TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

INDOLEAMINE 2,3-DIOXYGENASE IN CARDIOMETABOLIC HEALTH

Petri Niinisalo

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1797 | MEDICA – ODONTOLOGICA | TURKU 2024

University of Turku

Faculty of Medicine
Department of Clinical Medicine
Internal Medicine
Drug Research Doctoral Programme
Research Centre of Applied and Preventive Cardiovascular Medicine

Supervised by

Professor Risto Kaaja
Department of Clinical Medicine, University of Turku and Division of Medicine, Turku University Hospital, Turku, Finland

Professor Markus Juonala
Department of Clinical Medicine, University of Turku and Division of Medicine, Turku University Hospital, Turku, Finland

Reviewed by

Professor Jari Laukkanen
Institute of Clinical Medicine, School of Medicine, Faculty of Health Sciences, University of Eastern Finland, Kuopio, Finland

Professor Olavi Ukkola
Internal Medicine, Research Unit of Biomedicine and Internal Medicine, Faculty of Medicine, University of Oulu, Oulu, Finland

Opponent

Professor Risto Kerkelä
Molecular Cardiology, Research Unit of Biomedicine and Internal Medicine, Faculty of Medicine, University of Oulu, Oulu, Finland

The originality of this thesis has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

ISBN 978-951-29-9715-2 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)

UNIVERSITY OF TURKU
Faculty of Medicine
Department of Clinical Medicine
Internal Medicine
Drug Research Doctoral Programme
Research Centre of Applied and Preventive Cardiovascular Medicine
PETRI NIINISALO: Indoleamine 2,3-dioxygenase in cardiometabolic health
Doctoral Dissertation, 96 pp.
May 2024

ABSTRACT

Regulation of immune response is an important factor in determining progress of cardiometabolic diseases that may ultimately lead to atherosclerotic endpoints and premature death. Previous investigations have emphasized immunosuppressive and therefore protective role of intracellular enzyme – indoleamine 2,3-dioxygenase (IDO) – in the development of atherosclerosis. Recently this perspective has been challenged, consequently creating uncertainty of the true function of the enzyme.

The aim of the current study was to elucidate the role and functional mechanisms of IDO in the development of cardiometabolic diseases, especially visceral obesity, metabolic syndrome and atherosclerosis.

Data from three different human cohort studies was used in this thesis; 921 participants from Health 2000 -study, 30 patients from Tampere Vascular Study (TVS) and 927 participants from the Cardiovascular Risk in Young Finns Study (YFS). Association between IDO activity and atherosclerotic risk factors was evaluated by correlation analyses from Health 2000 and YFS-cohorts. Association of IDO in visceral obesity was also investigated in risk ratio analysis from YFS-cohort. Functional role of the enzyme was characterized in gene expression and immunohistochemical analyses executed from tissue samples of TVS.

Based on the Health 2000 and YFS cohort analyses, considerable variation was observed in statistically significant associations between IDO and risk factors. The age and gender of the patients seemed to affect the significance of the connections. In female risk ratio analysis, IDO activity potentially delayed progression of obesity in the premenopausal state. In males, IDO forecasted an increased risk of obesity already after 30 years. Based on tissue samples, the location of IDO was determined in atherosclerotic plaques. In addition, an enhanced expression of IDO and related inflammatory components in plaques was demonstrated.

Presence and operation of IDO in atherosclerosis is unconditional. However, the exact function and role is still cryptic. Under these circumstances, obtained results do not support the use of IDO as a therapeutic target or diagnostic marker; exclusive use as a marker do not provide sufficient evidence to evaluate the state of atherosclerosis in humans, whereas controversial results suggest that the nature of the enzyme may be volatile and regulated by external immunological signals.

KEYWORDS: IDO, cardiometabolic health, obesity, atherosclerosis

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Kliininen laitos
Sisätautioppi
Lääketutkimuksen tohtoriohjelma
Sydäntutkimuskeskus
PETRI NIINISALO: Indoliamiini 2,3-dioksigenaasi kardiometabolisessa terveydessä
Väitöskirja, 96 s.
Toukokuu 2024

TIIVISTELMÄ

Immuunivasteen säätely on tärkeä tekijä määritettäessä ateroskleroosiin ja ennenaikaiseen kuolemaan johtavien kardiometabolisten sairauksien etenemistä. Julkaistut tutkimukset ovat korostaneet solunsisäisen entsyymin – indoleamiini-2,3-dioksigenaasin (IDO) – immunosuppressiivista ja täten suojaavaa roolia ateroskleroosin kehittymisessä. Tämä näkökulma on kyseenalaistettu, mikä on aiheuttanut epävarmuutta entsyymin todellisesta toiminnasta.

Tämän tutkimuksen tavoitteena oli selvittää IDO:n roolia ja toimintamekanismeja kardiometabolisten sairauksien, erityisesti viskeraalisen lihavuuden, metabolisen oireyhtymän ja ateroskleroosin kehittymisessä.

Väitöskirjatyössä käytettiin kolmen eri kohorttitutkimuksen tietoja: Terveys 2000 -tutkimuksesta 921 osallistujaa, Tampereen verisuonitutkimuksesta (TVS) 30 potilasta ja Lasten Sepelvaltimotautien Riskitekijät (Laseri) -tutkimuksesta 927 osallistujaa. IDO-aktiivisuuden ja ateroskleroottisten riskitekijöiden välistä yhteyttä arvioitiin korrelaatioanalyyseillä Terveys 2000 ja Laseri-kohorteista. IDO:n yhteyttä viskeraaliseen lihavuuteen tutkittiin Laseri-kohortista tehdyssä riskisuhdeanalyysissä. Entsyymin toiminnallista roolia karakterisoitiin TVS:n kudosnäytteistä immunohistokemiallisilla värjäyksillä ja geenieksprессioanalyysillä.

Terveys 2000 ja Laseri-kohorttianalyysien perusteella IDO:n ja riskitekijöiden välisissä tilastollisesti merkitsevissä yhteyksissä havaittiin huomattavaa vaihtelua. Potilaiden ikä ja sukupuoli näytti vaikuttavan yhteyksien merkitsevyyteen. Naisten IDO-aktiivisuudella oli potentiaalinen yhteys lihavuuden etenemisen hidastumiseen premenopausaalisessa tilassa. Miehillä IDO ennusti lisääntynyttä lihavuusriskiä jo 30-vuoden jälkeen. IDO:n sijainti määritettiin ateroskleroottisissa plakeissa. Lisäksi osoitettiin IDO:n ja tulehdukseen liittyvien komponenttien korostunut ekspressio.

IDO:n esiintyminen ja toiminta ateroskleroosissa on varmaa, mutta tarkka tehtävä ja rooli on kuitenkin edelleen epäselvä. Saadut tulokset eivät tue IDO:n käyttöä terapeuttisena kohteena tai diagnostisena merkkiaineena; eksklusiivinen käyttö merkkiaineena ei anna riittäviä todisteita ateroskleroottisen tilan arvioimiseksi, kun taas ristiriitaiset tulokset viittaavat siihen, että entsyymin luonne saattaa vaihdella ja olla ulkoisten immunologisten signaalien säätelyn alainen.

AVAINSANAT: IDO, kardiometabolinen terveys, lihavuus, ateroskleroosi

Table of Contents

Abbreviations 8

List of Original Publications 10

1 Introduction 11

2 Review of the Literature 12
2.1 Cardiovascular diseases and pathogenesis of atherosclerosis 12
2.2 Clinical outcomes and the main diagnostic alternatives in atherosclerosis 14
2.3 Atherosclerotic risk factors 16
2.4 Indoleamine 2,3-dioxygenase (IDO) 18
2.4.1 Biochemical function 18
2.4.2 Association of IDO with atherosclerosis 19
2.4.3 Role in other medical conditions 20
2.5 IDO and atherosclerotic risk factors 24
2.5.1 Obesity 25
2.5.2 Metabolic syndrome (MetS) 26
2.5.3 Insulin resistance 27
2.5.4 Non-alcoholic fatty liver disease (NAFLD) 28
2.5.5 Type 2 diabetes 29
2.6 Clinical relevance of IDO in atherosclerosis 31

3 Aims 33

4 Materials and Methods 34
4.1 Description of the study cohorts 34
4.1.1 Health 2000 -study 34
4.1.2 Tampere Vascular Study (TVS) 35
4.1.3 The Cardiovascular Risk in Young Finns Study (YFS). 35
4.2 Study design and participants 35
4.3 Data acquisition 36
4.3.1 Data acquisition in Health 2000 -study 36
4.3.2 Data acquisition in TVS 37
4.3.3 Data acquisition in YFS 39
4.4 Statistical methods 40

5 Results 42
5.1 Characteristics of the participants 42

5.2 IDO activity and atherosclerotic risk factors between studies . 44
5.3 IDO activity and atherosclerotic risk factors in females 45
5.4 IDO activity and atherosclerotic risk factors in males 47
5.5 IDO and obesity 49
5.6 IDO mRNA expression in atherosclerotic tissue 50
5.7 Immunohistochemistry of IDO 51
5.8 IDO-pathway gene expression and pathway construction 53

6 Discussion 56
6.1 Participants 57
6.2 Methods 58
6.2.1 Determination of IDO activity 58
6.2.2 CVD risk factors 58
6.2.3 Immunohistochemistry, GWEA, quantitative RT-PCR.. 59
6.3 Results 59
6.3.1 IDO activity and atherosclerotic risk factors between studies 59
6.3.2 IDO and obesity 62
6.3.3 IDO in human atherosclerotic plaques 64
6.4 Strengths and limitations 67
6.5 Clinical implications and future research prospectives 68

7 Conclusions 70

Acknowledgements 71

References 72

Original Publications 85

Abbreviations

AHA	American Heart Association
APC	antigen-presenting cells
β cells	beta cells
BMI	body mass index
CAC	coronary-artery compliance
CD	cluster of differentiation
cDNA	complementary deoxyribonucleic acid
CpG-ODN	cytosine-phosphate-guanosine oligodeoxynucleotides
CRP	C-reactive protein
CVD	cardiovascular diseases
CTLA-4	cytotoxic T lymphocyte-associated antigen 4
FDR	false discovery rate
FoxP3	fork-head box protein 3
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GSEA	gene-set enrichment analysis
GWEA	genome-wide expression analysis
HDL	high-density lipoproteins
HPLC	high-performance liquid chromatography
IDF	International Diabetes Federation
IDO	indoleamine 2,3-dioxygenase
ICOS	inducible T-cell co-stimulator
IFN-γ	interferon gamma
IL	interleukin
IMT	intima-media thickness
Kyn	kynurenine
LADA	Latent Autoimmune Diabetes in Adults
LDA	low-density array
LDL	low-density lipoproteins
log	logarithmic transformation
MHCII	class II major histocompatibility complex
MetS	metabolic syndrome

mRNA	messenger ribonucleic acid
NAFLD	non-alcoholic fatty liver disease
NSTEMI	non-ST-elevation myocardial infarction
p-value	probability value
qPCR	quantitative polymerase chain reaction
SI	stiffness index
STEMI	ST-elevation myocardial infarction
SLE	systemic lupus erythematosus
TCR	T-cell receptor
TDO	tryptophan 2,3-dioxygenase
TGF-β	transforming growth factor beta
Th1-cell	T helper type 1 cell
TLR9	toll-like receptor 9
Treg-cell	regulatory T-cell
Trp	tryptophan
TVS	Tampere Vascular Study
UAP	unstable angina pectoris
YEM	Young's elastic modulus
YFS	Cardiovascular Risk in Young Finns Study

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Niinisalo, P., Raitala, A., Pertovaara, M., Oja, S. S., Lehtimäki, T., Kähönen, M., Reunanen, A., Jula, A., Moilanen, L., Kesäniemi, Y. A., Nieminen, M. S., Hurme, M. Indoleamine 2,3-dioxygenase activity associates with cardiovascular risk factors: The Health 2000 study. *Scand J Clin Lab Invest*, 2008; 68: 767-770.

II Niinisalo, P., Oksala, N., Levula, M., Pelto-Huikko, M., Järvinen, O., Salenius, J. P., Kytömäki, L., Soini, J. T., Kähönen, M., Laaksonen, R., Hurme, M. & Lehtimäki, T. Activation of indoleamine 2,3-dioxygenase-induced tryptophan degradation in advanced atherosclerotic plaques: Tampere vascular study. *Ann Med*, 2010; 42: 55-63.

III Niinisalo, P., Raitakari, O. T., Kähönen, M., Hurme, M., Lehtimäki, T., Magnussen, C., Viikari, J., Juonala, M. & Kaaja, R. IDO activity forecasts obesity in males and premenopausal females in a 10-year follow-up study:The Cardiovascular Risk in Young Finns Study. *Atherosclerosis*, 2021; 336: 32-38.

1 Introduction

Cardiometabolic health is of cardinal importance in determining physiological capability and maintaining quality of life. If compromised, outcomes are deleterious resulting in developing cardiometabolic diseases due to severe malfunction in metabolism and potentially even premature death. It is widely recognized and accepted that cardiovascular diseases (CVD) constitute the most life-threatening cluster from all cardiometabolic diseases and from CVD, atherosclerosis is the most prevalent cause of heart attacks, strokes and chronic coronary artery disease.

Perhaps a less noticed entity in cardiometabolic diseases, and therefore, also in atherosclerosis is the extensive presence of chronic inflammation, which naturally stimulates activation of immune response. Intracellular enzyme, indoleamine 2,3-dioxygenase (IDO), has traditionally been considered to have an immunosuppressive, and therefore protective role in the development of atherosclerosis (Polyzos et al. 2015), however, these conceptions have also been challenged (Laurans et al. 2018). As such, current understanding of the function is ambiguous. This thesis aimed to elucidate the role and functional mechanisms of IDO in the development of cardiometabolic diseases, especially visceral obesity, metabolic syndrome and atherosclerosis.

The Health 2000 -study consisted of 8,028 participants in a large Finnish cross-sectional health examination survey conducted in the catchment areas of the Finnish university hospitals, while the Cardiovascular Risk in Young Finns Study represented 3,596 randomly chosen participants in a multi-center cohort study in which presence and incidence of cardiovascular risk factors were investigated in the Finnish population over time. Tampere Vascular Study utilized atherosclerotic tissue samples from 30 patients undergoing vascular surgery in Tampere University Hospital. The main objectives of this thesis were to 1) examine correlation of IDO activity with atherosclerotic risk factors in subpopulations from Health 2000 -study and the Cardiovascular Risk in Young Finns Study; 2) elucidate the role and function of IDO in influencing visceral obesity over time; 3) investigate expression of IDO and related inflammatory components in advanced atherosclerotic plaques obtained from Tampere Vascular Study, and to construct a hypothetical IDO-mediated T-cell suppression pathways in human advanced atherosclerosis.

2 Review of the Literature

2.1 Cardiovascular diseases and pathogenesis of atherosclerosis

CVD are the leading cause of death. In 2016 alone, over 17,6 million lives were lost globally because of this infamous cluster of diseases (Murray et al. 2017). From all CVD, atherosclerosis is the most prevalent cause of heart attacks, strokes and chronic coronary artery disease. Atherosclerosis is associated with chronic inflammation, in which activation of the immune system plays a major role. Interaction of components in blood, such as mononuclear cells and lipids, and vascular wall cells is pivotal in initiation and development of atherosclerosis. This interaction is also known to be an important factor regulating genetic control in the development of the disease (Ross 1999; Hansson 2005, Roy et al. 2022; Vuong et al. 2022).

Clinical ramifications from atherosclerotic process are caused due to the narrowing of arterial lumens by atherosclerotic plaques that eventually become calcified (McGill et al. 1997). In this process lipid accumulation in the arterial wall evolve over time causing slow narrowing of the lumen and consequently blockage of blood flow. It has been stated that complete atherosclerotic process in a single human takes over 40 years (Stary 1990) and that clinically life-threatening stage is reached in approximately 55 years-of-age (Insull 2009). Prior to that, human physiology is able to withstand many of the possible adverse events although this is always not the case. Therefore, the risk for atherosclerosis increases with age. It has been found that 66 % of males and 47 % of females over 65 years-of-age have calcification in their coronary arteries (Nasir et al. 2007; Lowenstern et al. 2020). However, atherosclerosis cannot be considered exclusively as the disease of aged population. Previous studies have uncovered that childhood obesity and risk for developing atherosclerosis in adulthood are linked (Raitakari et al. 2005; Juonala et al. 2006).

Atherosclerotic plaque formation does not always happen by coincidence. First, numerous systemic risk factors, such as adverse lifestyle choices, metabolic syndrome (MetS), and type 2 diabetes, increase likelihood of plaque development.

Second, formation occur in branched and/or curvature sections of arterial tree. In these locations vessel wall is exposed to low shear stress or turbulent flow of blood indicating that hemodynamic characteristics play a major role in the development of the plaque (Cheruvu et al. 2007; Caro 2009). Atherosclerotic process progresses in several stages according to American Heart Association (AHA) staging system for atherosclerotic lesions (from type I to type VI) (Stary et al. 1994) (Figure 1). Prior to actual lesion development, a normal physiological process called diffuse intimal thickening develops into atherosclerosis prone arteries, which, however, may also play a role in the atherosclerotic development (Nakishima et al. 2007). These thickenings serve as locations for slow but steady lipid particle and macrophage accumulation (type I). Over time, first visible lesions called fatty streaks containing foam cells are formed (type II). In the next phase, extracellular space is slowly filling with lipids (type III) followed by formation of completely developed atheromatous plaque (type IV), which is covered by intimal tissue. This tissue is later replaced by thick layers of fibrous connective tissue called cap (type V) (Stary et al. 1995).

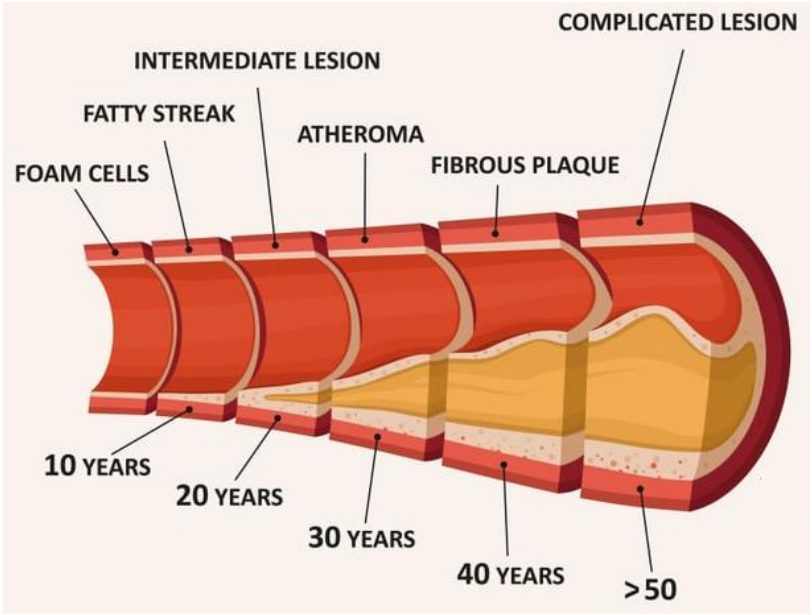

Figure 1. Development of atherosclerosis over time. Foam cells remain in the artery wall in diffuse intimal thickening (type I). First lesions become visible as fatty streaks (type II). Lipids fill extracellular space and form intermediate lesion (type III) followed by development of atheroma (type IV). Fibrous plaque is characterized by a cap made of connective tissue (type V). Finally, a complicated lesion prone to rupture is formed. Modified from https://pharmacygyan.com/atherosclerosis/

2.2 Clinical outcomes and the main diagnostic alternatives in atherosclerosis

Prior to rupture, type IV and V plaques may already produce warning signs by reducing the normal blood flow in coronary arteries. These signs may be identified as clinical symptoms such as chest pain, dyspnea, and nausea for instance during or after physical activity. Manifestation of these symptoms is, however, not self-evident since plaque may also grow from intima into adjacent media and adventitia thereby distorting these layers of artery. In this case clinical symptoms may be absent since the diameter of lumen remains largely undisturbed. However, this compensation by the vascular wall halts when approximately 40 % of the artery area is occupied by the plaque. From then on diameter of arterial lumen is reduced by the plaque growth that may eventually lead to stenosis in which blood flow and consequently oxygen supply is blocked or significantly restricted without the actual rupture of the plaque (Insull 2009).

Ultimate endpoint from atherosclerotic process is reached when the plaque ruptures. In this process low shear stress has been identified as a major cause of plaque instability, which in turn is a result of increased necrotic core volume of the plaque, reduction of stabilizing collagen fibers and endothelial cell coverage, and thinning of fibrous cap due to proteolytic enzyme activity (Chatzizisis et al. 2007; Insull 2009). In addition to low shear stress, tensile stress in the artery wall has been found to be a key factor determining plaque rupture (Slager et al. 2005; Chen et al. 2013). Combined effect of these factors with external triggers such as temporary physiological stress and/or sudden physical activity may be the final provocation for the rupture (Bentzon et al. 2014). An interesting fact in terms of the plaque rupture site is its ability to maintain as a potential rupture site also in the future. Initial rupturing is followed by healing process in which fibrous tissue matrices are reformed (Virmani et al. 2000; Burke et al. 2001). This process may, however, lead to second rupturing. In fact, cycle of rupturing and healing may occur as many as four times at one location. Naturally this results in multiple layers of healed tissue while simultaneously calcium deposits accumulate in the artery wall as large nodules. Further rupturing may expose these nodules that may then become sites of thrombosis (Insull 2009).

In the event of rupture, core of the plaque, containing of hematoma, hemorrhage and/or thrombotic deposits, is disengaged into lumen (type VI) (Hansson & Libby 2006). Contents from the core form a blood clot called thrombus, which then begins to travel inside the lumen due to constant blood flow eventually blocking the flow and hence oxygen supply partially or completely. In some rare cases vasospasm or coronary embolism may also limit the blood flow significantly and cause clinical

symptoms (Bentzon et al. 2014). Regardless of mechanism, sudden loss of oxygen caused by stenosis or blockage is called acute coronary syndrome in which clinical implications appear as perceptible symptoms such as radiating pain (angina pectoris) on the chest, shortness of breath, nausea, and even loss of consciousness. In the most severe cases the endpoint of the process may result in immediate myocardial infarction, ischemia and consequently partial necrosis of heart tissue. This is a severe medical condition since heart muscle is unable to regrow or regenerate. Therefore, damage from necrosis is irreversible unless the blood flow is rapidly restored.

Atherosclerosis may emerge in three different locations: coronary artery, carotid artery and peripheral artery. From these alternatives acute coronary syndromes are divided into three main categories: ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI), and unstable angina pectoris (UAP). STEMI is often caused by a total blockage of one of the main epicardial coronary arteries that provide blood flow to heart muscle, thus resulting in abnormal increase in ST segment of the electrocardiogram wave. When ST segment does not elevate regardless of symptoms of infarction, status is called NSTEMI. It is possible, though, that some changes in electrical patterns may be observed. The underlying cause in this case may be partial blockage in coronary artery or blockage in a branch of arterial tree, often responsible of the subendocardial vascularization, that is not the main coronary artery. Further, in UAP blood flow is compromised due to stenosis or blockage either partially or only for a short period of time. Chest pain and other symptoms may be present for a moment, however, it is possible that the flow is regained with the help of medication or if, for instance, thrombus dissolves by itself. The key difference between these alternative conditions is that necrosis may be absent in UAP, whereas in STEMI/NSTEMI it is present.

The simplest method to evaluate risk for atherosclerosis is to use risk calculators. By entering risk factor data required by the calculator, an estimate of the risk for the disease and potential need for further investigations is produced. In case the risk is only marginal, the need for time-consuming, resource demanding, and expensive investigations may be avoided completely. However, estimate from calculator should always be treated as indicative only.

Preclinical stage of atherosclerosis may be evaluated by measuring intima-media thickness (IMT) using ultrasound. IMT technique was first demonstrated by Pignoli and co-workers in 1986 (Pignoli et al. 1986). In IMT the distance between leading edges of the lumen–intima and media–adventitia interfaces are measured using non-invasive vascular ultrasound (Raitakari et al. 2003). This forms so-called double line pattern due to the two echogenic lines in the arterial wall that correlates strongly with IMT and can be used as a reliable measurement to evaluate clinical stage of atherosclerosis (de Groot et al. 2004). Risk of myocardial infarction has been found to increase 12 to 15 % by each 0.10 mm increment in IMT (Lorenz et al. 2007),

however, direct evidence uniting IMT and death due to cardiovascular event explicitly has not been found (Suzuki et al. 2020).

Another outcome from atherosclerotic development is calcification of artery and consequently decline of arterial elasticity, which, however, can be determined. Elasticity is generally maintained due to the high elastin to collagen ratio, but normal ageing reverses this ratio in addition to calcification process, thus causing arterial stiffness (Avolio et al. 1998). Association of stiffness to CVD risk has been verified previously (Koskinen et al. 2012; van Sloten et al. 2014). Stiffness can be measured ultrasonically as coronary-artery compliance (CAC), Young's elastic modulus (YEM) or stiffness index (SI). CAC evaluate the ability of arteries to expand as a response to pulse pressure caused by cardiac contraction and relaxation, while YEM measures change in arterial diameter during cardiac cycle, thereby producing an estimate of arterial stiffness independent of wall (intima-media) thickness. SI has been generated to reduce the impact of the curvilinear pressure-stiffness relationship on arterial stiffness and is therefore considered to be relatively independent of blood pressure (Juonala et al. 2005).

Recent advances in diagnostic alternatives have made it possible to evaluate the stage of the disease using more sophisticated techniques. Today, suspected coronary artery disease and the severity of the disease can be first determined by using a computed tomography scan, which can detect the stenosis with high certainty (Saraste & Knuuti 2018; Fusaro & Tessarin 2021). The evaluation can be continued with myocardial perfusion imaging techniques, such as positron emission tomography scan, which shows the blood flow in the coronary arteries and the heart muscle (Kazakauskaitė et al. 2018; Li & Kronenberg 2021). It is also possible to combine the images produced by these two methods in a technique called cardiac hybrid imaging to improve interpretation of the results morphologically and functionally (Gaemperli et al. 2011). Based on the results, the patient's need for, for example, percutaneous coronary intervention or bypass surgery can be assessed. However, despite of these recent advances, invasive coronary angiography is still widely used and in certain situations it defends its position as a go-to methodology. For instance, computed tomography is not suitable if operational procedures must be performed for morbidly obese patient.

2.3 Atherosclerotic risk factors

As stated previously, numerous systemic risk factors serve as possible sources promoting plaque development. Factors can be roughly classified into those that can be controlled and those beyond of control. In addition to age and sex, also hereditary

factors cannot be controlled. Both, previous family incidence as well as polygenic risk score have been found to be predisposing factors for future atherosclerotic events (Nasir et al. 2007; Lieb et al. 2020; Mars et al. 2020; Christiansen et al. 2021). Root causes for factors that can be controlled, however, are mostly related to lifestyle choices, especially the amount and quality of consumed nutrition (Torres et al. 2015). In many cases causal relationship is straight-forward; vast amounts of fat-containing food will consequently increase a chance for obesity, unless this process is somehow compensated, for instance, by enhanced level of physical activity. Detrimental nutritional choices are likely to lead into increased subcutaneous fat directly under the skin, but also accumulation of more hazardous visceral fat inside the body.

Other lifestyle choices, such as smoking and alcohol consumption, also play a significant role in promoting atherosclerotic process. Smoking causes deleterious effects on the cardiovascular system for instance by inducing oxidative stress, promoting vascular inflammation and dysfunction, and also by compromising serum lipid profile (Siasos et al. 2014). Consumption of excess alcohol results in anatomical damage of the cardiovascular system in the form of changes in circulation, inflammatory response, oxidative stress, and programmed cell death (Piano 2017). All previously mentioned adverse lifestyle choices are predisposing factors for dyslipidemia, hypertension, obesity, and increase in IMT. Simultaneously with these processes, excess amounts of inflammatory cells accumulate between visceral fat cells causing chronic low-grade inflammation in adipose tissue. Due to initiation of atherosclerotic plaque development, namely lipid particle and macrophage accumulation, inflammation extends to artery wall (Ross 1999; Hansson 2005).

Interactions between dyslipidemia, hypertension, and obesity operate as an omnidirectional complex network causing stresses of local hemodynamics and blood flow patterns (Insull 2009). As such, it cannot be stated which of the independent risk factors is more adverse in a single human because this depends on individual properties and characteristics, such as hereditary factors, physiological status, level of physical activity, and drug response. What can be stated, though, is that dyslipidemia, hypertension, and obesity together form a clinically defined risk cluster – MetS – which is also related to insulin resistance. This cluster may serve as a pathway to non-alcoholic fatty liver disease (NAFLD), type 2 diabetes and eventually to atherosclerotic endpoints. It should be noted that even though relationships between risk factors is presented here as a cascade, in reality some of the factors may have a diminished role or even be absent. For instance, not all diabetics have all metabolic diseases (Åberg et al. 2018). In addition, acute coronary syndrome is just one out of several potentially developing diseases; in type 2 diabetes many other organs may damage and fail prior to heart. Risk factors, their influence and implications on IDO activity are elaborated in section 2.5, while Table 1 summarises risk factors related to cardiometabolic health.

2.4 Indoleamine 2,3-dioxygenase (IDO)

IDO is an intracellular enzyme expressed mainly in human antigen-presenting cells (APC), namely dendritic cells, macrophages, and B cells that can be found in blood, connective and lymphoid tissues, and also in skin and mucosal epithelium. Previous studies have widely recognized IDO as one of the regulators of immune response (Mellor & Munn 1999; Mellor & Munn 2003) that also correlates with early signs of atherosclerotic risk factors (Pertovaara et al. 2007). However, the function of IDO is not only limited to atherosclerosis. Indeed, the role of IDO – especially how it influences human physiology, how it is regulated, and the role it has in different medical conditions – has been extensively characterized and discussed. It is generally recognized that expression of IDO is regulated by a complex array of immunological signals and as stated above IDO-expressing cells are found in several locations throughout human body (Mellor & Munn 2004). Together these form several potential impact avenues for operation in humans. Furthermore, activity of IDO is divided in IDO1, expressed in adipose tissue (Favennec et al. 2015), and IDO2, expressed for instance in the liver and kidneys (Merlo et al. 2020). Focus of this thesis is in function of IDO1 (named "IDO" from now on), which is the most plausible influencer in initiation and progression of atherosclerosis.

2.4.1 Biochemical function

The main function of IDO is to promote degradation of an essential amino acid tryptophan to kynurenine. This process is initiated by activation of IDO in mature IDO+ dendritic cells (APC). An array of pro-inflammatory mediators is likely to induce expression (Mellor & Munn 2004), and the whole process is presumably regulated by an intricate interplay of innate and adaptive immune responses (Hansson & Libby 2006; Nilsson & Hansson 2008). Metabolites from the process suppress T-cell activity due to decreased concentration of tryptophan in local microenvironments. The process, as a whole, is often regarded as "suppression by starvation" (Hwu et al. 2000; Terness et al. 2002; Mellor & Munn 2003; Mellor & Munn 2004). In terms of atherosclerosis, reduced T-cell activity may lead to diminishing inflammatory response in the artery wall, consequently slowing down development of the disease (Hansson 2005; Hansson & Libby 2006; Gisterå & Hansson 2017).

In addition to IDO, tryptophan degradation is also regulated by hepatic enzyme tryptophan 2,3-dioxygenase (TDO) expressed mainly in the liver (Mellor & Munn 2004; Ball et al. 2007). Compared to IDO, signals from the immune system do not induce or regulate TDO expression, and therefore expression is believed to focus on regulation of basal serum tryptophan concentrations. Consequently, TDO is regarded primarily as a homeostatic or "housekeeping" gene (Mellor & Munn 2004).

Even though the function of IDO may seem rather straight-forward, it is not. While reduced level of tryptophan has often been regarded as a defence mechanism regulating immunity by limiting growth of intracellular pathogens and proliferation of tumour cells (Pfefferkorn 1984; Ozaki et al. 1988; Mellor & Munn 2003; Mellor & Munn 2004), in fact elaborate regulatory functions and *in vivo* significance of IDO are still being debated and investigated today. From evolutionary perspective the function of IDO may have been focused on protecting primitive organisms from microbes probably using the same type of signal cascade as is seen today in mammalian systems. Therefore, historical standpoint favours the role of IDO as a host-defence system aiming to promote immunological status, thereby functioning as a protective enzyme in humans (Mellor & Munn 2004). On the other hand, suppression of T-cell activity does not always generate positive outcomes. Indeed, some functions of IDO may under certain conditions also produce harmful effects, namely, promotion of atherosclerosis (Hansson & Libby 2006; Laurans et al. 2018). This underlines complexity and even unpredictability of regulatory systems controlling up- and down-regulation of IDO.

2.4.2 Association of IDO with atherosclerosis

Previous studies have verified a connection between increased IDO activity and atherosclerosis both in mouse models (Polyzos et al. 2015; Laurans et al. 2018) and in humans (Wirleitner et al. 2003; Wongpraparut et al. 2021). Activation is already promoted by early signs of atherosclerotic risk factors (Pertovaara et al. 2007; Wolowczuk et al. 2012; Favennec et al. 2015) and as the disease progresses, association is further encouraged by chronic inflammation caused by complex interplay and signalling cascades between low-density lipoproteins (LDL), pro-inflammatory mediators, monocytes, macrophages, T-cells and other components in the artery wall (Hansson & Libby 2006). Especially the role of pro-inflammatory mediators is essential in this process offering alternative pathways for promotion of IDO activity. Also elaborate signalling relationships between regulatory T-cells (Treg-cells), APC, and T helper type 1 cells (Th1-cells) are likely to play a part in activation (Boasso et al. 2005; Mahnke et al. 2007; Vuong et al. 2022). Outcome

from activation may result in decreased vascular inflammation and consequently delayed progression of atherosclerosis. Furthermore, activation may also be promoted via some other previously uncharacterized mechanisms, which would be plausible due to complex interplay between various components in regulation of IDO activity.

Even though the function of IDO in atherosclerosis has traditionally been linked to beneficial outcomes, namely atheroprotective mechanism by reducing T-cell activity and by eliminating potentially harmful microbial agents and waste products (Nilsson & Hansson 2008), it should be noted that under certain conditions IDO may also promote adverse atherosclerotic events due to complexity and unpredictability of immune response (Hansson & Libby 2006). In relation to this, in a mouse model study IDO deletion or inhibition decreased chronic inflammation along with other beneficial effects (Laurans et al. 2018). Even though this result appeared in mice, the possibility cannot be excluded that this finding could also be observed in humans. In such case, it could imply a dual role for IDO; under certain conditions IDO activity could attenuate aspects of atherosclerosis and its complications whereas in others it could promote initiation and progression of the disease. Speculatively, this would suggest that the role and function of IDO could deviate depending on characteristics and physiological properties of an individual. Furthermore, fluctuation of IDO activity could even occur in a single human depending on the immunological status at different phases during lifespan. Identified and admitted interplay of innate and adaptive immune responses in atherosclerosis may support this hypothesis (Hansson & Libby 2006; Nilsson & Hansson 2008). To summarize, inflammation in the pathogenesis of atherosclerosis – and participation of IDO in the process – is widely recognized, however, the exact function and significance of the enzyme is still enigmatic.

2.4.3 Role in other medical conditions

In addition to atherosclerosis, IDO is also known to have a crucial role in several medical conditions in humans. Due to its ability to suppress T-cell activity, IDO activation increases a possibility for allograft survival after transplantation (Sucher et al. 2012). This activity is not limited to cardiac allograft survival (Jianping et al. 2007; Li et al. 2007; Yu et al. 2008), but is also observed in other cases such as renal, kidney, skin and liver transplantations (Cook et al. 2008; Wang et al. 2010; He et al. 2015; Khosravi-Maharlooei et al. 2017). The beneficial outcome is achieved by modulation of activation mechanisms, and consequently inflammatory responses, between IDO, T-cells and APC (Boasso et al. 2005; Mahnke et al. 2007). Utility of

IDO could be further enhanced by developing mechanisms that directly and selectively modify operation of IDO through activation or suppression. One example of successful modification has been achieved in a murine model, in which gene delivery of IDO prolonged cardiac allograft survival by shaping the types of T-cell responses (Yu et al. 2008).

Another role of IDO is its function in autoimmune diseases and further operation in complications related to pregnancy, which both are also indirectly related to atherosclerosis. From autoimmune diseases, systemic lupus erythematosus (SLE) is highly prevalent in young females (Bruce 2005) and also associated with IDO activity (Widner et al. 2000). The cause of the disease is unknown, but genetics and sex hormones are considered predisposing factors. Significance of symptoms vary from mild to severe. Occasionally, females may also have a simultaneous antiphospholipid syndrome and as a result, pathogenic antiphospholipid antibodies. These antibodies, combined with SLE, are associated with higher prevalence of thrombosis and consequently, atherosclerosis (Bruce 2005; Smith et al. 2016; Khan et al. 2017; Yelnik et al. 2017), but also pregnancy morbidity even though exact activation mechanism is still uncertain (Ünlü et al. 2016; Pons-Estel et al. 2017). It has been suggested, though, that oxidative stress may play a significant role both in the pathogenesis of SLE and atherosclerosis (Smith et al. 2016; Yang et al. 2016) and that macrophages in SLE may actually contribute to the development of accelerated atherosclerosis (Lewandowski & Kaplan 2016). In addition, SLE and antiphospholipid antibodies are also risk factors for pre-eclampsia (Simard et al. 2017) and prognostic factors for subsequent atherosclerosis in mother (Giguère et al. 2012; Brown et al. 2013; Gamble et al. 2019) and child (Kajantie et al. 2009, Mongraw-Chaffin et al. 2010).

Even though limited amount of research data has characterized the role of IDO in autoimmune diseases and pregnancy in detail, existing evidence have confirmed the presence and expression of IDO in placenta and serum during pregnancy (Zong et al. 2016), which may lead to pregnancy-related detriments if expression is compromised (Chang et al. 2018). Indeed, decreased IDO expression has been linked to recurrent spontaneous abortion (Ban et al. 2013; Zong et al. 2016) suggesting that IDO activity has a crucial role in maintaining immunological tolerance between mother and fetus during pregnancy (Obayashi et al. 2016). Further evidence has also suggested that IDO may be involved in preventing vascular rupture in calcified placenta during pregnancy, and that suppression of T-cell activity may reduce a possibility for acute placental inflammation and consequently prevent spontaneous abortion of fetus (Goldstein et al. 2020).

In addition to clinical relevance, these findings also suggest that IDO activity between females and males is likely to vary from operational standpoint; there may be differences either in intensity, activation mechanism, or combination of these. For

instance, in females, hormonal status is presumably playing a significant role, and impact of IDO activity may also be age-dependent and related other risk factors leading to increased risk for atherosclerotic endpoints (Bruce 2005; Sacre et al. 2015). One possible explanation for differences could be elevated estrogen levels in premenopausal females. Estrogen has been found to up-regulate dendritic cells (APC) to express IDO, which consequently may suppress T-cell responses (Xiao et al. 2004). This proposal is in line with previous research; female physiology may be using estrogen as a mediator and IDO as a weapon in its struggle to diminish the impact of adverse consequences of autoimmune diseases and complications in pregnancy.

Table 1. Summary of risk factors related to cardiometabolic health and potential influence and activation mechanism of IDO.

Risk factor	Description of risk factor and potential implications	Influence and mechanism of IDO	References
Uncontrollable	Family incidence	Unknown	Nasir et al. 2007; Lieb et al. 2020
Uncontrollable	Polygenic risk score	Unknown	Mars et al. 2020; Christiansen et al. 2021
Controllable	Detrimental nutritional choices; quality and quantity of consumed food	Promotion of subcutaneous and visceral obesity	Torres et al. 2015
Controllable	Smoking	Unknown	Siasos et al. 2014
Controllable	Alcohol	Unknown	Piano 2017
Dyslipidemia	Up-regulation of LDL and triglycerides	Connected, probably indirect mechanism	Pertovaara et al. 2007; Cole et al. 2015
Dyslipidemia	Down-regulation of HDL	Connected, probably indirect mechanism	Pertovaara et al. 2007; Cole et al. 2015
Hypertension	Unfavourable food consumption	Probably indirect by vascular relaxation	Wang et al. 2010; Nagy et al. 2017
Hypertension	Hereditary predisposition	Unknown	N/A
Obesity	Accumulation of visceral fat tissue	Promotion of BMI, expression in abdominal fat content, especially white adipose tissue of females	Wolowczuk et al. 2012; Mangge et al. 2014; Favennec et al. 2015; Groer et al. 2018; Larqué et al. 2019
Obesity	Control of body weight and insulin resistance	Shaping gut microbiota	Laurans et al. 2018
MetS	Characterized by obesity, dyslipidemia and hypertension, pathways to NAFLD, type 2 diabetes and CVD	Presence in inflammatory cells among visceral fat cells	Brandacher et al. 2007; Mangge et al. 2014; Mallmann et al. 2018
NAFLD	Abdominal obesity, hypertriglyceridemia, accumulation of excess fat in liver cells, insulin resistance, metabolic disorder	Indirect, perhaps a pro-inflammatory role related to obesity	Milić et al. 2014; Teunis et al. 2022
NAFLD	Abdominal obesity, hypertriglyceridemia, accumulation of excess fat in liver cells, insulin resistance, metabolic disorder	Neutral or promotion of insulin resistance	Laurans et al. 2018; Arora et al. 2022
NAFLD	Abdominal obesity, hypertriglyceridemia, accumulation of excess fat in liver cells, insulin resistance, metabolic disorder	Protective role against hepatic fibrosis	Nagano et al. 2013
Type 2 diabetes	Cluster of metabolic diseases, characterized by compromised insulin production, elevated blood glucose and risk for CVD	Probably via previously described obesity-related mechanisms	Alberti et al. 1998; Grundy 1999; Huxley et al. 2006
CVD	Pro-inflammatory response and related cellular components, development and progression of atherosclerotic lesion	Protection: immunosuppression, down-regulation of Th1-cells and potentially diminished vascular inflammation	Wirleitner et al. 2003; Nilsson & Hansson 2008; Polyzos et al. 2015; Wongpraparut et al. 2021
CVD	Pro-inflammatory response and related cellular components, development and progression of atherosclerotic lesion	Promotion: deletion or inhibition decrease chronic inflammation	Laurans et al. 2018

Definitions: IDO (indoleamine 2,3-dioxygenase), MetS (metabolic syndrome), NAFLD (non-alcoholic fatty liver disease), CVD (cardiovascular diseases), LDL (low-density lipoprotein), HDL (high-density lipoprotein), BMI (body mass index), Th1-cell (T helper type 1 cell), N/A (non-applicable; data not available).

2.5 IDO and atherosclerotic risk factors

Even though influence of IDO in atherosclerosis has clearly been established, interplay between IDO and various factors promoting risk for atherosclerotic endpoints are still largely unknown. What is known though is that smoking, alcohol consumption, level of exercise or consumed nutrition are not influenced by IDO as all of these are lifestyle choices made by a single individual. Rather, these risk factors, along with possible uncontrollable factors, serve as starting points of what may result in up-regulation of other pro-atherogenic risk factors such as dyslipidemia and hypertension.

Indeed, in dyslipidemia increased levels of LDL-cholesterol and triglycerides, and decreased level of high-density lipoprotein (HDL) -cholesterol in plasma are mainly an outcome of high fat diet. This results in accumulation of cholesterol in artery walls. Cholesterol is also operational in accrual of subcutaneous and visceral fat tissue, consequently promoting development of obesity. Unsurprisingly, extensive scientific evidence has shown a strong relationship between dyslipidemia and risk for atherosclerosis (Ference et al. 2017; Borén et al. 2020). IDO is also likely to play a part in this process since its presence has been characterized in obesity (Wolowczuk et al. 2012), especially in abdominal fat content (Mangge et al. 2014). From previous scientific evidence, Pertovaara and co-workers have found statistically significant correlations between IDO and components of dyslipidemia in 24-39-year-old females. They also detected statistically significant negative correlation with HDL-cholesterol in females and males (Pertovaara et al. 2007). Presence of IDO, however, does not automatically mean that it has a direct influence in promoting dyslipidemia. Indeed, it has been suggested that at least moderate dyslipidemia is required for activation of immunoregulatory effects of the IDO pathway (Cole et al. 2015), which may suggest an indirect connection with lipid components. Hence, a question remains whether dyslipidemia up-regulates IDO activity or vice versa.

Similarly, hypertension is also a result of lifestyle choices such as unfavourable food consumption, but also hereditary predisposition has a role. Connection between hypertension and increased risk for atherosclerotic outcomes is related since over the years hypertension burdens arteries and heart (Lewington et al. 2002; Franco et al. 2005; Tocci et al. 2008). Furthermore, presence of other atherosclerotic risk factors multiply adverse effects of hypertension (Berry et al. 2012). For instance, hypertriglyceridemia and even normal ageing both advance hypertension. Even though undisputed scientific evidence combining direct relationship between hypertension and IDO activity is absent, it has been suggested that IDO may contribute to regulation of blood pressure in systemic inflammation by vascular relaxation (Wang et al. 2010), more specifically, by playing a role in relaxation of

pulmonary artery (Nagy et al. 2017). Still, IDO as an independent predictor of up- or down-regulation of hypertension in atherosclerosis is poorly justified. It should be noted, though, that in addition to previously described lifestyle choices, also obesity is known to have an increasing impact on hypertension, and because IDO activity is known to have a role in obesity, a connection between IDO and hypertension may be indirectly established.

2.5.1 Obesity

Obesity is one of the most common long-term diseases among population in the western world and can be considered as a pathway for several life-threating diseases including MetS, type 2 diabetes and atherosclerosis. In obesity excess fat tissue is accumulated under the skin as a subcutaneous fat, but also for instance in abdominal cavity, thus forming "hidden" visceral fat stored inside the body. Visceral fat is more hazardous to health than subcutaneous fat and it is also more metabolically active (Bigaard et al. 2005). Even though genes may in some cases influence accumulation of fat tissue (Loos 2018), in most situations obesity is directly related to lifestyle choices, especially imbalance between food consumption and level of physical activity, and it can be identified already in childhood (Larqué et al. 2019). Childhood obesity has a high tendency to persist into adulthood, however, adverse outcomes might be alleviated by losing weight (Juonala et al. 2011; Juonala et al. 2013; Seidell & Halberstadt 2015; Styne et al. 2017). Unsurprisingly though, early prevention is the most efficient method to avoid raising problems (Schwartz et al. 2017). Body mass index (BMI) and waist circumference are generally used measurements of obesity. Further, according to Current Care Guidelines by Duodecim, BMI $\geq$30 measured as weight in kg divided by height square in m^2, and waist circumference of 100 cm for males and 90 cm for females are considered as thresholds for substantial obesity among Finnish population. Numerous cross-sectional and longitudinal studies have verified a connection between obesity and risk for atherosclerosis in children (Dalla Valle et al. 2015; Skinner et al. 2015) and in adults (Wilson et al. 2002; Romero-Corral et al. 2006; Guh et al. 2009). IDO also has a prominent role in promoting BMI (Favennec et al. 2015; Groer et al. 2018) and influence obesity (Wolowczuk et al. 2012) among females. More specifically, key location for high expression of IDO has been identified as abdominal fat content (Mangge et al. 2014), especially white adipose tissue of females (Wolowczuk et al. 2012). In addition, recent investigations have demonstrated that IDO has an important role in shaping gut microbiota, which is required to control both body

weight and insulin resistance (Laurans et al. 2018) also during pregnancy (Priyadarshini et al. 2022).

Involvement of IDO in obesity may be mediated via various complex biochemical pathways. For instance, promotion of BMI could take place in adipose tissue by kynurenine 3-monooxygenase activation, which could lead to the production of 3-hydroxykynurenine and xanthurenic acid in kynurenine pathway (Favennec et al. 2015). Alternatively, IDO activity may advance BMI during pregnancy by contributing TDO activity (Groer et al. 2018). These alternatives underline general acceptance that even though connection between obesity and IDO is present and the enzyme presumably has an important part in promoting obesity, exact mechanism of action and its further role in MetS are still not well-understood (Wolowczuk et al. 2012; Mallmann et al. 2018).

2.5.2 Metabolic syndrome (MetS)

MetS has been described as an asymptomatic risk cluster characterized by the presence of obesity, hypertension, and dyslipidemia, especially hypertriglyceridemia. Formally MetS is defined by The International Diabetes Federation (IDF) classification as waist circumference ≥94 cm for males and ≥80 cm for females plus any two of the following four factors: 1) raised triglycerides: >1.70 mmol/l, or specific treatment for this lipid abnormality, 2) reduced HDL-cholesterol: <1.04 mmol/l in males and <1.30 mmol/l in females, or specific treatment for this lipid abnormality, 3) raised blood pressure: blood pressure ≥130/85 mm Hg, or treatment of previously 1 diagnosed hypertension, 4) raised fasting plasma glucose ≥5.6 mmol/L, or previously diagnosed type 2 diabetes (Alberti et al. 2005). As previously disclosed, obesity and MetS are tightly linked since metabolically active visceral fat tissue, present in abdominal cavity, is one of the key elements promoting development of insulin resistance and therefore, influencing progression of MetS. Cascade from MetS is likely to result in NAFLD, type 2 diabetes and ultimately atherosclerotic endpoints. As with obesity, early changes in respect of MetS indicators in childhood may predict MetS in adulthood (Mattsson et al. 2008).

A connection between IDO, obesity, and MetS has been widely established especially in cross-sectional studies. Further, studies have indicated that inflammation is also involved in these relationships (Brandacher et al. 2007; Mangge et al. 2014; Mallmann et al. 2018), which is no surprise given the presence of inflammatory cells among visceral fat cells. Still, detailed interaction between IDO and MetS remains a mystery. However, since it is known that visceral obesity is one of the main features influencing MetS, it would be reasonable to assume that

influence of IDO in progression of MetS is transmitted via fat tissue, more specifically via gut microbiota, and that inflammation play also a part in this activity. This speculation is supported by the fact that dysbiosis is linked to type 2 diabetes, atherosclerosis and MetS (Tang et al. 2017), and that IDO is involved in shaping gut microbiota (Laurans et al. 2018).

2.5.3 Insulin resistance

Insulin is a peptide hormone secreted into blood by the beta cells (β cells) of the pancreatic islets and required for normal regulation of carbohydrate metabolism, especially down-regulation of blood glucose level. This function is disturbed by considerable weight gain and accumulation of fat leading to visceral obesity.

From clinical perspective, excess amounts of inflammatory cells accumulate between visceral fat cells. These inflammatory cells secrete, for instance, cytokines, which are then carried through the portal vein directly to the centre of human metabolism, liver, consequently disturbing normal functions of the organ and promoting formation of insulin resistant fatty liver. Insulin resistance leads to secretion of increasing levels of insulin from the pancreatic islets since insulin is required to transfer excess glucose from blood to liver cells. Eventually, this process leads to deficit in insulin supply when β cells fail to compensate the demand, thereby increasing blood glucose level.

One of the most detrimental outcomes of insulin resistance is accumulation of free fatty acids in the liver cells. Also hypertriglyceridemia is developed alongside with this function since compromised level of insulin is unable to inhibit production of triglycerides effectively in insulin resistant liver. Over the years this condition may result in type 2 diabetes, which also increases risk for the development of atherosclerosis. The risk is increased due to hypertriglyceridemia, which decreases HDL-cholesterol level simultaneously with transformation of LDL-cholesterol into small and thick atherogenic particles. These modified particles are susceptible in participating in the progression of atherosclerosis. Furthermore, excess free fatty acids and chronic low-grade inflammation, namely, increase in C-reactive protein (CRP) level, from visceral adipose tissue are considered two of the most important factors contributing progression of liver injury in NAFLD.

Despite its traditional atheroprotective role, in adverse scenario operation of IDO could also participate in violation of normal insulin production, thereby, disturbing glucose transfer and promoting insulin resistance, which then could lead to expedited accumulation of fat tissue. This accumulation could in turn aggravate interaction between IDO, obesity, and MetS, and result in further buildup of fat tissue, thus

becoming a vicious cycle that would be increasingly difficult to stop. Over time accumulation could lead to obesity-related diseases such as type 2 diabetes and atherosclerosis. Even though this might be a speculative consideration, recently challenged mechanism by Laurans and co-workers could potentially reveal previously undiscovered functions of IDO that also may indicate volatility in operation of the enzyme.

2.5.4 Non-alcoholic fatty liver disease (NAFLD)

In addition to MetS, indirect connection between IDO and NAFLD may also be present. NAFLD, being the most common liver disease in the world, is also a hepatic manifestation of MetS. This means widespread, detrimental clinical and histological changes in liver, consequently developing NAFLD. In NAFLD excess fat is accumulated into liver cells increasing the size of the organ and causing widespread metabolic disorder, which – as stated previously – is a predisposing factor for type 2 diabetes and atherosclerosis (Anstee et al. 2013; Yki-Järvinen 2014). NAFLD itself, however, is often asymptomatic (Pais et al. 2016). In this sinister condition, the distribution of fat tissue is essential as it plays a greater role in insulin resistance than BMI. Consequently, the disease is characterized by abdominal obesity, especially waist circumference, along with hypertriglyceridemia and components of insulin resistance (Milić et al. 2014; Younossi et al. 2016). In addition to insulin resistance, NAFLD may also induce inflammation along with multiple systemic adverse effects that jointly may encourage development of hypertension (Lonardo et al. 2018; Oikonomou et al. 2018). Unsurprisingly, commonness of NAFLD has been found to be dependent on the number of components of MetS present (Kotronen et al. 2007).

NAFLD is typically exposed in the age between 40-60, but due to current tendency of obesity it can already be detected among children and young adults (Anderson et al. 2015; Zhang et al. 2015). Hence, it is no surprise that up to 80 % of patients with NAFLD are obese, defined by BMI $\geq$30 kg/m^2. In some cases, hereditary causes influence fat accumulation into liver, however, presence of the gene variant does not in fact increase the risk for atherosclerosis (Holmen et al. 2014; Yki-Järvinen 2014). Luckily, adverse events from NAFLD are reversible if the initial reason for outcomes, visceral fat tissue, is eliminated (Musso et al. 2012; Kenneally et al. 2017). Furthermore, beneficial outcomes are even more prominent if physical activity and favourable food consumption are combined with weight loss (Romero-Gómez et al. 2017).

Recently a pro-inflammatory role of IDO in NAFLD has been proposed (Teunis et al. 2022). Indeed, due to presence of components characterizing NAFLD, it seems

plausible that high IDO activity and NAFLD share common clinical and biological outcomes, and that IDO could be an indirect risk factor of NAFLD especially in females (Pertovaara et al. 2007; Milić et al. 2014). Should this be true, mechanism of IDO may be transmitted via the same pathway as with MetS. Laurans and co-workers have found that lack of IDO ameliorated insulin resistance and protected mice from liver steatosis (Laurans et al. 2018). A recent investigation from mouse model also provided evidence for connection; IDO deficiency did not aggravate liver disease (Arora et al. 2022) although also a protective role of IDO against hepatic fibrosis has been suggested (Nagano et al. 2013). These controversial results underline complexity of IDO function. It seems that outcomes from activity are unpredictable or highly delicate and dependent on specific organisms and conditions being investigated. Therefore, further research is required to elucidate varying roles of IDO in NAFLD and to fully understand underlying mechanisms (Zhou et al. 2021).

2.5.5 Type 2 diabetes

Consequence of all previously described detrimental activities related to obesity, MetS and NAFLD may eventually result in type 2 diabetes. In addition, heredity plays a major role in susceptibility of the disease. This infamous cluster of metabolic diseases is united by a disorder in the production of insulin and incessantly elevated blood glucose concentration (Alberti et al. 1998). In addition, pathway to diabetes may also occur through Latent Autoimmune Diabetes in Adults (LADA) in which requirement for insulin treatment is not immediate, but in which a significant insulin deficiency is developed later (Tuomi et al. 1999).

According to IDF estimation, there were 537 million diabetes cases all over the world in 2021. Largely due to expansion of socio-economic development, numbers are expected to increase into 643 million by the year of 2030 and further into 783 million by the year of 2045 (International Diabetes Federation 2021). The reason for concern is not solely related to increase in volume of diabetic cases. In fact, there are several additional diabetes-related diseases that must be considered. Indeed, diabetes may act as a portal for sudden complications such as hypoglycemia or ketoacidosis (Cryer 2013; Cashen & Petersen 2019). Long-term complications may include retinopathy, neuropathy, and nephropathy (Cheung et al. 2010; Vinik et al. 2013; Samsu 2021). Naturally the most fatal outcomes are related to CVD and development of atherosclerosis (Grundy 1999; Huxley et al. 2006). It should be noted, though, that not all diabetics have all metabolic diseases. For instance, approximately 60-70

% of all type 2 diabetics have NAFLD and further, NAFLD can also develop after diabetes have already been diagnosed (Åberg et al. 2018).

In addition to type 2 diabetes, females may develop diabetes during pregnancy due to weight gain and up-regulation of pregnancy-related hormones. In this form of diabetes glucose metabolism is abnormal due to insulin resistance and insufficient insulin secretion by β cells (Buchanan & Xiang 2005; Plows et al. 2018). Often blood glucose level normalizes post-pregnancy, however, the risk for developing type 2 diabetes later has nonetheless increased. Furthermore, in some cases previously undiagnosed type 2 diabetes is diagnosed during pregnancy when glucose level is monitored routinely. This may be due to diminished insulin sensitivity that has taken place prior to pregnancy (Buchanan & Kojos 1999; Catalano et al. 1999).

As the number of obese pregnant females has increased over the past years, also the number of type 2 diabetes incidence can be expected to rise. Naturally this will become an increasing issue especially since females are not only endangering own health, but also the life of fetus during pregnancy (Herrera et al. 2018) or post-pregnancy (Pirkola et al. 2010; Yu et al. 2019; Josefson et al. 2020). Therefore, controlling body weight is crucially important and can be achieved by appropriate food consumption and physical activity that ultimately will result in weight loss (Morisset et al. 2010; Stephenson et al. 2018; Kaaja & Eriksson 2021). The same basic measures apply to all type 2 diabetes risk groups, although also other appropriate medical activities, such as medication, and surgical operations may be used in conjunction with health guidance.

Limited amount of data exists on how IDO may affect initiation and progression of type 2 diabetes. What can be said, though, is that IDO is probably not influencing diabetes directly *per se* but via previously described obesity-related mechanisms. Interestingly, a recent study revealed that IDO activity measured from urine was strongly related to type 2 diabetes risk in individuals with coronary artery disease, whereas plasma IDO activity remained unrelated (Rebnord et al. 2017). Also age-related association has been recognized; type 2 diabetes patients had reduced IDO production, which was associated with older age (Kartika et al. 2020). In relation to this, IDO has been reported to have a more direct impact on type 1 diabetes (Oxenkrug et al. 2015). Further, IDO has also been found to reverse the progression of diabetes in non-obese diabetic mice suggesting potential value of IDO in cell therapy (Zhang et al. 2016). These detached research results reinforce the perception of widespread nature of IDO and are probably echoes from previous health events from which alignment of direct connections to type 2 diabetes is challenging.

2.6 Clinical relevance of IDO in atherosclerosis

IDO enzyme is operational in various medical conditions in humans and its presence among atherosclerosis-related risk factors is well-established. Even though the role has been clearly identified, present research results do not support this role to be significant enough to determine the outcome of the disease independently. Instead, it is more likely that IDO is involved in the progression of the disease along with many other components that together may participate in up- or down-regulation of atherosclerosis. This, however, does not automatically mean that activity of IDO is insignificant; IDO may have a minor role but nevertheless this role could under certain conditions prove to be important in alleviating or retarding appearance of clinical symptoms. It is also important to notice that pathway to atherosclerosis often requires many injurious and long-term physiological and anatomical changes. As such, the role of any single risk factor alone is not significant enough to determine the fate of the disease.

Still, a question remains: what is the exact role and function of IDO? Simple answer is that it is not fully understood. Even though traditionally IDO has been considered to have a protective role due to its immunosuppressive properties, previous investigation proposes an opposite function (Laurans et al. 2018). This suggests that the nature and operation of IDO may be volatile. In addition, since IDO is known to operate under various medical conditions, its activity may be influenced by biochemical components related to age, sex, inflammatory response, accumulation of visceral fat tissue, pregnancy status and susceptibility to autoimmune diseases, or a mixture of these (Bruce 2005; Widner et al. 2000; Pertovaara et al. 2007; Wolowczuk et al. 2012; Mangge et al. 2014; Favennec et al. 2015; Groer et al. 2018; Young & Cho 2019). It is clear that the sheer volume of possible impact avenues forms a complex network for operation. Further, it is plausible that endpoints are at least partly determined by interactions between these alternatives that may vary due to heterogeneity and variability among individuals. This may also indicate that prognostic value of IDO activity in atherosclerosis may fluctuate or be time-dependent, as demonstrated by conflicting research outcomes; elevated level of IDO measured from urine or plasma both associated with increased risk for infarction (Pedersen et al. 2013; Pedersen et al. 2015), whereas decreased IDO level demonstrated a trend predicting one-year mortality (Wongpraparut et al. 2021), thus further underlining incompatibility of IDO as a definitive marker for CVD.

However, prognostic value may be enhanced if IDO is used alongside with other markers. Primarily based on these reasons, the use of IDO as an independent marker for CVD, or therapeutic agent or target cannot be justified with present knowledge, although also this has been proposed (Cole et al. 2015). Therefore, additional

research and novel human studies are required in the future to fully reveal function, mechanisms, and outcomes of IDO in various populations and medical conditions. Lastly, it must be remembered that initiation of atherosclerosis and its fatal outcomes are still mainly determined by individual lifestyle choices. Not promotion or inhibition by a single enzyme.

3 Aims

This thesis is based on the findings from Health 2000, Tampere Vascular Study (TVS), and the Cardiovascular Risk in Young Finns Study (YFS). The purpose was to investigate the role and function of IDO enzyme in initiation and progression of atherosclerosis using cross-sectional and longitudinal data collected from humans.

The specific aims were:

1. To examine correlation of IDO activity with atherosclerotic risk factors in young to middle-aged population (Study III) and older population with presumably more advanced stage of the disease (Study I)

2. To elucidate the role and function of IDO in influencing visceral obesity, and atherosclerotic risk factors related to NAFLD in 6- and 10-year follow-up (Study III)

3. To investigate expression of IDO and related inflammatory components in advanced atherosclerotic plaques, and to construct hypothetical IDO-mediated T-cell suppression pathways in human advanced atherosclerosis (Study II)

4 Materials and Methods

4.1 Description of the study cohorts

In this thesis three different study cohorts were used: Health 2000, TVS, and YFS. The study protocol of the Health 2000 -study was approved by the Epidemiology Ethics Committee of the Helsinki and Uusimaa hospital region, whereas TVS study was accepted by the Ethics Committee of Tampere University Hospital. YFS protocols were approved by local ethical committees depending on location where the data was collected. In each study all participants gave signed informed consent and the study protocols were conducted according to ethical guidelines of the 1975 Declaration of Helsinki principles.

4.1.1 Health 2000 -study

The Health 2000 -study was conducted from 2000 to 2001. Total number of 8,028 individuals participated in this large Finnish cross-sectional health examination survey (the Health 2000 Survey) as a two-stage stratified cluster sample. The study was carried out in the catchment areas of the Finnish university hospitals (Helsinki, Turku, Tampere, Kuopio, and Oulu) and represented the entire Finnish population aged 30 years and above. To examine CVD and diabetes in detail, a supplemental study from participants between 46 and 76 years of age was performed in which sample size was 1,867 participants and participation rate 82 %. Tryptophan and kynurenine concentrations determining the level of IDO activity along with thorough risk factor data was measured as a part of supplemental study.

4.1.2 Tampere Vascular Study (TVS)

In TVS, tissue samples were collected from 30 patients, aged 45-93, undergoing vascular surgery. Samples were collected from femoral artery, carotid artery, and aorta in the Division of Vascular Surgery, Tampere University Hospital followed by classification according to AHA recommendation. Control samples were collected from internal thoracic arteries of six patients in Tampere Heart Centre, Tampere University Hospital. Expression level of IDO and related inflammatory components were determined from these samples.

4.1.3 The Cardiovascular Risk in Young Finns Study (YFS)

YFS was a multi-center cohort study in which presence and incidence of cardiovascular risk factors were investigated in the Finnish population over time. The study was conducted in five university hospital cities (Turku, Tampere, Helsinki, Kuopio, and Oulu) and their rural surroundings in Finland. Altogether 3,596 randomly chosen participants, aged from 3 to 18 years, participated in the first cross-sectional survey that was launched in 1980. Follow-up studies were conducted in 1983, 1986, 1989, 1992, 2001, 2007 and 2011. CVD risk factor data used in this thesis was collected in 2007 and in 2011. These data were analyzed together with IDO activity determined from tryptophan and kynurenine concentrations of 24-39-year-old participants (females n=506, males n=421) in a 2001 sub-study.

4.2 Study design and participants

Study I examined correlation of IDO activity with atherosclerotic risk factors in an older population and, hence, with presumably more advanced stage of the disease. The study included 921 participants (females n=508, males n=413) from Health 2000 -study population aged 46-76 years (mean age for females 58.9 ± 8.4 years and for males 58.5 ± 7.8), who also had participated in supplemental CVD and diabetes study. The study included carotid ultrasound examination, inquiry of current smoking status and laboratory measurements for CVD risk factors, which were utilized in study I.

Study II investigated messenger ribonucleic acid (mRNA) and protein expression of IDO in advanced atherosclerotic plaques followed by expression

analysis of inflammatory components influencing promotion or inhibition of IDO. Previously performed genome-wide expression analysis (GWEA) had exposed differential expression of approximately 250 genes from which inflammatory components influencing IDO activity were selected based on existing research results from literature. A hypothetical IDO-mediated tryptophan-dependent T-cell suppression pathway was constructed from the results. In addition, gene-set enrichment analysis (GSEA) was used to test whether selected pathway genes function in a cooperating manner. Study utilized 24 collected tissue samples from TVS.

Study III clarified the role and function of IDO in influencing visceral obesity, and atherosclerotic risk factors related to NAFLD in 6- and 10-year follow-up. This was first accomplished by investigating correlations between IDO activity and atherosclerotic risk factors. Factors were selected in conjunction with other researchers participating in the study. Investigation was followed by risk ratio analysis to determine whether IDO activity associates with BMI and potentially forecasts risk for obesity. Study was performed using longitudinal data from YFS. A sub-study from 2001 was used as a baseline for IDO activity. Activity was determined from 927 participants, aged 24-39 years, along with comprehensive array of CVD risk factor profiles.

4.3 Data acquisition

4.3.1 Data acquisition in Health 2000 -study

For determination of IDO activity, previously described protocol was followed in which tryptophan (mmol/l) and kynurenine (μmol/l) concentrations were measured by reverse-phase high-performance liquid chromatography (HPLC) from peripheral blood (Laich et al. 2002). In detail, kynurenine separation was performed with a Hewlett Packard 1100 liquid chromatograph (Palo Alto, CA, USA) using Merck LiChroCart 55–4150 mm cartridge containing a Purospher STAR RP-18 3 μm column (Merck Co, Darmstadt, Germany) followed by determination using ultraviolet absorption at 360 nm wavelength with a Hewlett Packard G13144 detector. For tryptophan separation, Shimadzu liquid chromatograph LC-10AD VP (Shimadzu Co, Kyoto, Japan) using a 50-mm BDS Hypersil C18 5 μm column (Thermo Electron Co, Bellefonte, PA, USA) was first used. This was followed by fluorescence monitoring using Shimadzu RF-10A XL detector at 266 nm excitation

and 366 nm emission wavelengths. A numerical value for IDO activity was achieved by dividing kynurenine (μmol/l) by tryptophan (mmol/l).

CVD risk factors were determined as follows: current smoking habit was inquired using a questionnaire. Current smokers were defined as "smokers" and the rest as "non-smokers". Protocols outlined by Aromaa and co-workers were executed for measurements (Aromaa et al. 2004). Serum total cholesterol and triglycerides were measured by commercial automated enzymatic methods (Olympus system reagent, Germany). HDL- and LDL-cholesterol were determined by direct enzymatic methods (Roche Diagnostics, Mannheim, Germany) followed by analysis using Olympus AU400 (Germany) clinical chemistry autoanalyzer. Further, LDL-cholesterol concentration was calculated by using Friedewald formula (Friedewald et al. 1972). BMI was determined by a spring scale (Biospace, Inbody 3.0) that automatically calculated BMI after measured height was entered. For blood pressure measurement, a Mercuro 300 mercury manometer was used. Measurement was performed from the person's right arm on a sitting position and after a five-minute rest using a cuff size of 15 x 43 cm. Larger cuff was used only when necessary. Diastolic pressure was determined at the fifth phase of the Korotkoff sounds. CRP was analyzed from blood samples. Prior to drawn, subjects had fasted for four hours. An ultrasensitive immunoturbidometric test (Orion Diagnostica, Espoo, Finland) with Optima analyzer (Thermo Electron Corporation, Vantaa, Finland) was used for analysis.

Standardized protocol for a high-resolution B-mode carotid ultrasound examination of the right carotid artery was conducted first on the distal 1 cm of the common carotid artery and then on the carotid artery bulb using a 7.5-MHz linear array transducer. Image was focused on the posterior wall. Centrally trained and certified sonographers executed examinations. A single reader was responsible for reading all ultrasound images. IMT measurements were performed off-line by using automated image processing software. Measures of the IMT were determined as follows: 1. mean of the three average IMTs of the carotid bulb; 2. mean of the three average IMTs of the common carotid artery; 3. mean of these two means. The mean of these measurements was used in this study as the measure of carotid IMT.

4.3.2 Data acquisition in TVS

Isolation of RNA was performed first by soaking fresh tissue samples immediately into RNALater solution (Ambion Inc., Austin, TX, USA) followed by isolation of total-RNA using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and RNAeasy Kit (Qiagen, Valencia, CA, USA). Spectrophotometric (BioPhotometer, Eppendorf,

Wesseling-Berzdorf, Germany) analysis was carried out to confirm quality and determine concentration of RNA.

In GWEA, Sentrix Human-8 Expression BeadChip (Illumina, San Diego, CA, USA) was used according to the manufacturer's instructions to analyze more than 23,000 known and candidate genes. In practice, Ambion's Illumina RNA Amplification kit (cat. no I1755; Ambion, Inc.) was used to amplify 200 ng aliquot of total RNA from each sample to complementary deoxyribonucleic acid (cDNA). Next, each sample of cRNA (1,500 ng) was hybridized to Illumina Sentrix Human-8 Expression BeadChip arrays (Illumina, San Diego, CA, USA). Hybridized biotinylated cRNA was detected using 1 µg/mL Cyanine3-streptavidine (Amersham Biosciences, Pistacataway, NJ, USA) followed by scanning of BeadChips by Illumina BeadArray Reader.

Confirmatory gene expression analysis for tissue samples was performed using real-time quantitative polymerase chain reaction (qPCR). This was accomplished by using quantitative TaqMan low-density arrays (LDA) (Applied Biosystems, Foster City, CA, USA). First, High-Capacity cDNA Kit was used according to the manufacturer's instructions (Applied Biosystems) to reverse-transcribe total-RNA (500 ng) to cDNA. Then, LDA were loaded with 8 µL undiluted cDNA, 42 µL H_2O, and 50 µL PCR Universal Master Mix followed by operation of PCR according to the manufacturer's instructions (Applied Biosystems). Samples were analyzed as duplicates. Both cDNA synthesis and PCR reactions were validated for inhibition. A housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a control sample. Lastly, results were analyzed using SDS 2.2 software (Applied Biosystems).

In immunohistochemistry, paraffin-embedded vascular samples without any counterstain were used for ABD-method (Vectastain Elite kit; Vector Laboratories, Burlingame, CA, USA). Mouse monoclonal anti-human IDO antibody (AB9212; Upstate 05-840, Millipore, Billerica, MA, USA) was used to detect IDO in vascular wall. Primary antibodies used for detection of vascular cell markers were muscle actin (mouse anti-human muscle actin, clone HHF35; DakoCytomation, Glostrup, Denmark) for detection of smooth muscle cells, cluster of differentiation (CD) 68 (mouse anti-human CD68, clone PG-M1 (DakoCytomation)) for detection of monocytes and macrophages, and CD31 (mouse anti-human CD31, endothelial cell, clone JC70A; DakoCytomation) for detection of endothelial cells. Actual laboratory process began with microwave antigen retrieval treatment of the sections as previously described (Shi et al. 1991), followed by extinguishing endogenous peroxidase activity by treating the section with 0.3 % H_2O_2 for 30 minutes. Next, overnight incubation of the sections with the primary antibodies was executed followed by 30 minutes treatment using ABC-complex and biotinylated sheep antimouse (1:300; Amersham Int., Buckinghamshire, UK) in which

diaminobenzidine was used as the chromogen. Dilution of antibodies was carried out in PBS containing 1 % BSA and 0.3 % of Triton X-100. In terms of controls, primary antibody was omitted or replaced with non-immune sera. No staining was observed in the controls. Nikon Microphot FXA microscope equipped with PCO Sensicam digital camera (PCO, Kelheim, Germany) was used for examination of the sections. Co-localization of IDO and CD68 was examined in 5 µm adjacent paraffin sections (mirror image sections) and the sections were stained with ABD-method.

4.3.3 Data acquisition in YFS

Determination of IDO activity was performed as described in section 4.3.1. Only participants who had previously determined IDO activity, characterized by kynurenine-tryptophan ratio, and also complete CVD risk factor data from 2007 and 2011 were selected for investigation. Standardized protocols were used to measure waist circumference in cm and BMI in kg/m^2 (Raitakari et al. 2008). Triglyceride and serum cholesterol concentrations were determined enzymatically (Olympus System Reagent; Olympus Diagnostica GmbH, Hamburg, Germany) by using a clinical chemistry analyzer (Olympus AU400; Olympus Optical Ltd, Mishima, Japan) (Juonala et al. 2004). Dextran sulphate 500,000 was used to precipitate LDL-cholesterol from serum HDL-cholesterol. HDL-cholesterol concentration was measured from serum supernatants (Porkka et al. 1997) whereas indirect calculation of LDL-cholesterol levels using the Friedewald formula for participants with <4 mmol/l triglycerides was performed (Friedewald et al. 1972; Raitakari et al. 2003). Olympus AU400 clinical chemistry analyzer was used to measure glucose concentrations by standard enzymatic methods, and also CRP with a latex turbidimetric immunoassay kit (CRP-UL assay, Wako Chemicals, Neuss, Germany) (Koskinen et al. 2009). Thickness of carotid IMT and status of fatty liver were determined by ultrasound (Raitakari et al. 2003; Suomela et al. 2015). More specifically, liver fat content was based on the overall assessment of several measurements and classified as an index (units). Ultrasound studies were also used to assess carotid artery elasticity factors. Measurements for each factor were conducted as followed: $CAC=([D_s-D_d]/D_d)/(P_s-P_d)$, $YEM=([P_s-P_d]\times D_d)/([D_s-D_d]/IMT)$ and $SI=\ln(P_s/P_d)/([D_s-D_d]/D_d)$, where D_d is the diastolic diameter, D_s is the systolic diameter, P_s is systolic blood pressure, P_d is diastolic blood pressure (Juonala et al. 2005).

For definition of MetS, IDF classification was used: waist circumference ≥94 cm for males and ≥80 cm for females and any two of the following four factors: 1) raised triglycerides: >1.70 mmol/l, or specific treatment for this lipid abnormality, 2)

reduced HDL-cholesterol: <1.30 mmol/l in females and <1.04 mmol/l in males, or specific treatment for this lipid abnormality, 3) raised blood pressure: blood pressure ≥130/85 mm Hg, or treatment of previously 1 diagnosed hypertension, 4) raised fasting plasma glucose ≥5.6 mmol/L, or previously diagnosed type 2 diabetes.

4.4 Statistical methods

For all statistical analysis normality assumptions were assessed by examining histograms and normal probability plots. Statistical significance was inferred at a 2-tailed probability value (p-value) <0.05.

Study I

Student's t-test or non-parametric Mann-Whitney U test were used to compare continuous variables as appropriate, whereas the χ^2 test was used to compare current smokers versus non-smokers (dichotomous variable). Correlation between IDO and each risk factor was determined by the Pearson correlation coefficient. All statistical tests were performed with SPSS 15.0 for Windows (SPSS Inc., IL, USA).

Study II

Comparison of mRNA expression between atherosclerotic and control tissues was carried out by non-parametric Mann-Whitney U test. Data was presented as mean ± standard deviation unless otherwise stated. R language was used to normalize raw intensity data obtained from the Illumina platform. Single-probe analysis, including fold-change calculations and filtering the probes, was performed using R related Bioconductor module. Pathway analysis of the gene expression data was conducted using the GSEA implemented in GSEA java desktop application version 2.0 and Molecular Signature Database version 2.0. In order to avoid duplicates in the analysis, probes representing the same gene symbol in Illumina data were replaced with their maximum intensity. A self-constructed IDO pathway gene set was utilized in GSEA. Cooperation of genes in the set was determined according to recommendation by Subramanian et al. 2005. Statistically significant cooperation

was determined when false discovery rate (FDR) was less than 0.25. Analysis of data was performed using SPSS 14.00 for Windows (SPSS Inc., IL, USA).

Study III

Logarithmic transformation for CRP and triglyceride values were performed prior to analysis to correct skewed distribution. Pearson correlation coefficient was used to determine correlation between IDO and each risk factor, and also when the data was adjusted with age. To reduce possibility for incorrect conclusions, multiple testing adjustment with Bonferroni correction for each p-value from correlation analysis was carried out. Each p-value was multiplied by the number of IDO–risk factor pairs in 2007 and 2011 for females and males individually. To assess connection between IDO and risk for obesity, a risk ratio analysis using Poisson regression model with robust error variance was performed. In this analysis, data was adjusted with age, and with age and BMI both of which were measured at baseline in 2001. BMI value of 30 kg/m^2 was set as a threshold for obesity. Analysis of data was performed using SAS software version 9.4 (SAS Institute, Cary, NC).

5 Results

5.1 Characteristics of the participants

Table 2 combines descriptive characteristics of study populations from YFS and Health 2000. IDO activity, characterized by kynurenine-tryptophan ratio, was measured only in 2001 from 986 participants aged from 24 to 39. Weight of females (n=544) varied from 40.5 to 134.4 kg and males (n=442) from 55.2 to 157.0 kg. Only participants having measurement of kynurenine and tryptophan, as well as risk factor data were included in analysis.

Health 2000 -study population included 921 participants (females n=508, males n=413) aged 46-76 years (mean age for females 58.9 ± 8.4 years and for males 58.5 ± 7.8), who also had participated in supplemental CVD and diabetes study, whereas in YFS, mean age for females (n=506) was 31.5 ± 4.9 years and for males (n=421) 31.9 ± 5.0 years in 2001. Follow-up risk factor surveys were conducted in 2007 (n=2,204) and in 2011 (n=2,063). In 2007 age of the participants ranged from 30 to 45. Mean age for females (n=435) was 37.9 ± 4.8 years and for males (n=342) 37.9 ± 4.9 years. In 2011 age varied from 34 to 49. Mean age for females (n=394) was 41.8 ± 4.8 years and for males (n=312) 42.3 ± 5.1 years.

In TVS study, the age of participants (n=30) ranged from 45 to 93 years (median age 70 years). 75.9 % were males. Type V–VI advanced plaques were found from 73.3 % of the tissue samples based on histological classification by AHA. Prevalence of risk factors included dyslipidemia 40.7 %, hypertension 77.8 %, diabetes 18.5 %, history of smoking 86.2 %, alcohol usage more than once a week 42.8 %. Further, six non-atherosclerotic control samples from internal thoracic arteries were collected from patients undergoing coronary artery bypass grafting.

Table 2. Baseline characteristics of YFS and Health 2000 -study populations. Data are mean ± standard deviation. Modified from Original Publications I and III.

	Females			Males		
Variable	YFS 2007	YFS 2011	Health 2000	YFS 2007	YFS 2011	Health 2000
N	435	394	508	342	312	413
Age, years	37.9 ± 4.84	41.84 ± 4.84	58.9 ± 8.4	37.89 ± 4.94	42.26 ± 5.06	58.5 ± 7.8
Current smokers (%)	15.0	13.5	19.9	22.9	17.6	28.3
BMI, kg/m^2	25.07 ± 4.96	25.78 ± 5.34	27.19 ± 4.71, n=460	26.69 ± 4.28	26.78 ± 4.35	27.70 ± 3.95, n=375
Waist, cm	82.5 ± 12.38	86.8 ± 12.9	N/A	94.25 ± 12.37	97.02 ± 12.51	N/A
MetS, IDF classification	0.15 ± 0.36	0.17 ± 0.37	N/A	0.28 ± 0.45	0.34 ± 0.48	N/A
Glucose, mmol/L	5.15 ± 0.8	5.28 ± 1.03	N/A	5.44 ± 0.79	5.54 ± 0.86	N/A
Trigycerides, mmol/L	1.09 ± 0.52	1.03 ± 0.49	1.24 ± 0.58	1.50 ± 0.74	1.43 ± 0.7	1.46 ± 0.78
HDL-C, mmol/L	1.47 ± 0.33	1.46 ± 0.32	1.68 ± 0.32	1.19 ± 0.28	1.23 ± 0.28	1.40 ± 0.28
LDL-C, mmol/L	2.88 ± 0.71	3.12 ± 0.77	3.41 ± 0.82, n=422	3.28 ± 0.82	3.44 ± 0.91	3.47 ± 0.90, n=329
Total cholesterol, mmol/l	4.93 ± 0.86	5.07 ± 0.89	5.61 ± 0.89	5.19 ± 0.95	5.32 ± 1.01	5.48 ± 0.96
CRP, mg/L	2.00 ± 3.4	1.97 ± 2.93	3.09 ± 4.82	1.59 ± 2.9	1.76 ± 5.24	2.85 ± 4.35
IMT (mean), mm	0.6 ± 0.08	N/A	0.90 ± 0.21, n=496	0.64 ± 0.11	N/A	0.97 ± 0.24, n=397
Systolic blood pressure, mmHg	113.11 ± 12.97	114.63 ± 14.34	138 ± 22, n=506	123.21 ± 12.23	121.81 ± 13.28	142 ± 20, n=412
Diastolic blood pressure, mmHg	71.14 ± 11.08	71.2 ± 9.25	83 ± 10, n=507	76.97 ± 10.84	76.18 ± 10.81	87 ± 11, n=412
CAC, %/10 mm Hg	2.05 ± 0.74	N/A	N/A	1.77 ± 0.62	N/A	N/A
YEM, mm Hg - mm	375.56 ± 471.37	N/A	N/A	437.98 ± 319.87	N/A	N/A
SI (units)	6.29 ± 6.83	N/A	N/A	6.48 ± 3.81	N/A	N/A
Fatty liver (units)	N/A	1.08 ± 0.34	N/A	N/A	1.31 ± 0.56	N/A
Kyn/trp (2001), µmol/mmol	26.94 ± 7.02	26.94 ± 7.02	32.37 ± 9.09	28.37 ± 7.51	28.37 ± 7.51	31.89 ± 8.74

Definitions: YFS (the Cardiovascular Risk in Young Finns Study), N (total number of participants), BMI (body mass index), MetS (metabolic syndrome), HDL-C (high-density lipoprotein cholesterol), LDL-C (low-density lipoprotein cholesterol), CRP (C-reactive protein), IMT (intima-media thickness), CAC (coronary-artery compliance), YEM (Young's elastic modulus), SI (stiffness index), Kyn/trp (kynurenine/tryptophan) measured in 2001, N/A (non-applicable; data not available).

5.2 IDO activity and atherosclerotic risk factors between studies

Investigation of correlations between IDO enzyme and atherosclerosis risk factors were evaluated in studies I and III. In study III, young to middle-aged population of females and males from YFS was investigated in a longitudinal set-up. In 2007, the age of participants was 30-45 years, and in 2011 from 34-49 years-of-age. IDO measurements were performed only in 2001. In study I, cross-sectional measurements from 46-76-year-old females and males who participated in Health 2000 -study were examined. In addition, in a prior study by Pertovaara et al. cross-sectional correlations between IDO and several atherosclerotic risk factors were examined in YFS (Pertovaara et al. 2007).

As shown in Figure 2, among females 82 % of risk factors correlated with IDO in cross-sectional YFS analyses and 80 % in Health 2000 -study. In longitudinal analyses, 38 % of risk factors correlated at 6 years and 27 % at 10 years. In males, cross-sectional analyses demonstrated statistically significant correlations for 45 % (YFS) or 60 % (Health 2000) of risk factors. In 6- and 10-year intervals 11 % and 13 % of risk factors were correlated, respectively.

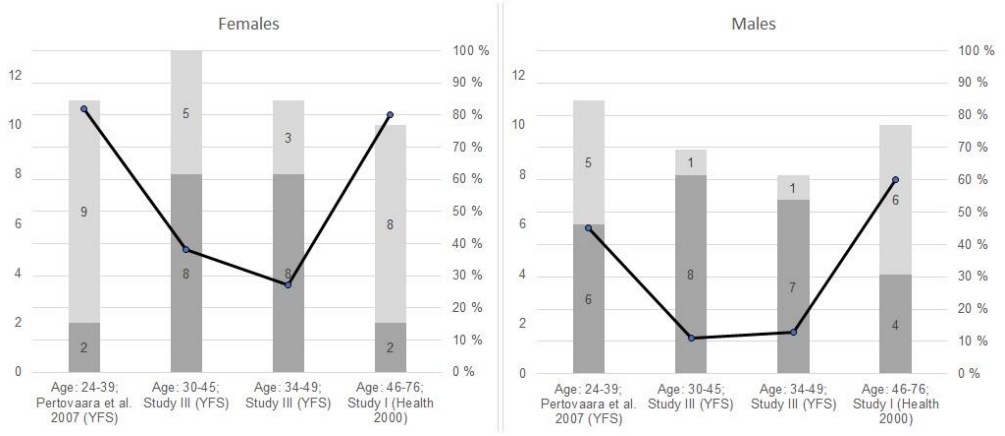

Figure 2. IDO activity and atherosclerotic risk factors between females and males in different age groups. For Study III, unadjusted values were used. Proportion of statistically significant correlations between IDO activity and atherosclerotic risk factors presented as percentages in black line (right axis). Absolute number of all investigated risk factors in each study presented as stacked columns (left axis); the number of uncorrelated risk factors (dark grey) and the number of correlated risk factors (light grey). Definition: YFS (the Cardiovascular Risk in Young Finns Study).

5.3 IDO activity and atherosclerotic risk factors in females

The results from female participants are presented in Table 3. In Health 2000 -study, significant cross-sectional correlations were found between IDO and age, BMI, IMT, logarithmically modified triglycerides and CRP ($p<0.0001$ for all), and negatively with HDL ($p=0.005$). In addition, correlations with systolic ($p=0.0001$) and diastolic ($p=0.032$) blood pressures were observed.

Longitudinal analyses were executed from YFS cohort. Risk factor data from 2007 represented 6-year follow-up, while IDO was measured only in 2001. IDO correlated with BMI ($p=0.0004$), waist circumference ($p=0.0003$), MetS ($p=0.024$) and logarithmically modified CRP ($p=0.0031$) in unadjusted analysis, and with mean IMT ($p=0.0452$). After Bonferroni correction, correlations with BMI ($p=0.0052$), waist ($p=0.0039$), and CRP ($p=0.0403$) remained significant. BMI ($p=0.0008$), waist ($p=0.0009$) and CRP ($p=0.0014$) correlated statistically significantly with IDO when the data was adjusted with age, and this outcome remained significant after Bonferroni correction ($p=0.0104$, $p=0.0117$, $p=0.0182$ for BMI, waist and CRP, respectively). In contrast to unadjusted analysis, a statistically significant correlation with logarithmically modified triglycerides ($p=0.0488$) was also observed, which, however, was absent after Bonferroni correction.

Similarly, risk factor data from 2011 represented 10-year follow-up. Statistically significant correlations were discovered between IDO and BMI as well as IDO and waist in both unadjusted ($p=0.0001$ for BMI and $p=0.0006$ for waist) and age-adjusted ($p=0.0007$ for BMI and $p=0.0063$ for waist) analysis. Correlation remained significant with these risk factors after Bonferroni correction in unadjusted ($p=0.0011$ for BMI and $p=0.0066$ for waist) analysis, and with BMI ($p=0.0077$) in age-adjusted analysis. Further, IDO also correlated with fatty liver ($p=0.0275$) in unadjusted analysis, which, however, did not remain significant after age-adjustment or Bonferroni correction.

Table 3. Pearson correlations between female IDO activity and atherosclerotic risk factors in different studies and age groups. Modified from Original Publications I and III.

	YFS 2007				YFS 2011				Health 2000
	Age: 30-45				Age: 34-49				Age: 46-76
	Pearson correlation (r)				Pearson correlation (r)				Pearson correlation (r)
Variable	Unadjusted with IDO	Multiple testing adjustment	Adjusted with age	Multiple testing adjustment	Unadjusted with IDO	Multiple testing adjustment	Adjusted with age	Multiple testing adjustment	Unadjusted with IDO
N	435	435	434	434	394	394	384	384	508
BMI	0.169, p=0.0004	p=0.0052	0.1598, p=0.0008	p=0.0104	0.192, p=0.0001	p=0.0011	0.1727, p=0.0007	p=0.0077	0.281, p<0.0001, n=423
Waist	0.1734, p=0.0003	p=0.0039	0.1587, p=0.0009	p=0.0117	0.1719, p=0.0006	p=0.0066	0.1393, p=0.0063	p=0.0693	N/A
MetS	0.1082, p=0.024	p=0.312	0.0876, p=0.0685	p=0.8905	0.0441, p=0.3828	p=4.2108	0.0118, p=0.8175	p=8.9925	N/A
Glucose	0.0897, p=0.0617	p=0.8021	0.0683, p=0.1559	p=2.0267	0.0719, p=0.1545	p=1.6995	0.0721, p=0.1591	p=1.7501	N/A
Trigycerides (Log)	0.092, p=0.0551	p=0.7163	0.0948, p=0.0488	p=0.6344	0.0017, p=0.9727	p=10.6997	-0.0351, p=0.4935	p=5.4285	0.154, p<0.0001
HDL-C	-0.0842, p=0.0795	p=1.0335	-0.069, p=0.1513	p=1.9669	-0.044, p=0.384	p=4.224	-0.0427, p=0.4049	p=4.4539	-0.124, p=0.005
LDL-C	0.0167, p=0.7282	p=9.4666	-0.0099, p=0.8371	p=10.8823	-0.0308, p=0.5422	p=5.9642	-0.0487, p=0.3414	p=3.7554	0.039, p=ns
CRP (Log)	0.1416, p=0.0031	p=0.0403	0.1535, p=0.0014	p=0.0182	0.0803, p=0.1117	p=1.2287	0.0853, p=0.0954	p=1.0494	0.293, p<0.0001
IMT (mean)	0.0961, p=0.0452	p=0.5876	0.0447, p=0.354	p=4.602	N/A	N/A	N/A	N/A	0.208, p<0.0001, n=496
Systolic BP	0.0084, p=0.8617	p=11.2021	-0.003, p=0.9513	p=12.3669	0.0899, p=0.0747	p=0.8217	0.0349, p=0.4957	p=5.4527	0.213, p=0.0001, n=506
Diastolic BP	0.0067, p=0.8889	p=11.5557	-0.0032, p=0.9464	p=12.3032	0.0581, p=0.2504	p=2.7544	-0.004, p=0.9382	p=10.3202	0.095, p=0.032, n=507
CAC	-0.0185, p=0.6998	p=9.0974	0.0385, p=0.4244	p=5.5172	N/A	N/A	N/A	N/A	N/A
YEM	0.0497, p=0.3009	p=3.9117	0.0189, p=0.6956	p=9.0428	N/A	N/A	N/A	N/A	N/A
Fatty liver	N/A	N/A	N/A	N/A	0.111, p=0.0275	p=0.3025	0.0939, p=0.0664	p=0.7304	N/A

Definitions: YFS (the Cardiovascular Risk in Young Finns Study), Unadjusted (unadjusted data), Multiple testing adjustment (Bonferroni corrected p-values), Adjusted with age (data adjusted with age), BMI (body mass index), MetS (metabolic syndrome), Log (logarithmically transformed), HDL-C (high-density lipoprotein cholesterol), LDL-C (low-density lipoprotein cholesterol), CRP (C-reactive protein), IMT (intima-media thickness), BP (blood pressure), CAC (coronary-artery compliance), YEM (Young's elastic modulus), ns (not significant), N/A (non-applicable; data not available).

5.4 IDO activity and atherosclerotic risk factors in males

The results from male participants are presented in Table 4. In cross-sectional Health 2000 -study, multiple statistically significant correlations were present. IDO correlated with age, logarithmically modified CRP ($p<0.0001$ for both) and IMT ($p=0.001$). Further, negative correlations were found with LDL ($p=0.026$) and diastolic blood pressure ($p<0.0001$). Interestingly, statistically significant correlation with BMI was absent.

In longitudinal analyses from YFS, limited number of statistically significant correlations were found compared to females. After 6-year follow-up (data from 2007), only waist correlated significantly with IDO in both unadjusted ($p=0.0148$) and age-adjusted ($p=0.0367$) analysis. IDO was also negatively correlated with HDL-cholesterol ($p=0.0489$) when the data was adjusted with age even though statistically significant correlation was absent in unadjusted analysis.

Furthermore, when the data was analyzed after 10-year follow-up (data from 2011), statistically significant negative correlation between IDO and HDL-cholesterol was present in both unadjusted ($p=0.0418$) and age-adjusted ($p=0.0348$) analysis. However, no further correlations were observed. After Bonferroni correction none of the correlations remained statistically significant.

Table 4. Pearson correlations between male IDO activity and atherosclerotic risk factors in different studies and age groups. Modified from Original Publications I and III.

	YFS 2007				YFS 2011				Health 2000
	Age: 30-45				Age: 34-49				Age: 46-76
	Pearson correlation (r)				Pearson correlation (r)				Pearson correlation (r)
Variable	Unadjusted with IDO	Multiple testing adjustment	Adjusted with age	Multiple testing adjustment	Unadjusted with IDO	Multiple testing adjustment	Adjusted with age	Multiple testing adjustment	Unadjusted with IDO
N	342	342	342	342	312	312	300	300	413
BMI	0.1037, p=0.0554	p=0.6094	0.0872, p=0.1079	p=1.1869	0.0698, p=0.2188	p=2.188	0.072, p=0.2144	p=2.144	-0.008, p=ns, n=335
Waist	0.1317, p=0.0148	p=0.1628	0.1132, p=0.0367	p=0.4037	0.0686, p=0.2273	p=2.273	0.0692, p=0.2326	p=2.326	N/A
MetS	-0.0146, p=0.7883	p=8.6713	-0.0264, p=0.6273	p=6.9003	-0.0095, p=0.8671	p=8.671	-0.0162, p=0.7799	p=7.799	N/A
Glucose	0.0063, p=0.907	p=9.977	-0.0155, p=0.7762	p=8.5382	-0.0579, p=0.3082	p=3.082	-0.0544, p=0.3485	p=3.485	N/A
Trigycerides (Log)	-0.0167, p=0.7585	p=8.3435	-0.0256, p=0.6375	p=7.0125	0.0218, p=0.7014	p=7.014	0.0265, p=0.6479	p=6.479	0.020, p=ns
HDL-C	-0.0943, p=0.0816	p=0.8976	-0.1067, p=0.0489	p=0.5379	-0.1153, p=0.0418	p=0.418	-0.1221, p=0.0348	p=0.348	-0.060, p=ns
LDL-C	0.0225, p=0.6789	p=7.4679	0.0058, p=0.915	p=10.065	-0.0029, p=0.9595	p=9.595	-0.0127, p=0.8273	p=8.273	-0.123, p=0.026
CRP (Log)	0.0091, p=0.8675	p=9.5425	-0.0009, p=0.9869	p=10.8559	0.0438, p=0.4411	p=4.411	0.0471, p=0.4174	p=4.174	0.184, p<0.0001
IMT (mean)	0.0434, p=0.4239	p=4.6629	0.0121, p=0.8242	p=9.0662	N/A	N/A	N/A	N/A	0.168, p=0.001, n=397
Systolic BP	-0.0613, p=0.2451	p=2.6961	-0.0864, p=0.1112	p=1.2232	-0.0187, p=0.7294	p=7.294	-0.0393, p=0.4982	p=4.982	-0.018, p=ns, n=412
Diastolic BP	-0.0363, p=0.492	p=5.412	-0.0607, p=0.2635	p=2.8985	-0.0406, p=0.4539	p=4.539	-0.0584, p=0.3143	p=3.143	-0.187, p<0.0001, n=412

Definitions: YFS (the Cardiovascular Risk in Young Finns Study), Unadjusted (unadjusted data), Multiple testing adjustment (Bonferroni corrected p-values), Adjusted with age (data adjusted with age), BMI (body mass index), MetS (metabolic syndrome), Log (logarithmically transformed), HDL-C (high-density lipoprotein cholesterol), LDL-C (low-density lipoprotein cholesterol), CRP (C-reactive protein), IMT (intima-media thickness), BP (blood pressure), ns (not significant), N/A (non-applicable; data not available).

5.5 IDO and obesity

To further examine potential influence of IDO activity in visceral obesity, and consequently cardiometabolic diseases over time, investigation whether IDO activity associates, and therefore forecasts risk for obesity was conducted. Examination was performed in study III by the means of longitudinal risk ratio analysis in which influence of IDO, measured in 2001, was determined for obesity in 2007 and in 2011, hence, in 6- and 10-year follow-up. Threshold for obesity was set as BMI $\geq$ 30 kg/m^2 both in 2007 and 2011. Data was first adjusted with age, and then with age and BMI, both of which were measured at baseline in 2001. Results from analysis for females and males are presented on Table 5.

Table 5. Risk ratio analysis for obesity. From Original Publication III.

Outcome	Age-adjusted, RR (95% CI)	Age- and BMI-adjusted, RR (95% CI)
Female		
Obesity in 2007	1.037 (1.012-1.062), P=0.0033	0.996 (0.965-1.028), P=0.8057
Obesity in 2011	1.057 (1.037-1.077), P <0.0001	1.026 (1.005-1.047), P=0.0147
Male		
Obesity in 2007	1.027 (1.008-1.046), P=0.0044	1.019 (1.005-1.033), P=0.0091
Obesity in 2011	1.024 (1.005-1.044), P=0.0148	1.015 (1.001-1.03), P=0.0404

Female and male risk ratio was determined separately in 6- and 10-year follow-up. The data was adjusted for age, and also for age and BMI (all measured at baseline in 2001). Definitions: RR (risk ratio), CI (confidence interval).

When the data was adjusted only with age, IDO activity associated statistically significantly with obesity in all investigated combinations. In females, presence of IDO increased risk for obesity 3.7 % (p=0.0033) and 5.7 % (p<0.0001) in 2007 and 2011, respectively. In males, increased risk was 2.7 % (p=0.0044) and 2.4 % (p=0.0148) in 2007 and 2011, respectively. Further, when the influence of age and BMI was eliminated, IDO was only associated with obesity in female participants after 10-year follow-up, consequently increasing risk for obesity 2.6 % (p=0.0147). By contrast, this phenomenon was absent in males in which increased risk was 1.9 % (p=0.0091) and 1.5 % (p=0.0404) in 2007 and 2011, respectively.

5.6 IDO mRNA expression in atherosclerotic tissue

In addition to correlation and risk ratio analysis, also presence and role of IDO in human atherosclerotic plaques was examined. To demonstrate the presence of IDO mRNA in advanced atherosclerotic plaques (AHA types V-VI), arterial samples (n=20) and non-atherosclerotic controls (n=6) were first pooled. Expression differences were then determined in GWEA as normalized average gene intensity for each group using Illumina Expression BeadChips. IDO expression level in plaques was approximately 1.3-fold relative to control ($p<0.0001$ for difference in Mann-Whitney U test). Median IDO expression level in arbitrary units was 152.5 (119.1-185.1) in plaques and 114.8 (102.6-125.7) in controls. The results are presented in Figure 3.

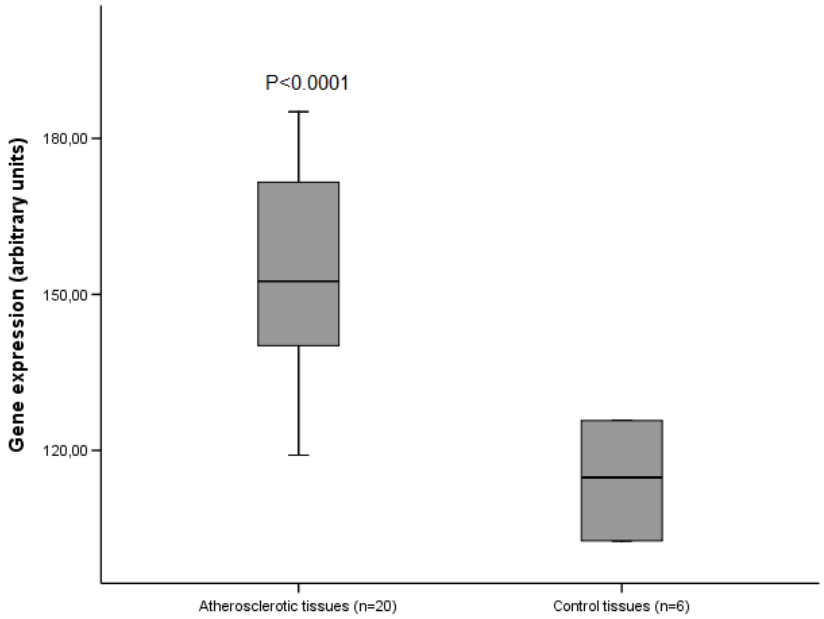

Figure 3. Indoleamine 2,3-dioxygenase (IDO) expression in atherosclerotic and control artery tissues. Gene expression value is the normalized average gene intensity for each group measured in the Illumina Expression BeadChip. Median IDO expression levels in atherosclerotic and non-atherosclerotic control tissues were 152.5 (119.1–185.1) and 114.8 (102.6–125.7), respectively. Mann-Whitney U-test relative to control tissues. From Original Publication II.

5.7 Immunohistochemistry of IDO

To investigate immunoreactivity of IDO in human carotid artery plaques, immunohistochemistry staining was performed with various markers. The results are visualized in Figures 4-5. A tissue sample from internal thoracic artery was used as a control. IDO-positive macrophages (arrows) and smaller cells (arrow-heads) were identified after IDO staining in Figure 4 (left). Similarly, labeled macrophage (arrow) and monocytes (arrow-heads) were observed after staining with monocyte marker CD68 in Figure 4 (right). In contrast, labelled monocytes and macrophages were not be found after samples were treated with smooth muscle cell marker HHF35 (Figure 5, left) or with endothelial cell marker CD31 (Figure 5, right). Further analysis using mirror image sections exposed co-localization of IDO and CD68 immunoreactivities in the same cells (arrows in Figure 6).

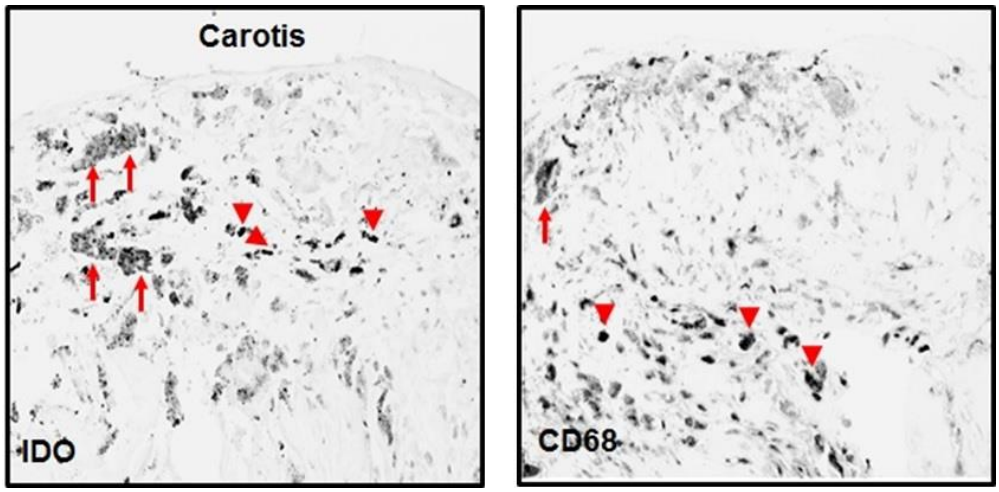

Figure 4. IDO immunoreactivity in human atherosclerotic plaques. In IDO staining (left), arrows point to IDO-positive macrophages. Also smaller cells (arrow-heads) were labelled. Staining with monocyte marker CD68 (right) demonstrated labelled macrophage (arrow) and monocytes (arrow-heads). The stage of atherosclerosis was classified according to American Heart Association classification. 100 x magnification. Modified from Original Publication II.

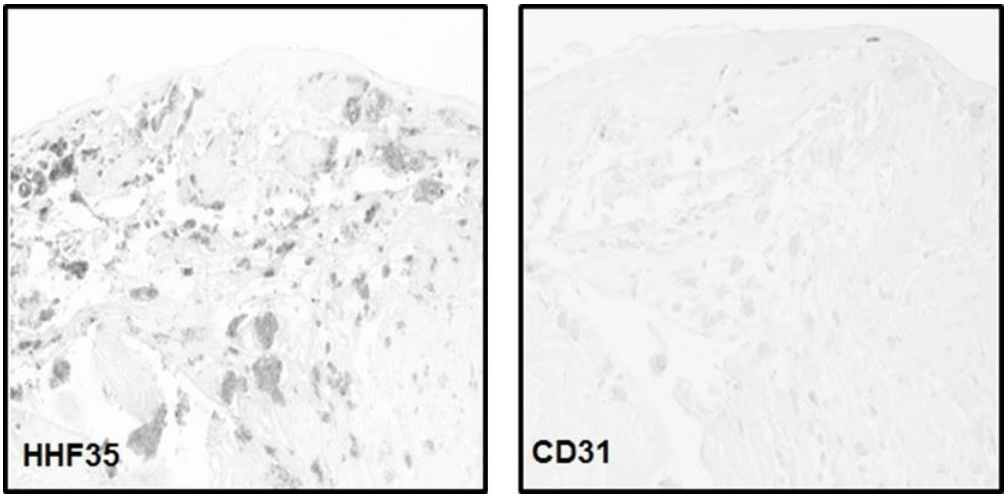

Figure 5. IDO immunoreactivity in human atherosclerotic plaques. Staining with smooth muscle cell marker HHF35 (left) did not show labelled monocytes or macrophages. No staining was observed with endothelial cell marker CD31 (right) in human carotid artery plaques. The stage of atherosclerosis was classified according to American Heart Association classification. 100 x magnification. Modified from Original Publication II.

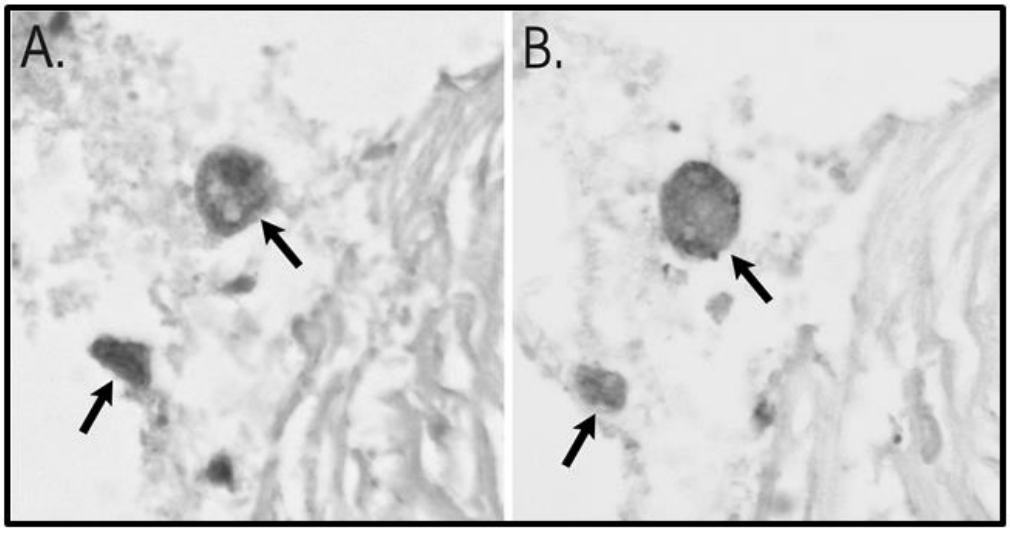

Figure 6. Mirror image sections illustrating co-localization of IDO and CD68 immunoreactivities in the same cells (arrows). 200 x magnification. Modified from Original Publication II.

5.8 IDO-pathway gene expression and pathway construction

Gene expression levels of IDO and various inflammatory components potentially influencing activity of the enzyme were further examined in a self-constructed pathway. GWEA had already exposed differential expression of approximately 250 genes from more than 23,000 known and candidate genes. Initial selection of inflammatory components was based on the existing research results from literature. Criteria for selection was scientifically verified influence on IDO activity. After selection, it was confirmed that the component gene had been expressed differentially in GWEA. Next, an additional analysis for IDO and each component was executed using qPCR for verification of the expression. This was followed by analysis in which expression level of each component was compared independently between atherosclerotic tissue sample (n=24) and control sample (n=6) to obtain fold change and p-value. Expression levels in artery tissues are tabulated on Table 6.

Table 6. Gene expression analysis of IDO-pathway from quantitative RT-PCR analysis. Interquartile range ranges (q1-q3) are presented in parenthesis. Modified from Original Publication II.

	Type of artery tissue			
Pathway gene	Atherosclerotic, n=24	Control, n=6	Fold change*	p-value**
CD80	16.83 (9.17-24.02)	1.00 (0.59-1.00)	16.83	<0.001
CD4	13.42 (8.62-18.86)	1.18 (1.00-1.95)	11.37	<0.001
CD86	9.69 (7.61-11.48)	1.06 (1.00-1.39)	9.14	0.001
IL-10	5.38 (3.30-7.41)	0.70 (0.57-1.00)	7.69	<0.001
CD28	9.16 (4.85-10.53)	1.21 (1.00-1.46)	7.57	0.001
IDO	4.97 (2.84-6.23)	1.02 (1.00-1.08)	4.87	0.001
FoxP3	4.72 (2.78-10.11)	1.00 (0.89-1.54)	4.72	0.001
CTLA-4	4.72 (2.89-20.46)	1.00 (0.79-1.05)	4.72	<0.001
ICOS	4.33 (2.71-14.30)	1.00 (0.80-1.41)	4.33	0.001
IFN-γ	2.13 (1.37-5.91)	1.00 (0.92-1.59)	2.13	0.022
TGF-β	2.16 (1.90-2.44)	1.07 (1.00-1.57)	2.01	<0.001

* Median expression values were used to calculate fold changes between advanced atherosclerotic and non-atherosclerotic control tissue. ** Mann-Whitney U-test was used to determine p-values between study groups. Definitions: Atherosclerotic (AHA type IV-VI atherosclerotic tissue), Control (non-atherosclerotic vessel tissue). Definitions: CD (cluster of differentiation), IL (interleukin), IDO (indoleamine 2,3-dioxygenase), FoxP3 (fork-head box protein 3), CTLA-4 (cytotoxic T lymphocyte-associated antigen 4), ICOS (inducible T-cell costimulatory), IFN-γ (interferon gamma), TGF-β (transforming growth factor beta).

All pathway genes in the table demonstrated increased expression in atherosclerotic tissue samples compared controls. Furthermore, fold change was statistically significant in all cases. Particularly high expression was observed in CD80 (16.83-fold, $p<0.001$) and CD4 (11.37-fold, $p<0.001$), which also demonstrated elevated expression in control vessel (1.18-fold). In contrast, expression of interleukin-10 (IL-10) was decreased in control (0.70-fold), which consequently explained high fold change observed (7.69-fold, $p<0.001$). Also CD86, which operates as a counteracting surface molecule of CD80, was significantly expressed (9.14-fold, $p=0.001$).

As expected, IDO was also highly expressed (4.87-fold, $p=0.001$). Interestingly though, IDO expression activating molecule interferon gamma (IFN-γ) was expressed only modestly (2.13-fold, $p=0.022$) as was transforming growth factor beta (TGF-β) (2.01-fold, $p<0.001$). Components present in Treg-cells, namely fork-head box protein 3 (FoxP3) (4.72-fold, $p=0.001$), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) (4.72-fold, $p=0.001$), and inducible T-cell co-stimulator (ICOS) (4.33-fold, $p=0.002$) were all notably up-regulated. Further, CD28, also present in Treg-cells, was highly expressed (7.57-fold, $p=0.001$). In addition to expression in atherosclerotic tissue, the component was also highly expressed in control vessel (median value of 1.21-fold) indicating elevated activity even in the absence of atherosclerosis.

Since independent examination of each gene expression provided limited amount of information, another aim was to elucidate relationships and cooperation of IDO and related components as a whole. This was achieved by constructing hypothetical pathways through which promotion of IDO could be transmitted. Due to participation of Treg-cells, APC and Th1-cells in elaborate signaling relationships in the artery wall, visualization of pathways was based on these three cell types. In a simplistic description illustrated in Figure 7, initiation of hypothetical signaling is began from Treg-cell followed by signal transmissions to IDO+ APC leading to activation of IDO. This, in turn, results in decreased concentration of tryptophan and subsequent suppression of Th1-cell activity. The final outcome of this whole process may be a diminished inflammatory response in the artery wall and consequently decreased development of atherosclerosis.

To include data from gene expression analysis in signal transmissions between these three cell types, altogether three hypothetically viable expression pathways, all of which may modify Th1-cell function, were characterized: 1) IDO activation via CD28–CTLA-4–ICOS molecule complex, 2) IDO activation via T-cell receptor (TCR)–CD74, and 3) Toll-like receptor 9 (TLR9) -mediated IDO activation via naive APC. These pathways are shown in Figure 7 in which the width of the arrows is proportional to signal strength. Further, in the figure some of the previously characterized components and their respective fold changes are presented, however,

the figure also contains four previously undescribed components: CD25, TCR, CD74 and TLR9. Of these components, TCR and CD74 form a signal transmission pair between Treg-cell and IDO+ APC, while TLR9 operate on APC and CD25 on Treg-cell. CD74, known as class II major histocompatibility complex (MHCII) invariant polypeptide chain, had a 2.4-fold expression (p=0.002), whereas TCR (0.9-fold, p=0.543), TLR9 (1.0-fold, p=0.885) and CD25 (0.9-fold, p=0.543) all had insignificant expression in atherosclerotic tissue. Detailed considerations of these hypothetical pathways and their potential role in influencing development of atherosclerosis are elaborated in Discussion.

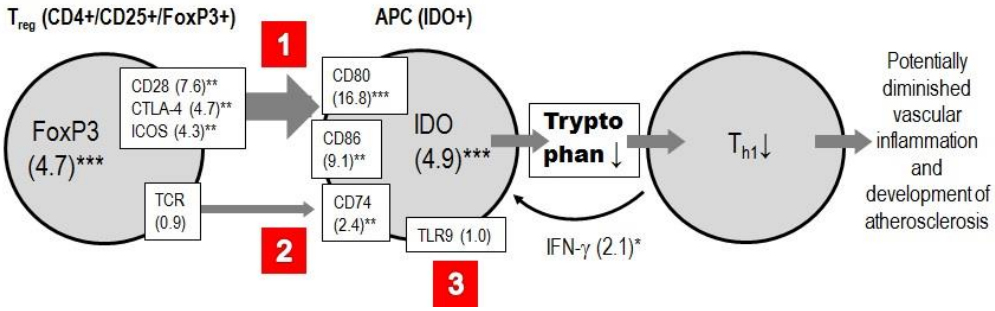

Figure 7. Hypothetical signal transmission pathways 1-3 each of which may cause suppression of Th1-cells and consequently decreased inflammatory response in atherosclerosis. Width of the arrows in pathways 1 and 2 is proportional to signal strength also expressed in folds. Data obtained from TaqMan LDA. Statistical significances: *p<0.05, **p<0.01, ***p<0.001 relative to control, Mann-Whitney U test. Definitions: APC (tolerogenic antigen-presenting cell), CD (cluster of differentiation), IDO (indoleamine 2,3-dioxygenase), FoxP3 (fork-head box protein 3), CTLA-4 (cytotoxic T lymphocyte-associated antigen 4), ICOS (inducible T-cell costimulatory), IFN-γ (interferon gamma), TCR (T-cell receptor), TLR9 (Toll-like receptor 9). Modified from Original Publication II.

Finally, GSEA was used to test whether selected and characterized pathway genes function in a co-operating manner according Subramanian et al. 2005 in which statistically significant cooperation is present when FDR is below 0.25. The result from analysis generated FDR-value of 0.07, which, therefore, indicated that selected genes appeared to cooperate harmoniously in the atherosclerotic tissue samples collected in this study. Overall, these outcomes suggested various alternative Treg-cell driven pathways leading to potential suppression of Th1-cell activity and consequently inhibition of vascular atherosclerosis.

6 Discussion

Intracellular IDO enzyme has been traditionally considered to have an immunosuppressive and therefore protective role in the development of atherosclerosis (Polyzos et al. 2015), however, these conceptions have been challenged (Laurans et al. 2018). This thesis aimed to elucidate ambiguous function of the enzyme. Considerable variation in statistically significant associations between IDO and atherosclerotic risk factors was observed. IDO was also associated with increased risk for visceral obesity over time. In females, premenopausal status, and up-regulated estrogen levels, seemed to influence IDO activity and delay progression of obesity and MetS, while in males the mechanism was presumably different. The results suggested, but did not explicitly support, age-dependent activation of the enzyme. Additionally, presence of IDO in macrophages and monocytes of human atherosclerotic plaques was confirmed. IDO and related inflammatory components were highly expressed in atherosclerotic tissue. Expression data was utilized to construct hypothetical expression pathways that may modify Th1-cell function, and consequently, suppress progression of vascular atherosclerosis. Figure 8 outlines key findings of the examinations.

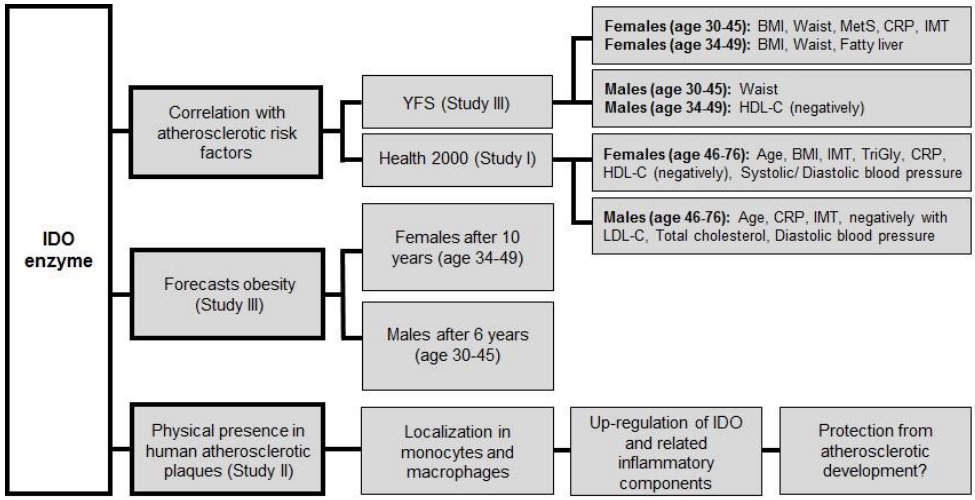

Figure 8. The main findings of the study.

6.1 Participants

This thesis consisted of three different cross-sectional and longitudinal study cohorts: Health 2000, TVS, and YFS. IDO activity along with CVD and diabetes risk factors were determined from 921 participants in a supplemental, cross-sectional study from Health 2000 -cohort. Similarly, atherosclerotic tissue samples and non-atherosclerotic control samples were collected cross-sectionally in TVS study. By contrast, longitudinal investigation was conducted from YFS. Began as a cross-sectional survey in 1980, YFS represented total random sample according to Åkerblom et al. 1985. Random selection equal from both sexes and different geographical areas was performed to comprehensively represent Finnish children and adolescents. A sub-study was performed in 2001 (2,284 participants) to determine IDO activity. Analysis conducted in this thesis utilized IDO activity along with complete CVD risk factor data from 927 participants at baseline, followed by further CVD risk factor measurements from 777 and 706 participants in 2007 and 2011, respectively.

Even though different study cohorts were used and compared in this thesis, they nevertheless appeared to represent original study cohorts. In the Health 2000 -study, satisfactory sample size from well-randomized and characterized population was used, whereas in TVS the sample size was small but highly relevant. Furthermore, classification of samples was conducted according to AHA standards. Lastly, participants in YFS sub-study had been previously utilized in scientific analysis, thus, their further utilization in this thesis presented no issues. In longitudinal examination, the number of dropouts naturally must be considered, however, in this thesis the number of participants was satisfactory. As such, it can be stated that statistical analyses were not influenced by bias, may be considered scientifically valid and appeared to be representative of the original study populations. Generalization of the results in this study may therefore be extended in wider populations.

6.2 Methods

6.2.1 Determination of IDO activity

Activity of IDO enzyme can be determined numerically by measuring concentrations of amino acids tryptophan and kynurenine. Repeatability of measurement was acceptable. Slight increase in IDO activity was observed as the population aged. Interestingly, when studies I and III were compared, in females the rate of increase was higher (from 26.94 ± 7.02 (age 24-39) to 32.37 ± 9.09 (age 58.9 ± 8.4)) compared to males (from 28.37 ± 7.51 (age 24-39) to 31.89 ± 8.74 (age 58.5 ± 7.8)).

The protocol characterizing this measurement has been outlined in 2002 by Laich and co-workers (Laich et al. 2002) and was generally accepted as the best alternative to determine IDO activity when the laboratory work for this research was performed. This protocol defends its position even today despite the fact that current gold standard method, liquid chromatography-mass spectrometry (Huang et al. 2013; Adu-Gyamfi et al. 2019), is available. Indeed, even though HPLC ultraviolet detection lack in some cases selectivity, the method used in this thesis is still sensitive and specific enough to measure IDO activity reliably. According to Laich and co-workers, HPLC ultraviolet detection method allows measurement of kynurenine and tryptophan concentrations with high sensitivity and without loss of specificity (Laich et al. 2002). Additionally, previously evidence has shown that extraction efficiencies for kynurenine and tryptophan were similar regardless of whether HPLC ultraviolet detection or liquid chromatography-mass spectrometry was used (Huang et al. 2013).

6.2.2 CVD risk factors

Risk factor measurements for CVD were outlined in Health 2000 and YFS protocols. These protocols have been considered and created over a long period of time by medical professionals and, therefore, are based on valid scientific evidence published in numerous high impact factor journals. As such, protocols can be considered well-standardized and reliable assessments of the measurements performed for each risk factor in question, which also enables valid comparison of results between studies. Clearly, determination of current smoking habits by questionnaire may involve limitations, which, however, did not play a major role in this thesis. Similarly, despite that determination of fatty liver status by ultrasound has a low sensitivity to

steatohepatitis (Saadeh et al. 2002), it is nevertheless an appropriate method in population studies (Suomela et al. 2015) even though accuracy could be improved by using magnetic resonance imaging techniques (Tan & Venkatesh 2016).

6.2.3 Immunohistochemistry, GWEA, quantitative RT-PCR

Immunohistochemical methodology and analysis was performed by experienced laboratory personnel using well-standardized protocols. The personnel have previously executed extensive amount of similar analyses for other research projects continuously producing reproducible, high-quality stainings that have been published in numerous scientific journals.

Protocols involving isolation of RNA and further analysis in GWEA using Sentrix Human-8 Expression BeadChip were considered golden standard methods at the time when the laboratory work for this research was performed. Consequently, it can be stated that protocols produced the most reliable results in gene expression levels that may be generalized in wider populations. This statement is consolidated by the fact that the accuracy of gene expression measurement had been verified previously by real-time quantitative TaqMan PCR in which expression of 20 genes with both methods was quantified with 95 % concordance (Laaksonen et al. 2006). Similarly, quantitative TaqMan LDA was widely used, high-quality technique that had been generally accepted for gene expression analyses. Commercial manufacturer of the products is a well-recognized company with established high-quality control. Therefore, the results from gene expression analyses can be considered scientifically reliable.

6.3 Results

6.3.1 IDO activity and atherosclerotic risk factors between studies

Macrolevel examination of statistically significant correlations between IDO enzyme and atherosclerosis risk factors in studies I and III, and comparison to previous investigation by Pertovaara and co-workers produced interesting observations. In cross-sectional settings comparability of the results between two

cohorts were justified even though the cohorts represented two different populations. In longitudinal analyses of YFS, the number of statistically significant associations decreased notably that could be explained by time and population differences of conducted measurements. Although this was not an ideal approach to compare overall results, high number of correlations in populations aged 24-39 and followed decline in 30-45 and 34-49 age groups were nevertheless unexpected and could not be explained explicitly. Therefore, definite conclusions would require further examinations to be conducted in adult populations.

Correlation differences between sexes was no surprise given the fact that in previous investigation IDO correlated with increased number of risk factors in females compared to males (Pertovaara et al. 2007). This suggested variation in immunological and hormonal status between sexes, which is in line with literature. For instance, elevated estrogen levels of females have been found to up-regulate dendritic cells (APC) to express IDO (Widner et al. 2000; Xiao et al. 2004; Bruce 2005; Sacre et al. 2015).

Table 3 presented Pearson correlations between female IDO activity and atherosclerotic risk factors in different studies and age groups. Statistically significant correlations were seen with BMI and waist circumference throughout YFS population, although coefficients were only modest. It may be that the impact of IDO is directly targeted to BMI and fat accumulation, consequently increasing waist circumference while collaboration with other risk factors may vary among females under investigation. In young population molecular level changes may fluctuate considerably or may not be observed because physiological alterations have not occurred. A certain number of females may already be in the process of developing MetS, however, adverse consequences related to clinical factors such as dyslipidemia, hypertension or IMT may take some time, whereas simple fat accumulation is likely to be much faster process. Regarding young females, also hormonal status must be considered. Indeed, even when the age of females is 34-49 years, some of them are still in premenopausal stage, and consequently influenced by elevated estrogen maintaining elasticity of arteries, thereby protecting females. This could also explain why artery elasticity variables did not correlate significantly with IDO in 2007. Obvious alternative is of course that IDO have no influence on elasticity factors. Possible influence of these risk factors could not be evaluated in 2011 due to lack of data. Direct comparison between YFS and Health 2000 -populations could not be performed due to longitudinal and cross-sectional data.

In Table 4, statistically significant correlations between IDO and risk factors produced markedly different results among males since considerably decreased number of correlations were found in study III compared to females. In fact, the only major finding was statistically significant correlation between IDO and waist circumference in both unadjusted and age-adjusted analysis in 30-45-year-old age

group that did not remain significant after Bonferroni correction. Interestingly, this correlation was absent in males aged 34-49 years. It therefore remained inconclusive whether IDO has a role in deleterious atherosclerotic development in males as they are approaching middle age. Overall, possible explanations follow the guidelines presented among females, in terms of rapid fat accumulation without the presence of molecular level changes in young population. However, absence of significant correlation with waist circumference in older age group and BMI altogether suggested that the mechanism of IDO may be different. Also hormonal differences between sexes must be recalled.

In cross-sectional Health 2000 -population analyses, the number of statistically significant correlations in females were almost identical and in males relatively equal compared to numbers obtained by Pertovaara and co-workers (Pertovaara et al. 2007) in cross-sectional YFS analyses. Furthermore, in both studies almost identical risk factor profiles were assessed. Based on the age profile in study I, it can be stated that the number of significant correlations were in line with expected outcome as this population is likely to have advanced stage in the development of cardiometabolic diseases. Having said this, the numbers in Pertovaara and co-workers investigation are surprisingly high considering the young age of the population. In females, age, HDL, LDL, BMI, waist circumference, waist-to-hip ratio, IMT, and logarithmically modified triglycerides and CRP correlated significantly and in males, HDL, BMI, waist circumference, waist-to-hip ratio, and logarithmically modified CRP. Overall number of investigated risk factors in both sexes were 11. This could indicate volatile and unpredictable nature of IDO enzyme activity, hormonal and metabolic changes that young population undergo, or perhaps a combination of these. Also influence of some currently unknown factors cannot be excluded.

Connections between IDO, BMI and waist circumference were unsurprising. This result is also in line with current understanding in which IDO, obesity, and MetS are connected even though the exact mechanism of action and further role of IDO in MetS are still not well-understood (Wolowczuk et al. 2012; Mallmann et al. 2018). The process occurs most likely via gut microbiota and involvement of IDO may be important (Laurans et al. 2018). This conclusion is supported by the fact that IDO is highly expressed in abdominal fat content (Mangge et al. 2014), especially in white adipose tissue of females (Wolowczuk et al. 2012). Therefore, influence of IDO in progression of MetS may be transmitted via fat tissue and also inflammation is likely to play a part in this activity. In fact, further evidence to support atherosclerotic development is gained from significant correlation between IDO and CRP that indicated presence of inflammation among females. In addition, previous cross-sectional studies have demonstrated involvement of inflammation between IDO, obesity, and MetS, and the presence of inflammatory cells among visceral fat cells (Brandacher et al. 2007; Mangge et al. 2014; Mallmann et al. 2018). Further

measurements including MetS and waist circumference would have given further information of the possible development of atherosclerosis in this population.

Differences between males and females are interesting and important. Again, differences in physiology and hormonal status between sexes could give reasons for these varying outcomes. For instance, even though the expression of IDO has been established in abdominal fat of females (Wolowczuk et al. 2012; Mangge et al. 2014), it does not automatically mean that expression is present in males.

6.3.2 IDO and obesity

Previously analyzed correlation data produced cross-sectional point estimates whether each risk factor was influenced by IDO activity at different moments in time. To widen this rather one-sided viewpoint, longitudinal risk ratio analysis was performed in study III to further examine association between IDO and obesity in 6- and 10-year follow-up. This approach generated evidence whether IDO activity forecasted risk for visceral obesity, which, of course, operate as a pathway for MetS and further cardiometabolic diseases.

After age-adjustment, statistically significant increase in risk was observed in all combinations, which, however, was only modest. In females, risk increased from 3.7 % to 5.7 % demonstrating only minor elevation over time. When data was additionally adjusted with BMI, in order to eliminate impact of BMI measured in 2001 and therefore to reinforce assessment of progression of obesity, the risk increased only 2.6 % in females aged 34-49 years, whereas significant association was absent among younger participants aged 30-45. The outcome in younger population was contradictory when compared to risk factor analysis in which both BMI and waist correlated significantly with IDO. However, since BMI value of 30 kg/m^2 was used as a threshold for obesity, it may be that majority of females had BMI-values close, but nevertheless below this threshold even though fat accumulation had already begun. In fact, data from baseline characteristics supported this speculation as BMI range in 2007 was 25.1 ± 5.0 kg/m^2 (n=435). Hence, overweight was present whereas obesity was mostly not. This conclusion, therefore, supports previous proposal that physiological development of adverse events leading to MetS is a slow process. This result might also suggest that that the borderline age when IDO begins to be active in female obesity is approximately 34–49 years. Until then female physiology may be able to combat against obesity, and consequently development of cardiometabolic diseases due to hormonal status, especially by the influence of estrogen. Alternatively, IDO enzyme may not be fully activated until female physiology changes as females begin to approach menopausal stage. Overall

though, based on present data, IDO is not forecasting risk for obesity substantially in females even though association is statistically significant and therefore established. This suggests only a minor role for IDO in the progression of obesity development. Naturally, further analysis using waist circumference would have produced more in-depth understanding of the process.

In males, results were straight-forward. After age adjustment IDO increased risk for obesity 2.7 % and 2.4 % in 2007 and 2011, respectively. Further, after additional BMI adjustment, increase was 1.9 % and 1.5 % in 2007 and 2011, respectively. All values were statistically significant. Also, percentages related to up-regulation remained nearly steady over time. This outcome was controversial in relation to risk factor analysis. IDO was forecasting risk for obesity to some extent even though this was not evident when statistically significant correlations were investigated. Ultimate reason for this difference could not be explained as both examinations used BMI values measured in 2007 and 2011. In risk ratio analysis using Poisson regression model with robust error variance, threshold for obesity was set as 30 kg/m^2 whereas in risk factor analysis such definition was not specified. Further, baseline characteristics indicated that BMI was only slightly above set threshold both in 2007 (26.7 ± 4.3 kg/m^2 (n=342)) and in 2011 (26.8 ± 4.4 kg/m^2 (n=312)) even though waist circumference clearly demonstrated that certain number of males were substantially obese in 2007 (94.3 ± 12.4 kg/m^2 (n=342)) and in 2011 (97.0 ± 12.5 kg/m^2 (n=312)). Absolute number of obese males, however, could not be clarified. Additionally, baseline characteristics also showed that BMI values did not change dramatically over time, thus explaining why obtained values remained mostly steady.

Overall, the impact of IDO in BMI and consequently role in fat accumulation leading to obesity remained a mystery. It was speculated that perhaps influence of IDO is volatile and may be transmitted via risk factors, but this may not always be the case. What can be said, though, was that influence is likely to be rather insignificant as shown by risk percentages. Furthermore, the mechanism in males appeared to be different compared to females. Indeed, this phenomenon might be explained by differences in physiology between sexes, especially low estrogen levels; males may not have the same protection capability as females, thus, presence of IDO could be associated with increased risk for obesity already in their early life. However, increased risk does not automatically mean that IDO has a functional role in influencing obesity that would explain lack of statistically significant correlations. Although the overall influence on BMI may be small, insignificant or volatile, males are likely to gain weight, and consequently visceral obesity, via some other mechanisms as shown by waist circumference figures. As with females, investigation using waist circumference would have probably proven to be useful.

Still, these observations are in line with CVD morbidity outcomes between sexes: adverse CVD outcomes appear later in females compared to males.

6.3.3 IDO in human atherosclerotic plaques

In study II a different approach was taken. The main aim was to investigate presence and role of IDO in human atherosclerotic plaques. First, the presence of IDO mRNA in advanced atherosclerotic plaques was determined. Based on gene expression profiles, IDO was expressed 1.3-fold in plaques relative to control samples indicating statistically significant up-regulation of IDO. In majority of previous studies, IDO activity has been measured from blood. Therefore, this outcome demonstrated that IDO is also physically present in actual human plaques further suggesting that IDO is likely to have a functional role in atherosclerotic development. Unfortunately, though, due to pooling and small sample size, possible expression differences between females and males, and also various arterial samples could not be investigated further.

Next, the aim was to determine immunoreactivity of IDO by characterizing the cell types in which IDO is potentially expressed. This was achieved by immunohistochemical stainings. Tissue samples were from human carotid artery plaques, whereas a sample from internal thoracic artery was used as a control. Samples were stained first with IDO followed by staining using monocyte marker (CD68), smooth muscle cell marker (HHF35), and endothelial cell marker (CD31). The results were straight-forward: only staining with CD68 produced positive outcomes indicating expression of IDO in macrophages and monocytes in the atheromatous core of carotid artery plaques. Detailed examination further revealed co-localization of IDO and CD68 immunoreactivities in the same cells. However, current approach could not distinguish possible differences in immunoreactivity between sexes. Although not surprising, overall, these outcomes confirmed and consolidated the presence of IDO in human atherosclerotic plaques and demonstrated that IDO is expressed in monocytes, therefore, producing confirmation to previously uncertain information of IDO expressing cell types.

In addition, gene expression analysis of IDO along with inflammatory components potentially influencing promotion or inhibition of IDO was performed from atherosclerotic plaques. Intention was to characterize gene expression profiles in IDO-related pathway and identify expression intensities, followed by construction of a hypothetical pathway through which influence of IDO could be transmitted. Each investigated gene in the pathway demonstrated elevated expression and statistically significant fold change when atherosclerotic tissue samples were

compared to control vessels, although considerable variation existed in expression folds. On general level, examined genes can be divided into four main categories based on their fold changes; CD80 and CD4 that were expressed over 10-times; CD86, IL-10 and CD28 expressed 7-9 -times; IDO, FoxP3, CTLA-4 and ICOS each expressed between 4-5 -times; IFN-γ and TGF-β expressed roughly 2-times. It is likely that differences in fold changes indicated varying influence of each component in the atheromatous core of plaques, though, this cannot be stated with certainty. Indeed, any of these components may potentially have an important role regardless of gene expression level. However, plain examination of each gene expression level independently holds little value. More appropriate approach is to understand relationships and cooperative networks of components. This was the reason for construction of hypothetical Treg-driven, IDO-mediated tryptophan-dependent T-cell suppression pathways in human advanced atherosclerosis. Although presentation in Figure 7 is greatly simplified, these pathways nonetheless offered some interesting considerations. Three hypothetically viable gene expression pathways were characterized all of which may suppress Th1-cell function. In these pathways, promotion of IDO may occur via 1) CD28–CTLA-4–ICOS molecule complex; 2) TCR–CD74 -pair; 3) TLR9-expression in naive APC.

In the first alternative, activation may initiate from CD28–CTLA-4–ICOS complex in which CD28 may have a predominant role based on the higher expression value. Still, this complex is likely to operate simultaneously and synergistically since in humans these three genes form a tightly linked cluster (Ling et al. 2001). Moreover, CD28 and ICOS may share overlapping signaling pathways suggesting that their gene expression may be co-ordinately regulated (Carreno & Collins 2002). Further, essential role of transcription factor FoxP3 in the development and immune regulation of Treg-cells has been characterized previously (Fontenot et al. 2003; Mallat et al. 2007; Nik Tavakoli et al. 2008) and may also play a role influencing this signaling pathway. Interaction is likely to occur on the surface of naive IDO+ APC via CD80/86 -pair (Carreno & Collins 2002; Collins et al. 2005; Greenwald et al. 2005). Even though ICOS is not directly involved in contacting CD80 or CD86 (Yoshinaga et al. 1999), it may nevertheless participate in synergistic amplification by assisting CD28 and CTLA-4 in signal transmission, thus causing accumulation of signals and increased gene activation. This potential route for signal transmission may be of importance as demonstrated by the width of the arrow, which is proportional to signal strength in Figure 7.

In the second option, speculated signal may propagate from TCR to CD74 even though direct evidence regarding participation of TCR–CD74 in IDO activation has not been described. Nevertheless, since CD74 is a part of MHCII and therefore a major player in signal transmission between T-cells and APC, it was considered that the route may be involved in activation of IDO. Statistically significant and positive

expression of CD74, which may also contribute to signals transmitted via CD80/86, supported this conclusion, whereas its signal transmission counterpart TCR did not based on fold change and statistically insignificant p-value. Further, participation of FoxP3 in this pathway could not be clarified. Width of the arrow in Figure 7 indicated questionable importance of this potential route for signal transmission.

In the third pathway, IDO may be activated on APC and therefore exclude the involvement of Treg-cells. In this scenario, TLR9 on the surface of naive APC is bound by cytosine-phosphate-guanosine oligodeoxynucleotides (CpG-ODN), which then may activate a signaling pathway for IDO promotion (Fallarino & Puccetti 2006). However, due to insignificant expression of TLR9, it is likely that this alternative does not promote activation of IDO directly or at least its influence is limited. In contrast, previously TLR9 activation has been found to initiate production of certain proinflammatory cytokines and co-stimulatory molecules, including CD80 and CD86 (Fallarino & Puccetti 2006). It is therefore possible that TLR9 participate in activation via some alternative, yet currently unknown, routes.

Regardless whether signal transmission route was one or some of the described pathways, or none of them, the outcome nonetheless was 4.97-fold promotion of IDO in atherosclerotic tissue. Unsurprisingly, IFN-γ, a proinflammatory cytokine and one of the key factors in the pathogenesis of atherosclerosis (Harvey & Ramji 2005), was also found to be up-regulated. Since its secretion originates from activated Th1-cells (Voloshyna et al. 2014), it was speculated that perhaps IFN-γ also tries to inhibit activated IDO to suppress Th1-lymphocytes, thus, forming a negative feedback loop. In fact, CpG-ODN-TLR9 binding, which have been found to enhance IFN-γ production (Krieg & Kline 2000), may play a role in this process. Should this be the case, it can be stated that the impact of IFN-γ is likely to be limited based on its modest expression. The end result of this whole process could be inhibition of vascular atherosclerosis; normal tryptophan-depleting function of IDO leads to locally decreased tryptophan levels and subsequent suppression of Th1-cells that consequently decrease inflammation in the artery wall. IDO-mediated suppression was supported by statistically significant negative correlation between IDO expression and Th1-cell associated chemokine receptor CXCR3 from GWEA in study II. Regarding signal transmission, width of the arrows in Figure 7 produced indicative information of preferred pathway, however, they should not be considered evidence as such. Even though certain pathway may be favored in activation, it is more likely that possible inhibition of atherosclerosis is a summary outcome of all genes described in various pathways. Indeed, FDR value of 0.07 demonstrated that genes selected for analysis functioned in cooperation. Furthermore, regulation of IDO is also likely to be influenced by some other components or signals not described here, or alternatively any possible impact of IDO could be so insignificant that its role is simply submerged by other, more vigorous and influential mechanisms

of atherosclerotic development. Therefore, information on Figure 7 should only be considered as a simplistic description of an extremely complex process.

6.4 Strengths and limitations

A definite strength in this thesis was the possibility to examine human-based data collected from various cohorts and populations at different time points, and also from different sources, namely blood samples and atherosclerotic plaques. In addition, utilized data provided a possibility for both cross-sectional and longitudinal investigation. This also allowed risk factor evaluation almost throughout the life of females and males. Investigated populations were well-characterized and large enough to allow justified scientific examination. Methods were widely used and well-established. All previously mentioned facts provided validity to draw credible conclusions from the data. Having said this, this thesis also had certain limitations. First, investigations were performed from data collected over 10-20 years ago. Even though this should not present a problem as such, it still raises a question, how valid the results are today? Second, studies used different approaches in terms of study protocols and methods. For instance, variation in selected risk factors was present, thus, complicating direct comparison of data between studies. Third, IDO activity was measured only in 2001. Later measurements for instance in 2007 and 2011 would have demonstrated how IDO activity changes as the population ages. Fourth, using BMI as the only determinant for obesity is problematic, because it provides limited amount of information of body composition (Romero-Corral et al. 2006). While not perfect either, waist circumference will produce better understanding, because it produces uncompromised evidence of actual obesity. However, since waist was not measured or used in all investigations, risk or existence of obesity was not fully revealed. Fifth, joint impact of all or certain groups of examined risk factors would have produced further evidence regarding risk profiles for cardiometabolic diseases compared to current approach in which correlation between IDO and each risk factor was analyzed independently. This would have been rather straight-forward analysis using multivariable models. Sixth, regarding study II, cell-specific localization of all IDO-related pathway markers were absent. Also, mechanistic insights regarding presented hypothesis of the IDO-related activation mechanism in atherosclerotic plaques were not discussed since such data would have required more specific functional studies in the form of *in vitro* experiments, which were impossible to organize at the time when the study was conducted. Lastly, further follow-up of participants as they age would have provided evidence on how many participants

were actually developing MetS and possibly other cardiometabolic diseases. This approach was completely overlooked for instance in study I. Such information would have elucidated the role of IDO in this process.

6.5 Clinical implications and future research prospectives

This thesis has investigated the role and function of IDO enzyme in human blood and tissue samples, and its potential influence and cooperation with multiple risk factors. Further, also physical presence in cell types was characterized along with potential influence of the enzyme in forecasting risk for visceral obesity. Overall, the results indicated localization of IDO in plaques and its involvement in progression of atherosclerosis. Despite these outcomes, the clinical value of the enzyme as an independent factor or marker in atherosclerosis is highly questionable based on controversial results obtained from the studies. In fact, also current understanding of the role is ambiguous; traditional immunosuppressive function has been challenged suggesting perhaps a volatile nature of the enzyme (Polyzos et al. 2015; Laurans et al. 2018). Naturally this presents a problem as stimulation of the enzyme may lead to adverse consequences. Another possible issue is raised due to multifunctional role of IDO, i.e. its operational function in various medical conditions. In practice this could mean that promotion or inhibition of the enzyme may result in unpredictable outcomes. Hypothetically, targeted stimulation to a certain organ or spot could potentially solve this dilemma, however, according to the best available information, such methodology is currently not available although proposals have been made especially in cancer immunotherapy (Park et al. 2023). Therefore, with present information, the results obtained in this thesis do not support the use of IDO as a therapeutic target *per se*, nor do they suggest that the diagnostic value of IDO alone is justified; exclusive use of the enzyme as a diagnostic marker do not provide sufficient evidence to evaluate the state of atherosclerotic development in humans. Instead, the optimal clinical value could be achieved if IDO is analyzed alongside with other atherosclerotic risk factors. This information could provide evidence of immunological status. However, even in such use, the results from any analysis should be treated with caution and any possible treatment decisions should be based on wider scale of well-established methods and generally accepted examinations.

All previously stated justifications are based on findings available today. Therefore, there is always a possibility that future research may reveal currently unknown mechanisms and treatment pathways. In such scenario, the importance of

IDO must be reevaluated. Perhaps using highly targeted triggering mechanisms operation of IDO could be enhanced to alleviate or retard chronic inflammation related to atherosclerosis. That is, if the ultimate function of the enzyme is found to be protective, which, like mentioned previously, is not self-evident. It would not actually be surprising if IDO was found to have a dual role in humans depending on individual characteristics. Obviously, the logical way forward is to conduct further investigations, especially human studies since results obtained from animal models may only be indicative. These studies should ideally focus on mechanisms and outcomes of IDO in various populations and medical conditions over time to elucidate true clinical relevance of the enzyme. Having this information would enable development of efficient treatment protocols and possible therapeutic alternatives if the role of IDO is found to be crucial in a pathway leading to atherosclerosis.

7 Conclusions

1. Evidence from correlation analyses between IDO and atherosclerotic risk factors revealed variation between sexes. Statistically significant associations were apparent in cross-sectional analysis while contradictory in longitudinal analysis.

2. IDO activity associated with BMI and forecasted risk for obesity both in females and males. However, increased risk was only marginal from clinical perspective. Functional role of IDO as an independent promoter of obesity is presumably minor.

3. IDO and related inflammatory components were present and up-regulated in human atherosclerotic plaques. Activity of these components may participate in modifying Th1-cell function, consequently suppressing vascular atherosclerosis.

Acknowledgements

This study was executed at the Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku.

Financial support was kindly provided by Urmas Pekkala Foundation, Turku University Foundation, Alfred Kordelin Foundation, University of Turku and Emil Aaltonen Foundation.

I am deeply thankful to my supervisor, Professor Risto Kaaja for his patience and friendly support, and the always encouraging attitude, wide knowledge and insightful ideas he has devoted to my work. I am deeply indebted to my supervisor, Professor Markus Juonala for all the support and time he has committed to my thesis. His expertise, passion for science, and visionary ideas were invaluable for this study.

The official reviewers, Professor Jari Laukkanen and Professor Olavi Ukkola, are warmly acknowledged for their valuable comments and constructive criticism, which improved the quality of this study.

The co-authors of the original publications in this thesis, Mari Levula, Markku Pelto-Huikko, Simo S. Oja, Annika Raitala, Jorma Viikari, Markku S. Nieminen, Juhani T. Soini, Mikko Hurme, Niku Oksala, Leena Moilanen, Juha-Pekka Salenius, Marja Pertovaara, Costan Magnussen, Terho Lehtimäki, Yrjö A. Kesäniemi, Olli T. Raitakari, Antti Reunanen, Mika Kähönen, Antti Jula, Reijo Laaksonen, Otso Järvinen and Leena Kytömäki are acknowledged for their collaboration.

I am deeply grateful to Irina Lisinen for her patient and hands-on approach to process data and all statistical assistance she has provided over the years. I wish to thank Nina Ruotsalainen for her assistance. I would also like to thank Nina Peltonen, Sinikka Repo-Koskinen and Eija Spåre for skilful technical assistance.

Personally, I want to express my deepest gratitude to my mother Leila for countless tough but educational discussions and keeping my head straight. This would have not been possible without your endless support. Love and respect.

Helsinki, April 2024

Petri Niinisalo

References

Adu-Gyamfi, C. G., Savulescu, D., George, J. A. & Suchard, M. S., 2019. Indoleamine 2,3-Dioxygenase-Mediated Tryptophan Catabolism: A Leading Star or Supporting Act in the Tuberculosis and HIV Pas-de-Deux? *Front Cell Infect Microbiol*, 9, p. 372.

Alberti, K. G. & Zimmet, P. Z., 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med*, 15(7), p. 539-553.

Alberti, K. G, Zimmet, P., Shaw, J. & IDF Epidemiology Task Force Consensus Group., 2005. The metabolic syndrome--a new worldwide definition. *Lancet*, 366(9491), p. 1059-1062.

Anderson, E. L., Howe, L. D., Jones, H. E., Higgins, J. P., Lawlor, D. A. & Fraser, A., 2015. The Prevalence of Non-Alcoholic Fatty Liver Disease in Children and Adolescents: A Systematic Review and Meta-Analysis. *PLoS One*, 10(10), p. e0140908.

Anstee, Q. M., Targher, G. & Day, C. P., 2013. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat Rev Gastroenterol Hepatol*, 10(6), p. 330-344.

Aromaa, A. & Koskinen, S., 2004. Health and functional capacity in Finland. Baseline results of the Health 2000 Health Examination Survey. *Publications of the National Public Health Institute*, B12/2004, p. 1-175.

Arora, A., Tripodi, G. L., Kareinen, I., Berg, M., Forteza, M. J., Gisterå, A., Griepke, S., Casagrande, F. B., Martins, J. O., Abdalla, D. S. P., Cole, J., Monaco, C. & Ketelhuth, D. F. J., 2022. Genetic Deficiency of Indoleamine 2,3-dioxygenase Aggravates Vascular but Not Liver Disease in a Nonalcoholic Steatohepatitis and Atherosclerosis Comorbidity Model. *Int J Mol Sci*, 23(9), p. 5203.

Avolio, A., Jones, D. & Tafazzoli-Shadpour, M., 1998. Quantification of alterations in structure and function of elastin in the arterial media. *Hypertension*, 32(1), p. 170–175.

Ball, H. J., Sanchez-Perez, A., Weiser, S., Austin, C. J., Astelbauer, F., Miu, J., McQuillan, J.A., Stocker, R., Jermiin, L. S. & Hunt, N. H., 2007. Characterization of an indoleamine 2,3-dioxygenase-like protein found in humans and mice. *Gene*, 396(1), p. 203–213.

Ban, Y., Chang, Y., Dong, B., Kong, B. & Qu, X., 2013. Indoleamine 2,3-dioxygenase levels at the normal and recurrent spontaneous abortion fetal-maternal interface. *J Int Med Res*, 41(4), p.1135-1149.

Bentzon, J. F., Otsuka, F., Virmani, R. & Falk, E., 2014. Mechanisms of plaque formation and rupture. *Circ Res*, 114(12), p. 1852-1866.

Berry, J. D., Dyer, A., Cai, X., Garside, D. B., Ning, H., Thomas, A., Greenland, P., Van Horn, L., Tracy, R. P. & Lloyd-Jones, D. M., 2012. Lifetime risks of cardiovascular disease. *N Engl J Med*, 366(4), p. 321-329.

Bigaard, J., Frederiksen, K., Tjønneland, A., Thomsen, B. L., Overvad, K., Heitmann, B. L. & Sørensen, T. I., 2005. Waist circumference and body composition in relation to all-cause mortality in middle-aged men and women. *Int J Obes (Lond)*, 29(7), p. 778-784.

Boasso, A., Herbeuval, J. P., Hardy, A. W., Winkler, C. & Shearer, G. M., 2005. Regulation of indoleamine 2,3-dioxygenase and tryptophanyl-tRNA-synthetase by CTLA-4-Fc in human CD4+ T cells. *Blood*, 105(4), p. 1574-1581.

Borén, J., Chapman, M. J., Krauss, R. M., Packard, C. J., Bentzon, J. F., Binder, C. J., Daemen, M. J., Demer, L. L., Hegele, R. A., Nicholls, S. J., Nordestgaard, B.G. , Watts, G. F., Bruckert, E., Fazio, S., Ference, B. A., Graham, I., Horton, J. D., Landmesser, U., Laufs, U., Masana, L., Pasterkamp, G., Raal, F. J., Ray, K. K., Schunkert, H., Taskinen, M. R., van de Sluis, B., Wiklund, O., Tokgozoglu, L., Catapano, A. L. & Ginsberg, H. N., 2020. Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J*, 41(24), p. 2313-2330.

Brandacher, G., Hoeller, E., Fuchs, D. & Weiss, H. G., 2007. Chronic immune activation underlies morbid obesity: is Ido a key player? *Curr Drug Metab*, 8(3), p. 289-295.

Bruce, I. N., 2005. 'Not only...but also': factors that contribute to accelerated atherosclerosis and premature coronary heart disease in systemic lupus erythematosus. *Rheumatology (Oxford)*, 44(12), p. 1492-1502.

Buchanan, T. A. & Kjos, S. L., 1999. Gestational diabetes: risk or myth? *J Clin Endocrinol Metab*, 84(6), p. 1854-1857.

Buchanan, T. A. & Xiang, A. H., 2005. Gestational diabetes mellitus. *J Clin Invest*, 115(3), p. 485-491.

Burke, A. P., Kolodgie, F. D., Farb, A., Weber, D. K., Malcom, G. T., Smialek, J. & Virmani, R., 2001. Healed plaque ruptures and sudden coronary death: evidence that subclinical rupture has a role in plaque progression. *Circulation*, 103(7), p. 934-940.

Brown, M. C., Best, K. E., Pearce, M. S., Waugh, J., Robson, S. C. & Bell, R., 2013. Cardiovascular disease risk in women with pre-eclampsia: systematic review and meta-analysis. *Eur J Epidemiol*, 28(1), p. 1-19.

Caro, C. G., 2009. Discovery of the role of wall shear in atherosclerosis. *Arterioscler Thromb Vasc Biol*, 29(2), p. 158–161.

Carreno, B. M. & Collins, M., 2002. The B7 family of ligands and its receptors: new pathways for costimulation and inhibition of immune responses. *Annu Rev Immunol*, 20, p. 29–53.

Cashen, K. & Petersen, T., 2019. Diabetic Ketoacidosis. *Pediatr Rev*, 40(8), p. 412-420.

Catalano, P. M., Huston, L., Amini, S. B. & Kalhan, S. C., 1999. Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and gestational diabetes mellitus. *Am J Obstet Gynecol*, 180(4), p. 903-916.

Chang, R. Q., Li, D. J. & Li. M. Q., 2018. The role of indoleamine-2,3-dioxygenase in normal and pathological pregnancies. *Am J Reprod Immunol*, 79(4), p. e12786.

Chatzizisis, Y. S., Coskun, A. U., Jonas, M., Edelman, E. R., Feldman, C. L. & Stone, P. H., 2007. Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior. *J Am Coll Cardiol*, 49(25), p. 2379–2393.

Chen, Y. C., Bui, A. V., Diesch, J., Manasseh, R., Hausding, C., Rivera, J., Haviv, I., Agrotis, A., Htun, N. M., Jowett, J., Hagemeyer, C. E., Hannan, R. D., Bobik, A. & Peter, K., 2013. A novel mouse model of atherosclerotic plaque instability for drug testing and mechanistic/therapeutic discoveries using gene and microRNA expression profiling. *Circ Res*, 113(3), p. 252–265.

Cheruvu, P. K., Finn, A. V., Gardner, C., Caplan, J., Goldstein, J., Stone, G. W., Virmani, R. & Muller, J. E., 2007. Frequency and distribution of thin-cap fibroatheroma and ruptured plaques in human coronary arteries: a pathologic study. *J Am Coll Cardiol*, 50(10), p. 940–949.

Cheung, N., Mitchell, P. & Wong, T. Y., 2010. Diabetic retinopathy. *Lancet*, 376(9735), p. 124-136.

Christiansen, M. K., Winther, S., Nissen, L., Vilhjálmsson, B. J., Frost, L., Johansen, J. K., Møller, P. L., Schmidt, S. E., Westra, J., Holm, N. R., Jensen, H. K., Christiansen, E. H., Guðbjartsson, D. F., Hólm, H., Stefánsson, K., Bøtker, H. E., Bøttcher, M. & Nyegaard, M., 2021. Polygenic Risk Score-enhanced Risk Stratification of Coronary Artery Disease in Patients with Stable Chest Pain. *Circ Genom Precis Med*, 14(3), p. e003298.

Cole, J. E., Astola, N., Cribbs, A. P., Goddard, M. E., Park, I., Green, P., Davies, A. H., Williams, R. O., Feldmann, M. & Monaco, C., 2015. Indoleamine 2,3-dioxygenase-1 is protective in

atherosclerosis and its metabolites provide new opportunities for drug development. *Proc Natl Acad Sci U S A*, 112(42), p.13033-13038.

Collins, M., Ling, V. & Carreno, B. M., 2005. The B7 family of immune-regulatory ligands. *Genome Biol*, 6(6), p. 223.

Cook, C. H., Bickerstaff, A. A., Wang, J. J., Nadasdy, T., Della Pelle, P., Colvin, R. B. & Orosz, C. G., 2008. Spontaneous renal allograft acceptance associated with "regulatory" dendritic cells and IDO. *J Immunol*, 180(5), p. 3103-3112.

Cryer, P. E., 2013. Mechanisms of hypoglycemia-associated autonomic failure in diabetes. *N Engl J Med*, 369(4), p. 362-372.

Dalla Valle, M., Laatikainen, T., Kalliokoski, T., Nykänen, P. & Jääskeläinen, J., 2015. Childhood obesity in specialist care--searching for a healthy obese child. *Ann Med*, 47(8), p. 639-654.

de Groot, E., Hovingh, G. K., Wiegman, A., Duriez, P., Smit, A. J., Fruchart, J. C. & Kastelein, J. J., 2004. Measurement of arterial wall thickness as a surrogate marker for atherosclerosis. *Circulation*, 109(23 Suppl 1), p. III33-38.

Fallarino, F. & Puccetti, P., 2006. Toll-like receptor 9-mediated induction of the immunosuppressive pathway of tryptophan catabolism. *Eur J Immunol*, 36(1), p. 8–11.

Favennec, M., Hennart, B., Caiazzo, R., Leloire, A., Yengo, L., Verbanck, M., Arredouani, A., Marre, M., Pigeyre, M., Bessede, A., Guillemin, G. J., Chinetti, G., Staels, B., Pattou, F., Balkau, B., Allorge, D., Froguel, P. & Poulain-Godefroy, O., 2015. The kynurenine pathway is activated in human obesity and shifted toward kynurenine monooxygenase activation. *Obesity (Silver Spring)*, 23(10), p. 2066–2074.

Ference, B. A., Ginsberg, H. N., Graham, I., Kausik, K. R., Packard, C. J., Bruckert, E., Hegele, R. A., Krauss, R. M., Raal, F. J., Schunkert, H., Watts, G. F., Borén, J., Fazio, S., Horton, J. D., Masana, L., Nicholls, S. J., Nordestgaard, B. G., van de Sluis, B., Taskinen, M. R., Tokgözoglu, L., Landmesser, U., Laufs, U., Wiklund, O., Stock, J. K., Chapman, M. J. & Catapano, A. L., 2017. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J*, 38(32), p. 2459-2472.

Fontenot, J. D., Gavin, M. A. & Rudensky, A. Y., 2003. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol*, 4(4), p. 330–336.

Franco, O. H., Peeters, A., Bonneux, L. & de Laet, C., 2005. Blood pressure in adulthood and life expectancy with cardiovascular disease in men and women: life course analysis. *Hypertension*, 46(2), p. 280-286.

Friedewald, W. T., Levy, R. I. & Fredrickson, D. S., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*, 18(6), p. 499-502.

Fusaro, M. & Tessarin, G., 2021. Prevention of Coronary Artery Disease-Related Heart Failure: The Role of Computed Tomography Scan. *Heart Fail Clin*, 17(2), p. 187-194.

Gaemperli, O., Bengel, F. M. & Kaufmann, P. A., 2011. Cardiac hybrid imaging. *Eur Heart J*, 32(17), p. 2100-2108.

Gamble, D. T., Brikinns, B., Myint, P. K. & Bhattacharya, S., 2019. Hypertensive Disorders of Pregnancy and Subsequent Cardiovascular Disease: Current National and International Guidelines and the Need for Future Research. *Front Cardiovasc Med*, 6, p. 55.

Giguère, Y., Charland, M., Thériault, S., Bujold, E., Laroche, M., Rousseau, F., Lafond, J. & Forest, J. C., 2012. Linking preeclampsia and cardiovascular disease later in life. *Clin Chem Lab Med*, 50, p. 985-993.

Gisterå, A. & Hansson, G. K., 2017. The immunology of atherosclerosis. *Nat Rev Nephrol*, 13(6), p. 368-380.

Greenwald, R. J., Freeman, G. J. & Sharpe, A. H., 2005. The B7 family revisited. *Annu Rev Immunol*, 23, p. 515–548.

Groer, M., Fuchs, D., Duffy, A., Louis-Jacques, A., D'Agata, A. & Postolache, T. T., 2018. Associations among Obesity, Inflammation, and Tryptophan Catabolism in Pregnancy. *Biol Res Nurs*, 20(3), p. 284–291.

Grundy, S. M., 1999. Primary prevention of coronary heart disease: integrating risk assessment with intervention. *Circulation*, 100(9), p. 988-998.

Goldstein, J. A., Gallagher, K., Beck, C., Kumar, R. & Gernand, A. D., 2020. Maternal-Fetal Inflammation in the Placenta and the Developmental Origins of Health and Disease. *Front Immunol*, 11, p. 531543.

Guh, D. P., Zhang, W., Bansback, N., Amarsi, Z., Birmingham, C. L. & Anis, A. H., 2009. The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. *BMC Public Health*, 9, p. 88.

Hansson, G. K., 2005. Inflammation, atherosclerosis and coronary artery disease. *N Engl J Med*, 352(16), p. 1685–1695.

Hansson, G. K. & Libby, P., 2006. The immune response in atherosclerosis: a double-edged sword. *Nat Rev Immunol*, 6(7), p. 508–519.

Harvey, E. & Ramji, D. P., 2005. Interferon-gamma and atherosclerosis: pro- or anti-atherogenic? *Cardiovasc Res*, 67(1), p. 11–20.

He, Y., Zhou, S., Liu, H., Shen, B., Zhao, H., Peng, K. & Wu, X., 2015. Indoleamine 2, 3-Dioxygenase Transfected Mesenchymal Stem Cells Induce Kidney Allograft Tolerance by Increasing the Production and Function of Regulatory T Cells. *Transplantation*, 99(9), p. 1829-1838.

Herrera, E. & Ortega-Senovilla, H., 2018. Implications of Lipids in Neonatal Body Weight and Fat Mass in Gestational Diabetic Mothers and Non-Diabetic Controls. *Curr Diab Rep*, 18(2), p. 7.

Holmen, O. L., Zhang, H., Fan, Y., Hovelson, D. H., Schmidt, E. M., Zhou, W., Guo, Y., Zhang, J., Langhammer, A., Løchen, M. L., Ganesh, S. K., Vatten, L., Skorpen, F., Dalen, H., Zhang, J., Pennathur, S., Chen, J., Platou, C., Mathiesen, E. B., Wilsgaard, T., Njølstad, I., Boehnke, M., Chen, Y. E., Abecasis, G. R., Hveem, K. & Willer, C. J., 2014. Systematic evaluation of coding variation identifies a candidate causal variant in TM6SF2 influencing total cholesterol and myocardial infarction risk. *Nat Genet*, 46(4), p. 345-351.

Huang, Y., Louie, A., Yang, Q., Massenkoff, N., Xu, C., Hunt, P. W. & Gee, W., 2013. A simple LC–MS/MS method for determination of kynurenine and tryptophan concentrations in human plasma from HIV-infected patients. *Bioanalysis*, 5(11), p. 1397-1407.

Huxley, R., Barzi, F. & Woodward, M., 2006. Excess risk of fatal coronary heart disease associated with diabetes in men and women: meta-analysis of 37 prospective cohort studies. *BMJ*, 332(7533), p. 73-78.

Hwu, P., Du, M. X., Lapointe, R., Do, M., Taylor, M. W. & Young, H. A., 2000. Indoleamine 2,3-dioxygenase production by human dendritic cells results in the inhibition of T cell proliferation. *J Immunol*, 164, p. 3596–3599.

Insull, W Jr., 2009. The pathology of atherosclerosis: plaque development and plaque responses to medical treatment. *Am J Med*, 122(1 Suppl), p. S3-S14.

International Diabetes Federation, 2021. IDF diabetes atlas. 10th edition. < https://diabetesatlas.org/atlas/tenth-edition/> Read: October 5th, 2023.

Josefson, J. L., Catalano, P. M., Lowe, W. L., Scholtens, D. M., Kuang, A., Dyer, A. R., Lowe, L. P. & Metzger, B. E., 2020. The Joint Associations of Maternal BMI and Glycemia with Childhood Adiposity. *J Clin Endocrinol Metab*, 105(7), p. 2177-2188.

Juonala, M., Viikari, J. S., Hutri-Kähönen, N., Pietikäinen, M., Jokinen, E., Taittonen, L., Marniemi, J., Rönnemaa, T. & Raitakari, O. T., 2004. The 21-year follow-up of the Cardiovascular Risk in Young Finns Study: risk factor levels, secular trends and east-west difference. *J Intern Med*, 255(4), p. 457-468.

Juonala, M., Järvisalo, M. J., Mäki-Torkko, N., Kähönen, M., Viikari, J. S. & Raitakari, O. T., 2005. Risk factors identified in childhood and decreased carotid artery elasticity in adulthood: the Cardiovascular Risk in Young Finns Study. *Circulation*, 112(10), p. 1486-1493.

Juonala, M., Raitakari, M., Viikari, J. S. A., Raitakari, O. T., 2006. Obesity in youth is not an independent predictor of carotid IMT in adulthood. The Cardiovascular Risk in Young Finns Study. *Atherosclerosis*, 185(2), p. 388-393.

Juonala, M., Magnussen, C. G., Berenson, G. S., Venn, A., Burns, T. L., Sabin, M. A., Srinivasan, S. R., Daniels, S. R., Davis, P. H., Chen, W., Sun, C., Cheung, M., Viikari, J. S., Dwyer, T. & Raitakari, O. T., 2011. Childhood adiposity, adult adiposity, and cardiovascular risk factors. *N Engl J Med*, 365(20), p. 1876-1885.

Juonala, M., Viikari, J. S. & Raitakari, O. T., 2013. Main findings from the prospective Cardiovascular Risk in Young Finns Study. *Curr Opin Lipidol*, 24(1), p. 57-64.

Kaaja, R. & Eriksson, J., 2021. Rasvamaksatauti osana raskausdiabetesta, pääkirjoitus. *Duodecim*, 137, p. 1853-1855.

Kajantie, E., Eriksson, J. G., Osmond, C., Thornburg, K. & Barker, D. J., 2009. Pre-eclampsia is associated with increased risk of stroke in the adult offspring: the Helsinki birth cohort study. *Stroke*, 40(4), p. 1176-1180.

Kartika, R., Wibowo, H., Purnamasari, D., Pradipta, S. & Larasati, R. A., 2020. Altered Indoleamine 2,3-Dioxygenase Production and Its Association to Inflammatory Cytokines in Peripheral Blood Mononuclear Cells Culture of Type 2 Diabetes Mellitus. *Int J Tryptophan Res*, 13, p. 1178646920978236.

Kazakauskaitė, E., Žaliaduonytė-Pekšienė, D., Rumbinaitė, E., Keršulis, J., Kulakienė, I. & Jurkevičius, R., 2018. Positron Emission Tomography in the Diagnosis and Management of Coronary Artery Disease. *Medicina (Kaunas)*, 54(3), p. 47.

Kenneally, S., Sier, J. H. & Moore, J. B., 2017. Efficacy of dietary and physical activity intervention in non-alcoholic fatty liver disease: a systematic review. *BMJ Open Gastroenterol*, 4(1), p. e000139.

Khan, A., Arbab-Zadeh, A., Kiani, A. N., Magder, L. S. & Petri, M., 2017. Progression of noncalcified and calcified coronary plaque by CT angiography in SLE. *Rheumatol Int*, 37(1), p. 59-65.

Khosravi-Maharlooei, M., Pakyari, M., Jalili, R. B., Kilani, R. T. & Ghahary, A., 2017. Intraperitoneal injection of IDO-expressing dermal fibroblasts improves the allograft survival. *Clin Immunol*, 174, p. 1-9.

Koskinen, J., Kähönen, M., Viikari, J. S., Taittonen, L., Laitinen, T., Rönnemaa, T., Lehtimäki, T., Hutri-Kähönen, N., Pietikäinen, M., Jokinen, E., Helenius, H., Mattsson, N., Raitakari, O. T. & Juonala, M., 2009. Conventional cardiovascular risk factors and metabolic syndrome in predicting carotid intima-media thickness progression in young adults: the cardiovascular risk in young Finns study. *Circulation*, 120(3), p. 229-236.

Koskinen, J., Magnussen, C. G., Viikari, J. S., Kähönen, M., Laitinen, T., Hutri-Kähönen, N., Lehtimäki, T., Jokinen, E., Raitakari, O. T. & Juonala, M., 2012. Effect of age, gender and cardiovascular risk factors on carotid distensibility during 6-year follow-up. The Cardiovascular Risk in Young Finns Study. *Atherosclerosis*, 224(2), p. 474–479.

Kotronen, A., Westerbacka, J., Bergholm, R., Pietiläinen, K. H. & Yki-Järvinen, H., 2007. Liver fat in the metabolic syndrome. *J Clin Endocrinol Metab*, 92(9), p. 3490-3497.

Krieg, A. M. & Kline, J. N., 2000. Immune effects and therapeutic applications of CpG motifs in bacterial DNA. *Immunopharmacology*, 48(3), p. 303–305.

Laaksonen, R., Katajamaa, M., Päivä, H., Sysi-Aho, M., Saarinen, L., Junni, P., Lütjohann, D., Smet, J., Van Coster, R., Seppänen-Laakso, T., Lehtimäki, T., Soini, J. & Oresic, M., 2006. A systems biology strategy reveals biological pathways and plasma biomarker candidates for potentially toxic statin-induced changes in muscle. *PLoS One*, 1(1), p. e97.

Laich, A., Neurauter, G., Widner, B. & Fuchs, D., 2002. More rapid method for simultaneous measurement of tryptophan and kynurenine by HPLC. *Clin Chem*, 48(3), p. 579-581.

Larqué, E., Labayen, I., Flodmark, C. E., Lissau, I., Czernin, S., Moreno, L. A., Pietrobelli, A. & Widhalm, K., 2019. From conception to infancy - early risk factors for childhood obesity. *Nat Rev Endocrinol*, 15(8), p. 456-478.

Laurans, L., Venteclef, N., Haddad, Y., Chajadine, M., Alzaid, F., Metghalchi, S., Sovran, B., Denis, R. G. P., Dairou, J., Cardellini, M., Moreno-Navarrete, J. M., Straub, M., Jegou, S., McQuitty, C., Viel, T., Esposito, B., Tavitian, B., Callebert, J., Luquet, S. H., Federici, M., Fernandez-Real, J. M., Burcelin, R., Launay, J. M., Tedgui, A., Mallat, Z., Sokol, H. & Taleb, S., 2018. Genetic deficiency of indoleamine 2,3-dioxygenase promotes gut microbiota-mediated metabolic health. *Nat Med*, 24(8), p. 1113-1120.

Lewandowski, L. B. & Kaplan, M. J., 2016. Update on cardiovascular disease in lupus. *Curr Opin Rheumatol*, 28(5), p. 468-476.

Lewington, S., Clarke, R., Qizilbash, N., Peto, R., Collins, R. & Prospective Studies Collaboration., 2002. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet*, 360(9349), p. 1903-1913.

Li, D. L. & Kronenberg, M. W., 2021. Myocardial Perfusion and Viability Imaging in Coronary Artery Disease: Clinical Value in Diagnosis, Prognosis, and Therapeutic Guidance. *Am J Med*, 134(8), p. 968-975.

Li, J., Meinhardt, A., Roehrich, M. E., Golshayan, D., Dudler, J., Pagnotta, M., Trucco, M. & Vassalli, G., 2007. Indoleamine 2,3-dioxygenase gene transfer prolongs cardiac allograft survival. *Am J Physiol Heart Circ Physiol*, 293(6), p. H3415-H3423.

Lieb, W., Song, R. J., Vasan, R. S. & Xanthakis, V., 2020. Premature Parental Cardiovascular Disease and Subclinical Disease Burden in the Offspring. *J Am Heart Assoc*, 9(18), p. e015406.

Ling, V., Wu, P. W., Finnerty, H. F., Agostino, M. J., Graham, J. R., Chen, S., Jussiff, J. M., Fisk, G. J., Miller, C. P. & Collins, M., 2001. Assembly and annotation of human chromosome 2q33 sequence containing the CD28, CTLA4, and ICOS gene cluster: analysis by computational, comparative, and microarray approaches. *Genomics*, 78(3), p. 155-168.

Lonardo, A., Nascimbeni, F., Mantovani, A. & Targher, G., 2018. Hypertension, diabetes, atherosclerosis and NASH: Cause or consequence? *J Hepatol*, 68(2), p. 335–352.

Loos, R. J., 2018. The genetics of adiposity. *Curr Opin Genet Dev*, 50, p. 86-95.

Lorenz, M. W., Markus, H. S., Bots, M. L., Rosvall, M. & Sitzer, M., 2007. Prediction of clinical cardiovascular events with carotid intima-media thickness: A systematic review and meta-analysis. *Circulation*, 115(4), p. 459–467.

Lowenstern, A., Alexander, K. P., Hill, C. L., Alhanti, B., Pellikka, P. A., Nanna, M. G., Mehta, R. H., Cooper, L. S., Bullock-Palmer, R. P., Hoffmann, U. & Douglas, P. S., 2020. Age-Related Differences in the Noninvasive Evaluation for Possible Coronary Artery Disease: Insights From the Prospective Multicenter Imaging Study for Evaluation of Chest Pain (PROMISE) Trial. *JAMA Cardiol*, 5(2), p. 193-201.

Mahnke, K., Bedke, T. & Enk, A. H., 2007. Regulatory conversation between antigen presenting cells and regulatory T cells enhance immune suppression. *Cell Immunol*, 250(1-2), p. 1-13.

Mallat, Z., Ait-Oufella, H. & Tedgui, A., 2007. Regulatory T-cell immunity in atherosclerosis. *Trends Cardiovasc Med*, 17(4), p. 113–118.

Mallmann, N. H., Lima, E. S. & Lalwani, P., 2018. Dysregulation of tryptophan catabolism in metabolic syndrome. *Metab Syndr Relat Disord*, 16(3), p. 135-142.

Mangge, H., Summers, K. L., Meinitzer, A., Zelzer, S., Almer, G., Prassl, R., Schnedl, W. J., Reininghaus, E., Paulmichl, K., Weghuber, D. & Fuchs, D., 2014. Obesity-related dysregulation of the tryptophan-kynurenine metabolism: role of age and parameters of the metabolic syndrome. *Obesity (Silver Spring)*, 22(1), p. 195-201.

Mars, N., Koskela, J. T., Ripatti, P., Kiiskinen, T. T. J., Havulinna, A. S., Lindbohm, J. V., Ahola-Olli, A., Kurki, M., Karjalainen, J., Palta, P.; FinnGen, Neale, B. M., Daly, M., Salomaa, V., Palotie, A., Widén, E. & Ripatti, S., 2020. Polygenic and clinical risk scores and their impact on age at onset and prediction of cardiometabolic diseases and common cancers. *Nat Med*, 26(4), p. 549-557.

Mattsson, N., Rönnemaa, T., Juonala, M., Viikari, J. S. & Raitakari, O. T., 2008. Childhood predictors of the metabolic syndrome in adulthood. The Cardiovascular Risk in Young Finns Study. *Ann Med*, 40(7), p. 542-552.

McGill, H. C. Jr., McMahan, C. A., Malcom, G. T., Oalmann, M. C. & Strong, J. P., 1997. Effects of serum lipoproteins and smoking on atherosclerosis in young men and women. The PDAY Research Group. Pathobiological Determinants of Atherosclerosis in Youth. *Arterioscler Thromb Vasc Biol*, 17(1), p. 95-106.

Mellor, A. L. & Munn, D. H., 1999. Tryptophan catabolism and T-cell tolerance: immunosuppression by starvation? *Immunol Today*, 20(10), p. 469–473.

Mellor, A. L. & Munn, D. H., 2003. Tryptophan catabolism and regulation of adaptive immunity. *J Immunol*, 170(12), p. 5809–5813.

Mellor, A. L. & Munn, D. H., 2004. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nature Rev Immunol*, 4(10), p. 762–774.

Merlo, L. M. F., DuHadaway, J. B., Montgomery, J. D., Peng, W. D., Murray, P. J., Prendergast, G. C., Caton, A. J., Muller, A. J. & Mandik-Nayak, L., 2020. Differential roles of Ido1 and Ido2 in T and β cell inflammatory immune responses. *Front Immunol*, 11, p. 1861.

Milić, S., Lulić, D. & Štimac, D., 2014. Non-alcoholic fatty liver disease and obesity: biochemical, metabolic and clinical presentations. *World J Gastroenterol*, 20(28), p. 9330-9337.

Mongraw-Chaffin, M. L., Cirillo, P. M. & Cohn, B. A., 2010. Preeclampsia and cardiovascular disease death: prospective evidence from the child health and development studies cohort. *Hypertension*, 56(1), p. 166-171.

Morisset, A. S., St-Yves, A., Veillette, J., Weisnagel, S. J., Tchernof, A. & Robitaille, J., 2010. Prevention of gestational diabetes mellitus: a review of studies on weight management. *Diabetes Metab Res Rev*, 26(1), p. 17-25.

Murray, C. J. L. & GBD 2016 Causes of Death Collaborators., 2017. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet*, 390(10100), p. 1151–1210.

Musso, G., Cassader, M., Rosina, F. & Gambino, R., 2012. Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. *Diabetologia*, 55(4), p. 885-904.

Nagano, J., Shimizu, M., Hara, T., Shirakami, Y., Kochi, T., Nakamura, N., Ohtaki, H., Ito, H., Tanaka, T., Tsurumi, H., Saito, K., Seishima, M. & Moriwaki, H., 2013. Effects of indoleamine 2,3-dioxygenase deficiency on high-fat diet-induced hepatic inflammation. *PLoS One*, 8(9), p. e73404.

Nagy, B. M., Nagaraj, C., Meinitzer, A., Sharma, N., Papp, R., Foris, V., Ghanim, B., Kwapiszewska, G., Kovacs, G., Klepetko, W., Pieber, T. R., Mangge, H., Olschewski, H. & Olschewski, A., 2017. Importance of kynurenine in pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol*, 313(5), p. L741-L751.

Nakashima, Y., Fujii, H., Sumiyoshi, S., Wight, T. N. & Sueishi, K., 2007. Early human atherosclerosis: accumulation of lipid and proteoglycans in intimal thickenings followed by macrophage infiltration. *Arterioscler Thromb Vasc Biol*, 27(5), p. 1159-1165.

Nasir, K., Budoff, M. J., Wong, N. D., Scheuner, M., Herrington, D., Arnett, D. K., Szklo, M., Greenland, P. & Blumenthal, R. S., 2007. Family history of premature coronary heart disease and coronary artery calcification: Multi-Ethnic Study of Atherosclerosis (MESA). *Circulation*, 116(6), p. 619-626.

Nik Tavakoli, N., Hambly, B. D., Sullivan, D. R. & Bao, S., 2008. Forkhead box protein 3: Essential immune regulatory role. *Int J Biochem Cell Biol*, 40(11), p. 2369–2373.

Nilsson, J. & Hansson, G. K., 2008. Autoimmunity in atherosclerosis: a protective response losing control? *J Intern Med*, 263(5), p. 464-478.

Obayashi, Y., Ozaki, Y., Goto, S., Obayashi, S., Suzumori, N., Ohyama, F., Tone, S. & Sugiura-Ogasawara, M., 2016. Role of Indoleamine 2,3-Dioxygenase and Tryptophan 2,3-Dioxygenase in Patients with Recurrent Miscarriage. *Am J Reprod Immunol*, 75(1), p. 69-77.

Oikonomou, D., Georgiopoulos, G., Katsi, V., Kourek, C., Tsioufis, C., Alexopoulou, A., Koutli, E. & Tousoulis, D., 2018. Non-alcoholic fatty liver disease and hypertension: coprevalent or correlated? *Eur J Gastroenterol Hepatol*, 30(9), p. 979–985.

Ozaki, Y., Edelstein, M. P. & Duch, D. S., 1988. Induction of indoleamine 2,3-dioxygenase: a mechanism of the antitumor activity of interferon gamma. *Proc Natl Acad Sci U S A*, 85(4), p. 1242-1246.

Oxenkrug, G., van der Hart, M. & Summergrad, P., 2015. Elevated anthranilic acid plasma concentrations in type 1 but not type 2 diabetes mellitus. *Integr Mol Med*, 2(5), p. 365-368.

Pais, R., Barritt, A. S. 4th., Calmus, Y., Scatton, O., Runge, T., Lebray, P., Poynard, T., Ratziu, V. & Conti, F., 2016. NAFLD and liver transplantation: Current burden and expected challenges. *J Hepatol*, 65(6), p. 1245-1257.

Park, J., Nah, Y. & Kim, W. J., 2023. IDO-triggered swellable polymeric micelles for IDO inhibition and targeted cancer immunotherapy. *J Control Release*, 363, p. 496-506.

Pedersen, E. R., Svingen, G. F., Schartum-Hansen, H., Ueland, P. M., Ebbing, M., Nordrehaug, J. E., Igland, J., Seifert, R., Nilsen, R. M. & Nygård, O., 2013. Urinary excretion of kynurenine and tryptophan, cardiovascular events, and mortality after elective coronary angiography. *Eur Heart J*, 34(34), p. 2689-2696.

Pedersen, E. R., Tuseth, N., Eussen, S. J., Ueland, P. M., Strand, E., Svingen, G. F., Midttun, Ø., Meyer, K., Mellgren, G., Ulvik, A., Nordrehaug, J. E., Nilsen, D. W. & Nygård, O., 2015. Associations of plasma kynurenines with risk of acute myocardial infarction in patients with stable angina pectoris. *Arterioscler Thromb Vasc Biol*, 35(2), p. 455-462.

Pertovaara, M., Raitala, A., Lehtimäki, T., Karhunen, P. J., Oja, S. S., Jylhä, M., Hervonen, A. & Hurme, M., 2006. Indoleamine 2,3-dioxygenase activity in nonagenarians is markedly increased and predicts mortality. *Mech Ageing Dev*, 127(5), p. 497-499.

Pertovaara, M., Raitala, A., Juonala, M., Lehtimäki, T., Huhtala, H., Oja, S. S., Jokinen, E., Viikari, J. S., Raitakari, O. T. & Hurme, M., 2007. Indoleamine 2,3-dioxygenase enzyme activity correlates with risk factors for atherosclerosis: the Cardiovascular Risk in Young Finns Study. *Clin Exp Immunol*, 148(1), p. 106-111.

Pfefferkorn, E. R., 1984. Interferon gamma blocks the growth of Toxoplasma gondii in human fibroblasts by inducing the host cells to degrade tryptophan. *Proc Natl Acad Sci U S A*, 81(3), p. 908-912.

Piano, M. R., 2017. Alcohol's Effects on the Cardiovascular System. *Alcohol Res*, 38(2), p. 219-241.

Pignoli, P., Tremoli, E., Poli, A., Oreste, P. & Paoletti, R., 1986. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation*, 74(6), p. 1399–1406.

Pirkola, J., Pouta, A., Bloigu, A., Hartikainen, A. L., Laitinen, J., Järvelin, M. R. & Vääräsmäki, M., 2010. Risks of overweight and abdominal obesity at age 16 years associated with prenatal exposures to maternal prepregnancy overweight and gestational diabetes mellitus. *Diabetes Care*, 33(5), p. 1115-1121.

Plows, J. F., Stanley, J. L., Baker, P. N., Reynolds, C. M. & Vickers, M. H., 2018. The Pathophysiology of Gestational Diabetes Mellitus. *Int J Mol Sci*, 19(11), p. 3342.

Polyzos, K. A., Ovchinnikova, O., Berg, M., Baumgartner, R., Agardh, H., Pirault, J., Gisterå, A., Assinger, A., Laguna-Fernandez, A., Bäck, M., Hansson, G. K. & Ketelhuth, D. F., 2015. Inhibition of indoleamine 2,3-dioxygenase promotes vascular inflammation and increases atherosclerosis in Apoe-/- mice. *Cardiovasc Res*, 106(2), p. 295-302.

Pons-Estel, G. J., Andreoli, L., Scanzi, F., Cervera, R. & Tincani, A., 2017. The antiphospholipid syndrome patients with systemic lupus erythematosus. *J Autoimmun*, 76, p. 10-20.

Porkka, K. V., Raitakari, O. T., Leino, A., Laitinen, S., Räsänen, L., Rönnemaa, T., Marniemi, J., Lehtimäki, T., Taimela, S., Dahl, M., Uhari, M., Akerblom, H. K. & Viikari, J. S., 1997. Trends in

serum lipid levels during 1980–1992 in children and young adults. The Cardiovascular Risk in Young Finns Study. *Am J Epidemiol*, 146(1), p. 64-77.

Priyadarshini, M., Navarro, G., Reiman, D. J., Sharma, A., Xu, K., Lednovich, K., Manzella, C. R., Khan, M. W., Garcia, M. S., Allard, S., Wicksteed, B., Chlipala, G. E., Szynal, B., Bernabe, B. P., Maki, P. M., Gill, R. K., Perdew, G. H., Gilbert, J., Dai, Y. & Layden, B. T., 2022. Gestational Insulin Resistance Is Mediated by the Gut Microbiome-Indoleamine 2,3-Dioxygenase Axis. *Gastroenterology*, 162(6), p. 1675-1689.e11.

Raitakari, O. T., Juonala, M., Kähönen, M., Taittonen, L., Laitinen, T., Mäki-Torkko, N., Järvisalo, M. J., Uhari, M., Jokinen, E., Rönnemaa, T., Akerblom, H. K. & Viikari, J. S., 2003. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: The Cardiovascular Risk in Young Finns Study. *JAMA*, 290(17), p. 2277–2283.

Raitakari, O. T., Juonala, M. & Viikari, J. S. A., 2005. Obesity in childhood and vascular changes in adulthood: insights into the Cardiovascular Risk in Young Finns Study. *Int J Obes (Lond)*, 29(Suppl 2), p. S101-S104.

Raitakari, O. T., Juonala, M., Rönnemaa, T., Keltikangas-Järvinen, L., Räsänen, L., Pietikäinen, M., Hutri-Kähönen, N., Taittonen, L., Jokinen, E., Marniemi, J., Jula, A., Telama, R., Kähönen, M., Lehtimäki, T., Åkerblom, H. K. & Viikari, J. S., 2008. Cohort profile: the cardiovascular risk in Young Finns Study. *Int J Epidemiol*, 37(6), p. 1220-1226.

Rebnord, E. W., Strand, E., Midttun, Ø., Svingen, G. F. T., Christensen, M. H. E., Ueland, P. M., Mellgren, G., Njølstad, P. R., Tell, G. S., Nygård, O. K. & Pedersen, E. R., 2017. The kunurenine:tryptophan ratio as predictor of incidence type 2 diabetes mellitus in individuals with coronary artery disease. *Diabetologia*, 60(9), p. 1712-1721.

Romero-Corral, A., Montori, V. M., Somers, V. K., Korinek, J., Thomas, R. J., Allison, T. G., Mookadam, F. & Lopez-Jimenez, F., 2006. Association of bodyweight with total mortality and with cardiovascular events in coronary artery disease: a systematic review of cohort studies. *Lancet*, 368(9536), p. 666-678.

Romero-Gómez, M., Zelber-Sagi, S. & Trenell, M., 2017. Treatment of NAFLD with diet, physical activity and exercise. *J Hepatol*, 67(4), p. 829-846.

Ross, R., 1999. Atherosclerosis--an inflammatory disease. *N Engl J Med*, 340(2), p. 115-126.

Roy, P., Orecchioni, M. & Ley, K., 2022. How the immune system shapes atherosclerosis: roles of innate and adaptive immunity. *Nat Rev Immunol*, 22(4), p. 251-265.

Saadeh, S., Younossi, Z. M., Remer, E. M., Gramlich, T., Ong, J. P., Hurley, M., Mullen, K. D., Cooper, J. N. & Sheridan, M. J., 2002. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology*, 123(3), p. 745-750.

Sacre, K., Escoubet, B., Zennaro, M. C., Chauveheid, M. P., Gayat, E. & Papo, T., 2015. Overweight Is a Major Contributor to Atherosclerosis in Systemic Lupus Erythematosus Patients at Apparent Low Risk for Cardiovascular Disease: A Cross-Sectional Controlled Study. *Medicine (Baltimore)*, 94(48), p. e2177.

Samsu, N., 2021. Diabetic Nephropathy: Challenges in Pathogenesis, Diagnosis, and Treatment. *Biomed Res Int*, 2021, p. 1497449.

Saraste, A. & Knuuti, J., 2018. Evaluation of coronary artery disease after computed tomography angiography. *Eur Heart J Cardiovasc Imaging*, 19(4), p. 378-379.

Schwartz, M. W., Seeley, R. J., Zeltser, L. M., Drewnowski, A., Ravussin, E., Redman, L. M. & Leibel, R. L., 2017. Obesity Pathogenesis: An Endocrine Society Scientific Statement. *Endocr Rev*, 38(4), p. 267-296.

Seidell, J. C. & Halberstadt, J., 2015. The global burden of obesity and the challenges of prevention. *Ann Nutr Metab*, 66(Suppl 2), p. 7-12.

Shi, S. R., Key, M. E. & Kalra, K. L., 1991. Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J Histochem Cytochem*, 39(6), p. 741-748.

Siasos, G., Tsigkou, V., Kokkou, E., Oikonomou, E., Vavuranakis, M., Vlachopoulos, C., Verveniotis, A., Limperi, M., Genimata, V., Papavassiliou, A. G., Stefanadis, C. & Tousoulis, D., 2014. Smoking and atherosclerosis: mechanisms of disease and new therapeutic approaches. *Curr Med Chem*, 21(34), p. 3936-3948.

Simard, J. F., Arkema, E. V., Nguyen, C., Svenungsson, E., Wikström, A. K., Palmsten, K. & Salmon, J. E., 2017. Early-onset Preeclampsia in Lupus Pregnancy. *Paediatr Perinat Epidemiol*, 31(1), p. 29-36.

Skinner, A. C., Perrin, E. M., Moss, L. A. & Skelton, J. A., 2015. Cardiometabolic Risks and Severity of Obesity in Children and Young Adults. *N Engl J Med*, 373(14), p. 1307-1317.

Slager, C. J., Wentzel, J. J., Gijsen, F. J., Thury, A., van der Wal, A. C., Schaar, J. A. & Serruys, P. W., 2005. The role of shear stress in the destabilization of vulnerable plaques and related therapeutic implications. *Nat Clin Pract Cardiovasc Med*, 2(9), p. 456–464.

Smith, C. K., Seto, N. L., Vivekanandan-Giri, A., Yuan, W., Playford, M. P., Manna, Z., Hasni, S. A., Kuai, R., Mehta, N. N., Schwendeman, A., Pennathur, S. & Kaplan, M. J., 2017. Lupus high-density lipoprotein induces proinflammatory responses in macrophages by binding lectin-like oxidised low-density lipoprotein receptor 1 and failing to promote activating transcription factor 3 activity. *Ann Rheum Dis*, 76(3), p. 602-611.

Stary, H. C., 1990. The sequence of cell and matrix changes in atherosclerotic lesions of coronary arteries in the first forty years of life. *Eur Heart J*, 11(Suppl E), p. 3-19.

Stary, H. C., Chandler, A. B., Glagov, S., Guyton, J. R., Insull, W Jr., Rosenfeld, M. E., Schaffer, S. A., Schwartz, C. J., Wagner, W. D. & Wissler, R. W., 1994. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation*, 89(5), p. 2462-2478.

Stary, H. C., Chandler, A. B., Dinsmore, R. E., Fuster, V., Glagov, S., Insull, W Jr., Rosenfeld, M. E., Schwartz, C. J., Wagner, W. D. & Wissler, R. W., 1995. 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*, 92(5), p. 1355-1374.

Stephenson, J., Heslehurst, N., Hall, J., Schoenaker, D. A. J. M., Hutchinson, J., Cade, J. E., Poston, L., Barrett, G., Crozier, S. R., Barker, M., Kumaran, K., Yajnik, C. S., Baird, J. & Mishra, G. D., 2018. Before the beginning: nutrition and lifestyle in the preconception period and its importance for future health. *Lancet*, 391(10132), p. 1830-1841.

Styne, D. M., Arslanian, S. A., Connor, E. L., Farooqi, I. S., Murad, M. H., Silverstein, J. H. & Yanovski, J. A., 2017. Pediatric Obesity-Assessment, Treatment, and Prevention: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*, 102(3), p. 709-757.

Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., Paulovich, A., Pomeroy, S. L., Golub, T. R., Lander, E. S. & Mesirov, J. P., 2005. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A*, 102(43), p. 15545-15550.

Sucher, R., Fischler, K., Oberhuber, R., Kronberger, I., Margreiter, C., Ollinger, R., Schneeberger, S., Fuchs, D., Werner, E. R., Watschinger, K., Zelger, B., Tellides, G., Pilat, N., Pratschke, J., Margreiter, R., Wekerle, T. & Brandacher, G., 2012. IDO and regulatory T cell support are critical for cytotoxic T lymphocyte-associated Ag-4 Ig-mediated long-term solid organ allograft survival. *J Immunol*, 188(1), p. 37-46.

Suomela, E., Oikonen, M., Virtanen, J., Parkkola, R., Jokinen, E., Laitinen, T., Hutri-Kähönen, N., Kähönen, M., Lehtimäki, T., Taittonen, L., Tossavainen, P., Jula, A., Loo, B. M., Mikkilä, V., Younossi, Z., Viikari, J. S., Juonala, M. & Raitakari, O. T., 2015. Prevalence and determinants of fatty liver in normal-weight and overweight young adults. The Cardiovascular Risk in Young Finns Study. *Ann Med*, 47(1), p. 40-46.

Suzuki, T, Wang, W., Wilsdon, A., Butler, K. R., Adabag, S., Griswold, M. E., Nambi, W., Rosamond, W., Sotoodehnia, N. & Mosley, T. H., 2020. Carotid Intima-Media Thickness and the Risk of Sudden Cardiac Death: The ARIC Study and the CHS. *J Am Heart Assoc*, 9(19), p. e016981.

Tan, C. H. & Venkatesh, S. K., 2016. Magnetic Resonance Elastography and Other Magnetic Resonance Imaging Techniques in Chronic Liver Disease: Current Status and Future Directions. *Gut Liver*, 10(5), p. 672-686.

Tang, W. H., Kitai, T. & Hazen, S. L., 2017. Gut Microbiota in Cardiovascular Health and Disease. *Circ Res*, 120(7), p. 1183-1196.

Terness, P., Bauer, T. M., Röse, L., Dufter, C., Watzlik, A., Simon, H. & Opelz, G., 2002. Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites. *J Exp Med*, 196(4), p. 447-457.

Teunis, C., Nieuwdorp, M. & Hanssen, N., 2022. Interactions between Tryptophan Metabolism, the Gut Microbiome and the Immune System as Potential Drivers of Non-Alcoholic Fatty Liver Disease (NAFLD) and Metabolic Diseases. *Metabolites*, 12(6), p. 514.

Tocci, G., Sciarretta, S. & Volpe, M., 2008. Development of heart failure in recent hypertension trials. *J Hypertens*, 26(7), p. 1477-1486.

Torres, N., Guevara-Cruz, M., Velázquez-Villegas, L. A. & Tovar, A. R., 2015. Nutrition and Atherosclerosis. *Arch Med Res*, 46(5), p. 408-426.

Tuomi, T., Carlsson, A., Li, H., Isomaa, B., Miettinen, A., Nilsson, A., Nissén, M., Ehrnström, B. O., Forsén, B., Snickars, B., Lahti, K., Forsblom, C., Saloranta, C., Taskinen, M. R. & Groop, L. C., 1999. Clinical and genetic characteristics of type 2 diabetes with and without GAD antibodies. *Diabetes*, 48(1), p. 150-157.

Ünlü, O., Zuily, S. & Erkan, D., 2016. The clinical significance of antiphospholipid antibodies in systemic lupus erythematosus. *Eur J Rheumatol*, 3(2), p. 75-84.

van Sloten, T. T., Schram, M. T., van den Hurk, K., Dekker, J. M., Nijpels, G., Henry, R. M. & Stehouwer, C. D., 2014. Local stiffness of the carotid and femoral artery is associated with incident cardiovascular events and all-cause mortality: The Hoorn Study. *J Am Coll Cardiol*, 63(17), p. 1739-1747.

Vinik, A. I., Nevoret, M. L., Casellini, C. & Parson, H., 2013. Diabetic neuropathy. *Endocrinol Metab Clin North Am*, 42(4), p. 747-787.

Virmani, R., Kolodgie, F. D., Burke, A. P., Farb, A. & Schwartz, S. M., 2000. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. *Arterioscler Thromb Vasc Biol*, 20(5), p. 1262-1275.

Voloshyna, I., Littlefield, M. J. & Reiss, A. B., 2014. Atherosclerosis and interferon-γ: new insights and therapeutic targets. *Trends Cardiovasc Med*, 24(1), p. 45-51.

Vuong, J. T., Stein-Merlob, A. F., Nayeri, A., Sallam, T., Neilan, T. G. & Yang, E. H., 2022. Immune Checkpoint Therapies and Atherosclerosis: Mechanisms and Clinical Implications: JACC State-of-the-Art Review. *J Am Coll Cardiol*, 79(6), p. 577-593.

Wang, C., Tay, S. S., Tran, G. T., Hodgkinson, S. J., Allen, R. D., Hall, B. M., McCaughan, G. W., Sharland, A. F. & Bishop, G. A., 2010. Donor IL-4-treatment induces alternatively activated liver macrophages and IDO-expressing NK cells and promotes rat liver allograft acceptance. *Transpl Immunol*, 22(3-4), p. 172-178.

Wang, Y., Liu, H., McKenzie, G., Witting, P. K., Stasch, J. P., Hahn, M., Changsirivathanathamrong, D., Wu, B. J., Ball, H. J., Thomas, S. R., Kapoor, V., Celermajer, D. S., Mellor, A. L., Keaney, J. F. Jr., Hunt, N. H. & Stocker, R., 2010. Kynurenine is an endothelium-derived relaxing factor produced during inflammation. *Nat Med*, 16(3), p. 279-285.

Widner, B., Sepp, N., Kowald, E., Ortner, U., Wirleitner, B., Fritsch, P., Baier-Bitterlich, G. & Fuchs, D., 2000. Enhanced tryptophan degradation in systemic lupus erythematosus. *Immunobiology*, 201(5), p. 621-630.

Wilson, P. W., D'Agostino, R. B., Sullivan, L., Parise, H. & Kannel, W. B., 2002. Overweight and obesity as determinants of cardiovascular risk: the Framingham experience. *Arch Intern Med*, 162(16), p. 1867-1872.

Wirleitner, B., Rudzite, V., Neurauter, G., Murr, C., Kalnins, U., Erglis, A., Trusinskis, K. & Fuchs, D., 2003. Immune activation and degradation of tryptophan in coronary heart disease. *Eur J Clin Invest*, 33(7), p. 550-554.

Wolowczuk, I., Hennart, B., Leloire, A., Bessede, A., Soichot, M., Taront, S., Caiazzo, R., Raverdy, V., Pigeyre, M., ABOS Consortium, Guillemin, G. J., Allorge, D., Pattou, F., Froguel, P. & Poulain-Godefroy, O., 2012. Tryptophan metabolism activation by indoleamine 2,3-dioxygenase in adipose tissue of obese women: an attempt to maintain immune homeostasis and vascular tone. *Am J Physiol Regul Integr Comp Physiol*, 303(2), p. R135-R143.

Wongpraparut, N., Pengchata, P., Piyophirapong, S., Panchavinnin, P., Pongakasira, R., Arechep, N., Kasetsinsombat, K. & Maneechotesuwan, K., 2021. Indoleamine 2,3 dioxygenase (IDO) level as a marker for significant coronary artery disease. *BMC Cardiovasc Disord*, 21(1), p. 353.

Xiao, B. G., Liu, X. & Link, H., 2004. Antigen-specific T cell functions are suppressed over the estrogen-dendritic cell-indoleamine 2,3-dioxygenase axis. *Steroids*, 69(10), p. 653-659.

Yang, J., Yang, X., Zou, H. & Li, M., 2016. Oxidative Stress and Treg and Th17 Dysfunction in Systemic Lupus Erythematosus. *Oxid Med Cell Longev*, 2016, p. 2526174.

Yelnik, C. M., Urbanski, G., Drumez, E., Sobanski, V., Maillard, H., Lanteri, A., Morell-Dubois, S., Caron, C., Dubucquoi, S., Launay, D., Duhamel, A., Hachulla, E., Hatron, P. Y. & Lambert, M., 2017. Persistent triple antiphospholipid antibody positivity as a strong risk factor of first thrombosis, in a long-term follow-up study of patients without history of thrombosis or obstetrical morbidity. *Lupus*, 26(2), p. 163-169.

Yki-Järvinen, H., 2014. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. *Lancet Diabetes Endocrinol*, 2, p. 901-910.

Yoshinaga, S. K., Whoriskey, J. S., Khare, S. D., Sarmiento, U., Guo, J., Horan, T., Shih, G., Zhang, M., Coccia, M. A., Kohno, T., Tafuri-Bladt, A., Brankow, D., Campbell, P., Chang, D., Chiu, L., Dai, T., Duncan, G., Elliott, G. S., Hui, A., McCabe, S. M., Scully, S., Shahinian, A., Shaklee, C. L., Van, G., Mak, T. W. & Senaldi, G., 1999. T-cell co-stimulation through B7RP-1 and ICOS. *Nature*, 402(6763), p. 827-832.

Young, L. & Cho, L., 2019. Unique cardiovascular risk factors in women. *Heart*, 105(21), p. 1656-1660.

Younossi, Z. M., Koenig, A. B., Abdelatif, D., Fazel, Y., Henry, L. & Wymer, M., 2016. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*, 64(1), p. 73-84.

Yu, G., Dai, H., Chen, J., Duan, L., Gong, M., Liu, L., Xiong, P., Wang, C. Y., Fang, M. & Gong, F., 2008. Gene delivery of indoleamine 2,3-dioxygenase prolongs cardiac allograft survival by shaping the types of T-cell responses. *J Gene Med*, 10(7), p. 754-761.

Yu, Y., Arah, O. A., Liew, Z., Cnattingius, S., Olsen, J., Sørensen, H. T., Qin, G. & Li, J., 2019. Maternal diabetes during pregnancy and early onset of cardiovascular disease in offspring: population based cohort study with 40 years of follow-up. *BMJ*, ;367, p. l6398.

Zhang, H., Yang, H., Lai, C., Xu, X., Huang, K. & Fu, J., 2015. Quantitative relationship between liver fat content and metabolic syndrome in obese children and adolescents. *Clin Endocrinol (Oxf)*, 83(1), p. 43-49.

Zhang, Y., Jalili, R. B., Kilani, R. T., Elizei, S. S., Farrokhi, A., Khosravi-Maharlooei, M., Warnock, G. L., Ao, Z., Marzban, L. & Ghahary, A. J., 2016. IDO-Expressing Fibroblasts Protect Islet Beta Cells From Immunological Attack and Reverse Hyperglycemia in Non-Obese Diabetic Mice. *Cell Physiol*, 231(9), p. 1964-1973.

Zhou, Q., Shi, Y., Chen, C., Wu, F. & Chen, Z., 2021. A narrative review of the roles of indoleamine 2,3-dioxygenase and tryptophan-2,3-dioxygenase in liver diseases. *Ann Transl Med*, 9(2), p. 174.

Zong, S., Li, C., Luo, C., Zhao, X., Liu, C., Wang, K., Jia, W., Bai, M., Yin, M., Bao, S., Guo, J., Kang, J., Duan, T. & Zhou, Q., 2016. Dysregulated expression of IDO may cause unexplained recurrent spontaneous abortion through suppression of trophoblast cell proliferation and migration. *Sci Rep*, 6, p. 19916.

Åberg, F., Helenius-Hietala, J., Puukka, P., Färkkilä, M. & Jula, A., 2018. Interaction between alcohol consumption and metabolic syndrome in predicting severe liver disease in the general population. *Hepatology*, 67(6), p. 2141-2149.

Åkerblom, H. K., Viikari, J., Uhari, M., Räsänen, L., Byckling, T., Louhivuori, K., Pesonen, E., Suoninen, P., Pietikäinen, M. & Lähde, P. L., 1985. Atherosclerosis precursors in Finnish children and adolescents. I. General description of the cross-sectional study of 1980, and an account of the children's and families' state of health. *Acta Paediatr Scand Suppl*, 318, p. 49-63.

Original Publications

Niinisalo, P, Raitala, A, Pertovaara, M, Oja, SS, Lehtimäki, T, Kähönen, M, Reunanen, A, Jula, A, Moilanen, L, Kesäniemi, YA, Nieminen MS & Hurme, M (2008)
Indoleamine 2,3-dioxygenase activity associates with cardiovascular risk factors: The Health 2000 study.
The Scandinavian Journal of Clinical & Laboratory Investigation

I

Niinisalo, P, Oksala, N, Levula, M, Pelto-Huikko, M, Järvinen, O, Salenius JP, Kytömäki, L, Soini, JT, Kähönen, M, Laaksonen, R, Hurme, M & Lehtimäki, T (2010)
Activation of indoleamine 2,3-dioxygenase-induced tryptophan degradation in advanced atherosclerotic plaques: Tampere vascular study.
Annals of Medicine

II

Niinisalo, P, Raitakari, OT, Kähönen, M, Hurme, M, Lehtimäki, T, Magnussen, C, Viikari, J, Juonala, M & Kaaja, R (2021)
IDO activity forecasts obesity in males and premenopausal females in a 10-year follow-up study:The Cardiovascular Risk in Young Finns Study.
Atherosclerosis

III