to an increased risk of all-cause mortality. Monounsaturated and polyunsaturated fatty acids (MUFAs and PUFAs) have shown protective effects against CVD and mortality. In contrast, saturated fatty acids (SFAs) and trans fats are associated with higher CVD risk.

Dietary intake, whether inadequate or excessive, significantly influences adipokines, thereby impacting the development of atherosclerosis. However, research on how nutritional factors affect serum adipokine levels in T2DM is notably limited. Therefore, assessing the dietary habits of individuals with T2DM, their adherence to general nutritional guidelines, and the influence of dietary adherence on the relationship between adipokines and subclinical atherosclerosis could fill the gap in the literature.

6. AIMS OF THE STUDY

The aim of the thesis was to identify factors and markers that predict subclinical atherosclerosis in individuals with type 2 diabetes.

The specific objectives of the present thesis:

1. To evaluate the predictive ability of the ankle-brachial index for carotid artery subclinical atherosclerosis in T2DM patients without previously diagnosed atherosclerosis (Paper I).
2. To find if associations exist between circulating adipokines and carotid artery subclinical atherosclerosis in individuals with T2DM (Paper III).
3. To assess adherence to general nutritional recommendations in individuals with T2DM (Paper IV).
4. To determine whether an imbalance in the intake of micronutrients influences the risk of subclinical atherosclerosis (Paper II).
5. To identify factors influencing the association between circulating adipokines and carotid artery subclinical atherosclerosis (Papers III and IV).

7. SUBJECTS AND METHODS

7.1 Study subjects

A population-based cross-sectional multicentre study was conducted in Estonia from November 2014 to March 2017. Two hundred sixteen participants diagnosed with T2DM were included. The patients were recruited from 13 general practitioner practices in Tartu County. Adult patients aged 30 to 70 years with T2DM who met the study criteria were invited to participate in the study by their own general practitioners. T2DM was defined according to the WHO 2006/2011 recommendations as having a fasting plasma glucose level ≥ 7.0 mmol/l, a 2-hour plasma glucose level ≥11.1 mmol/l, or an HbA1c ≥6.5% (WHO 2006, 2011). Eligible subjects had T2DM, were able to visit outpatient clinics independently, had never been diagnosed with atherosclerosis or its complications, and weighed less than 140 kg (the upper limit of the DEXA). The exclusion criteria included other types of diabetes, pregnancy or lactation, history of coronary artery, carotid artery, lower limb peripheral artery, or CVD, and background of chronic inflammatory disease, malignant tumour, or other severe illness. The research adhered to the principles of the Declaration of Helsinki and its subsequent amendments. The study was approved by the Research Ethics Committee of the University of Tartu (protocol number: 223/T-17; 25 February 2013).

7.2 Methods

7.2.1 Medical interview

Papers I–IV: Participants were scheduled for morning appointments after a minimum of 10 hours of fasting. During the appointment, they provided written informed consent and attended individual face-to-face interviews. Information on medical history, sociodemographic traits, smoking habits, and current medication was gathered using a non-validated questionnaire. A general practitioner verified the participants' medical histories and current prescription medication usage through the national electronic health information and prescription system. The analysis only considered medications dispensed through pharmacies.

7.2.2 Physical examination

Papers I-IV: Height, weight, and waist and hip circumference of the participants wearing normal light indoor clothing without shoes were measured. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m2). Blood pressure was measured using a mercury sphygmomanometer after the patient had been sitting for at least 5 min. The mean of three consecutive measurements was used for analysis.

7.2.3 Laboratory analysis

Papers I–IV: Peripheral venous blood samples were collected for analysis of serum concentrations of HbA1c and high-sensitivity C reactive protein (immunoturbidimetric method) and total cholesterol, triglycerides, LDL-C, HDL- C (enzymatic colourimetric assay) according to standard methodology in SYNLAB laboratory diagnostic services. Blood samples underwent centrifugation to separate the serum, which was subsequently stored at -80°C until required for analysis.

Papers III–IV: A comprehensive panel of metabolic markers, including high molecular weight (a large multimer of 12–18 subunits) adiponectin, leptin, RBP-4, resistin and visfatin, were quantified utilising Luminex xMAP® technology, manufactured by Luminex Corporation. SYNLAB laboratory diagnostic services were used. Non-HDL-C was then calculated from the lipid profiles (total cholesterol minus HDL-C).

7.2.4 Dietary assessment and the identification of misreporting of energy intake and the handling of data from under-reporters

Papers II and IV: All study participants underwent a 24-hour dietary recall session conducted by the investigator. The NutriData food composition database and dietary analysis software was utilised to incorporate the average nutritional composition of foods commonly consumed in Estonia (Nutridata 2024). Considering various cooking methods, the software translated this information into 60 nutrient equivalents.

Papers II and IV: As overall nutrient intake is closely correlated with the amount of energy consumed, we used implausibly low energy intake to indicate a general under-reporting of food eaten. Under-reporters were excluded from the analysis to reduce bias. The Goldberg cut-off method was applied (Black 2000). Reported energy intake (EIrep) was derived from the 24-hour dietary recall. The Katch-McArdle Formula [BMR = 370 + 21.6 x weight x (100 - body fat)/100] was used to calculate the estimated basal metabolic rate (BMRest). In this formula, bodyweight is reported in kilograms and body fat is reported as a percentage. Body fat was measured with a Lunar Prodigy Advance Dual Energy X-Ray absorptiometer (GE Healthcare, USA). As energy intake should be equal to energy expenditure (Black 2000), the ratio of EIrep and BMRest was used to indicate the physical activity level (PAL = EIrep:BMRest). The International Physical Activity Questionnaires (IPAQ) were used to assess the subjects' individual physical activity. The physical activity level was calculated as a continuous variable (metabolic equivalent of task minutes in a week) and then reported in categories as a "low", "moderate", or "high" activity level (IPAQ Research Committee 2005). The IPAQ-based categories were converted to PAL categories (low, moderate, and high activity as PAL values of 1.4, 1.6 and 1.8, respectively). Thresholds (95% lower and upper confidence limit) for each physical activity category by age were calculated. For individuals aged 16 to 70, PAL ranges of

0.872 to 2.249, 0.996 to 2.570, and 1.120 to 2.892 were established for low, moderate, and high activity levels, respectively (EFSA Panel 2013). Individual values of PAL were calculated and compared with those of the corresponding activity category. Subjects with EIrep: BMRest below the lower limit of individual PAL were classified as under-reporters. Subjects with EIrep:BMRest between the lower and upper limits were classified as plausible reporters. Subjects with an EIrep:BMRest ratio above the upper limit were classified as over-reporters.

7.2.5 Measurement of the Ankle–Brachial Index

Papers I and III: The technique laid out in a scientific statement from the American Heart Association was used to measure ABI (Aboyans et al. 2012). An Atys Microflow S 8 MHz Doppler device (Atys Medical, 17, Parc d'Arbora, 69510, Soucieuc en Jarrest, France) was used to locate the arterial pulse after the patient had rested in the supine position for at least 10 min. A blood pressure cuff was inflated proximal to the artery under investigation. Two systolic pressure measurements were obtained from each arm and ankle, and the average value of the two measurements was recorded for both sides. The straight wrapping method was used to place the cuff around the ankle. The lower edge of the cuff was placed 2 cm above the superior aspect of the medial malleolus. To calculate ABI, the highest ankle blood pressure (dorsalis pedis or posterior tibial artery) was divided by the highest blood pressure in the arms (right or left). For statistical analysis, we used the highest ABI for each side. Lower limb PAD was defined as ABI values below 0.9.

7.2.6 Measurement of Carotid Artery Intima–Media Thickness

Papers I-IV: The measurements were performed with a single sonographer, using a high-resolution B-mode tomographic ultrasound system (Philips Affiniti 70 G, Philips Healthcare, USA) with a linear 12 MHz transducer. For an overview of the vessel orientation, plaque and surrounding structures, a transverse scan was taken of the proximal part of the CCA throughout the bulb to the distal ICA and the external carotid artery. Longitudinal scanning techniques were used to create two distinct parallel echogenic lines representing the intima and media layers. Measurements were taken from anterior, lateral, and posterior imaging planes in the CCA (10 mm proximal), bulb and ICA (5–10 mm from the bulb) in plaque-free areas. In each section of the carotid artery, three measurements were taken manually at a lower resolution (i.e., the zoom function was not used) from the far wall on both sides of the artery (54 measurements per patient). Images and cine-loops were obtained using a three-lead electrocardiogram and saved as dynamic sequences using Digital Imaging and Communications in Medicine (DICOM) for offline analysis with a RadiAnt DICOM Viewer. All recordings were reviewed and measured by the same expert. The intra-observer variability for measuring IMT was 3.5% (according to Bland–Altman analysis). Based on the American

Society of Echocardiography and Mannheim Carotid Intima–Media Thickness and Plaque consensus statement, subclinical atherosclerosis was defined as IMT ≥1 mm, and the presence of plaque was defined as focal wall thickening that was at least 50% greater than the surroundings or as focal regions with IMT greater than 1.5 mm (Stein et al. 2008; Touboul et al. 2012). The presence of plaque was recorded on a "yes or no" basis. Atherosclerosis was defined as IMT ≥1 mm or the presence of plaque.

7.2.7 Measurement of the android to gynoid tissue fat percent ratio

Paper III: A Lunar Prodigy Advance dual-energy X-ray absorptiometry machine (GE Healthcare, Waukesha, WI, USA) was used to evaluate the percentages of android and gynoid fat. All scans were performed in accordance with the manufacturer's recommended positioning, and all measurements were carried out by a qualified and experienced technician. The regions of interest for regional body composition were defined using the software provided by the manufacturer. The android fat to gynoid fat ratio (A/G) was calculated as android fat divided by gynoid fat. An A/G ratio of more than one was defined as a risk factor.

7.2.8 Visceral adiposity dysfunction calculation

Paper II: Visceral adiposity indices (VAI) were calculated by the following formulas:

$$\text{Males: VAI} = (\text{WC}/\,[39.68 + (1.88 \times \text{BMI})] \times (\text{TG}/1.03) \times (1.31/\text{HDL})$$
$$\text{Females: VAI} = (\text{WC}/\,[39.58 + (1.89 \times \text{BMI})] \times (\text{TG}/0.81) \times (1.52/\text{HDL})$$

The optimal VAI thresholds for detecting visceral adipose dysfunction are 2.23 (age ≥30 and <42 years), 1.92 (age ≥42 and <52 years), 1.93 (age ≥52 and <66 years) and 2.00 (age ≥66 years) (Amato et al. 2010). Higher VAI values indicate visceral adipose dysfunction.

7.2.9 Statistical Analysis

Papers I–IV: The sample size was determined based on a margin of error of 0.05, a population size of 1.329 million, and an anticipated population proportion of 8%. The calculations for sample size were performed using the following formulas:

$$n = [z^2 \times \Phi(1 - \Phi)]/\varepsilon^2$$
$$n' = n/\{1 + [\,^{z2} \times \Phi(1 - \Phi)]/\varepsilon^2 N\}$$

In these formulas, z represents the z-score (2.58 for a confidence level of 99%), ε the margin of error, n the sample size within an unlimited population, n′ the finite sample size, N the population size, and F the population proportion. The

sample size was sufficient for all measured variables to achieve a statistical power greater than 0.99.

Statistical analysis was performed using SPSS v. 26–29 (IBM Corp., USA). All statistical tests were two-tailed with a 5% significance level, and adjustments were made for multiple comparisons using Bonferroni correction.

The Shapiro–Wilk test was used to test the normality distributions of the variables. The descriptive results were expressed as the median and interquartile range (for parameters that did not follow the normal distribution), the mean ± standard deviation (for parameters that followed the normal distribution) or as numbers and percentages (for categorical variables). Differences between categorical variables among groups were assessed using the chi-square test or Fisher's exact test (if the assumptions of the chi-square test were not met). ANOVA or student t-test was applied to compare means of parametrically distributed variables.

Paper I: A linear regression model was created to analyse the association between IMT and risk factors. Binary logistic regression analysis was applied to identify the association between ultrasound-visualised atherosclerosis, ABI, and CV risk factors; the combination of IMT and plaque status was used as the dependent variable.

Paper II: A linear regression model was created to analyse the association between IMT, Zn:Cu ratio, and other risk factors.

Paper III: Cook's distance and leverage values were used to exclude outliers in adipokines. Linear regression was used to examine the associations between IMT or ABI and adipokines. The correlations between adipokines and markers of subclinical atherosclerosis were further explored using binary logistic regression analysis. Receiver operating characteristics curves were used to determine the sensitivity and specificity of visfatin for predicting subclinical atherosclerosis (IMT ≥1 mm or plaque).

Papers III and IV: Single-factor correlations between two independent variables were analysed using Pearson coefficient analysis. A linear regression model was constructed to examine the association between IMT, adherence to dietary recommendations, and other risk factors.

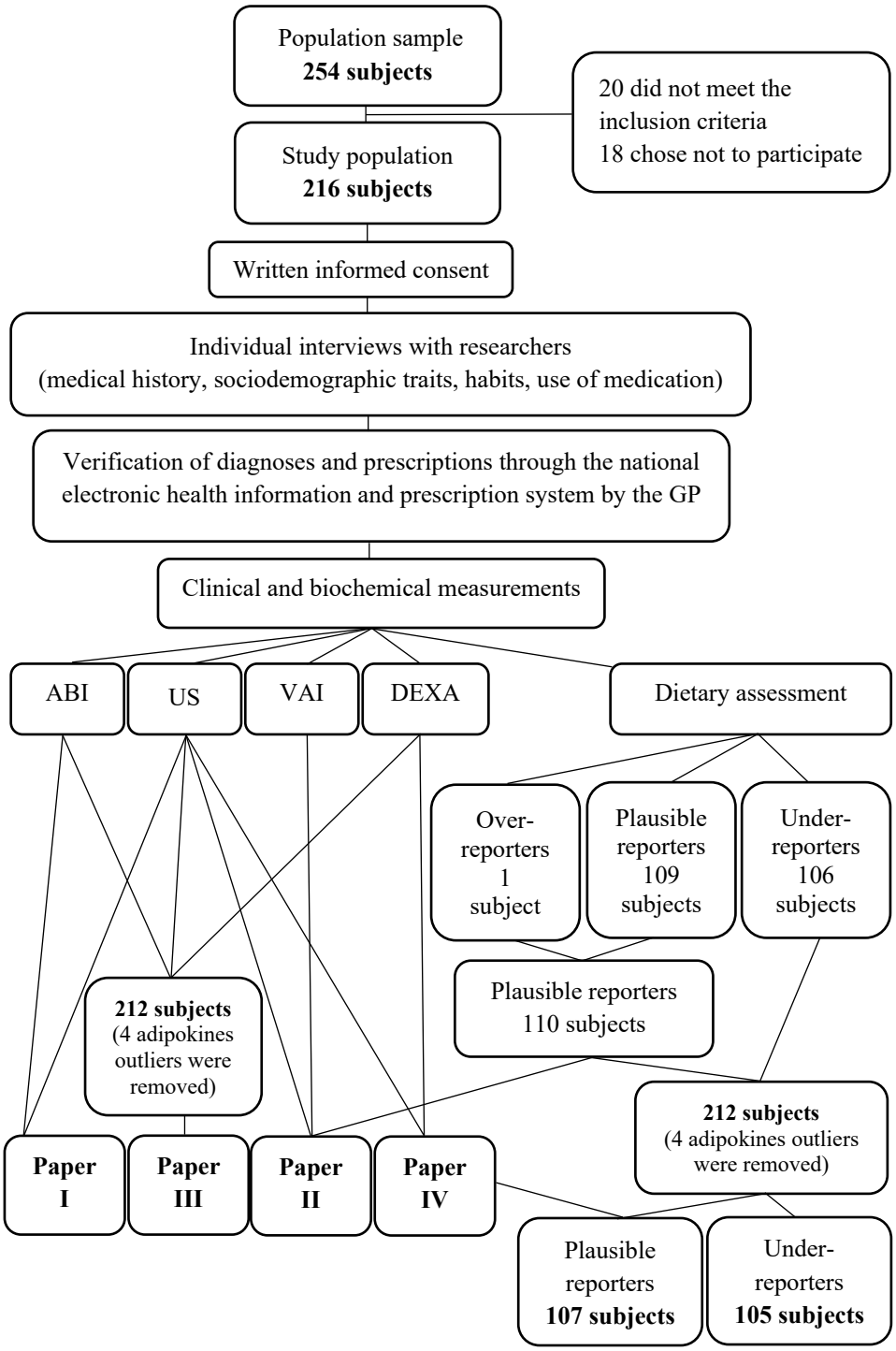

Figure 7. Study subject allocation according to different papers.

8. RESULTS

8.1 Characteristics of the Study Population

Paper I: All 216 subjects were analysed (Paper I, Table 1). The mean age was 59.0 ± 8 years, and the mean duration of T2DM was 7.1 ± 6 years. Hypertension was diagnosed in 87% of subjects, more prevalent and more often treated in women than men (accordingly, 90% vs. 81% and 83% vs. 64%). Hyperlipidaemia was present in 51%; 27% of the subjects were on statins. LDL-C levels exceeded 2.6 mmol/L in 72%. Women had higher HDL-C levels and lower triglycerides than men. Fifty-six percent had never smoked (33% men vs. 74% women), 25% were former smokers (45% vs. 10%), and 19% were current smokers (22% vs. 17%). Men had a higher ABI (p=0.012), mean IMT (p=0.030), and frequency of an IMT >1 mm compared to women (p=0.001).

Paper II: Using Goldberg's cut-off method, the same study population as in Paper I was equally divided between under-reporters and plausible reporters (49.1% vs. 50.9%). There were no statistical differences in sociodemographic factors, lifestyle behaviours, past medical history, medication history, laboratory analyses and atherosclerosis markers between under-reporters and plausible reporters (Paper II, Table 1). Plausible reporters had a longer duration of DM (6.0 ± 6.3 vs 5.0 ± 5.5 years) and used more oral antidiabetic drugs and insulin than under-reporters (89.1% vs 85.8% and 13.6% vs 6.6%, respectively).

Paper III: The exclusion of outliers (N=4) who had significantly higher concentrations of visfatin or resistin did not alter the mean age of participants (N=212), duration of DM, prevalence of hypertension or hyperlipidaemia, blood pressure values, medication usage, physical activity levels, common laboratory analyses or subclinical atherosclerosis markers (Paper III, Table 1). These findings were consistent with the findings observed in Paper I.

Paper IV: After excluding the outliers, 212 subjects were included in the analysis, with under-reporters and plausible reporters evenly distributed (49.5% vs 50.5%). A higher percentage of plausible reporters than under-reporters had high education levels (32.7% vs 19.0%, respectively). Plausible reporters had a lower average BMI (32.5 vs 35.6) and a lower total tissue fat percentage (38.2% vs 40.7%). The exclusion of outliers did not affect any of the various health parameters or differences between under-reporters and plausible reporters (Paper IV, Table 1), aligning with the findings of Paper I.

8.2 Associations between ABI and carotid artery subclinical atherosclerosis (Paper I)

In logistic regression analysis, an ABI ≤1.1 predicted subclinical atherosclerosis in the carotid arteries (odds ratio, OR 1.8; p=0.037). This association persisted even after adjusting for hypertension, hyperlipidaemia, DM duration, BMI, HbA1c, and smoking (p=0.039). The predictive power was the strongest for males over the age of 50 (OR 2.2; p=0.013). The atherosclerotic population, identified by an IMT value ≥1.0 mm and/or the presence of carotid artery plaque, tended to be older, have a longer duration of diabetes, and included more smokers, individuals with lower physical activity levels, and higher rates of peripheral artery disease (Table 2). Table 3 presents correlations between subclinical atherosclerosis markers and the most utilised CV risk factors in statistical models. As anticipated, the primary risk factors for subclinical atherosclerosis included advanced age, male gender, hypertension, hyperlipidaemia, and smoking.

Table 2. Characteristics of the study population, according to IMT ≥1 mm and plaque status (Paper I).

	IMT ≥1 mm and/or plaque			
	Total	Yes	No	*p*-value
N (%)	216	96 (44.4%)	120 (55.6%)	
Male [N (%)]	91 (42.1%)	44 (46.3%)	45 (38.5%)	NS
Age	59.0±8.0	60.9±6.9	57.4±8.6	0.001
BMI	34.0±5.7	33.5±5.7	34.5±5.8	NS
VAI	3.11±4.74	3.49±6.52	2.74±2.53	NS
A/G ratio	1.24±0.23	1.23±0.25	1.18±0.19	NS
Hypertension	187 (87%)	86 (89.6%)	101 (84.2%)	NS
Hyperlipidaemia	110 (51%)	48 (50.0%)	62 (51.7%)	NS
Duration of DM (years)	7.05±6.0	7.96±6.89	6.32±4.99	0.044
Statins	58 (26.9%)	33 (34.4%)	25 (20.8%)	0.026
Antiplatelet agents	54 (25.0%)	24 (25.0%)	30 (25%)	NS
Antihypertensive agents	162 (75.0%)	75 (78.1%)	87 (72.5%)	NS
Smoking (former and current)	94 (43.5%)	44 (45.8%)	50 (41.7%)	NS
Smoking pack years	19.6±16.4	27.4±15.0	15.8±12.2	0.014
Physical activity (MET mins)	4614±266	4021±366	5088±375	0.043
Systolic blood pressure (mmHg)	145±18	149±20	143±15	0.010
Diastolic blood pressure (mmHg)	89±9	89±9	88±10	NS
HbA1c (%)	6.9±1.2	6.87±1.27	6.89±1.14	NS
LDL-C (mmol/L)	3.4±1.2	3.45±1.23	3.48±1.11	NS
LDL-C >2.6 mmol/L	156 (72.2%)	67 (69.8%)	89 (74.2%)	NS
Triglyceride (mmol/L)	2.2±1.8	2.23±2.19	2.07±1.26	NS
hsCRV (mg/L)	3.9±5.1	4.53±5.76	3.39±4.53	NS
ABI ≤0.9	7 (3.2%)	6 (6.3%)	1 (0.8%)	0.046
ABI 0.91–0.99	7 (3.2%)	12 (12.5%)	15 (12.5%)	NS
ABI <1.0	38 (17.6%)	20 (20.8%)	18 (15.0%)	NS
ABI 1.00–1.09	57 (26.4%)	30 (31.3%)	27 (22.5%)	NS
ABI ≤1.1	91 (42.2%)	48 (50.0%)	43 (35.8%)	0.036
ABI 1.1–1.3	113 (52.3%)	44 (45.8%)	69 (57.5%)	NS
ABI ≥1.3	12 (5.6%)	4 (4.2%)	8 (6.7%)	NS
ABI	1.12±0.13	1.09±0.14	1.14±0.12	0.017

The values are expressed as means (± SD) or prevalence (%). Abbreviations: IMT, intima-media thickness; N, number; NS, non-specific; DM, diabetes mellitus; BMI, body mass index; VAI, visceral adiposity index; A/G, android/ gynoid fat ratio; hsCRP, high-sensitivity C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycated haemoglobin; ABI, ankle-brachial index.
Note: Adapted from Kärberg K, Lember M. Subclinical atherosclerosis in the carotid artery: can the ankle-brachial index predict it in type 2 diabetes patients? Scand J Clin Lab Invest. 2021

Table 3. Correlations between common cardiovascular risk factors and atherosclerosis markers.

		Mean IMT	CCA IMT	Bulb IMT	ICA IMT	IMT >1.0	Plaque	ABI	ABI ≤0.9	ABI ≤1.1	ABI ≥1.3
Age	r	0.297	0.433	0.254			0.214			0.156	
	p	<0.001	<0.001	<0.001	NS	NS	0.002	NS	NS	0.022	NS
Male	r	0.150			0.217	0.145		0.171		-0.158	
	p	0.027	NS	NS	0.001	0.033	NS	0.012	NS	0.020	NS
A/G	r									-0.153	
	p	NS	NS	NS	NS	NS	NS	NS	NS	0.026	NS
BMI	r										
	p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
VAI	r										
	p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
HT	r	0.149	0.141	0.149			0.158			0.148	
	p	0.029	0.039	0.028	NS	NS	0.021	NS	NS	0.029	NS
HL	r							0.137		-0.192	
	p	NS	NS	NS	NS	NS	NS	0.045	NS	0.005	NS
DM	r						0.140				
duration	p	NS	NS	NS	NS	NS	0.041	NS	NS	NS	NS
HbA1c	r										
	p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
hsCRV	r										
	p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Non-	r				-0.137			0.154	-0.193		
smoker	p	NS	NS	NS	0.046	NS	NS	0.025	0.005	NS	NS
Previous	r				0.14						
smoker	p	NS	NS	NS	0.041	NS	NS		NS	NS	NS
Current	r							-0.201	0.205	0.145	
smoker	p	NS	NS	NS	NS	NS	NS	0.003	0.003	0.035	NS
Pack-	r	0.317	0.452				0.419				
years	p	0.043	0.003	NS	NS	NS	0.006	NS	NS	NS	NS

Abbreviations: A/G, android to gynoid; BMI, body mass index; VAI, visceral adiposity index; HT, hypertension; HL, hyperlipidaemia, DM, diabetes mellitus; HbA1c, glycated haemoglobin; hsCRV, high-sensitivity C-reactive protein; IMT, intima-media thickness; r, Pearson's r; p, p-value; NS, non-specific; CCA, common carotid artery; ICA, internal carotid artery; ABI, ankle-brachial index.

* Definition of hypertension (diagnosis of hypertension, blood pressure more than 140/90 mmHg or antihypertensive treatment) and hyper-lipidaemia (diagnosis of hyperlipidaemia or LDL-C more than 2.6 mmol/L or statin therapy).

8.3 Associations between adipokines and subclinical atherosclerosis (Paper III)

In the univariate linear and binary logistic regression analyses, only visfatin and resistin showed statistically significant correlations with atherosclerotic vascular alterations. High molecular weight (HMW) adiponectin, leptin, and RBP4 were not correlated to atherosclerotic changes (Paper III, Table 2). Resistin had a positive correlation with mean IMT ($p=0.009$) and a modest association with plaque ($p=0.049$). Nevertheless, it failed to predict ABI or peripheral artery disease. Visfatin had a positive correlation with mean IMT ($p=0.002$), an IMT ≥ 1 ($p=0.002$) and an IMT ≥ 1 and/or the presence of plaque ($p=0.008$). Despite its modest inverse association with ABI, visfatin was not correlated with plaque or peripheral artery disease.

8.4 The daily dietary intake and adherence to dietary recommendations of subjects with T2DM (Paper II, IV)

Dietary recommendations were based on Estonia's general dietary recommendations and the guidelines from the Diabetes and Nutrition Study Group of the European Association for the Study of Diabetes (Aas et al. 2023; Pitsi et al. 2017).

Statistically significant differences were observed in the daily intake levels of both macro- and micronutrients between males and females (Table 3). Men had a higher caloric intake than women. Men also consumed more fat and protein quantitatively, leading to a higher intake of various fatty acids and certain micronutrients than women. The percentage of fat in the total energy intake was 3.8% higher than the recommended among men. The percentage of protein in the total energy intake was within the recommended range in both genders. The total intake of fibre and carbohydrates was similar between the sexes. Therefore, men consumed approximately 10 grams less dietary fibre than recommended. Unlike women, men consumed more salt than the recommended limit, exceeding it by 2 grams per day.

Most participants adhered to three out of the eight dietary recommendations (Figure 8; also, Paper IV, Figure 1). Two subjects did not comply with any recommendations, and no participant followed all the recommendations. Participants frequently exhibited adherence to the recommended intake of MUFA (70% of participants) and protein (67%) (Figure 9; also, Paper IV, Figure 2). These figures were followed by intakes of PUFA (59%), fibre (35%), fat (28%), carbohydrate (23%) and saturated fat acid (19%). The recommended salt intake limit had the lowest adherence (10%).

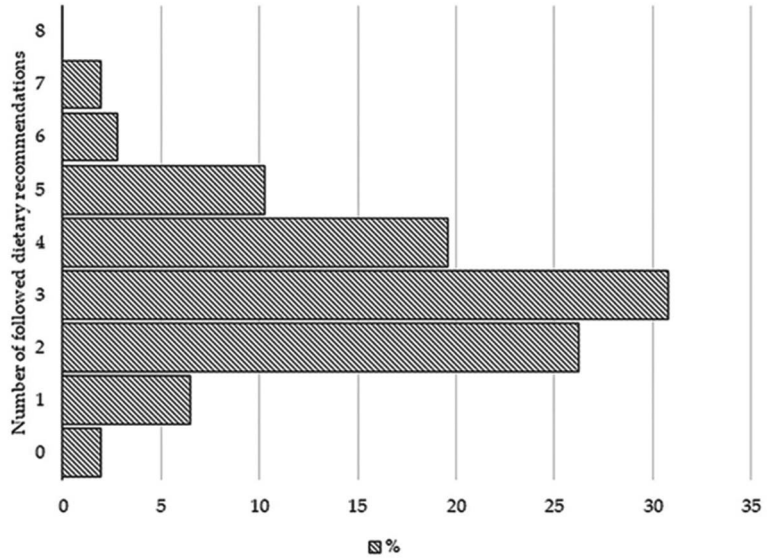

Figure 8. The number of followed recommendations.
Abbreviations: %; the percentage of subjects who adhered to recommendations. Recommendations that were evaluated are as follows: carbohydrate 50–60%, fat 25–35%, protein 10–20%, saturated fat acid ≤10%, monounsaturated fatty acids 10–20%, polyunsaturated fatty acids 5–10% of total energy, fibre ≥14 g/1000 kcal, and sodium ≤ 2.3 g/day.

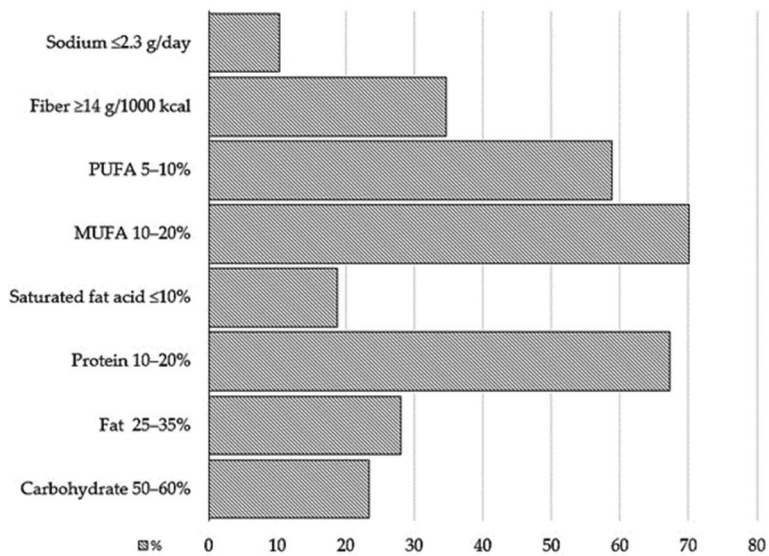

Figure 9. Adherence to specific dietary recommendations.
Abbreviations: PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; %, the percentage of subjects who adhered to recommendations.

Table 4. Mean daily dietary intake of plausible reporters by gender (Paper II).

		IMT ≥1 mm or plaque			
		Total	Men	Female	*p* value
Energy, kcal		1857±484	2098±339	1671±499	<0.001
Carbohydrates, g		211.7±72.3	222.9±72.2	203.0±71.6	NS
Fats, g		78.0±28.6	92.2±26.0	66.8±25.5	<0.001
Proteins, g	0.8 g/kg	82.8±33.5	96.2±34.0	72.4±29.5	<0.001
Alcohol, g	<40/<20	2.9±1.0	4.9±2.1	1.4±0.8	NS
Water, g	28–35 ml/kg	1554.7±435.9	1673.0 ± 435.8	1463.1±416.7	0.012
Ash, g		16.3±5.1	18.3±5.3	14.7±4.3	<0.001
Carbohydrates, %	50–60%	44.9±11.3	41.7±12.2	47.3±10.0	0.012
Fats, %	25–35%	36.8±9.11	38.8±9.3	35.2±8.7	0.043
Proteins, %	10–20%	17.5±5.16	18.0±5.6	17.1±4.8	NS
Alcohol, %		0.8±3.2	1.5±0.2	0.4±0.6	NS
Fibers, g	35/25	22.1±9.2	22.0±9.4	22.1±9.2	NS
Starch, g		101.8±51.1	117.0±47.9	90.1±50.8	0.005
Saccharose, g		32.8±28.0	32.6±27.3	33.0±28.7	NS
Lactose, g		12.2±9.05	14.5±10.7	10.4±7.2	0.024
Maltose, g		1.4±0.2	1.8±0.3	1.0±0.2	0.018
Glucose, g		13.0±9.5	10.5±6.0	14.9±11.1	0.008
Fructose, g		18.5±15.7	14.2±10.8	21.8±18.0	0.007
Galactose, g		0.6±0.1	0.6±0.1	0.6±0.1	NS
Fatty acids, total, g		40.1±20.0	47.3±21.7	34.6±16.8	0.001
Saturated fatty acids, g		30.6±12.8	36.3±13.3	26.2±10.4	<0.001
MUFA, g		29.5±12.6	35.5±11.5	24.9±11.5	<0.001
PUFA, g		12.8±6.4	14.7±6.4	11.3±6.0	0.004
Trans fatty acids, g		0.4±0.3	0.4±0.4	0.4±0.3	NS
Palmitic acid, g		15.6±6.1	18.5±5.8	13.4±5.4	<0.001
Stearic acid, g		6.8±3.2	8.1±2.9	5.7±3.1	<0.001

		IMT ≥1 mm or plaque			
		Total	Men	Female	*p* value
Linoleic acid, g		9.4±5.6	10.8±5.6	8.2±5.4	0.016
Linolenic acid, g		2.6±0.3	3.1±0.6	2.2±0.3	NS
Cholesterol, mg		339.2 ±228.6	418.2±278.9	277.9±157.3	0.003
Saturated fatty acids, %	<1 %	14.5±4.8	15.3±5.2	13.9±4.5	NS
MUFA, %	10–20%	13.8±4.3	14.9±4.4	13.0±4.0	0.020
PUFA, %	5–10%	6.1±2.6	6.2±2.6	5.9±2.7	NS
Potassium, mg	3500	3574.6±1121.5	3827.3±1132.1	3379.0±1082.1	0.038
Calcium, mg	800	646.7±319.1	767.5±367.9	553.1± 239.3	0.001
Magnesium, mg	380/320	325.7±95.6	350.3±88.2	306.7±97.3	0.016
Phosphorus, mg	600	1300.3±402.3	1490.3±440.4	1153.2±298.9	<0.001
Iron, mg	10/15	14.1±6.8	15.5±5.9	13.0±7.4	NS
Zinc, mg	9–10/9	11.2±5.2	13.2±4.3	9.7±5.3	<0.001
Copper, mg	0.9	1.3±0.6	1.4±0.6	1.2±0.5	NS
Zn/Cu ratio		9.5±7.5	11.0±6.2	8.4±3.4	0.011
Manganese, mg	10	8.1±8.3	8.1±5.7	8.1±9.9	0.034
Iodine, µg	150	114.9±54.8	134.8±69.7	99.5±32.7	0.002
Selenium, µg	60–90/50–75	64.7±36.6	74.3±38.2	57.3±33.8	0.017
Chrome, µg	30–35/20–25	26.0±17.5	29.6±23.1	23.2±10.9	NS
Nickel, µg		121.0±81.3	127.4±89.8	116.0±74.4	NS
Vitamin A, RE	800/700	511.5±122.6	555.5±249.5	485.5±94.9	NS
Retinol, µg		321.5±121.1	363.5±245.7	244.0±93.9	NS
BCE		1435.0±175.3	1235.0±250.7	1460.0±243.4	NS
Vitamin D, µg		7.80±1.8	11.9±4.0	4.9±0.6	NS
Vitamin D3, µg	10–20	3.7±1.7	7.0±3.9	1.3±0.4	NS
Vitamin E, αTE	11/9–10	9.0±6.3	9.9±6.3	8.3±6.3	NS
Vitamin K, µg	70–75/60–65	38.0±37.1	43.8±42.4	33.3±32.1	NS
Vitamin B1, mg	1.4–1.5/1.0–1.1	1.1±0.6	1.3±0.7	0.9±0.5	<0.001
Vitamin B2, mg	1.5–1.7/1.2–1.3	1.1±0.5	1.3±0.6	0.9±0.3	0.001

		IMT ≥1 mm or plaque			
		Total	Men	Female	*p* value
Niacin E, total	18–19/14–15	30.5±12.0	34.8±11.8	27.2±11.2	0.042
Niacin, mg	17–19/14–15	16.4±7.8	18.1±7.6	15.1±7.7	NS
NE tryptophan, mg		8.4±5.4	9.45±6.8	7.5±4.0	NS
Pantothenic acid, mg	5	4.5±1.7	4.9±2.0	4.1±1.2	0.012
Vitamin B6, mg	1.8/1.5–1.6	1.4±0.6	1.5±0.6	1.4±0.6	NS
Biotin, µg	30	25.7±14.8	28.0±17.7	24.0±11.9	NS
Folates, µg	300	173.0±61.8	182.0±62.0	166.1±61.2	NS
Vitamin B12, µg	3.0	6.5±6.1	8.1±7.4	5.2±4.5	0.021
Vitamin C, mg	95–100	65.1±63.5	52.0±46.9	75.2±72.6	0.046
Sodium, mg	2400	2568.2±147.2	3017.5±236.4	2220.4±175.6	0.008
Salt equivalent, mg	6000	6396.6±366.6	7519.8±586.8	5527.0±438.4	0.008

The values are expressed as mean ± SD. Abbreviations: RDA, Recommended Dietary Allowance; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Zn/Cu, Zinc to Copper Ratio; RE, retinol equivalents (preformed retinol and provitamin A carotenoids from all sources are converted into one unit); BCE, beta-carotene equivalent; αTE, α-tocopherol equivalent; NE, niacin equivalent.

Note: Adapted from Kärberg K, Forbes A, Lember M. Raised dietary Zn:Cu ratio increases the risk of atherosclerosis in type 2 diabetes. Clin Nutr ESPEN. 202

8.5 Micronutrient intake and subclinical atherosclerosis (Paper II)

As demonstrated in Table 3, the micronutrient consumption of individuals with T2DM tended to be below the recommended dietary allowance (RDA).

The most significant positive correlations were found between mean IMT and dietary intake of calcium (r=0.277, p=0.004), phosphorus (r=0.198, p=0.041), and vitamin A (r=0.195, p=0.044). Conversely, vitamin C exhibited a significant negative correlation (r= -0.214, p=0.027) with mean IMT. While dietary zinc and copper concentrations did not individually correlate with mean IMT, a significant correlation was present between IMT and the zinc-to-copper ratio (r=0.197, p=0.042). Additionally, significant positive correlations were observed between carotid artery plaque and intake of calcium (r=0.228, p=0.018), vitamin B1 (r=0.219, p=0.024), vitamin B2 (r=0.215, p=0.021), and biotin (r=0.191, p=0.039). In contrast, vitamin C was negatively correlated with carotid artery plaque (r= -0.207, p=0.032).

ABI exhibited correlations with dietary intake of phosphorus (r=0.204, p=0.035), vitamin D (r=0.362, p<0.001), vitamin B2 (r=0.225, p=0.020), vitamin B6 (r=0.220, p=0.023), biotin (r=0.210, p=0.030), and cholesterol (r=0.364, p<0.001). An ABI value of ≤0.9 was positively correlated with calcium (r=0.195, p=0.044) and negatively correlated with vitamin B6 (r= -0.215, p=0.026). Conversely, an ABI value of ≥1.3 was positively correlated with the intake of vitamin D (r=0.302, p=0.002), biotin (r=0.267, p=0.006) and cholesterol (r=0.312, p=0.001).

Table 5. Correlation between dietary zinc to copper ratio and dietary recommendations in plausible reporters.

	Zn:Cu	Zn:Cu	MD	95% CI		*p*-value
Carbohydrates >50% vs ≤50%						
Female	6.17	9.03	2.94	1.32	4.56	0.001
Male	6.73	10.13	4.61	0.31	8.90	0.036
Fats <35% vs ≥35%						
Female	6.38	9.14	2.64	1.02	4.26	0.002
Male	8.54	9.86	2.38	-1.44	6.20	0.216
Protein <20% vs ≥20%						
Female	7.38	8.81	0.96	-0.95	2.86	0.318
Male	8.02	13.01	6.73	3.33	10.14	<0.001

Abbreviations: Zn:Cu: zinc to copper ratio; MD: mean difference; CI: confidence interval. Note: Adapted from Kärberg K, Forbes A, Lember M. Raised dietary Zn:Cu ratio increases the risk of atherosclerosis in type 2 diabetes. Clin Nutr ESPEN. 2022

Given the existing description of associations in scientific literature, we focused on exploring the comparatively underexplored relationship between the indicators of atherosclerosis and the ratio of zinc and copper. We found that the correlation between carotid artery IMT and the Zn:Cu ratio (p=0.024) became stronger after adjusting for cardiovascular risk factors. The Zn:Cu ratio depended on adherence to dietary recommendations, with higher ratios in those not meeting recommended intakes of carbohydrates, fat, and protein. The Zn:Cu ratio increased significantly in both genders when carbohydrates constituted less than 50% of total energy intake. However, in women, the Zn:Cu ratio also increased when their fat consumption exceeded 35%, and in men, when their protein intake surpassed 20% of the total energy intake (Table 5).

8.6 Dietary effect on the association between adipokines and subclinical atherosclerosis (Paper IV)

No significant correlations were observed between HMW adiponectin, resistin or RBP4, and adherence to dietary recommendations. However, leptin displayed a negative correlation with MUFA intake comprising ≥20% of the total energy intake (r= -0.218, p=0.024). No other correlations were evident.

Among individuals with low carbohydrate intake and high consumption of total fat, saturated fatty acids, and salt, there was a statistically significant correlation between IMT and visfatin (Paper IV, Table 3). The same relationship was evident in subjects whose intake of PUFA and MUFA adhered to the recommended nutrition guidelines (Paper IV, Table 3). In a linear regression analysis, when the intake of PUFA was ≤10% or that of MUFA was ≤20% of the total energy. A statistically significant correlation was present between the visfatin concentration and carotid artery IMT (p=0.010 and p=0.006, respectively).

In the linear regression analysis, the relationship between visfatin and IMT was also statistically significant (p=0.009). In the multivariable linear regression analysis, after adjusting for common risk factors, medication usage, and non-adherence to dietary recommendations, the relationship between visfatin and IMT showed an even stronger level of significance (p=0.006) (Paper IV, Table 4).

8.7 Effect of medications on the associations between visfatin and subclinical atherosclerosis (Paper III)

Ninety-six point two percent of the participants were on CV-related therapy, with metformin, ACE inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs), and beta-blockers (BB) being the most common (Paper III, Table 1). Females used more antihypertensive treatments.

Statin users had lower visfatin levels (2.87 pg/ml vs 3.12 pg/ml), while BB users had higher visfatin levels (3.34 pg/ml vs 2.88 pg/ml); these differences were not significant. Monotherapy with statins or calcium channel blockers (CCBs)

resulted in lower visfatin levels (2.49 pg/mL and 1.91 pg/mL) compared to non-users and ACEI/ARB and BB users (3.37 pg/mL and 3.44 pg/mL).

Visfatin's predictive capacity for detecting mean IMT ≥1.0 mm or carotid artery plaque varied by medication (Paper III, Figures 2 and 3). Optimal visfatin concentration thresholds were 1.63 pg/ml for BB users (88% sensitivity, 70% specificity), 1.23 pg/ml for ACEI/ARB and CCB users (86% sensitivity, 83% specificity), and 1.41 pg/ml for those with unknown treatment (63% sensitivity, 60% specificity).

Visfatin levels were positively associated with mean IMT (p=0.003), an IMT ≥1 mm (p=0.038), an IMT ≥1 mm and the presence of plaque (p=0.005). An ABI ≤0.9 (p=0.029) had an inverse association with visfatin (p=0.038).

Metformin users had higher visfatin levels than non-users (3.20 pg/ml vs. 2.29 pg/ml); the difference was not significant. Visfatin levels ≥1.42 pg/ml predicted subclinical atherosclerosis in metformin users (64% sensitivity, 58% specificity) (Figure 10). No significant association was found for those on metformin, ACEI/ARBs, or BBs (Figure 11). For those on metformin, ACEIs, ARBs, and CCBs, a visfatin threshold of ≥1.15 pg/ml (94% sensitivity, 58% specificity) indicated subclinical carotid atherosclerosis (Figure 12).

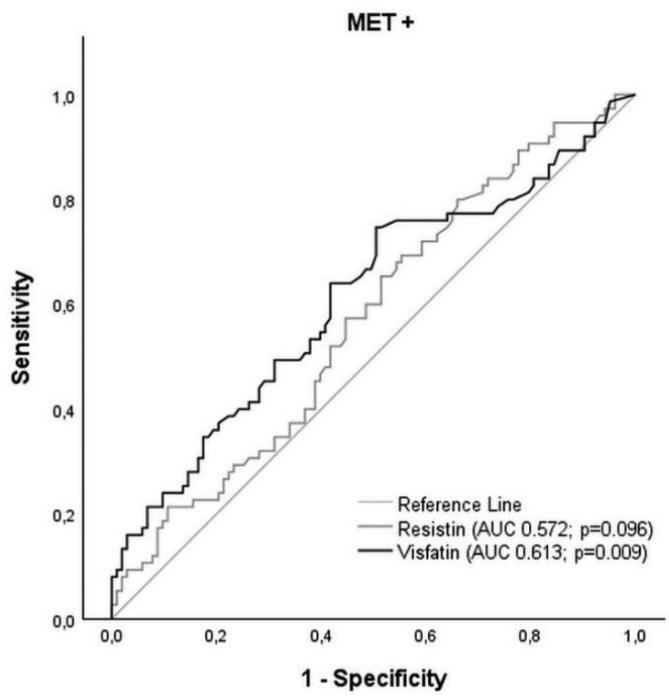

Figure 10. ROC curve analysis for the changing correlations between visfatin and resistin concentrations and subclinical atherosclerosis (IMT ≥1 or plaque) in the carotid artery by metformin without adjustment for other medications.
The p-value is based on logistic regression analysis. Abbreviations: MET+, metformin; AUC, area under the curve.

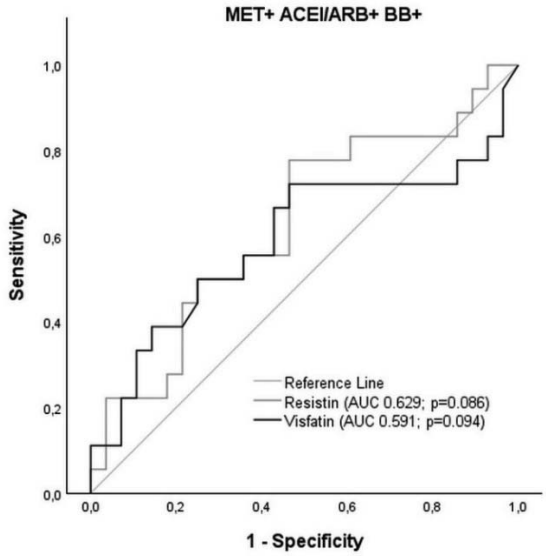

Figure 7. ROC curve analysis for the changing correlations between visfatin and resistin concentrations and IMT ≥1 or plaque in the carotid artery adjusted for metformin, ACEI/ARB and BB. The p-value is based on logistic regression analysis. Abbreviations: ACEI/ARB+, angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers; AUC, area under the curve; BB+, β-blocker.

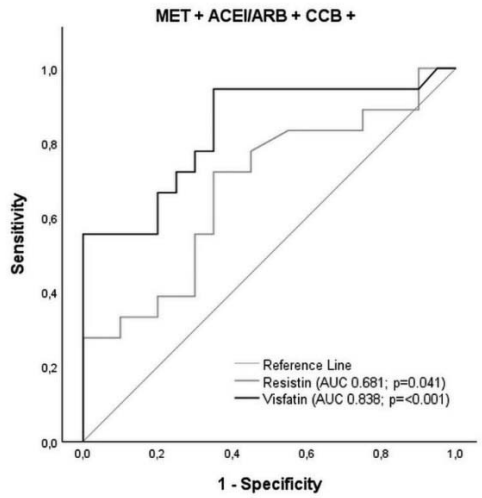

Figure 8. ROC curve analysis for the changing correlations between visfatin and resistin concentrations and subclinical atherosclerosis (IMT ≥1 or plaque) in the carotid artery adjusted for metformin, ACEI/ARB and CCB.
The p-value is based on logistic regression analysis. Abbreviations: ACEI/ARB+, angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers; AUC, area under the curve; CCB+, calcium channel blockers.

9. DISCUSSION

The primary objective of this thesis was to identify factors and markers that predict subclinical atherosclerosis in individuals with T2DM without a previous diagnosis of atherosclerosis or its complications. Atherosclerosis progresses silently, often without symptoms, until significant ischemic complications arise. Identifying factors and markers that predict subclinical atherosclerosis is important for enabling earlier diagnosis, providing more effective intervention, and increasing patient motivation. Ultimately, identifying these factors and markers can potentially improve patient outcomes and reduce the burden of CVD in this high-risk population. Detecting subclinical atherosclerosis through simple laboratory or radiology methods will make it possible to facilitate lifestyle changes and timely treatment.

We conducted a cross-sectional multicentre study in which patients with T2DM were recruited from 13 general practices across Estonia. The recruitment was done by general physicians using a sequential approach from their patient lists, ensuring a representative sample of T2DM patients in Estonia. The sample size was comparable to similar studies. Each participant provided informed consent and subsequently completed questionnaires, a 24-hour dietary recall, and underwent blood tests, ABI measurement, carotid artery ultrasound, and DEXA scanning.

9.1 The predictive ability of ankle-brachial index for carotid artery subclinical atherosclerosis in type 2 diabetes patients without prior atherosclerosis diagnosis (Paper I).

9.1.1 Atherosclerosis in lower limb peripheral arteries

We aimed to determine whether ABI can predict carotid artery atherosclerosis and identify a threshold value for early atherosclerotic changes in T2DM patients. We focused on ABI values at the upper limit of the “grey area”. We found that an ABI ≤1.1 strongly predicts subclinical atherosclerosis. Even after adjusting for risk factors, an ABI ≤1.1 remained a statistically significant predictor of IMT ≥1 ± plaque in male patients, particularly those over 50.

Lower limb PAD is defined as an obstructive atherosclerotic disease of the arteries from the distal aorta to the foot. It presents with clinical symptoms, signs, or abnormalities on non-invasive or invasive vascular testing or medical imaging and results in disturbed or impaired circulation to one or both lower extremities (Nordanstig et al. 2024). The literature shows different ABI threshold values for defining PAD. Older studies recommend ABI threshold values between 0.85 and 0.97 (Aboyans et al. 2012). In a primary healthcare study, an ABI <0.8 or a mean of three ABIs <0.9 strongly indicated lower limb PAD (positive predictive value

≥95%). Conversely, an ABI >1.1 or a mean of three ABIs >1.0 effectively ruled out the presence of advanced disease (negative predictive value ≥99%) (Stoffers et al. 1996). For patients with diabetes, 1.0 has been suggested as the best threshold value for PAD, with optimal sensitivity and specificity. However, it is important to note that the number of patients with diabetes was relatively small in the study (Clairotte et al. 2009).

Using ABI for diagnosing PAD is not a novel method. An ABI value of <0.9 is traditionally used as a diagnostic criterion for lower limb PAD, indicating advanced atherosclerosis (Nordanstig et al. 2024). Therefore, an ABI value of <0.9 indicates already clinically relevant atherosclerotic changes in the major arteries distal to the aortic arch. The 2024 European Society for Vascular Medicine guideline recommends using ABI screening for clinically asymptomatic individuals at higher risk of lower limb PAD to bolster secondary prevention strategies (Nordanstig et al. 2024). Thus, the assessment of ABI may serve a dual purpose: it provides patients with real evidence of atherosclerosis and encourages improved patient cooperation throughout the treatment process.

There is no definitive ABI value for detecting subclinical atherosclerosis. The ARIC study (9.4% with DM) showed an increased risk of coronary heart disease at ABI <1.0 and a decreased risk at ABI >1.0 (Newman et al. 1993). A Japanese study of patients with diabetes found that an ABI <1.0 correlated with atherosclerotic events (Hayashi et al. 2004). In contrast, the Rancho Bernardo Study (6% with DM) suggested considering ABI values between 1.10 and 1.26 as a new normal (Allison et al. 2006). The MESA study (13% with diabetes) found an ABI of <1.1 in men and an ABI of <1.0 in women to be associated with atherosclerosis (McDermott et al. 2005). In the ARIC study of middle-aged adults in the general population without clinical PAD symptoms, an ABI ≤0.90 showed a 2.4 to 2.7 times higher risk of critical limb ischaemia and ischemic leg amputation compared to ABI values of 1.11 to 1.20 (Paskiewicz et al. 2021). ABI values ranging from 0.91 to 1.00 and 1.01 to 1.10 also demonstrated adjusted hazard ratios of 1.7 to 2.0 (Paskiewicz et al. 2021). In our study, we focused on individuals without clinically apparent CVD and, therefore, set the ABI threshold at <1.1.

There are considerable differences in how studies measure blood pressure and calculate the ABI. Some measure ankle blood pressure from one leg and brachial blood pressure from one hand (Paskiewicz et al. 2021), while others take measurements from all four limbs. Calculations also vary, using either the average (Winckler et al. 2018) or the highest ankle and brachial systolic blood pressure (Bots et al. 1994; McDermott et al. 2005; Paskiewicz et al. 2021). Choosing the lower ankle pressure as the ABI numerator provides better sensitivity for PAD, while the higher ankle pressure provides greater specificity (Aboyans et al. 2012). Therefore, using lower ankle pressure should identify more patients at risk. Using the lower systolic pressure of peripheral arteries in the calculation resulted in the weakest association between lower limb PAD and CV risk factors, as well as subclinical atherosclerosis in the coronary or carotid arteries (Allison et al. 2010). However, the Cardiovascular Health Study found no notable differences

in CV event risks when using either the lower or higher ABI calculations (Newman et al. 1993). We chose to use the higher peripheral arterial pressure to enhance specificity to luminal stenosis and reflect lower-extremity perfusion.

Age-adjusted analyses indicate a stronger inverse association between IMT, ABI, and CVD in older populations, typically aged 60–75 (Polak et al. 2019). Few studies have involved subjects under 60 (Winckler et al. 2018; Zheng et al. 1997). Both ABI and IMT are influenced by age, likely due to the increased prevalence of atherosclerosis in older adults (McDermott et al. 2005). The Copenhagen City Heart Study (n=2744, 4.6% had DM) found a stronger inverse association between IMT and ABI in those with diabetes, especially those aged over 60 (Winckler et al. 2018). In our sample, with a mean age of under 60 years and all subjects having T2DM, ABI was found to be relevant for assessing subclinical atherosclerosis, with stronger correlations after adjusting for male gender and age over 50 years. Differences in the findings may have been caused by differences in population sizes, definitions of atherosclerosis, and the inclusion or exclusion of patients with macrovascular complications. We suggest an ABI threshold of ≤1.1 for predicting subclinical atherosclerosis in T2DM patients, particularly men over 50 years of age.

High ABI (>1.30) is indicative of an uncompressible artery. In our study, 5.6% of subjects had an ABI >1.3. An ABI of >1.3 did not have a statistically significant correlation with subclinical atherosclerosis in the carotid artery. The prevalence of diabetes among those with ABI >1.30 was 11% in the ARIC study (Paskiewicz et al. 2021). The presence of diabetes and or end-stage renal disease is a known risk factor for medial arterial calcification, which has been shown to increase ABI (Aboyans et al. 2012). The presence of vascular calcification does not necessarily indicate the presence of occlusive lesions, although these two conditions often occur together. Therefore, an ABI of ≥1.3 is inconclusive for lower limb PAD and needs further research in the context of diabetes.

9.1.2 The definition of subclinical atherosclerosis in carotid artery

Definitions of subclinical atherosclerosis in the carotid artery vary in the scientific literature (Spence 2006; Zhong et al. 2008). To reduce the risk of measuring an adaptive response of the vessel wall to changes in shear stress, tensile stress, and blood flow and subsequent changes in lumen diameter (Stary et al. 1992), we defined subclinical atherosclerosis as the carotid artery IMT ≥1 mm with or without plaque, the presence of focal wall thickening at least 0.5 mm or 50% greater than the surrounding vessel wall or a focal region with IMT greater than 1.5mm (Stein et al. 2008). This approach takes into account the age-related change in IMT and uses values higher than the 75th percentile based on the Atherosclerosis Risk in Communities Study and American Society of Echocardiography guideline for measuring IMT (Howard et al. 1993; Stein et al. 2008).

An increase in IMT does not directly indicate subclinical atherosclerosis. Increased IMT can also result from non-atherosclerotic processes such as smooth muscle cell hyperplasia and fibrocellular hypertrophy that have, in turn, led to medial hypertrophy and compensatory arterial remodelling (Al-Shali et al. 2005). Several factors are known to influence the IMT. The Rotterdam Study found significant positive associations between CCA IMT and age, male sex, body mass index, systolic blood pressure, hypertension, total cholesterol, and diabetes mellitus (Bots et al. 1997). The same correlations were present in our study. Therefore, the statistical models predicting IMT included all common and well-established CV risk factors.

The presence of carotid plaque indicates an advanced stage of atherosclerosis, in contrast to increased carotid IMT without plaque, thereby prompting the reclassification of patients from the intermediate to the high CV risk category (Polak et al. 2011). Regardless of the method of evaluation, the presence of carotid plaque has been shown to be significantly associated with an increased risk of stroke and coronary heart disease events (Powell et al. 2024). Consequently, the criterion for identifying the presence of plaque has been incorporated into the definition of atherosclerosis.

9.1.3 Correlation between ABI and carotid artery subclinical atherosclerosis

Our study found an inverse correlation between ABI and IMT, with 50% of subjects with subclinical atherosclerosis having an ABI ≤1.1; this was statistically significantly different from subjects without subclinical atherosclerosis. This inverse relationship between ABI and IMT is supported by previous research (Winckler et al. 2018). The Rotterdam study of subjects without symptomatic CVD showed a significant correlation between carotid IMT and ABI, with a higher likelihood of PAD in those with an IMT > 0.89 mm (Bots et al. 1994). The ARIC study also found a strong correlation between IMT >0.86 mm and PAD (Wattanakit et al. 2005). In the MESA study, men with borderline ABI (0.90–0.99) and low-normal ABI (1.00–1.09) had higher carotid IMT. In women, definite PAD and borderline ABI were linked to higher ICA IMT (McDermott et al. 2005). The Cardiovascular Health Study reported a strong correlation between carotid stenosis and decreasing ABI, starting at values <1.0 (Newman et al. 1993). Additionally, a Japanese study found that T2DM patients with an ABI <1.0 and an IMT >0.91 mm are more prone to atherosclerotic events (Hayashi et al. 2004). Despite variations in study demographics, the presence of clinically evident CVD and the proportion of patients with diabetes, a significant inverse relationship between ABI and IMT is a consistent finding, underscoring the value of ABI and IMT in detecting and stratifying atherosclerosis risk, particularly in patients with diabetes.

9.2 Associations between circulating adipokines and subclinical atherosclerosis in individuals with type 2 diabetes (Paper III)

Obesity and T2DM are closely linked, with excess fat increasing the risk of insulin resistance (Cavalcanti et al. 2022). Adipose tissue secretes adipokines that influence metabolism, inflammation, endocrine processes, and atherosclerosis progression (Dahl et al. 2007; Henning 2021; Liu et al. 2022; Romacho et al. 2020). We looked for several adipokines as potential markers of subclinical atherosclerosis and found significant positive correlations between visfatin levels and IMT, and resistin levels and IMT. No significant correlations were found for HMW adiponectin, leptin, or RBP-4. Due to their lack of significant association with subclinical atherosclerosis, we excluded HMW adiponectin, leptin and RBP-4 from further analysis and focused on visfatin, which had the strongest link with atherosclerosis and included resistin for comparison due to its moderate association.

After adjustments for risk factors, visfatin remained independently associated with mean IMT, carotid subclinical atherosclerosis, and lower limb PAD, especially in males. We could not find a clear correlation between visfatin and plaque. Studies have suggested that visfatin contributes to plaque development, progression, and instability (Chiu et al. 2012; Kadoglou et al. 2011, 2012; Spence 2006). Increased visfatin levels have been reported in symptomatic carotid stenosis (Kadoglou et al. 2012), stable asymptomatic coronary artery disease (Kadoglou et al. 2011), acute myocardial infarction (Chiu et al. 2012), higher incidence of major adverse CVD events, ischemic cerebrovascular disease, and atherosclerotic peripheral arterial obstructive disease compared to healthy controls (Kong et al. 2014; Pitoulias et al. 2017; Zheng et al. 2020). Additionally, patients with T2DM and atherosclerotic plaques, especially those with carotid plaques compared to femoral plaques, have been shown to have higher serum visfatin levels than those without (Zheng et al. 2019). In metabolic syndrome patients, especially those with carotid atherosclerosis, serum visfatin levels have been found to be higher than in control subjects (Zhong et al. 2008). In contrast to our study, the mentioned studies included patients with advanced atherosclerosis, shorter durations of diabetes, and younger average age. Therefore, research on visfatin and subclinical atherosclerosis is scarce.

Serum resistin has been independently linked to the progression of CIMT in hypertensive patients, smokers, and those with metabolic syndrome or diabetes (He et al. 2017; Shin et al. 2008). Our results are consistent with this statement. However, serum resistin failed to predict ABI and peripheral artery disease in our study, although it has previously been linked to PAD (Hsu et al. 2017). Based on our research, the assessment of visfatin concentration could aid in identifying individuals with T2DM who could benefit from implementing preventive measures against atherosclerosis.

9.3 Adherence to general nutritional recommendations in individuals with type 2 diabetes (Paper IV)

Patients with T2DM had poor compliance with dietary guidelines. We determined the target values of macronutrients based on the Estonian dietary guidelines (Pitsi et al. 2017), which were in line with the recommendations of the Diabetes and Nutrition Research Group of the European Association for the Study of Diabetes (Aas et al. 2023). Our findings revealed that none of the subjects adhered to all eight dietary recommendations simultaneously, with the majority following two to three of the dietary recommendations stated in the guidelines. Seventy percent of the patients met the recommended intake for MUFA; this was followed by the recommended intake of protein and PUFA. However, there were significant deviations in the intake of salt, saturated fatty acids, and carbohydrates. In contrast, a Polish study reported adherence to 7 out of 10 recommendations (Gętek-Paszek et al. 2020). This highlights the need for improved dietary management among T2DM patients.

We also identified a common dietary pattern marked by low carbohydrate and fibre but high fat, total fat, saturated fat, and salt intake. Low carbohydrate intake is commonly compensated by an increased intake of fat and/or protein in other studies of DM patients (Gętek-Paszek et al. 2020; Iwase et al. 2015). The dietary composition reported in our study was similar to the T2DM dietary survey by the Polish Society of Diabetology (Gętek-Paszek et al. 2020), although our patients had a higher fibre and fat intake and a lower saturated fatty acid and salt intake. Compared to Japan, our participants consumed less carbohydrate but more fat and protein, which may impact CVD prevalence and life expectancy outcomes (Iwase et al. 2015).

9.4 Imbalance in the intake of micronutrients influences the risk of atherosclerosis (Paper II)

In our study, patients with low carbohydrate and high fat and protein intake had a high dietary Zn:Cu ratio, which was statistically significantly correlated with CIMT. There was no correlation between the Zn:Cu ratio and plaque. The correlation between Zn:Cu ratio and IMT remained statistically significant after adjustment for common CV risk factors. An experimental investigation showed that when the Zn:Cu ratio surpasses 16, insufficient dietary copper levels are implicated in ischemic heart disease by modulating lipid metabolism (Klevay et al. 1984). Human studies with a Zn:Cu ratio exceeding 20 were terminated due to the emergence of cardiac irregularities (Altarelli et al. 2019). The Zn:Cu ratio is intricately linked to dietary composition, with meat and dairy products typically associated with elevated Zn:Cu ratios. However, meat and dairy products constitute an important component of the Estonian diet.

Studies investigating the link between trace elements and CVD have yielded contradictory results. One study, where 9.4% of patients had DM, found that

dietary Zn intake is inversely related to subclinical atherosclerosis (defined as IMT >1 mm) in the CCA, visualised by ultrasound (Yang et al. 2010). Another study, where 30% of the patients had DM, reported a higher serum Zn:Cu ratio (Kazemi-Bajestani et al. 2007) in patients with angiographically confirmed CAD compared to those without. The nutritional study did not exclude under- and over-reporters and reported a lower Zn intake (6 mg per day) compared to our study (10 mg per day). Although dietary Zn and Cu levels do not directly correlate with the content of Zn and Cu in the body, and IMT and plaques represent different aspects of atherosclerosis pathogenesis, it seems likely that the dietary Zn:Cu ratio correlates with angiographically confirmed atherosclerosis.

9.5 Factors influencing the association between circulating adipokines and subclinical atherosclerosis (Paper III and IV)

Visfatin is the most promising marker for subclinical atherosclerosis among the adipokines studied. Integrating visfatin into routine clinical practice requires establishing reference values, which has not yet been achieved. Some research has aimed to identify diagnostic thresholds for indicating pathology based on visfatin levels (Zheng et al. 2020). Understanding the factors that influence visfatin concentration remains crucial. Reported visfatin concentrations vary across studies due to differences in study populations and laboratory techniques. Various commercially available assays with different reliability metrics have been used, yet consensus on universal normal ranges for adipokine levels remains elusive. Therefore, we aimed to identify factors influencing visfatin concentration, which should be considered for future research and clinical applications.

We found a statistically significant correlation between IMT and visfatin among individuals adhering to dietary patterns characterised by low carbohydrate and fibre but high fat, total fat, saturated fat, and salt intake. Previous studies have demonstrated that hypocaloric diets and Okinawan-based Nordic diets reduce visfatin concentration in obese patients without diabetes, without considering the underlying modifications in food composition (de Luis et al. 2008; Ohlsson 2019). Studies have also demonstrated associations between visfatin levels and low carbohydrate intake (Öztürk et al. 2023), high salt intake (Chen et al. 2023), and diets rich in saturated fatty acids (Haghighatdoost et al. 2012). In contrast to the previous studies, protein intake did not appear to affect the association between visfatin and IMT in our study. We found a significant association between visfatin and IMT in individuals with inadequate dietary fibre. This is consistent with existing evidence about the CV benefits of fibre consumption (Burger et al. 2012). However, not all studies have found an association between fibre consumption and CV risk (Öztürk et al. 2023).

The positive association between visfatin and carotid artery IMT in individuals whose intake of MUFA and PUFA is at the recommended level stimulates

contemplation. The intriguing aspect is that MUFAs and PUFAs, particularly omega-3 fatty acids, should have anti-atherosclerotic effects (Garg 1998). Among overweight individuals, an inverse correlation has been described between visfatin and MUFAs, while no association has been identified in those with T2DM (Haghighatdoost et al. 2012; Öztürk et al. 2023). Some studies have found a positive correlation between visfatin and PUFAs without any correlations with omega-3 or omega-6 intake in patients with T2DM (Öztürk et al. 2023). Some have found a positive correlation between omega-6 intake and visfatin levels (Rahbar and Nabipour 2014). However, the debated omega-6 to omega-3 ratio may be the more important indicator to consider. We found that the ratio of ≥2 is linked to a positive association between visfatin and carotid artery IMT. From the above, it can be inferred that food may be pro-inflammatory and medical nutrition therapy should be one component of diabetes management.

Visfatin's ability to predict subclinical atherosclerosis was reduced in patients using ACE inhibitors, ARBs, and CCBs, likely due to the effects these drugs have on the thickness of the intima media. Previous research has suggested that there are differences not only between but also within drug classes (Derosa et al. 2010). For example, candesartan and amlodipine had no impact on the visfatin levels (Skoczylas et al. 2016), while telmisartan increased and amlodipine decreased them in hypertensive patients without diabetes (Lan et al. 2011). Losartan increased visfatin levels, while ramipril did not (Derosa et al. 2011), candesartan increased visfatin levels, while olmesartan had no effect (Derosa et al. 2010). Higher visfatin levels have been reported in resistant hypertension (Ozal et al. 2017). Our study found a significant correlation between visfatin and subclinical atherosclerosis, particularly in men, in whom hypertension had been less frequently diagnosed. This suggests that undiagnosed hypertension may affect visfatin levels. We found an optimal visfatin level threshold for detecting subclinical atherosclerosis in patients using β-blockers, ACE inhibitors/ARBs, CCBs, and those on unknown drugs.

We found that β-blocker users had higher visfatin concentrations than patients who did not use β-blockers; the correlation between visfatin and subclinical atherosclerosis was statistically significant among β-blocker users. However, metoprolol has been shown to increase intracellular visfatin activity in cardiomyocytes (Li et al. 2022), and bisoprolol to reduce visfatin levels in hypertension (Skoczylas et al. 2016). The impact of β-blockers on visfatin levels remains unclear and requires further research.

Statins, including atorvastatin, rosuvastatin, and simvastatin, decrease visfatin levels across various study populations and atherosclerosis severity levels (Kadoglou et al. 2012; Kostapanos et al. 2008; Petreanu et al. 2014). Our study findings align with existing literature, emphasising the importance of considering antidyslipidemic drug usage when interpreting visfatin concentrations.

9.6 Strengths and limitations

The strengths of this study include an ethnically homogeneous population, standardised radiological studies performed by a single investigator at a single centre to minimise variations, the deliberate exclusion of under-reporters, and the use of regional food-based software for precise 24-hour intake data.

This study had some limitations that should be acknowledged. Its cross-sectional and observational design precludes causal inferences regarding the relationships between adipokines, IMT, carotid plaque, and ABI. The origins of adipokines from multiple cell types introduce complexity and uncertainty into their associations with subclinical atherosclerosis. The relatively small sample size and absence of a healthy control group further constrain the generalizability of the findings. Without a radiological assessment of lower limb atherosclerosis, only ABI may not accurately confirm the diagnosis of lower limb PAD. However, our findings suggest that visfatin levels could offer a low-cost alternative for assessing CV risk.

Manual IMT measurements can vary due to subjective differences among observers, although efforts were made to minimize variability using consistent equipment and expert technicians. The absence of serum Zn and Cu data prevented performing a direct correlation analysis between serum Zn and Cu and dietary intake, which is essential for understanding their role in atherosclerosis. We cannot assume a direct correlation between dietary copper and zinc and their availability in the body. Tissue levels would be required to address this issue accurately and determine whether participants have an abnormal trace element status. Extrapolating from a single-day dietary record to long-term atherosclerosis development is speculative despite our findings being consistent with previous research.

The study focused on drug classes rather than specific medications, limiting conclusions about the effects of individual drugs. Changes in adipokine concentrations linked to age and sex-related variations in body fat distribution would require longitudinal assessment for comprehensive understanding. Different methods of calculating ABI yielded varying associations with CV risk factors and subclinical atherosclerosis; higher ABI demonstrated higher specificity but lower sensitivity, whereas lower ABI showed higher sensitivity but lower specificity, identifying an additional 10% of the population at increased CV risk.

10. FUTURE PERSPECTIVES

The present study demonstrated that an ABI value of less than 1.1 predicts subclinical atherosclerosis in patients with T2DM without clinically expressed atherosclerosis. This threshold should be used for screening for subclinical atherosclerosis and motivating patients to comply with statin therapy in general practice. Future studies should explore integrating the ABI threshold of less than 1.1 in routine clinical screenings for patients with T2DM. Large-scale multicentre trials could validate the effectiveness of this threshold in diverse populations and settings, potentially leading to its widespread adoption in general practice.

Our findings underscore the significant impact of dietary choices on health. While a low-carbohydrate diet helps regulate blood sugar levels, it also poses risks due to the compensatory increased fat and protein intake, imbalanced micronutrients and accelerated atherosclerosis in T2DM patients. Implementing programs to promote healthy dietary practices and regular screening for subclinical atherosclerosis could help provide early intervention and reduce the burden of CVD.

Visfatin levels were significantly associated with markers of subclinical atherosclerosis in patients with T2DM, even after adjusting for common risk factors and CV medications. Therefore, measuring visfatin concentrations could help identify individuals who might benefit from preventive measures against atherosclerosis. Future research should focus on standardising visfatin measurement techniques, exploring its interactions with other biomarkers, and determining its predictive value across different patient demographics and clinical conditions. Developing personalised treatment plans that incorporate ABI measurements, dietary adjustments and vitamin levels could enhance the prevention and management of atherosclerosis in T2DM patients.

11. CONCLUSIONS

1. The ankle-brachial index value can predict subclinical atherosclerosis in patients with T2DM without previously diagnosed atherosclerosis. In males aged 50 years and above, an ABI lower than 1.1 has a significant predictive value for subclinical atherosclerosis in the carotid artery.

2. Out of several tested adipokines, visfatin stands out as a marker of carotid artery subclinical atherosclerosis in patients with T2DM, particularly among males. Visfatin and also resistin mainly correlated with carotid artery atherosclerosis.

3. Patients diagnosed with T2DM exhibit limited adherence to conventional nutritional recommendations. Instead, they adhere to a dietary pattern characterised by low carbohydrate and fibre but high fat, total fat, saturated fat, and salt intake.

4. A high dietary Zn:Cu ratio derived from the dietary pattern in patients with T2DM, as described in our study, is related to worse carotid artery subclinical atherosclerosis.

5. Antihypertensive and statin therapies influence visfatin concentrations, and correcting for dietary patterns strengthens the statistical correlation between visfatin and carotid artery subclinical atherosclerosis.

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13. SUMMARY IN ESTONIAN

Subkliinilist ateroskleroosi ennustavad tegurid ja markerid 2. tüüpi diabeediga haigetel

Sissejuhatus

Teist tüüpi diabeedi (T2DM) ja sellega seotud veresoonte tüsistuste levimus on kasvutrendis kogu maailmas, mille tõttu tõuseb ka tervishoiusüsteemi koormus. Eestis diagnoositi 2022. aastal 5397 uut T2DM juhtu (tervisestatistika ja terviseuuringute andmebaas 2024) ja see trend on alates 2016. aastast püsinud stabiilsena. Sama andmebaasi alusel registreeriti 2016. aastal 8953 südame isheemiatõve, 5659 tserebrovaskulaarsete ja 465 alajäseme arterite haiguste esmajuhtumit.

Ateroskleroosiga seotud haigused, eriti alajäsemete arterite haigus, on aladiagnoositud. Andmebaasid peegeldavad ainult arstide diagnoositud juhtumeid radioloogiliste uuringute või väljendunud kliinilise leiu põhjal. Esmatasandi arstiabis aga alajäseme arterite haiguse tuvastamise võimalusi ei kasutata. 2024. aastal viidi Inglismaa ja Iirimaa perearstide seas läbi veebiküsitlus, et kajastada kasutatavaid diagnostikameetodeid, mida rakendatakse alajäsemete arterite haiguse kahtlusega patsientidel. Selgus, et hüppeliigese-õlavarre indeksit (ABI) ei kasuta 25% Inglismaa ning 55% Iirimaa perearstikeskustest (Konya *et al*. 2024). Euroopa patsientidel, kes ei ole kõrge kardiovaskulaarse riskiga, on asümptomaatilise PAD levimus 17,8% (Cimminiello *et al*. 2011). Hamburgi linna terviseuuringus oli alajäseme arterite haiguse levimus 23,6% ja unearterite ateroskleroosi (IMT ≥1 mm) levimus 30% (Behrendt *et al.* 2023). Subkliiniline ateroskleroos, mis eelneb kliiniliselt avaldunud kardiovaskulaarsetele haigustele, jääb sageli varjatuks, kuni see areneb ilmsemateks tüsistunud haigusvormideks.

Subkliinilise ateroskleroosi varajane avastamine on eriti oluline T2DM-iga patsientidel, kuna diabeet mitte ainult ei suurenda ateroskleroosi riski, vaid raskendab ka selle avastamist ja ravi. Varajases staadiumis avaldub ateroskleroos arteriseina paksenemise, naastude moodustumise ja endoteeli düsfunktsioonina, kuid ilmsed kliinilised sümptomid puuduvad. Seega on oluline leida haiguse varajaseks avastamiseks ja sekkumiseks usaldusväärsed markerid, eriti kõrge riskiga populatsioonides nagu T2DM-iga patsiendid.

Ateroskleroosi arengus mängib olulist rolli rasvumine, mis on sageli seotud T2DM-iga (Siiteri 1987). Rasvkude toimib endokriinse organina, eritades adipokiine, mis osalevad ateroskleroosi tekkes ja progresseerumises (Siiteri 1987; Liu *et al.* 2022). Adipokiinide ja subkliinilise ateroskleroosi vaheliste seoste uurimine on uute varajase ateroskleroosi riskimarkerite leidmisel paljulubav.

Tervislik toitumine ja kehaline aktiivsus on diabeediravi alustalad, mis aitavad vähendada T2DM-i ja sellega seotud tüsistuste riski (Aas *et al.* 2023). T2DM-iga inimeste toitumisharjumuste hindamine ning selle seose subkliinilise ateroskleroosi ja adipokiinidega uurimine võib aidata tuvastada spetsiifilisi toitumisega seotud riske.

Seoseid kaugele arenenud ateroskleroosi ja selle teket soodustavate riskitegurite vahel on püüdnud leida arvukad uuringud, kuid varajaste staadiumite uuringuid on vähe. Doktoritöö eesmärk on leida markerid, mis aitaksid kergemini tuvastada subkliinilist ateroskleroosi ja motiveeriksid patsiente muutma oma elustiili ning otsima sobivat ravi.

Uurimistöö eesmärgid

Uurimistöö üldeesmärk on leida tegurid ja markerid, mis ennustavad 2. tüüpi diabeediga patsientidel subkliinilist ateroskleroosi.

Uuringu konkreetsed eesmärgid on alljärgnevad:

1. Hinnata, kas 2. tüüpi diabeediga patsientidel on võimalik hüppeliigese-õlavarre indeksiga ennustada unearteri subkliinilist ateroskleroosi.
2. Hinnata, kas 2. tüüpi diabeediga haigetel esineb seos veres ringlevate adipokiinide kontsentratsiooni ja subkliinilise ateroskleroosi vahel.
3. Hinnata, kas ja millisel määral järgivad 2. tüüpi diabeediga haiged üldiseid toitumissoovitusi.
4. Teha kindlaks, kas 2. tüüpi diabeediga haigetel on seos ateroskleroosi ja toidus sisalduvate mikrotoitainete kõrvalekalletega soovituslikest kontsentratsioonidest.
5. Teha kindlaks tegurid, mis mõjutavad 2. tüüpi diabeediga haigetel adipokiinide ja subkliinilise ateroskleroosi seost.

Uuritavad ja meetodid

Läbilõikelisse prospektiivsesse uuringusse kaasati 216 patsienti kolmeteistkümnest Tartumaa perearstikeskusest. Uuritavad olid 30–70-aastased T2DM-ga patsiendid, kellel ei olnud varem diagnoositud ateroskleroosi ega sellega seonduvaid tüsistusi. Perearstid kutsusid oma nimistust uuringus osalema kõiki T2DM-ga haigeid, kes vastasid uuringu kriteeriumidele.

Pärast uuringusse kutsututelt informeeritud nõusoleku saamist toimus vastavates perearstikeskustes uuringu esimene etapp. Uuringupäeva hommikul võeti patsientidelt kümnetunnise paastu järel vereanalüüsid. Seejärel viidi läbi individuaalsed intervjuud tervise, sotsiaaldemograafiliste tunnuste, suitsetamise ja ravimite kasutamise kohta. Füüsilisel läbivaatusel fikseeriti kehapikkus, kaal ja ümbermõõdud. Alajäsemete arteriaalse vereringe hindamiseks mõõdeti ABI.

Uuringu teine etapp toimus Tartu Ülikooli Kliinikumis, kus viidi läbi ultraheliuuringud karotiidarterite *intima-media* paksuse (IMT) ja aterosklerootilise naastu olemasolu kohta ja DEXA uuring kehakoostise hindamiseks.

Viimase 24 tunni toitumise hindamiseks kasutati NutriData tarkvara. Kuna üldine toitainete tarbimine on tihedas korrelatsioonis tarbitud energia kogusega, siis toitumisega seotud analüüsidest eemaldati uuritavad, kes raporteerisid ebausutavalt madala energiatarbimise. Selleks kasutati Goldbergi väljalõike meetodit (Black 2000). Raporteeritud energiatarbimine (EIrep) oli energiatarbimine, mille

kohta saadi teave 24-tunnise toitumise meenutamise põhjal. Hinnangulise põhiainevahetuse kiiruse (BMRest) arvutamiseks kasutati Katch-McArdle'i valemit [BMR = 370 + 21,6 x kaal x (100 – keharasv)/100]. Selles valemis arvestati kehakaalu kilogrammides ja keharasva protsentides. Keha rasvasisaldus saadi DEXA uuringust (Lunar Prodigy Advance Dual Energy X-Ray absorptiomeeter, GE Healthcare, USA).

Kuna energiatarbimine peaks olema võrdne energiakuluga (Black 2000), näitab EIrep ja BMRest suhe kehalise aktiivsuse taset (PAL=EIrep:BMRest). Uuritavate individuaalse kehalise aktiivsuse hindamiseks kasutati rahvusvahelist kehalise aktiivsuse küsimustikku (IPAQ). Aktiivsus arvutati pideva muutujana (metaboolne ekvivalent tööminutite kohta nädalas) ja seejärel esitati kategooriates madala, mõõduka või kõrge aktiivsustasemena (IPAQ Research Committee 2005). IPAQ-põhised kategooriad teisendati PAL-kategooriateks (madal, mõõdukas ja kõrge aktiivsus vastavalt PAL väärtustele 1,4; 1,6 ja 1,8). Vanuse järgi arvutati iga kehalise aktiivsuse kategooria künnised (95% alumine ja ülemine usalduspiir). 16–70-aastaste inimeste jaoks määrati PAL-i vahemikud 0,872–2,249; 0,996–2,570 ja 1,120–2,892 vastavalt madala, mõõduka ja kõrge aktiivsustaseme jaoks (EFSA paneel 2013). PAL-i individuaalsed väärtused arvutati välja ja võrreldi vastava tegevuskategooria väärtustega. Katsealused, kellel oli EIrep:BMRest alla individuaalse PAL-i alampiiri, klassifitseeriti alaraporteerijateks. Usutavad raporteerijad olid katsealused, kelle EIrep:BMRest oli alumise ja ülemise piiri vahel. Üleraporteerijatena määratleti katsealused, kelle EIrep:BMRest suhe oli ülempiirist kõrgemal.

Perearstikeskustes koguti perifeersest veenist veri, mille analüüsimiseks kasutati SYNLABi laborit. Vastavalt SYNLABi standardmetoodikale mõõdeti glükeeritud hemoglobiini (HbA1c), kõrge tundlikkusega C-reaktiivse valgu ning üldkolesterooli, triglütseriide, madala ja kõrge tihedusega kolesterooli kontsentratsioonid. Vereproovid tsentrifuugiti seerumi eraldamiseks ja säilitati -80 °C juures kuni metaboolsete markerite paneeli (sh suure molekulmassiga (HMW) adiponektiin, leptiin, retinooli siduv proteiin-4 (RBP-4), resistiini ja visfatiini kontsentratsioon) teostamiseni, milleks kasutati Luminex Corporationi xMAP® tehnoloogiat.

Statistiliste analüüside läbi viimiseks kasutati SPSS versioone 26–29 (IBM Corp., USA). Muutujate normaaljaotuse kontrollimiseks kasutati Shapiro-Wilki testi. Andmeid, mis ei järgnenud normaaljaotusele, esitati mediaani ja interkvartiilvahemiku abil, samas kui normaaljaotusega andmeid kirjeldati keskmise ja standardhälbega. Kategooriliste muutujate rühmade vaheliste erinevuste hindamiseks kasutati kas Hii-ruut-testi või Fisheri täpset testi. Normaaljaotusega parameetrite keskmiste võrdlemiseks rakendati ANOVA-d või Studenti t-testi. Pidevate väärtuste ja riskifaktorite vahelisi seoseid analüüsiti lineaarse regressioonimudeli abil, samal ajal kui binaarsete väärtuste ja riskifaktorite vahelisi seoseid uuriti logistilise regressioonimudeli abil. Statistiliselt oluliste erindite (*outliers*) olemasolu hinnati võimendusväärtuse (*leverage values*) ja Cooki statistikut kasutades.

Tulemused ja järeldused

1. T2DM haigetel, kellel ei ole varem ateroskleroosi diagnoositud, ennustab ABI väärtus subkliinilist ateroskleroosi (p=0,037). See seos püsis ka pärast kohandamist hüpertensiooni, hüperlipideemia, diabeedi kestuse, KMI, HbA1c ja suitsetamisega (p=0,039). Ennustusvõime oli tugevaim üle 50-aastastel meestel (p=0,013). Uuritavad, kellel tuvastati ateroskleroos (IMT väärtusega ≥1,0 mm ja/või naast), olid madalama füüsilise aktiivsusega, pikema diabeedi kestusega, vanemad, sagedamini suitsetajad ja neil esines sagedamini alajäseme arterite haigust.
2. Visfatiin ennustab T2DM haigetel subkliinilist ateroskleroosi. Resistiin korreleerus küll positiivselt keskmise IMT-ga (p=0,009) ja sellel oli tagasihoidlik seos naastudega (p=0,049), kuid ei suutnud ennustada ABI-d ega alajäseme arterite haigust. Visfatiin korreleerus positiivselt keskmise IMT (p=0,002), IMT ≥1 (p=0,002) ja IMT ≥1 ja/või naastudega (p=0,008), kuid vaatamata tagasihoidlikule pöördvõrdelisele seosele ABI-ga ei näidanud visfatiin seost naastude ega alajäseme arterite haigusega. HMW adiponektiin, leptiin ja RBP4 ei näidanud statistiliselt olulist seost subkliinilise ateroskleroosiga.
3. T2DM-ga haiged jälgivad väga vähesel määral Eesti üldiseid ja Euroopa Diabeedi Uuringute Ühingu toitumissoovitusi (Aas *et al.* 2023; Pitsi *et al.* 2017). Enamik osalejatest järgis samal ajal kaheksast toitumissoovitust kolmest. Väljakujunenud toitumismustrit iseloomustab madal süsivesikute ja kiudainete sisaldus, kuid kõrge rasvade, küllastunud rasvade ja soola tarbimine.
4. Tuvastatud toitumismustrist lähtuvalt on T2DM haigete toidus kõrge tsingi ja vase suhe (Zn:Cu) ning see on seotud unearteri *intima-media* paksenemisega (p=0,024). See seos jäi püsima ka pärast traditsioonilistele kardiovaskulaarsetele riskiteguritele kohandamist.
5. Toitumisharjumused tugevdavad statistilist korrelatsiooni visfatiini ja subkliinilise ateroskleroosi vahel. Visfatiin ennustab *intima-media* paksust patsientidel, kelle süsivesikute tarbimine on madal (p=0,003), kuid rasva (p=0,020) ja soola (p=0,013) tarbimine on kõrge. Lisaks mõjutavad visfatiini kontsentratsiooni antihüpertensiivne ja statiinravi. Metformiini ja beeta-blokaatorite (BB) tarvitajatel oli kõrgem visfatiini kontsentratsioon kui nende ravimite mittetarvitajatel, samas statiinravi saajatel oli visfatiini kontsentratsioon madalam. Visfatiini võime ennustada subkliinilist ateroskleroosi (IMT ≥1,0 mm ja/või unearteri naast) varieerus ravimgrupiti. BB puhul oli optimaalne visfatiini piirväärtus 1,63 pg/ml (88% tundlikkus, 70% spetsiifilisus), angiotensiini konverteeriva ensüümi inhibiitorite (ACEI) ja/või angiotensiini retseptori blokaatorite (ARB) ja kaltsiumikanali blokaatorite (CCB) kasutajatel 1,23 pg/ml (86% tundlikkus, 83% spetsiifilisus) ja uuritavatel, kelle puhul kasutatav ravi ei olnud teada 1,41 pg/ml (63% tundlikkus, 60% spetsiifilisus) ja metformiini, ACEI/ARB ja CCB koos kasutavatel ≥1,15 pg/ml (94% tundlikkus, 58% spetsiifilisus).

Uuring näitas, et ABI väärtus ≤1,1 ennustab subkliinilist ateroskleroosi T2DM-ga patsientidel, kellel ei ole eelnevalt diagnoositud ateroskleroosi ega selle tüsistusi. Seda läve tuleks kasutada subkliinilise ateroskleroosi sõeluurimiseks ja selleks, et motiveerida patsiente muutma oma elustiili ja raviskeemi. Lisaks rõhutavad selle töö tulemused toitumisvalikute olulist mõju tervisele. Kuigi süsivesikutevaene dieet aitab reguleerida veresuhkru taset, kujutab see endast riske ka kompenseerivalt suurenenud rasva- ja valgutarbimise, tasakaalustamata mikrotoitainete ja T2DM-i kiirenenud ateroskleroosi tõttu. Tervislike toitumistavade ja regulaarsete sõeluuringute edendamise programmide rakendamine võib aidata varakult sekkuda ja vähendada südame-veresoonkonna haiguste koormust tervishoiusüsteemile. Visfatiini kontsentratsioonide mõõtmine verest aitab tuvastada isikuid, kes võivad saada ateroskleroosi vastastest ennetusmeetmetest kõige enam kasu.

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PUBLICATIONS

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2. **Kärberg K,** Forbes A, Lember M. Unlocking the Dietary Puzzle: How Macronutrient Intake Shapes the Relationship between Visfatin and Atherosclerosis in Type 2 Diabetes. Medicina. 2024;60(3):438.
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2021–	The World Leader Interactive Network Focused On Critical UltraSound (WINFOCUS)
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Teadustegevus: Minu uurimistöö keskendub peamiselt ateroskleroosi varajastele biomarkeritele ning dieedi ja mikroelementide mõjule kardiovaskulaarsele

riskile. Selle eesmärk on ka välja töötada kulutõhusad meetodid ateroskleroosi riski hindamiseks kliinilises töös.

Artiklid rahvusvahelistes eelretsenseeritavates ajakirjades:

1. Pietrantonio F, Florczak M, Kuhn S, **Kärberg K**, Leung T, Said-Criado I et al. Applications to augment patient care for Internal Medicine specialists: a position paper from the EFIM working group on telemedicine, innovative technologies & digital health. Front Public Health. 2024;12:1370555.
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DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

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2. **Mihkel Zilmer**. Na-pump in normal and tumorous brain tissues: Structural, functional and tumorigenesis aspects. Tartu, 1991.
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