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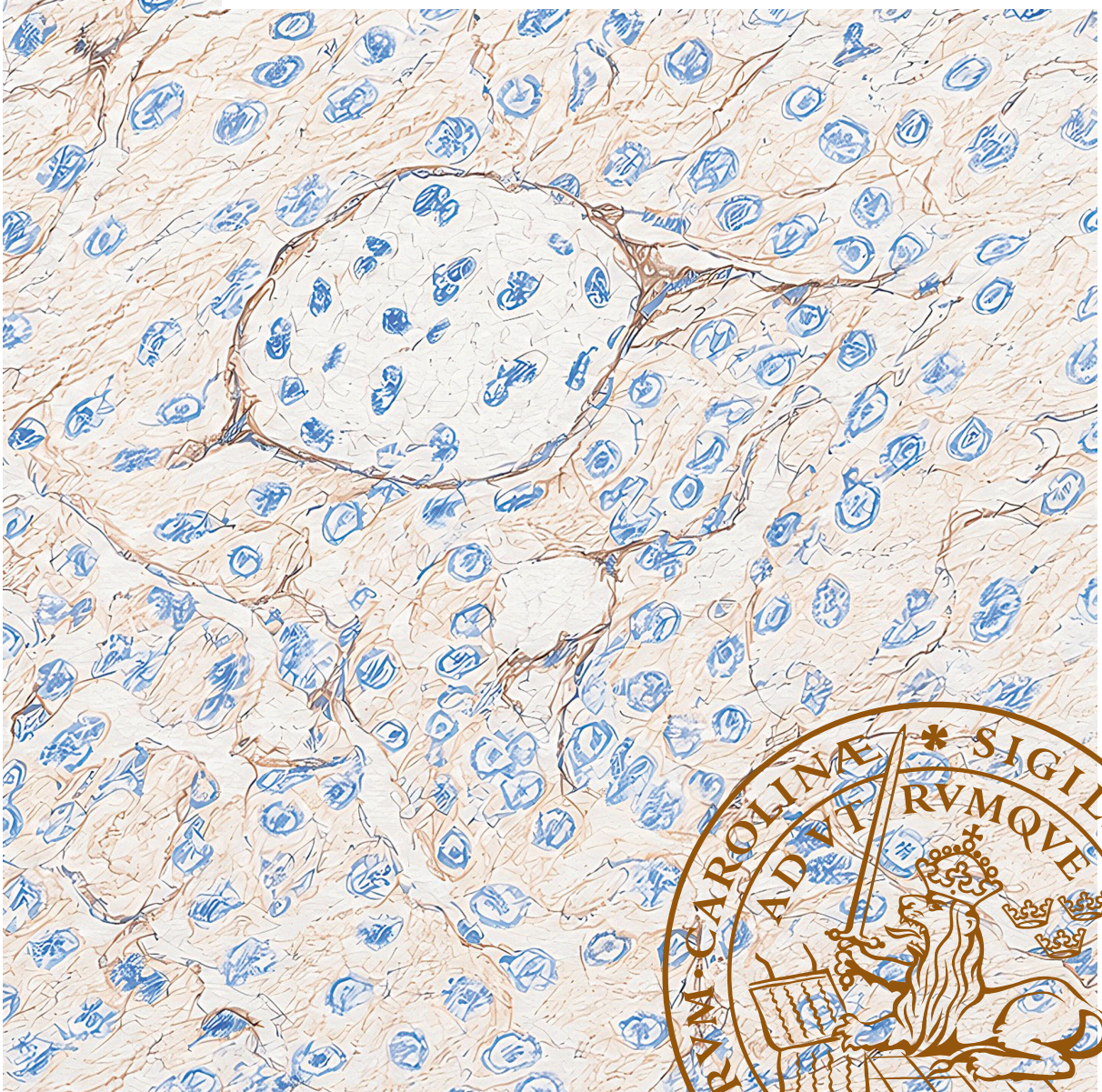

The Adrenomedullin system in acute dyspnea and cardiometabolic disease

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Kevin Bronton

LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Abstract:

Background
Adrenomedullin (ADM) is a vasoactive neurohormonal peptide involved in vascular integrity and fluid homeostasis. Even if elevated ADM is an established marker of acute vascular stress, it remains unclear how the ADM system should be measured and interpreted in the general population. In particular, it is uncertain which components of the pathway are most informative, when they should be measured, and how differences in ADM concentrations should be understood. Addressing these questions may help clarify why the same biological system is associated with both acute vascular dysfunction and longer term metabolic risk. This thesis therefore examined the ADM system across acute clinical and population-based settings using molecular epidemiological methods.

Methods
Four studies were conducted using observational clinical and population-based cohorts. Paper I evaluated ADM in patients presenting to the emergency department with acute dyspnea, with 90-day mortality and hospitalization as outcomes. Papers II and IV examined ADM and related precursor peptides glycine-extended ADM, stable prohormone fragment midregional-proADM and peptidylglycine α-amidating monooxygenase (PAM) activity, in relation to diabetes, insulin resistance and adiposity. Paper III used loss of function genetic variants to investigate associations between genetically impaired PAM function and metabolic and body-composition traits.

Results
In the clinical setting of acute dyspnea, higher circulating ADM concentrations were associated with greater risks of 90-day mortality and hospitalization. In the population-based setting ADM was associated with metabolic dysfunction and prospectively with incident diabetes mellitus. Genetically impaired PAM function was associated with worse glucose tolerance and lower muscle mass. Longitudinal analyses suggested that the ADM-system primarily reflected current adiposity and metabolic dysfunction, rather than future changes in weight.

Conclusion
Collectively, the findings support ADM as a context-dependent marker of vascular and metabolic stress, whose triggers and consequences vary across clinical settings and tissues. The predominant observational design does not establish causality,

Key words: adrenomedullin, peptidyl-glycine α-amidating monooxygenase, molecular epidemiology, acute dyspnea, metabolic dysfunction

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To my mother, Anna

Preface

Science is a very human form of knowledge. We are always at the brink of the known. We always feel forward for what is to be hoped. Every judgment in science is personal and stands on the edge of error. Science is a tribute to what we can know while also being capable of error.

Jacob Bornowski (1908-1974)

Polish-British mathematician and philosopher. Best known for developing a humanistic approach to science.

As a newly graduated doctor, what fascinated me most about medicine was the decision itself: the diagnostic puzzle, and the quiet satisfaction of watching it come together. Practice introduced the other half of that. Every decision belonged to a real person, with real symptoms and real worries, and none of it was ever certain. So I looked for certainty where I assumed it lived, in imaging and laboratory tests, hoping for something that would hold up both in the decision and in the conversation that followed it. That search travelled with me into Internal Medicine. Somewhere along the way the illusion gave out. Clinical judgement stayed where it had always been, at the centre, and the tests turned out to be nudges: targeted, probabilistic, and rarely more than that. With experience came trust in my own clinical gestalt, and with it the harder skill of accepting a calculated risk, managing it, and saying it plainly to colleagues and to patients.

That shift changed how I see my own research. Biomarkers no longer look like verdicts waiting to take over from judgement. They work alongside it. Not the piece that completes the puzzle, but a marker of where its edges lie: what can be done, what should be done, and, often enough, what should not.

What follows asks a narrow version of that question. It asks what molecules circulating in the blood can say about illness, acute and chronic, and how much of that is worth acting on. The answer emerging through these papers is that they narrow uncertainty without removing it. Learning to practice comfortably within that remaining uncertainty has become a source of great satisfaction over the years.

Disclosures

Statement on use of generative Artificial Intelligence (AI)

The use of generative AI was limited to (1) language editing, (2) text restructuring, (3) summarization, (4) formatting, and (5) assistance with visual presentation (e.g., figure design and caption refinement). Generative AI was not used to formulate the study aims, research questions, hypotheses, methodology, interpretation of results, or scientific conclusions. The large language model used was ChatGPT (OpenAI 2026). All AI-generated content was critically reviewed, revised, and approved by the author, who remains fully responsible for the accuracy, originality, interpretation, and final presentation of the work.

Parts of this work prepared with assistance from generative artificial intelligence tools will be flagged with **[AI]** with a connotation (1-5) specifying how the technology was applied.

For example:

Figure X. *Figure caption here...* **[AI^{5}]** for (5) assistance with visual presentation.

References were in part synthesized from digital pictures and formatted (to .ris format) for import to reference management software using ChatGPT (OpenAI. 2026. *ChatGPT*) [Large language model]. Also, generative AI was used to generate R-code to reformat figures adapted from the papers according to the LU color palette and ggplot figure template (Anthropic. (2024). Claude (Opus 4.6) [Large language model]).

Conflicts of Interest

SphingoTec GmbH is the manufacturer of and holds patent rights to the ADM assay (sphingotest®bio-ADM®). PAM Theragnostics holds patent rights and is the manufacturer of the PAM and ADM-Gly assays. J Schulte and O Hartmann, co-authors on Paper I, are employees of SphingoTec GmbH. A Bergmann, co-author on Paper III, is a shareholder in and managing director of both SphingoTec GmbH and PAM Theragnostics. P Kaufmann and Y Ilina, co-authors of Papers II, III and IV, are employees of PAM Theragnostics. The author's main supervisor Prof. Olle Melander is listed as an inventor together with A Bergmann on patents and patent applications owned by SphingoTec GmbH, among them a patent related to the use of Adrenomedullin in the prediction and diagnosis of dementia. The author has no conflicts of interest to declare.

Papers included in the thesis

i. Bronton K, Wessman T, Gränsbo K, Schulte J, Hartmann O, Melander O. Bioactive adrenomedullin a prognostic biomarker in patients with mild to moderate dyspnea at the emergency department: an observational study. Internal and Emergency Medicine. 2022;17(2):541-550.[1]

ii. Bronton K, Giontella A, Kaufmann P, Ilina Y, Melander O. Circulating Adrenomedullin is associated with and predicts metabolic disorder - a Swedish observational cohort study. Submitted Manuscript: Lund University; 2026.[2]

iii. Giontella A, Åkerlund M, Bronton K, et al. Deficiency of Peptidylglycine-α-amidating Monooxygenase, a Cause of Sarcopenic Diabetes Mellitus. J Clin Endocrinol Metab. 2025;110(3):820-829.[3]

iv. Bronton K, Kaufmann P, Ilina Y, Melander O. The adrenomedullin maturation system in metabolic dysfunction - a prospective longitudinal cohort study. Unpublished Manuscript: Lund University; 2026.[4]

Papers not included in the thesis

i. Rosenqvist M, Bronton K, Hartmann O, Bergmann A, Struck J, Melander O. Proenkephalin a 119-159 (penKid) - a novel biomarker for acute kidney injury in sepsis: an observational study. BMC Emerg Med. 2019;19(1):75.[5]

ii. Lundberg OHM, Rosenqvist M, Bronton K, Schulte J, Friberg H, Melander O. Bioactive adrenomedullin in sepsis patients in the emergency department is associated with mortality, organ failure and admission to intensive care. PLoS One. 2022;17(4):e0267497.[6]

iii. Egerstedt A, Czuba T, Bronton K, et al. Bioactive adrenomedullin for assessment of venous congestion in heart failure. ESC Heart Fail. 2022.[7]

Abbreviations

ADM Adrenomedullin

ADM-Gly Glycine-extended Adrenomedullin

ADYS Acute Dyspnea at the Emergency Department Study

AHT Antihypertensive drug treatment

ROC Receiver Operating Characteristic

bio-ADM bioactive Adrenomedullin, interchangeable with ADM

BMI Body mass index

CHF Congestive heart failure

CI Confidence interval

CKD Chronic kidney disease

COPD Chronic obstructive pulmonary disease

CRP C-reactive protein

CVD Cardiovascular disease

DM Diabetes mellitus

DMt2 Diabetes mellitus type 2

ED Emergency department

eGFR Estimated glomerular filtration rate

EHR Electronic health records

ELISA Enzyme-linked immunosorbent assay

fP-Glc Fasting plasma glucose

GFR Glomerular filtration rate

HDL High-density lipoprotein Cholesterol

HF Heart failure

HOMA-IR Homeostatic model assessment of insulin resistance

HR Hazard ratio

hs-cTn High-sensitivity cardiac troponin

HT Hypertension

ICD-10 International Classification of Diseases, 10th revision

ICU Intensive care unit

IFG Impaired fasting glucose

OGTT Oral glucose tolerance test

IQR Interquartile range

IR Insulin resistance

LDL Low-density lipoprotein Cholesterol

MDC Malmö Diet and Cancer Study

MDC-CC Malmö Diet and Cancer Study, the Cardiovascular Cohort

MetS Metabolic syndrome

METTS Medical Emergency Triage and Treatment System

MPP Malmö Preventive Project

MPP-RES Malmö Preventive Project - Re-Examination Study

MR-proADM Mid-regional pro-adrenomedullin

OR Odds ratio

PAM Peptidyl-glycine α-amidating monooxygenase

RETTS Rapid Emergency Triage and Treatment System

SBP Systolic blood pressure

SCB Statistiska Centralbyrån, the state agency for Statistics Sweden

SCr Serum creatinine

SD Standard deviation

SoS Socialstyrelsen, the Swedish National Board of Health and Welfare

SUS Skånes Universitetssjukhus, Skåne University Hospital

TG Triglycerides

WC Waist circumference

WHO World Health Organization

WHR Waist-to-hip ratio

Populärvetenskaplig Sammanfattning

Adrenomedullin i akut och kronisk sjukdom

Denna avhandling utforskar hur man genom att analysera små signalämnen i blodet, hormoner, kan utvinna information som kan hjälpa till i mötet med patienter och i de beslut som fattas i samband med deras vård.

Biomarkörer är mätbara biologiska signaler. De kan vara enkla kliniska tecken, som puls och kroppstemperatur, men också molekyler som cirkulerar i blodet och som speglar olika biologiska processer i kroppen. En sådan molekyl är Adrenomedullin (ADM), ett peptidhormon som identifierades på 1990-talet i samband med forskning på tumörvävnad från binjuremärgen från vilken den även fått sitt namn (Eng. adrenal medulla). Adrenomedullin har en viktig roll i kroppens förmåga att bilda och upprätthålla välfungerande blodkärl.

När kärlsystemet får utstå belastning och inte kan upprätthålla sin funktion kan vätska läcka ut från blodbanan och in i omkringliggande vävnad, vilket i sin tur rubbar vävnadens funktion. Samtidigt kan de många signalämnen som vanligtivs transporteras genom cirkulationssystemet få svårare att nå sina mål. I detta avseende kan ADM spegla belastning av kärlsystemet.

Resultaten i denna avhandling bygger delvis på ett urval av patienter som söker akutmottagningen med andningsbesvär och delvis på observationsstudier i den allmänna befolkningen. Det betyder att de mönster och förhållanden som träder fram främst bör tolkas som just samband på gruppnivå, snarare än som säkra slutsatser som rör enskilda individer.

Avhandlingens fyra delarbeten ställer frågan hur ADM-systemet förhåller sig till olika kliniska situationer.

Adrenomedullin vid andningssvårigheter

Det första delarbetet undersöker ADM hos patienter med akuta påkomna andningsbesvär, på medicinskt språk kallat dyspné. Vi ställer frågan huruvida ADM-hormonet, mätt under patientens första tid på akutmottagningen, kan bidra till att bättre förstå hur det kommer att gå för patienten.

Mer konkret undersöks om nivåerna av ADM-hormon i blodet kan skilja patienter med högre risk för död eller behov av sjukhusvård från de med lägre risk. I detta sammanhang fungerar ADM som en signal för akut belastning på kärlsystemet och kroppen som helhet.

Adrenomedullin vid övervikt och diabetes

De återstående tre delarbetena behandlar ett annat sammanhang; en av våra vanligaste folksjukdomar, diabetes typ 2 som ofta går hand i hand med övervikt. Här flyttas perspektivet från den akut sjuka patienten mot till synes friska och välmående personer i den allmänna befolkningen.

Delarbete 2 ställer frågan ifall ADM mätt i blodet hos individer ur ett urval i befolkningen kan spegla något meningsfullt om deras hälsa kopplat till ämnesomsättningen (metabolism). Framför allt undersöks om hormonet är kopplat till övervikt, diabetes och framtida risk för att insjukna i det.

Det fjärde delarbetet skiftar fokus från en ögonblicksbild till förändring med uppföljande mätpunkter över tid. Frågan vi söker svar på gäller huruvida nivåer av ADM vid början av studien är kopplade till förändring i kroppsvikt och kroppssammansättning under många års uppföljning.

ADM-systemet är ett pussel med flera bitar

Det tredje delarbetet skiljer sig från de övriga. Där läggs fokus inte direkt på ADM, utan på ett enzym som kallas PAM. Enzymet PAM är helt avgörande för att en uppsjö peptidhormoner, inklusive ADM, ska aktiveras och börja verka i blodbanan och vävnaderna.

Genom att analysera individers arvsmassa är det möjligt att identifiera genetiska varianter som är kopplade till lägre funktion i PAM. På så viss kan dessa varianter användas som ett slags naturligt experiment. Om livslång lägre PAM-aktivitet på grund av genetisk variation går att koppla till ökad sjukdomsrisk: talar det för att enzymet faktiskt spelar en avgörande roll i sjukdomsutvecklingen, snarare än att det enbart speglar sjukdomen.

I studien visar vi att individer med genetiska varianter kopplade till lägre PAM-funktion löper förhöjd risk att utveckla diabetes. Eftersom PAM också är kopplat till hormonsystem som är viktiga för muskeluppbyggnad, undersökte vi även hormonella förhållanden bortom diabetes. Vi fann att samma individer tenderar att ha svårare för att upprätthålla muskelmassa, och har i större utsträckning en betydande muskelförlust, sk. sarkopeni, vilket inte sällan ses som en komplikation vid diabetes.

Sammanvägd tolkning

En av de mest framträdande slutsatserna från avhandlingens fyra delarbeten är att adrenomedullin (ADM) fungerar som en i högsta grad sammanhangsberoende biologisk signal. Hormonets innebörd som kliniskt verktyg och biologiska verkan

bestäms framför allt av om det mäts under akut fysiologisk stress eller vid långvarig, kronisk påfrestning.

Vid akut påfrestning, som hos individer som söker akutmottagningen för andningsbesvär fungerar ökade ADM-nivåer som en varningssignal för belastning på kärlsystemet. Här ger mätning av hormonet oberoende information om sjukdomsförloppet, genom att spegla ökad sannolikhet av att behöva läggas in på sjukhus. Samtidigt verkar ADM inte på ett betydande sätt förbättra befintliga riskmarkörers sammantagna förmåga att förutse sjukdomsförlopp vid akut dyspné.

Vid kroniska tillstånd som övervikt och diabetes är bilden en annan. Där ses en mindre uttalad men sannolikt bestående ökning av ADM-nivåer över tid, vilket speglar en successiv försämring i kroppens ämnesomsättning. Resultaten talar för att ADM-systemet, på gruppnivå, är kopplat till både övervikt och diabetes. Samt att ADM i denna kontext främst speglar nuet, snarare än framtida förändringar i kroppsvikt och kroppssammansättning. Dessutom visar vi att enzymet Peptidylglycin alfa-amiderande monooxygenas (PAM), som ansvarar för aktivering av ADM och en uppsjö av andra peptider, har en direkt roll i ämnesomsättningen: då individer med genetisk predisposition för lägre aktivitet i PAM-enzymet uppvisar ökad risk för diabetes samt lägre muskelmassa. Fynden i delarbeten 2 och 4 stärker även tolkningen att PAM-enzymet inte bör ses som ett kugghjul begränsat till ADM-systemet. De talar för att PAM aktiviteten till stor del är oberoende från och med effekter som når bortom ADM-systemet.

Sammanfattningsvis visar avhandlingen att ADM inte kan tolkas som en enkel, universell biomarkör. Samma biologiska signal får helt olika betydelse beroende på om den speglar en akut kris i kärlsystemet eller en långsam, kronisk metabol förändring. För att ge klinisk nytta måste mätningen därför alltid tolkas mot rätt klinisk bakgrund.

Introduction

In this thesis, I direct my focus toward Adrenomedullin, a peptide hormone discovered three decades ago, and synthesize findings on how it is associated with acute dyspnea, a common presentation at the emergency department, and metabolic disease like obesity and diabetes, which belong to some of the most widespread diseases worldwide. All these conditions are commonplace in my daily practice at the Department of Internal Medicine. Despite significant progress being made regarding treatment and management of these conditions over the last decades, their wide-ranging complications are associated with major morbidity and hospitalization and pose difficult and complex clinical problems.

One can wonder how acute shortness of breath which can arise in minutes to hours and metabolic changes like obesity and diabetes that take years to cause irreparable damage in the body are intertwined. A common denominator is the vascular system, which is responsible for two, among many, related functions: containing and directing fluid to the appropriate spaces, and facilitating communication throughout the body by delivering signaling molecules to where they act. In acute conditions, like sudden shortness of breath, the underlying cause may manifest as containment failure, with fluid accumulation in the wrong spaces leading to organ dysfunction manifesting as dyspnea. Metabolic disease developing over longer timeframes tends to manifest as communication failure, with important signaling compounds like insulin failing to reach and signal to their target tissues. Adrenomedullin is implicated in both and offers a measurable readout of vascular function along both axes.

This thesis examines whether levels of circulating adrenomedullin reflect clinically important vascular dysfunction in acute breathlessness and metabolic disease, and evaluates whether it can predict outcomes and explain underlying disease processes at population level.

Table 1. Thesis Summary (TL;DR)

Paper	Research question	Materials and methods	Results and conclusion
I	Is circulating plasma adrenomedullin (ADM) associated with mortality in patients with acute dyspnea? Are they hospitalized more?	Baseline plasma ADM concentration was measured in 1402 patients with shortness of breath and associated with all-cause mortality and hospitalization using logistic regression models adjusted for established risk factors in heart failure (sex, age, NTproBNP creatinine and CRP). Added value to those risk factors was evaluated using ROC-curves and by likelihood ratio test on the deviances of the nested models.	We found that increasing ADM was associated with adverse outcomes (90-day all-cause mortality and hospitalization). Adding ADM to the reference model improved model performance, however it is unclear whether this improvement is clinically relevant.
II	How is adrenomedullin (ADM) associated with diabetes and obesity at the population level? Is there any association with ADM-precursor peptides and maturation enzyme, peptidyl-glycine α-amidating monooxygenase (PAM)?	ADM and the related peptides were analysed retrospectively in 5060 and 6094 individuals from two population-based cohorts based in Malmö, Sweden. Logistic regression models were fitted to study cross-sectional association between ADM and prevalent metabolic disease. We also prospectively evaluated diabetes risk using Cox Proportional Hazards model. The follow-up periods were 12 and 25 years respectively. Secondary analyses examined hormone maturation pathway components and ratios.	We found that mature ADM concentration cross-sectionally related to prevalent diabetes mellitus and obesity at the population level and that mature ADM concentration at baseline prospectively related to future risk of diabetes mellitus.
III	Does genetic variants that impair the function of the adrenomedullin maturation enzyme peptidyl-glycine α-amidating monooxygenase (PAM) lead to increased risk of metabolic disease, like diabetes?	Genetic and phenotypic data was analyzed from three population-based cohorts from Malmö and the UK. Two independent enzyme loss-of-function mutations were identified and a weighted allele score was created and associated with metabolic traits and clinical phenotypes in regression models.	Genetically impaired PAM function leads to worse glucose tolerance and less muscle mass. Identifying individuals carrying such mutations could improve preventive interventions for glucose tolerance and loss of muscle mass. Therapies targeted at restoring PAM function could be beneficial in carriers of such mutations.
IV	Are adrenomedullin (ADM) levels associated with changes in body composition and insulin resistance at the population level? How do precursor peptides in the ADM-system (ADM-Gly and MR-proADM, PAM activity) relate with these measures?	A prospective longitudinal observational study design where Adrenomedullin, related prohormone, precursor peptides and maturation enzyme activity were measured in 2674 individuals from a population-based cohort from Malmö, Sweden. Baseline peptide levels were associated with changes in BMI, waist-to-hip ratio, and insulin resistance using regression modelling. Complementary analyses characterized cascade markers including prohormone fragment and PAM activity.	ADM reflects current adiposity distribution rather than weight trajectory. MR-proADM emerges as a reliable longitudinal metabolic signal. PAM activity appears to represent a protective metabolic effect, independent and opposite from the ADM pathway.

Background

Epidemiology as a bridge from population to mechanism

Modern epidemiology has moved beyond solely describing disease occurrence. Large population-based cohorts with biological samples, genetic data, and registry linkage now allow us to study early biological variation before disease onset. Even if such population-based cohorts offer a high statistical resolution, they do not by themselves offer corresponding biological resolution. Hallmark epidemiological tools for capturing exposures are questionnaires, body measurements, or clinical history that all describe the phenotype of an individual, but only allow limited inference about biological pathways linking distal risk factors, like metabolic dysfunction, to subsequent disease outcomes on their own.[8]

Incorporating molecular measurements into population studies is formally described as molecular epidemiology[9, 10] and it intends to address this limitation, by characterizing participants at the level of circulating peptides and related biochemical analytes alongside the conventional epidemiological assessment. Within this framework, measurable biochemical signals, often circulating in our bloodstream, so called biomarkers, occupy a central position, both as the measurement substrate and as the conceptual object of interest.

In the work that follows, adrenomedullin is examined both as a potential indicator of future disease risk and as a potential indicator of prognosis among individuals with established disease. That is, as a blood marker measured in participants, first to describe how common different levels are in the population, and then to study whether those levels are linked to later health outcomes such as death, hospitalization, diabetes, or obesity.

Biomarkers

Biomarkers are measurements reflecting a biological system. It may be a chemical, physical or biological measurement and may reflect functional, physiological or cellular processes. In clinical practice, biomarkers range from observations (e.g. breathing rate, skin pallor) to analyses of blood and plasma enabled by biochemical assays. A defining characteristic of a clinical biomarker is that it should be both objective and reproducible.[11]

If a biomarker proves to reliably reflect a specific clinical condition, it may be used as an indirect replacement of that outcome, a so-called surrogate marker, and sometimes even comes to constitute the definition of that particular outcome. An example of a well-established clinical biomarker is Troponin, a marker of cardiac myocyte injury, and a cornerstone in the definition and diagnosis of acute coronary syndrome.[12-14]

Much has been invested into biomarker-related research with the intent of discovering new reliable and cost-effective biomarkers, like Troponin, into clinical practice for the benefit of patients[15, 16]. Other examples of biomarkers that have been successfully translated into clinical practice are Lipoprotein(a), a marker of dyslipidemia[17, 18]. However, these examples represent only a fraction of the total amount of candidate biomarkers that have, for any reason, failed to reach clinical practice[19]. The evidence-base of cardiovascular biomarkers has been put into question[20], where observational studies tend to estimate larger effects on prognosis of studied biomarkers than randomized controlled trials[21].

Given the papers in this thesis are based on observational cohort design, findings indicate associations and directional trends rather than direct causal relationships (**Figure 1**).

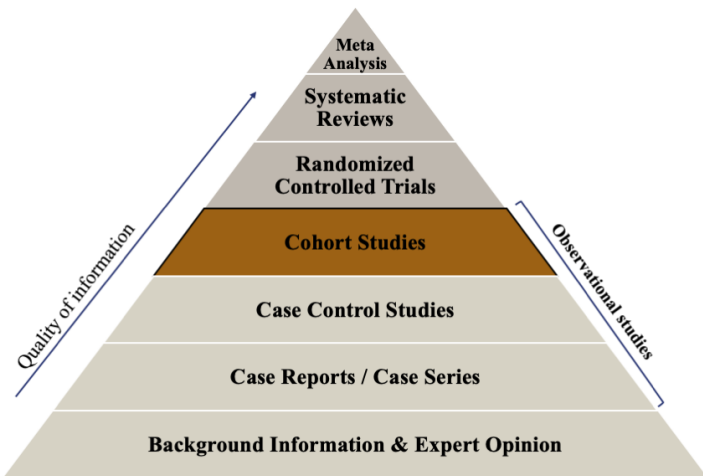

Figure 1. A schematic of the epistemic ladder which illustrates the hierarchy of evidence according to study design. Adapted from Oxford Centre for Evidence-Based Medicine[22].

Precision medicine

Precision medicine is a field dedicated to developing methods and approaches catered to the unique characteristics of individuals, as opposed to "the average patient" which much of currently adopted treatments and diagnostic tools are based on. It strives to develop personalized methods for preventive measures, diagnostics and treatments of disease for select groups of individuals. One of the overarching goals is to provide strategies to allow choosing the right approach, at the right time for each individual patient with regard to their unique circumstances and needs.[23]

Enrichment in precision medicine is the process of refining a varied group of patients into subgroups where treatment has potential to be effective[24]. This can be achieved by, on one hand, improving prognostication by identifying high risk patients, such as individuals at high risk of not responding to treatment. If the probability of unwanted events is very low, perhaps the important thing is to refrain from treating, sparing the patient any potential adverse effects in a "first, do no harm" approach. On the other hand, identifying individuals who are most likely to respond to treatment is called predictive enrichment.[25]

In this context, blood sampling is clinically readily available, and circulating biomarkers can aid the enrichment process by allowing clinicians to monitor disease states and response to treatment in near real time, fulfilling a complementary role to genetic information, comorbidities and environmental factors.[26]

Another approach in the field of precision medicine is the identification of genetic variants which are associated with either favorable or detrimental phenotypes in individuals carrying those genetic variants. The discovery of the PCSK9 gene and associated mutations which can both boost and inhibit genetic function, i.e. gain and loss of function mutations (**Figure 2**), provided the basis for a new treatment strategy targeting PCSK9 to treat hypercholesterolemia, a major risk factor for developing cardiovascular disease. PCSK9 inhibitors have been developed in record time and shown clinically significant LDL-C lowering and cardiovascular risk reduction.[27]

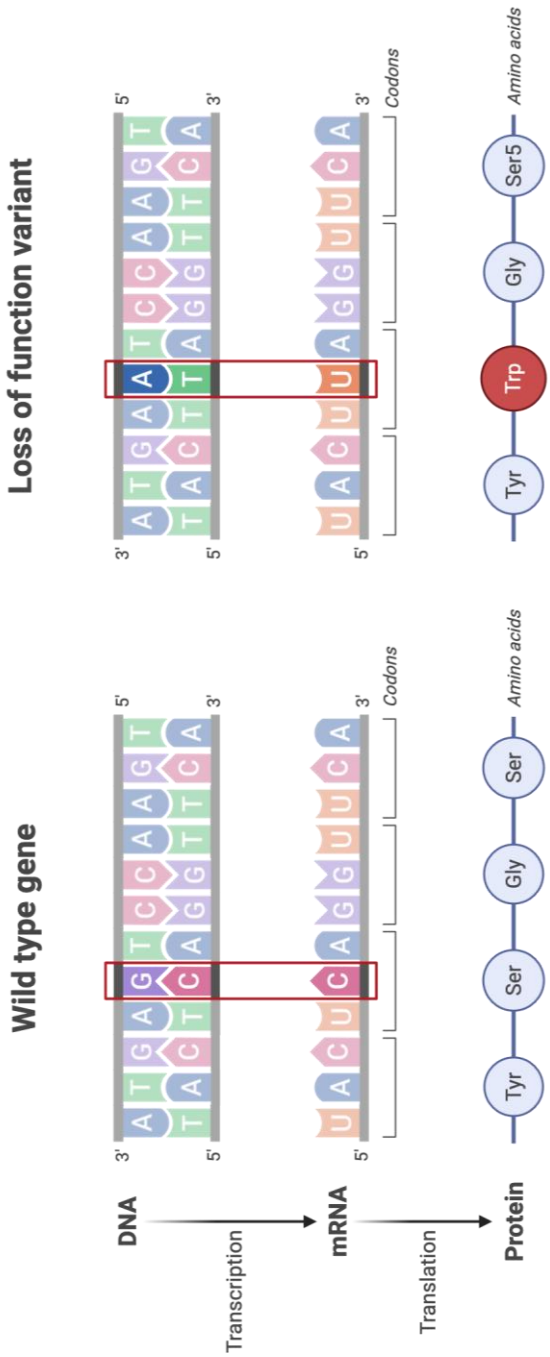

Figure 2. Schematic illustration of a functional wild-type gene and a loss of function variant with a different amino-acid in the end-product protein. Created in BioRender.

The vascular endothelial system

The vascular system plays a critical role in maintaining tissue fluid balance by preventing leakage of plasma. Conditions which disrupt the vascular barrier can lead to tissue swelling and following injury if uncontrolled. Several factors disrupt this barrier through mechanisms involving endothelial junctions. Understanding the signaling pathways associated with these disruptions and developing targeted therapies are key areas of focus in both heart failure and metabolic disease which are associated with frequent hospitalizations and increased morbidity.[28-32]

Agents that alter vascular permeability and tone might improve drug delivery to target organs and tissues, such as the kidneys, lungs or visceral fat depots. Focusing on targeting key signaling pathways offers avenues for managing diseases which involve disruption of the vascular barrier, like acute heart failure, sepsis and diabetes[33]. **Figure 3** illustrates the time frame of various vascular stressors.

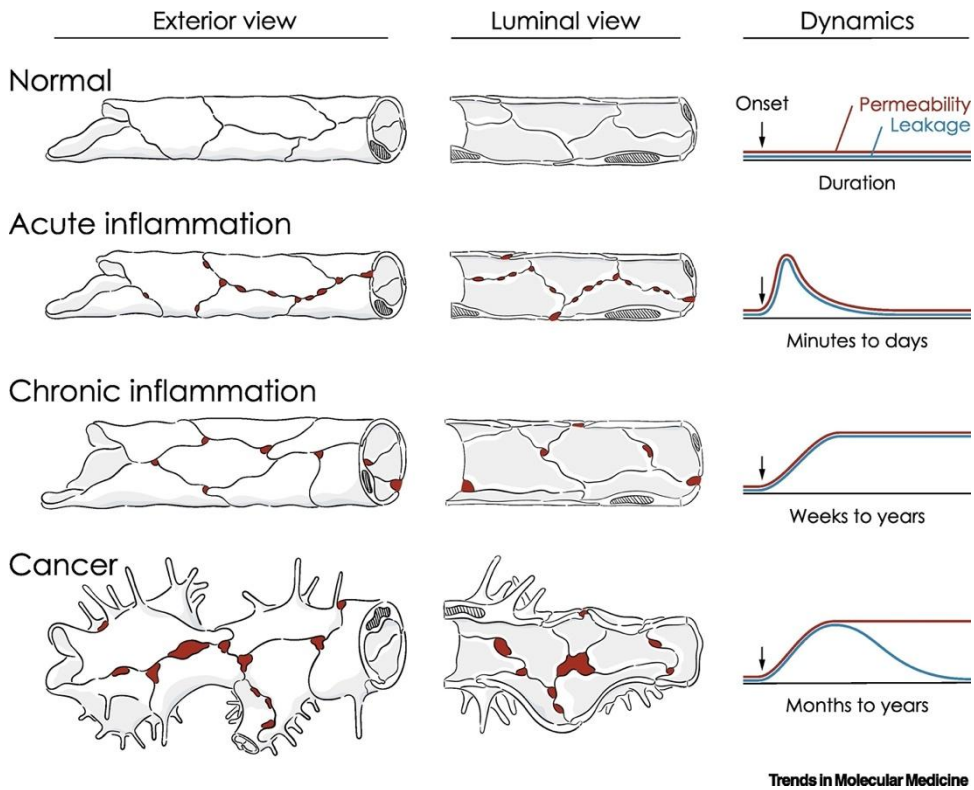

Figure 3. Vascular function and leakage in relation to duration. Reproduced with permission from Welsh-Claesson et al.[33] under CC BY-NC-ND 4.0.

The clinical spectrum of dyspnea

To manage an increasing number of patients with various constellations of symptoms in resource-constrained settings is a daily task for physicians working in acute care settings. Dyspnea is a frequent chief complaint, affecting approximately 10% of hospitalized patients and adequate evaluation and workup are key for the benefit of the patient. The complexity of dyspnea arises from multiple pathophysiological mechanisms, primarily chronic obstructive pulmonary disease (COPD) and congestive heart failure (CHF), often accompanied by co- and multimorbidity[34-36]. This heterogeneity complicates evaluation and treatment in the emergency department and is particularly prevalent in the elderly frail[37], who are also at highest risk for adverse drug events[38]. **Figure 4** illustrates the complexity and width of tools available in the diagnostic work-up in patients with dyspnea.

Prognosis is influenced by both chronic diseases and acute events, making it challenging to diagnose underlying causes early in emergency settings. Physicians must assess prognosis accurately to decide between discharge or admission, with biomarkers for unselected dyspneic patients being understudied despite high hospitalization rates.[39, 40]

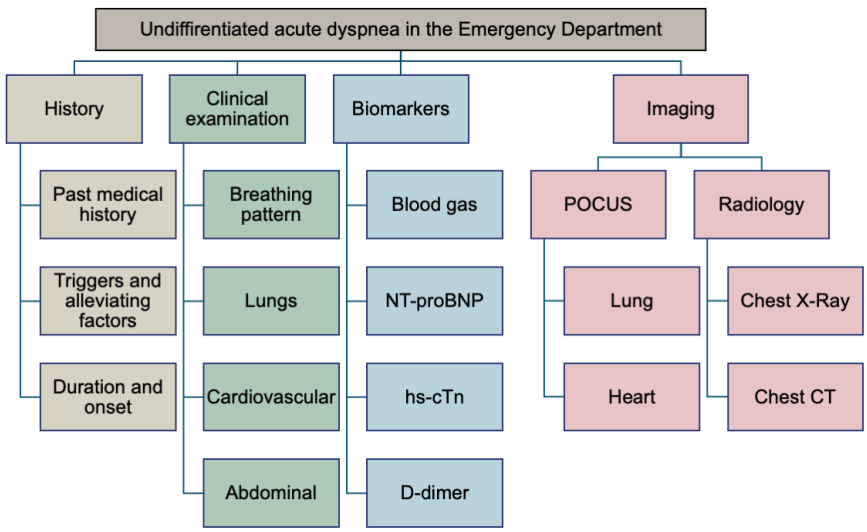

Figure 4. Approach to undifferentiated dyspnea in a non-critically ill patient in the ED. Adapted from De vos et al.[41]. Created in Microsoft PowerPoint. Abbreviations: NT-proBNP, N-terminal pro-B-type Natriuretic Peptide; hs-cTn, high-sensitivity cardiac Troponin; POCUS, point-of-care ultrasound; CT, Computed tomography.

Metabolic disease on the rise

Obesity, defined as a Body Mass Index (BMI) exceeding 30 kg/m^2, is a condition with increasing prevalence all parts of the world. At the center of this development are lifestyle factors and changing dietary habits. Calorie intake may have increased, but physical activity in everyday life of the average person has decreased substantially. This has resulted in more widespread obesity and metabolic syndrome (see Table 2 for definition) in the population. As a result, there has been a rise in glucose tolerance and diabetes mellitus (DM), in addition to being associated with significant morbidity, also predisposes the individual to cardiovascular disease (CVD). The increase of abdominal obesity is a natural consequence of this trend, as metabolic changes like insulin resistance and an increased chronic inflammatory response have detrimental effects on glucose metabolism[42].

Table 2. International Diabetes Federation criteria for Metabolic Syndrome in European individuals from 2006.[43]

Central Obesity	Waist circumference ≥94 cm in men or ≥80 cm in women
Elevated triglycerides	≥1.7 mmol/L, or lipid lowering treatment
Low HDL cholesterol	<1.0 mmol/L in men or <1.3 mmol/L in women
Elevated blood pressure (BP)	Systolic BP ≥130 mmHg or diastolic BP ≥85 mmHg or BP-lowering medication
Impaired fasting glucose or known DMt2	Fasting plasma glucose ≥5.6 mmol/L, or known DMt2

Accompanying the obesity epidemic, the prevalence of DM has risen from 4.7% in 1980 to 8.5% in 2021, which corresponds to 537 million adults living with DM worldwide[44, 45]. Current trends project that one in eight will be living with DM by 2045[45]. Among these, diabetes mellitus type 2 (DMt2) accounts for 90-95% of all cases globally. DMt2 is a progressive metabolic disease characterized by hyperglycemia because of insulin resistance. Successive worsening of pancreatic beta cell function and insulin secretion are common.[45-47]

In a Swedish context, it is also a widespread disease with a prevalence around 6% and increasing with age with one in every 5-10 (10-20%) individuals aged 65 or older are estimated to be living with DMt2.[48, 49] **Figure 5** illustrates the increasing prevalence of DMt2 in Sweden between 2006 and 2021.

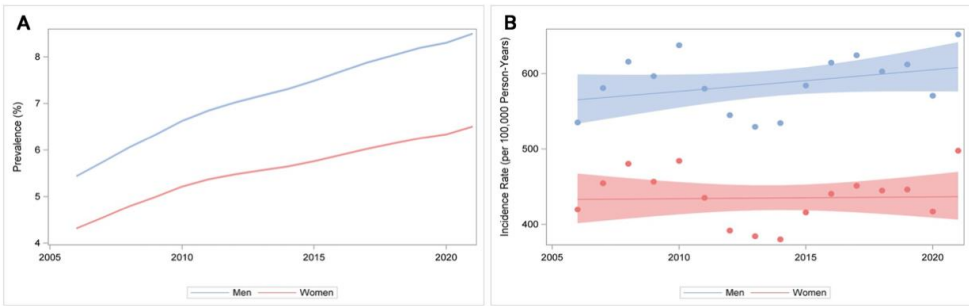

Figure 5. (A) Age-standardized prevalence (%) of type 2 diabetes in Sweden 2006–2021 by sex. The shaded areas represent 95% confidence intervals. (B) Age-standardized incidence (per 100 000 person-years) of type 2 diabetes in Sweden 2006–2021 by sex. The shaded areas represent 95% confidence intervals. Reproduced with permission from Carlsson et al[50] under CC-BY.

The adrenomedullin system

Adrenomedullin (ADM) is a vasoactive 52-amino-acid peptide hormone originally identified in 1993 by Kitamura and colleagues when studying pheochromocytoma tissue[51]. It belongs to the calcitonin super-family of peptides and binds Calcitonin Receptor-Like Receptor (CLR), a G-protein-coupled-receptor (GPCR), one of the largest drug target families[52, 53]. ADM binds its receptor by associating with a Receptor-Activity-Modifying Protein (RAMP), and can interact with receptors of related peptides, like CLR, adrenomedullin2-receptor but with lower affinity[54, 55].

The genetic information for the ADM peptide hormone is contained within the ADM-gene located on chromosome 11. Through a maturation cascade involving multiple processing steps (**Figure 6**), the prohormone Proadrenomedullin is cleaved into multiple peptides: Glycine-extended ADM (ADM-Gly), proadrenomedullin aminoterminal peptide (PAMP), adrenotensin and a stable prohormone fragment without any known biological function, Midregional-proADM (MR-proADM). ADM-Gly is the biologically mute precursor of mature ADM distinguished by a glycine residue at the end of the amino-acid chain (ie. the C-terminus) [56]. The glycine extension allows the peptide to be detected by the enzyme Peptidylglycine α-amidating monooxygenase (PAM), which converts ADM-Gly into mature ADM through amidation[57].

ADM is produced by almost every organ tissue and cell type, like endothelial cells, adipocytes and lung epithelial cells[58-61]. It circulates in plasma at low picomolar concentrations (2-3.5 pM, range: 1-10 pM) and has a short half-life in plasma of round 22 minutes, with the primary site of clearing being the lungs, rather than the kidneys[62].

The physiological effects of ADM depend on the tissue-type and compartment it interacts with. For example, ADM exerts vasodilatory effects through receptors on vascular endothelial cells (ECs) and vascular smooth muscle cells (VSMCs).[63-67]. In a study by Koyama and colleagues[68], where they studied the effect of ADM-gene modification in knockout mice and found that the ADM-system is crucial for appropriate vascular function and permeability. Mice without a functional ADM-system developed remarkable edema due to extensive vascular leakage. With ADM implicated in vascular barrier function, it has also been shown to be elevated in several conditions characterized by excessive fluid accumulation in tissues, like sepsis[69, 70], acute heart failure[71-73].

Other studies have suggested that ADM has role in regulating glucose metabolism, adipocyte tissue[60, 74, 75] and insulin homeostasis by inhibiting insulin secretion in pancreatic islets in a dose-dependent fashion[76, 77]. Adipose tissue is a significant source of ADM and exerts signaling functions specific to adipose tissue, leading to ADM being labelled an adipokine[78]. Also, disrupted crosstalk between organs is thought to be a key factor behind obesity[79, 80]. Adrenomedullin is also involved in regulation of insulin sensitivity[76] and blood flow to adipose tissue[60, 65, 81]. In the context of metabolic disease, obese and diabetic individuals have higher plasma ADM concentration compared to healthy individuals[82-85].

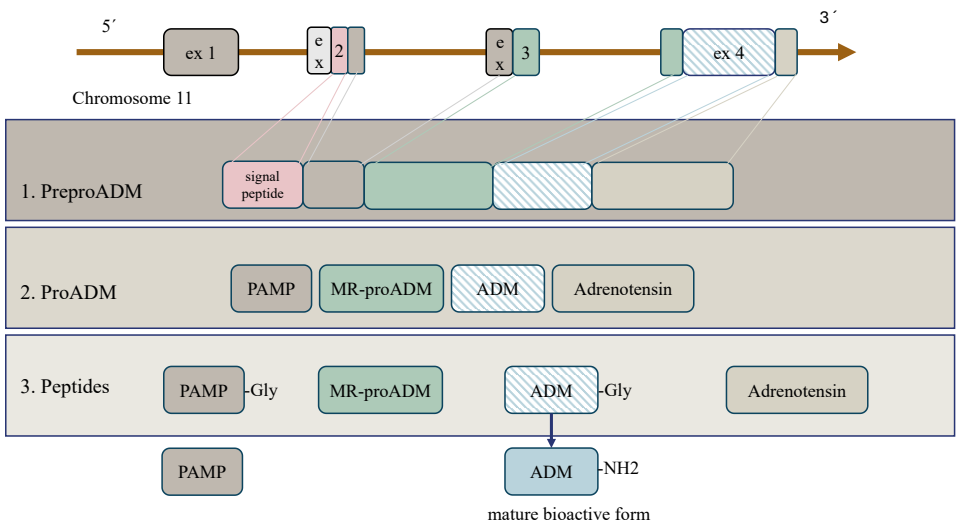

Figure 6. Adrenomedullin (ADM) maturation cascade with precursor peptides. Created in Microsoft PowerPoint.

Kato and colleagues[82] showed in animal subjects that rats infused with ADM had lower blood pressure and arterial stiffness, and that this response was blunted in

insulin-resistant rats, leading them to hypothesize that insulin resistance counteracts physiological effects of ADM. In another experimental study[78], it was shown that ADM concentration and plasma insulin levels were inversely correlated: With increasing insulin levels, ADM production was progressively inhibited, but whether ADM produced by adipocytes promotes or reduces obesity-associated disease was not clarified.

This would change in 2025 when Cho et al[86] describe a potentially unifying mechanism of ADM:s role in obesity and diabetes. In their study, they show that insulin delivery to tissues is dependent on insulin receptors expressed on endothelial cells and that this mechanism is impaired in obesity-induced diabetes and that ADM inhibits insulin-receptor through mediation of signaling pathways affecting vascular tone and endothelial insulin transport in a way that increases insulin resistance.

Adrenomedullin assays

Due to fast plasma turnover because of degradation by proteases, ADM proved difficult to study in humans for many years after its discovery. Most studies involving ADM were limited to in vitro and animal experimental designs. That would change in 2005, when Morgenthaler and colleagues presented a new antibody assay targeting the stable prohormone fragment released as a part of the ADM-peptide maturation cascade, Mid-regional pro-Adrenomedullin (MR-proADM)[87].

MR-proADM is a biologically inactive cleavage product from pro-adrenomedullin and thus not targeted by protease-degradation, and suggested to reflect ADM-pathway activation in a 1:1 ratio[88]. The assay was adapted and became commercially available in 2009 with assay reliability measures, like analyte stability, proving adequate for more than 72 hours in room temperature and to repeated freeze-thaw cycles[87]. MR-proADM concentration was determined to be below 0.55 nmol/L (range: 0.10-0.64 nmol/L) in a healthy reference population[87] and was later validated in a separate study[89].

However, there was still no practical method to reliably quantify the mature ADM peptide. An assay targeting mature bioactive ADM was first presented in 2014[90] and further improved in 2017[91]. The new assay was based on antibodies targeting the amidated peptide end-terminal (ie. C-terminal) unique to the mature ADM peptide with analyte stability shown to remain up to 24 hours and through repeated freeze-thaw cycles[91].

It has also been shown that the principal form of circulating ADM is the Glycine-extended biologically mute form (ADM-Gly), suggesting only a proportion of circulating ADM actually has potential for biological activity and signaling[92]. As a result, uncertainties regarding the stoichiometric relationship between MR-proADM and mature bioactive ADM were introduced, leading to arguments being

laid forth in favor of the newer method better reflecting the actual biological potential of adrenomedullin[91].

While ADM-Gly is not exerting any biological signaling, studies report that ADM-Gly levels are also elevated in individuals under a variety of clinical conditions like obesity[84], high blood pressure and chronic kidney disease[93], heart disease[94]or sepsis[95]. This has led to speculation as to whether this phenomenon of higher plasma concentrations of the inactive precursor could be related to insufficient conversion by PAM of ADM-Gly to mature ADM[96]. Kaufmann and colleagues further discuss potential benefits of interventions that increase the efficacy of PAM in converting inactive ADM-Gly to mature biologically active ADM. Figure 8 illustrates the ADM maturation axis with the PAM activation step integrated.

Peptidylglycine α-amidating monooxygenase (PAM)

PAM is a bifunctional precursor enzyme consisting of two components working in tandem, peptidylglycine α-hydroxylating monooxygenase (PHM) and peptidylglycine α-hydroxyglycine lyase (PAL). It is the only known enzyme capable of C-terminal amidation in mammals, and could be viewed as the gatekeeper of bioactive peptide maturation. In a two-step catalytic reaction dependent on the presence of copper (Cu) and zink (Zn), the enzyme cleaves the glycine-extended peptide leading to the formation of an amide group, corresponding to a “go-signal” for mature bioactive peptides, like ADM[97]. A schematic overview of a selection of peptide maturation pathways PAM is solely responsible for is shown in Figure 8.

In addition to its catalytic role, PAM has been shown to exert a non-catalytic function important to adequate formation and stability of secretory granules. In the heart, these granules store natriuretic peptides important for cardiac function and hemodynamic homeostasis[98]. A mechanistically similar role has been proposed in pancreatic beta cells, where PAM is implicated in insulin packaging and release, which is known to be relevant in development of DMt2[99].

Activity of the PAM enzyme has also been shown to be sensitive to hypoxia, with physiologically relevant oxygen levels inhibiting the enzyme[100], supporting a suggested role in tissue-specific responses to hypoxic states.

This dual role, involving both amidating activity and non-amidating granule function, PAM has been proposed as a potential therapeutic target across several conditions, including cancer[101], disorders of copper homeostasis[57] and conditions involving pituitary hypersecretion, like prolactinomas[102]

The current body of evidence supports a role for PAM in DMt2. In animal models, pancreatic beta cells from PAM-knockout mice show impaired insulin secretion through a mechanism involving islet amyloid polypeptide amidation. [103] In

humans, genetic studies have identified PAM-gene variants associated with increased risk of DMt2 and impaired insulin secretion, indicating that PAM activity plays a key role in pancreatic beta cell function.[104-106] Consistent with these findings, individuals living with diabetes display lower levels of PAM activity[107].

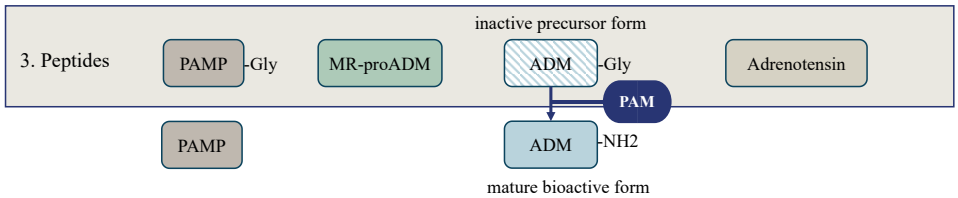

Figure 7. Adrenomedullin (ADM) maturation axis with integrated Peptidyl-glycine alpha-amidating monooxygenase enzyme (PAM) activation step. Created in Microsoft PowerPoint.

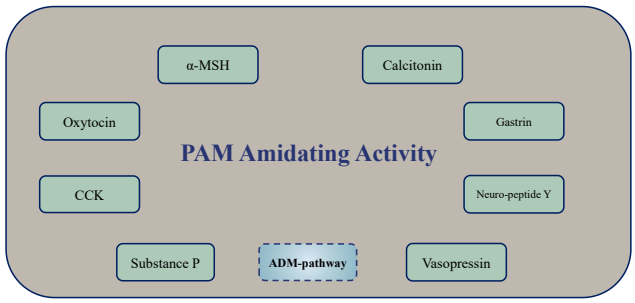

Figure 8. Peptidylglycine α-amidating monooxygenase (PAM) amidating activity and target substrates of which the ADM-pathway is one. Adapted from Eipper et al.[108]. Created in Microsoft PowerPoint.

Knowledge gap

It is still unclear how best to use and interpret ADM patterns in large populations. We do not yet know which component gives the most useful prediction, when they should be measured, or how to interpret conflicting changes between enzyme activity and ADM peptide levels. Studying these patterns epidemiologically may help explain why the same system can be linked both to acute vascular failure and to long-term metabolic risk.

Aims and Rationale

Rationale

The rationale for studying the Adrenomedullin system is that it may represent a shared vascular signaling pathway linking acute disease course and chronic disease susceptibility. In acute conditions, increased activity of the system may reflect a compensatory response to vascular leakage and impaired fluid containment, potentially supporting endothelial barrier function and intravascular stability. In chronic conditions, however, persistently elevated circulating levels may instead indicate dysregulated vascular signaling, impaired endothelial communication, or reduced biological responsiveness, with possible downstream consequences for metabolism and disease development.

As such, the same biomarker system may have different meanings across clinical contexts: as a marker of acute vascular stress and prognosis in established disease, and as a marker of long-term susceptibility to cardiometabolic outcomes in initially disease-free individuals. This thesis therefore examines the Adrenomedullin system as a context-dependent biomarker axis rather than as a uniformly protective or harmful exposure.

The overarching aim with the thesis is to elucidate the role of mature Adrenomedullin as a prognostic risk marker in both acute disease and cardiometabolic status at population-level.

Specific objectives

Paper I. To validate circulating ADM as an independent prognostic marker for acute congestion and mortality in undifferentiated dyspnea.

Paper II. To evaluate the epidemiological utility of plasma ADM and related precursor peptides, including maturation enzyme PAM, in cross-sectionally identifying and longitudinally predicting incident DMt2.

Paper III. To investigate the impact of deleterious, loss-of-function PAM genetic mutations on clinical metabolic hormones and anthropometric traits.

Paper IV. To map out how the longitudinal interaction of the entire ADM system, ie. mature ADM and related precursor peptides ADM-Gly, MR-proADM and activity of the maturation enzyme PAM relate to changes in body composition and insulin resistance over time.

Methodology

Author contributions

Author contributions were assigned using the CRediT taxonomy[109].

The author was not involved in baseline nor longitudinal follow-up data collection. Neither was the author involved in methodological design of any of the studied cohorts.

Paper I.

Contributed to conceptualization. Responsible for data curation, statistical analysis, and with contributing with collaborators to interpretation of findings. Writing the original draft and both reviewing and editing subsequent versions. Responsible for publication process listed as the corresponding author.

Paper II.

Contributed to conceptualization. Responsible for data curation, statistical analysis, and with contributing with collaborators to interpretation of findings. Writing the original draft and both reviewing and editing subsequent versions. Together with supervisor identified appropriate target journal, prepared and formatted manuscript for submission.

Paper III.

Had a marginal role in conceptualization of the original study idea, data curation and genomic data analysis. Contributed with interpretation of findings and reviewing and editing the manuscript draft in preparation for submission. Was not listed as the corresponding author during the publication process.

Paper IV.

Contributed to conceptualization. Responsible for data curation, statistical analysis, and with collaborators contributed significantly to interpretation of findings. Writing the original draft and both reviewing and editing subsequent versions.

Table 3. Methodological summary of the papers.

	Paper I	Paper II	Paper III	Paper IV
Design	Prospective cohort study, ADM measured retrospectively	Prospective two-cohort study, ADM-system markers measured retrospectively	Multi-cohort study with whole-exome sequencing performed biobanked baseline samples	Prospective cohort study, ADM-system markers measured retrospectively
Cohort	ADYS	MDC[1], MPP[2]	MDC[1], MPP[2], UKB[3]	MDC
Sample	N = 1402	[1]N = 6094, [2]N = 5060	[1]N = 4653, [2]N = 4769, [3]N = 331 215	N = 2674
Primary outcome	90-day mortality	Prevalent obesity and DMt2	PAM amidating activity	Change in BMI, WHR and HOMA-IR
ADM-system markers studied	ADM	ADM, ADM-Gly, MR-proADM, PAM	PAM amidating activity	ADM, ADM-Gly, MR-proADM, PAM
Statistical approach	Logistic regression, Cox regression, Kaplain-Meier estimates, Receiver operating characteristics	Logistic regression, Cox regression, Kaplan-Meier estimates	Genomic data pipeline with exome-wide sequencing, linear and logistic regression	Linear regression, Restricted Cubic Spline modelling, Logistic regression, Estimated predicted probabilities

ADM, adrenomedullin; ADM-Gly, Glycine-extended adrenomedullin; MR-proADM, Mid-regional pro-Adrenomedullin; PAM, peptidyl-glycine α-amidating monooxygenase; ADYS, Acute Dyspnea at the Emergency Department cohort; MDC, Malmö Dietary Cancer Study; MPP, Malmö Preventive Project; UKB, UK Biobank. DMt2, Diabetes mellitus type 2; BMI, Body mass index; WHR, waist-to-hip ratio; HOMA-IR, homeostatic model assessment for insulin resistance.

Overview of population and registry-based cohort studies

This thesis adopted a variety of epidemiological approaches. The basis for the data analyzed in the papers have been a selection of population cohorts geographically defined in the Malmö-region of southern Sweden and the United Kingdom. In **Paper I**, we studied the Acute dyspnea at the emergency department (ADYS) cohort. **Paper II** was based on data from the general population-based Malmö Dietary Cancer Study (MDC) and Malmö Preventive Project (MPP). **Paper III** involved data from both MDC, MPP as well as individuals from the UK Biobank (UKB). Lastly, **Paper IV** studied individuals from the MDC cohort longitudinally.

Outcome data for the cohorts studied was obtained from a combination of sources, mainly national and regional registries combined with electronic health records. Linkage of reliable registries is a major strength of Swedish population epidemiology. Maintenance population and health registers can provide long-term

follow-up for outcomes like mortality, hospitalization, migration and chronic diagnoses such as diabetes or heart failure.

The Acute dyspnea at the emergency department (ADYS) cohort

The ADYS Cohort entails adult individuals who visited the emergency department (ED) of Skåne University Hospital in Malmö (SUS Malmö) with a primary complaint of shortness of breath. Recruitment was led by a research nurse during office hours on working days between March 2013 and January 2019. The ED at SUS Malmö has a catchment area surpassing 400,000 inhabitants and services around 85,000 visits annually. A total of 1900 visits were registered in the ADYS cohort.

Individuals who presented with critical conditions, like unconsciousness or requiring mechanical ventilatory support at the Intensive Care Unit were excluded. Baseline data collection included triage parameters, blood samples secured within one hour of presentation to the ED and a questionnaire-guided interview by the research nurse including items such as past medical history, comorbidities and current medications. Complementary information was obtained through the EHR. The inclusion of participants in **Paper I** was based on ADYS participants with complete data including blood biomarkers or Swedish personal identity number for registry matching, which left 1402 individuals eligible for analysis. For a more detailed description please see methods section in **Paper I**.

The Malmö Preventive Project (MPP)

With the aim of gaining understanding of and identifying individuals at high risk of cardiovascular disease, alcohol use disorder and breast cancer in the general population, the Malmö Preventive Project (MPP) was launched in 1974 and ran through 1992 at the Department of Medicine in Malmö University Hospital (today Skåne University Hospital). Pre-specified birth cohorts of middle-aged men (1927-29, -44, -46, -48) and women (1928, -30, -38) were sent an invitation letter to take part in a screening program including physical examination, blood sampling for analysis and questionnaire with items focused on cardiovascular risk factors. More than 33,000 individuals attended (21,911 males, 8676 females) which equaled an overall attendance rate of 71% (range: 64-78%).[110]

Study participants who were still alive were invited for follow-up examinations between 2002 and 2006. Around 18,000 individuals were re-examined with blood sampling, body measurements and a follow-up questionnaire. Individuals who attended the re-examination were defined as a sub-cohort of MPP, the MPP Re-Examination Cohort (MPP-RES).[111]

This cohort was studied in **Paper II** (baseline data and metabolic disease outcomes) and **Paper III** (genomic and metabolic data). Since the study designs were different, individuals eligible for final analysis differ between the papers. Please see methods section of each paper for a detailed description.

The Malmö Diet and Cancer Study (MDC)

The Malmö Diet and Cancer study is a prospective population-based cohort based in the municipality of Malmö, Sweden. The purpose was to learn about the relationship between diet and cancer incidence, but combining baseline clinical phenotyping, extensive questionnaire on demographic, lifestyle and socio-economic factors, dietary patterns, biological samples, and long-term connection to Swedish health registers it also came to serve as a resource for hypothesis-generation. Study participants were recruited during 1991-1996, with all inhabitants born between 1925 and 1945, which was then extended to 1923-1950 in 1995, invited to participate through public advertisements and mail to randomly selected eligible individuals. By the end of 1996 when inclusion ended about 17,000 women and 11,000 men had been included, corresponding to a participation rate of 41%.[112]

In a bid to expand the scope of the study cohort to also include the development of atherosclerotic artery disease in the general population, starting in 1992 and leading through 1994 MDC participants were randomly selected in a ratio of 1:2 to be re-invited for further examination. These individuals formed what was named the cardiovascular cohort of MDC (MDC-CC) and consisted of more than 6000 individuals ($N_{MDC\text{-}CC}$ = 6,103, participation rate of 49%). Individuals in the MDC-CC sub-cohort were subject to two additional visits: one for ultrasound imaging of the carotid arteries and a second providing fasting blood samples, oral glucose tolerance test (OGTT) and anthropometric and blood pressure measurements.[113]

All participants in the MDC-CC cohort who had not deceased or emigrated from Sweden were invited for a follow-up examination between May 2007 and January 2012, 15 years after inception of the baseline study. Out of 4924 eligible individuals, 3734 attended the follow-up visit (attendance rate 76%) The proceedings of the follow-up examination were similar to the baseline visit procedure: with blood sampling, body measurements and collection of clinical data including past medical history through a questionnaire.[114]

Data from the MDC cohort were utilized in **Paper II** (baseline data and metabolic disease outcomes), **Paper III** (baseline and genetic data) and **Paper IV** (baseline and longitudinal data related to metabolic disease).

The UK Biobank (UKB)

The UK Biobank (UKB) is an easily accessible large-scale prospective cohort study comprising approximately 500,000 participants aged 40 to 69 recruited between 2006 and 2010 from 22 centers from all over the UK. Participants were recruited at that specific age interval so that the likelihood of already having developed disease would be low at the time of enrollment. During baseline assessment participants had to answer a questionnaire, be interviewed, and have anthropometric and functional measurements done and provide samples from blood, urine and saliva. After the initial assessment, selected participants have been invited to support collection of additional data to expand the study. Approximately 20,000 individuals were followed up with repeat examinations in 2012 to 2013.[115]**[AI^3]**

Between 2014 and 2020, existing cohort members were re-invited to undergo magnetic resonance imaging, dual-energy X-ray absorptiometry (DXA), and carotid ultrasound at dedicated imaging centers, as part of an imaging enhancement initiative. It aimed to include 100,000 individuals from the original cohort of ~500,000, with an effective attendance rate of 21% in line with meeting the goal. A repeat imaging visit was initiated in a subgroup of cohort members enabling longitudinal data of imaging phenotypes and disease progression.[116]

There has also been a gradual increase in genome-wide sequencing in the cohort, and whole-exome sequencing in more than 450,000 participants was released in 2022.[117, 118]

Health and genetic data, which includes questionnaire answers, past and current medical status, medications, lab results and bioimpedance data, from ~330,000 individuals from the baseline UKB and ~33,000 from the repeat imaging subcohort with both exome sequencing and longitudinal DXA data were used in **Paper III**.

Variable definitions

Anthropometric measurements

BMI was calculated as weight divided by height squared (kg/m^2). Waist and hip circumferences were measured with the individual standing upright without clothing (**Paper II** and **IV**). Body composition measures in **Paper III**, including body fat percentage, fat-free mass, and segmental predicted muscle mass, were assessed by bioelectrical impedance analysis. Total body mineral content and bone mineral density were measured by dual-energy X-ray absorptiometry in participants of the UKB imaging-study. Hand grip strength was assessed bilaterally using a hydraulic dynamometer.

Diabetes mellitus (DM)

In both Malmö Cohorts (MPP and MDC; **Papers II-IV**) DM was defined using data from six national and regional diabetes-related registers: the Malmö HbA1c Register, the Swedish National Diabetes Register, the regional Diabetes 2000 Register, the Swedish National Patient Register, the Swedish Cause of Death Register, and the Swedish Prescribed Drug Register. Additional cases were identified through self-reported diabetes in enrolment questionnaires. Participants were also classified as prevalent diabetes cases if fasting plasma glucose was ≥7.0 mmol/L at baseline or within 14 days after screening. Whereas in MDC (**Papers II-IV**), since glucose was analyzed in whole blood samples, study participants were defined as prevalent diabetics if they had a fasting whole blood glucose >6.1 mmol/L at inclusion and up to 14 days thereafter, self-reported diabetes diagnosis or use of anti-diabetic medications in study questionnaires.

In **Paper III**, with hemoglobin A1c (HbA1c) available and measured in UKB, the criterion of having HbA1c greater than or equal to 48 mmol/mol (6.5%) was also added for UKB cohort members.

Impaired fasting glucose (IFG)

IFG has been identified as a significant risk factor for developing diabetes and characteristic of a prediabetic state where intervention could be particularly valuable[119] As a result, non-diabetic participants were defined as having impaired fasting if their fasting blood glucose was 4.5 to 5.9 mmol/L in MDC, and fasting plasma glucose between 5.6 to 6.9 mmol/L in MPP.

Hypertension

Hypertension was defined as two repeated measurements of systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, or ongoing antihypertensive medication at screening in **Papers I**, **II** and **IV**.

Kidney function

Kidney function was based on plasma creatinine and presented differently across the papers. In **Paper I**, plasma creatinine is presented as a direct surrogate marker for kidney function presented plainly in μmol/L. In **Papers II** and **IV**, however, kidney function was presented as estimated glomerular filtration rate using the creatinine-based CKD-EPI 2021 formula[120]. Chronic kidney disease staging was done according to estimated glomerular filtration rate per KDIGO 2024 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease[121].

Obesity measures

Obesity was defined as a BMI ≥ 30 kg/m^2 (**Paper II, IV**). In **Paper IV**, we also included a broader definition of obesity with regard to excess adiposity by including

measures of visceral adiposity in addition to a plain BMI-based definition.[122] Excess adiposity was defined as having at least one additional body measurement indicating adiposity, unless BMI was > 40 kg/m^2: waist circumference (≥ 102 cm for men, ≥ 88 cm for women), waist-to-hip ratio (> 90 for men, > 0.85 for women), waist-to-height ratio (> 0.50 for both men and women), proposed as a broader definition relevant to clinical obesity by Rubino and colleagues in January 2025[122].

Sarcopenia

In **Paper III**, measures of muscle mass and muscle strength was available among UKB participants. A proxy variable for sarcopenia was defined as being in the lowest sex-specific quintile for all five measures: bilateral grip strength, mean arm lean mass, mean leg lean mass, and trunk lean mass estimated by bioelectrical impedance analysis. In line with a definition proposed by the European Working Group on Sarcopenia in Older People[123].

Insulin Resistance (IR)

In **Paper IV**, the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was used as a surrogate measure of insulin resistance. It is based on a formula combining fasting values of insulin and plasma glucose and dividing by a constant[124] and has been widely cited in epidemiological study settings and validated against multiple physiological methods. It has been used previously in the MDC cohort by Hedblad in colleagues[113], who defined individuals among the top 25% of sex-specific HOMA-IR value distribution as insulin resistant (In MDC sample ≥ 1.8 in females and ≥ 2.12 in males), and we chose to adopt the same definition to maintain consistency in **Paper IV**.

Congestive heart failure (CHF)

Specified as a secondary outcome in **Paper I**. CHF diagnosis was extracted from the EHR if either specified explicitly by admitting or discharging physician and based on diagnoses defined using International Classification of Diseases (ICD) codes in I50 corresponding to heart failure diagnoses recorded in electronic health records.

Endpoint data

For the study cohorts based in Sweden (ADYS, MPP, MDC), the Swedish personal identity number was used for linkage to official national and regional registries, such as the Swedish Hospital Discharge Register (**Paper I**), Swedish Cause of Death Register[125] (**Paper I**) and the Swedish National Diabetes Register[126] (**Papers II-IV**). In **Paper II**, endpoint data was collected from registries, whereas in **Paper IV**, endpoint data was based on re-examination with research nurse between 2009 and 2012.

For the UKB cohort (**Paper III**), primary care data for approximately 230,000 UK Biobank participants (up to 2016 or 2017 depending on the data supplier) was made available in 2019. This dataset contains clinical events and current medications. Hospital inpatient data, such as date of admission, diagnosis, during admission, procedures and discharge information were available for the full cohort. Linkage includes national registries like the Hospital Episode Statistics (England), the Scottish Morbidity Records, and the Patient Episode Database for Wales.

Follow up dates

In the ADYS cohort (**Paper I**) we retrieved mortality status for participants 90 days after inclusion (primary outcome) and recorded if any events occurred within 7 and 30 days (secondary outcomes). Recruitment in ADYS ended in January 2019 and last follow-up date was set to April 30th 2019. The 90-day cutoff for outcomes like all-cause mortality are widely used in the field of emergency medicine research.[127-129]

In **Paper II**, for the MPP sample last follow-up date was set to December 31st 2014, while for MDC last follow-up date was specified as December 31st 2019.

In **Paper III**, end of follow-up for MPP participants was set to December 31st 2006 at the end date of the re-examination, whereas for MDC-CC participants were re-screened until Januari 31st 2012. The UKB sample follow-up dates lead from 2012-2013 (re-examination) and 2014-2020 (imaging) after original assessment in 2009.

In **Paper IV** end of follow-up was also set to last re-screening date among MDC-CC participants ended in Januari 31st 2012.

Blood sample handling and analysis

Venous blood and plasma samples across the Swedish cohorts (ADYS, MDC and MPP) were processed via uniform pre-analytical protocols; samples were drawn during clinical triage or standardized fasting/stimulated timepoints (0 to 120 minutes), immediately stabilized or frozen within two hours of collection, and preserved at -80 °C until analysis. Routine clinical chemistry including plasma glucose, lipid panels, and serum creatinine was performed using certified automated platforms (Siemens Atellica, Roche Cobas, and Beckman Coulter) linked to national accreditation quality control systems, with low-density lipoprotein cholesterol derived via the Friedewald formula[130]. Endocrine, incretin, and vasoactive peptide profiles (insulin, glucagon, GIP, GLP-1 isoforms, GH, and IGF-1) were quantified using validated radioimmunoassays and enzyme-linked immunosorbent assays (ELISA).

Cardiovascular and ADM-system peptides, including NT-proBNP, MR-ANP, mature ADM, ADM-Gly and MR-proADM, were determined utilizing automated

sandwich electrochemiluminescence, time-resolved fluoroimmunoassays, and high-throughput microtiter plate chemiluminescence (Figure 9).

PAM activity was quantified via a two-step functional amidation assay[131], in which synthetic ADM-Gly substrate conversion was initiated at 37 °C, halted via EDTA-mediated inactivation, and the resulting amidated product isolated and detected using a C-terminally specific immunoluminometric assay. All specialized marker investigations were conducted by laboratory personnel blinded to clinical and phenotype data. Blood sampling procedure and biological sample handling in UKB participants is described in detail elsewhere[132]

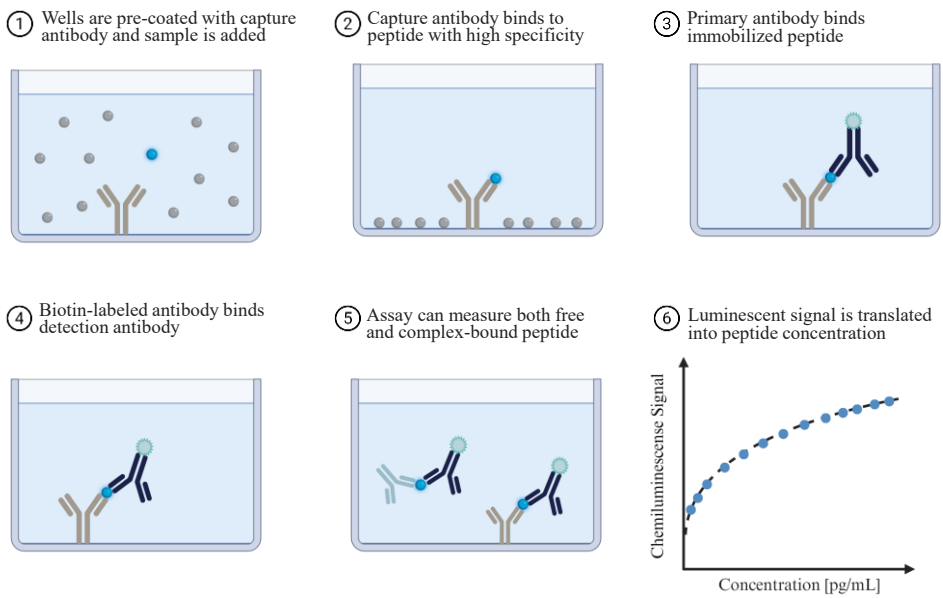

Figure 9. Schematic of the principles behind a sandwich ELISA (Enzyme-Linked Immunosorbent Assay) immunoassay, used to detect and quantify antigens, like adrenomedullin, by capturing it between two specific antibodies, known as the capture antibody and the detection antibody. The dose-response curve in panel (6) is for illustrative purposes only. Created in BioRender.

General statistical framework

Statistical analyses were performed using R (**paper I**: version 3.6.3, **papers II** and **IV**: v4.4.2 and **paper III**: v4.1.2; R Foundation for Statistical Computing, Vienna, Austria) and IBM SPSS Statistics v29 (**paper III**). All hypothesis testing was two-sided, with an alpha level of 0.05 chosen. Point estimates are reported with 95% confidence intervals (95% CIs).

Descriptive statistics

Cohort characteristics and descriptives are presented as frequencies and percentages for categorical variables, and as medians with interquartile ranges (IQR) for skewed variables, like raw biomarker levels.

In **paper I** we have included a statistical test for monotonic trends across exposure quartiles, reasoning at the time that it could give a hint with regard to a possible dose-response relationship. However, this should be viewed more as a stylistic convention and could embed assumptions that a descriptive table usually does not justify. The covariates in such a table are typically potential confounders and showing that they trend across exposure is arguably the point of displaying them, but a formal test adds little beyond what the displayed gradient already shows. Leading experts have also argued against statistical testing in descriptive tables in observational studies[133, 134], which is why we have refrained from this in **papers II-IV**.

Throughout the papers, the primary principle in selecting which covariates to present in descriptive tables has been factors with established clinical relevance or factors that could potentially introduce bias when studying the ADM-system and health outcomes, rather than selection through statistical methods.

Prognostic modelling

The predominant statistical approach was to use regression-based modelling to study the association between exposures (ADM-peptides, genetic variants) and outcomes. The choice of regression model depended on the outcome studied. Logistic regression was used for binary outcomes, such as prevalent diabetes and obesity (**Paper II**) or sarcopenia (**Paper III**). For incident outcomes where time-to-event data was available, like all-cause mortality (**Paper I**) or incident diabetes (**Paper II**), Cox Proportional-Hazards models were fitted. To associate exposure variables with continuous longitudinal outcomes, linear regression was chosen (**Papers III** and **IV**). Kaplan-Meier estimate curves (**Paper I** and **II**) and receiver operating characteristic (ROC) graphs (**Paper I**) were used mainly for illustrative purposes. In **Paper I**, we evaluate the incremental predictive value of mature ADM by comparing nested models, such as a basic model with and without the ADM peptide, using likelihood-based tests and changes in discrimination measures such as the C-index.

Models were fitted sequentially, first crude or minimally adjusted associations were evaluated, followed by more adjusted models. In this way, we could first confirm a statistical association between the exposure and selected outcome, and then assess robustness or attenuation after also accounting for potential confounding factors.

In time-to-event models, like the Cox Proportional Hazards model, there are two key concepts to keep in mind. The first being censoring, i.e. the timepoint at which the model stops receiving data on a given participant within the “at-risk” window

for an event. Censoring can occur due to follow-up ending without the event having occurred (for example if an individual survived throughout the follow-up period, or never got diagnosed with diabetes), or when individuals are lost to follow-up due to emigration, death or for some other reason not available for registering data regarding the event. The other concept being proportional hazards; for estimates yielded by the Cox regression model to be reliable, the risk (hazard) between an exposure and an event need to remain roughly stable over the specified follow-up period[135]. For this reason, before proceeding with interpreting Cox model estimates, it is important to test whether the assumption of proportionality holds true. This can be done through statistical tests and graphically by plotting the Schoenfeldt residuals of the model. They look at whether the effect of a variable changes over time. If the residuals show no clear time-related pattern, the proportional hazards assumption is considered acceptable. If there is a clear pattern, it suggests that the effect of that variable may not be constant over follow-up.

In the clinical setting, this assumption is rarely met, especially when an exposure variable or covariate is closely related to disease expression. Examples include glucose in diabetes prediction or troponin in acute coronary syndrome, where the risk is inherently higher for an event early in the trajectory. However, there are strategies to remedy the violation to still reliably interpret the estimates produced, such as stratification of the violating variable (with the downside of losing the hazard ratio for this particular variable), include time-varying coefficients by including an effect modification term between the violating covariate and the logarithm of time[136] or by proceeding with landmark analysis using pre-specified follow-up intervals (say instead of 0-15 years, use 0-5, 5-10 and 10-15 years). Landmark analysis could be particularly useful when determining whether a predictor is stronger in the short or long term, and account for underlying risk associated with preclinical disease at baseline[137].

Biomarker exposures

ADM-related peptide exposures were log-transformed due to two primary reasons: circulating biomarkers are often right-skewed (a long tail of spread out values toward the high end and much more concentrated values toward the low end) and because the log-scale allows for effect estimates to reflect relative rather than absolute change, such as a doubling in concentration, which is often more meaningful in biology. Quartile-based analyses were also used, with the lowest quartile set to be the reference, making it possible to describe patterns across increasing biomarker levels without relying on the assumption that the association was linear. However, categorization also has disadvantages, including loss of information, arbitrary cut-points, and reduced statistical power.[138, 139]

Effect estimates related to ADM-system peptide concentration or PAM Loss-of-function score were predominantly reported as distribution-based increments on the log-scale, per increase in interquartile range (**Paper I**) or standard deviation (**Papers**

II, **III**). In **Paper IV**, a conscious choice was made to instead use the log2-scale to express ADM-peptide estimates per doubling in concentration, which could be seen as more intuitive than distribution-based increments.

Restricted cubic splines or natural splines were considered useful for evaluating non-linear associations. Since spline coefficients are not directly interpretable, graphical presentation is essential.

Dose-response plots or predicted probability plots can show whether risk increases linearly, plateaus, or follows a threshold-like pattern. An approach modelling a non-linear association with restricted cubic splines was applied in **Paper IV**.

Genetic modelling

In **paper III**, we studied the genetics of thousands of individuals across MDC, MPP and UKB to find specific genetic variants associated with decreased efficacy of the PAM enzyme, expressed as amidating activity (AMA). The genetic variants associated with PAM AMA were first identified in MDC, which served as a discovery cohort. They were then evaluated in MPP, as an independent validation cohort to assess consistency of their association. Variants showing evidence of replication were then combined into a weighted allele score, which was applied in UKB, a much larger cohort to examine if genetically proxied PAM AMA was associated with metabolic traits.

*Genomic data pipeline**[AI^3]***

In MDC and UKB we looked at exome data, the specific 1.5% of the genome that contains instructions for making proteins[140]. We had access to exome data from ~30,000 individuals in MDC and ~470,000 people in UKB. For MPP, instead of reading the whole exome, we used a genetic array from ~5,000 people and missing genotypes were statistically estimated through imputation.

Genetic variants were divided into two main categories: Loss of function, which are severe changes that break a gene's ability to function, and missense, where a change in one amino acid (i.e. building block) of a protein causes it to work differently.

Five different computational tools were used to determine how detrimental the identified missense variants could be. Relying on a single such tool to predict the impact of missense variants runs the risk of introducing significant bias and increases the rate of misclassification. Using several tools in the computational pipeline leads to a more robust conclusion that minimizes systemic bias[141]

Given the excessive amount of data obtained through a genomic pipeline, the signal to noise ratio is extremely low. Consequently, low-quality data needs to be removed through a quality control procedure. For example, by removing individuals with missing data, exclude close relatives to prevent family bias, restrict the analysis to

individuals with certain ancestry to improve reliability (in our case European) and filter out rare anomalies (*e.g.* mismatched sex chromosomes).

After cleaning the genetic data, the next step in the pipeline is to understand which of the genetic variants actually matter. For that, a specialized software called REGENIE[142] was used, which cleanly finds genetic links in massive datasets without the influence of shared genetic noise through regression modelling. It is based on a two-layer approach, where in a first layer it looks at overall genetic background of the individuals to account for baseline differences. This prevents mistaking a common genetic trait for a cause of PAM AMA. The second layer then runs and tests individual genetic variants to look for matches with PAM AMA.

Since the genetic data is analyzed from two separate cohorts, final results were using inverse-variance weighting (IVW), which assigns weights to each studied cohort based on the variance of their estimates. Because variance is inherently determined by sample size, larger sample sizes yield smaller variances and greater reliability. In extension, larger cohorts are assigned higher weights than smaller cohorts with larger variances.

In genome-wide association studies (GWAS), each genetic variant is tested for association with the phenotype of interest, which in **Paper III** was PAM AMA. Because hundreds of thousands to millions of variants can be evaluated, using a conventional significance threshold such as $p < 0.05$ would produce many false-positive findings purely by chance. Because of this, GWAS analyses use much stricter significance thresholds to account for multiple testing and reduce the probability of type I errors. In this study, a threshold of $p < 5 \times 10^{-8}$ was used to identify candidate variants for follow-up, balancing false-positive control against the need to keep potentially relevant variants for validation in an independent cohort.

To account for differences in the strength and precision of genetic variant and PAM AMA associations, selected variants from MDC and replicated in MPP were combined into a weighted genetic score. Each variant was weighted by its estimated association with PAM AMA, with IVW used to give higher weight to variants with more precise effect estimates. The resulting score was used as a genetic representation for PAM AMA in traditional regression models after standardization, allowing interpretation of effect estimates as “per one standard deviation increase”.

Genetic sample processing

DNA for MPP and MDC sample was extracted from stored blood sample collected at the time of the baseline visits. DNA extraction was performed by SWEGENE Resource Center for Profiling Polygenic Disease, Skåne University Hospital, Malmö, Sweden. Biobank services were provided by Region Skåne Competence Centre (RSKC Malmö), Skåne University Hospital, Malmö, Sweden, and BD47

Region Skåne Biobank, Sweden. UKB DNA extraction was performed by laboratory staff at UKB and by Wellcome Trust Centre for Human Genetics[143].

Methodological Considerations

Observational Cohort study design

Cohorts are useful as the exposure is measured before any health event occurs. In a biomarker context, blood samples which are later analyzed for baseline biomarker levels have been secured before the outcome of interest affects the individual. Another strength of cohort studies is the availability of thorough data on conceivable sources of bias, plasma-derived measurements or genetics, while many study participants allow for appropriate study design and conclusions. While there are tangible strengths to studying cohorts, there are important limitations to keep in mind, including residual confounding, which will be elaborated upon in a later section.

Missing data

Missing data were handled using complete case analysis, meaning participants with missing data on the exposure, outcome, or covariates included in each model were excluded from that specific analysis. This approach is transparent and simple, but it assumes that missingness does not introduce substantial bias.

Missing data are expected in large-scale cohort studies, particularly when information is collected from multiple sources, including anthropometric measurements, questionnaires, clinical examinations, electronic health records, interviews, and biological samples. Missingness may occur at several analytical levels: in the biomarker exposure, in outcome registration, or in covariates considered to be relevant confounders. As such, the consequences of missing data do not only depend on the amount of missingness, but also on its pattern and relation to the exposure, outcome, and participant characteristics.

Complete case analysis may reduce statistical power and may introduce selection bias if the probability of being included in the analysis is related to the exposure or outcome. This is particularly relevant in observational cohort studies, where missingness may reflect health status, participation behavior, disease severity, socioeconomic factors, or differing follow-up.

To support interpretation, the extent of missingness should be described for key exposures, outcomes, and covariates. Where relevant, baseline characteristics of participants included in complete case analyses may be compared with those excluded due to missing data. This can help assess the direction and weight of selection mechanisms, but bias from unobserved differences cannot be fully excluded.

Effect modification and interaction terms

Interaction terms were used to evaluate whether the association between a biomarker and outcome differed across levels of another variable. This is relevant when biological mechanisms plausibly differ between subgroups.

One example is sex-specific effect modification in the relationship between ADM and body composition. Since visceral and peripheral fat distribution differ between men and women[144] it is plausible that the relationship between ADM and waist-to-hip ratio may vary by sex.

It is important to keep in mind that while interaction analyses potentially increase the precision of an estimate, they come at the cost of an increased number of statistical tests and are often underpowered unless specified beforehand.

Confounding

The scenario of exposure and outcome both being associated with a third variable is termed confounding[145]. An example of this is age, which is associated with both ADM-levels and diabetes. The consequence of this is that an association between ADM-levels and diabetes, in this case, might simply be due to the difference in age between an individual living with diabetes (case) and one without (control). When age is then accounted for in the association between ADM-levels and diabetes, the strength of the association may change and reflects the relationship between ADM-levels and diabetes in individuals of the same age (**Figure 10**).

When comparing groups of individuals, say those living with and without diabetes, differences between these groups may be due to uncaptured bias despite statistical adjustment. This uncaptured bias is termed residual confounding and occurs because confounders may be unmeasured, incompletely measured, or imperfectly modeled.

Residual confounding is a limitation ingrained into the design of observation association study design, which **Papers I**, **II**, and **IV** are based on, and cannot be excluded. This limitation is not mitigated by sample size, and in large-scale population cohorts like the Malmö Cohorts and UKB, the large numbers can make the findings look more convincing but may all the same be misleading if important differences between the groups are not accounted for.

There are approaches that may reduce this problem, but cannot entirely remove it. For example, matching or propensity-score methods can make comparison groups more similar with regard to measured characteristics. However, these methods only help with the factors that have been measured. They cannot account for unknown or unmeasured differences in the way that randomization can. Matching and propensity-score methods were not used in any of the papers of this thesis, and randomization was not feasible. As a result, it is important to remember that the findings are associations rather than evidence of direct causal effects. Residual

confounding remains a possible explanation and should be considered when interpreting the results.

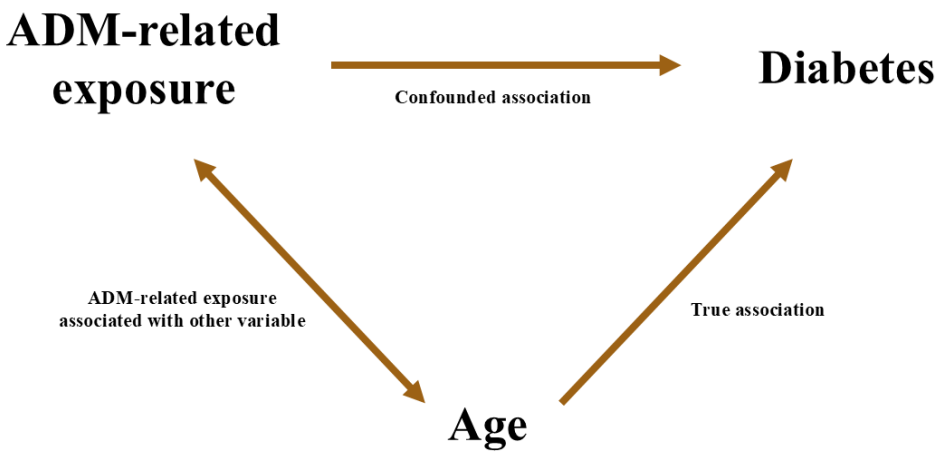

Figure 10. Schematic illustrates the associations between variables and the concept of confounding. Created in Microsoft PowerPoint.

Population drift

Using distribution-based percentile cutoff for incident outcomes, as we have chosen with the incident variable for insulin resistance in **Paper IV**, might be protected against population-level drift. An alternative approach could be to recalculate the 75th percentile cutoff at follow-up. A study participant classified as having insulin resistance at follow-up would be in the top quartile of the follow-up distribution. Opting for a baseline-only cutoff applied to both timepoints anchors the definition so that shifts in population distribution don't obscure individual-level change.

However, the second approach could arguably be preferred for incident analyses as it prevents the scenario of population-level drift between timepoints redefining who is "resistant".

Multiple testing

Biomarker studies often involve many exposures, outcomes, subgroups, and model specifications. This increases the probability of false-positive findings due to running multiple statistical tests.

A conventional significance threshold such as $p < 0.05$ is not enough when many related tests are performed. To mitigate this risk, there are statistical procedures that are useful. One of them, which we use in **Paper IV**, is the the Benjamini–Hochberg procedure to control the false discovery rate. This approach is less conservative than

the often used Bonferroni correction and balances the risk of false positives against the risk of overlooking potentially meaningful associations. Nevertheless, statistical correction does not remove the need for cautious interpretation.

Ethical Considerations

The bigger picture

In the aftermath of the COVID-19 pandemic in 2023, Salerno and colleagues published an overview of ethical considerations in "the post-pandemic era" of epidemiological research involving both genetic epidemiology and health informatics, which they argued was necessary to reflect on after the notable shift in studying disease at population level made apparent by the pandemic[146].Traditional epidemiological ethical principles involve obtaining valid and informed consent, appropriate protection of privacy and confidentiality, striving after balance between maximizing benefit and minimizing risk of conducted research, all while maintaining public trust.[147]

Genetic epidemiology made possible by large-scale genome-wide sequencing, combined with clinical data through electronic health records, both characteristics of the design in **Paper III**, are the backbone of personalized interventions for patients. There are various potential benefits of this field: earlier risk factor modification by identifying individuals at higher-than-average risk which could benefit from directed preventive intervention, like coaching to realize lifestyle-changes, or interpreting results in the presence of a particular genetic variant which could guide clinical decisions. On the other hand, there are also risks of such large-scale genetic research.

Examples include unintended access with the risk of identifying sensitive information, permitted but unwanted use of information for objectional topics or attempts at financially profiting from the data (like when UKB data was found for sale at an international e-commerce site[148]) or the emergence of new risks that are unfamiliar and difficult to grasp for individuals and researchers alike.

What about this thesis?

This thesis primarily leans on studies applying observational study design, drawing on biobanked plasma and cohort data rather than on prospectively assigned treatment. As a result, the principal ethical weight falls not on the risks related to intervention, but on the management and handling of data and material entrusted by participants, and on the discipline with which the findings are described.

In observational biomarker research, our primary duty is to act as responsible custodians. Participants donate their data and biological samples trusting they will be kept secure and used ethically. Fulfilling this trust relies less on the consent form

and more on strict technical and organizational controls, like anonymization through participant ID coding and designated data managers, which is applicable to all cohorts in the thesis.

Considerations in acutely ill individuals

In the acute setting, which is the context of **Paper I**, a major ethical issue is whether a patient with acute onset of respiratory symptoms can provide a truly voluntary consent. Such patients may be frightened, physically unwell, and dependent on the clinical team. The responsibility is therefore to design the study in a way that protects voluntariness as much as possible, rather than assuming that an acutely ill patient can decline participation as freely as a healthy volunteer.

The design of **Paper I** tried to reduce this pressure. Patients were approached to participate first after initial triage. The research nurse responsible was separate from the treating clinical team. This separation was intended to make clear that participation was not required for receiving care, and would not lead to better or faster treatment. This approach does not remove the imbalance between patients and healthcare system. A person who is acutely unwell is not in the same position as a healthy volunteer considering research participation under more collected conditions.

The aim is therefore not to claim that all pressure has been removed, but to reduce it as much as possible. The responsibility for protecting voluntary consent lies mainly in the study design, rather than in expecting a sick patient to carry the full burden of saying no.

The caveats of large numbers and passing of many years

In the population cohorts (**Papers II-IV**), the ethical issue is different. Participants in MDC, MPP and UKB gave broad consent many years ago, often before the current research questions or methods existed. Perhaps it is necessary for long-term cohort research, but it places a responsibility on researchers and governance bodies to use the data and samples carefully. Stored plasma is limited and cannot be replaced, so each analysis should be justified and unnecessary measurement avoided.

There is also an ethical responsibility in how results are presented. Observational studies show associations, not proof of causation. Researchers should therefore avoid wording that makes findings sound more causal than they are. Overstated claims can influence the literature and may be difficult to correct later.

Responsible translation

These concerns must be weighed against the possible benefits. Most biomarkers never become clinically useful, so the justification for biomarker research must be realistic. However, some biomarkers can become important as treatment targets or

clinical tests. The adrenomedullin system is relevant because it may have both roles: adrenomedullin and PAM may be therapeutic targets, while measured adrenomedullin may provide diagnostic or prognostic information. This potential benefit can justify the work, provided the conclusions remain cautious and aligned with the strength of the evidence.

Ethical approval

Ethical approval was sought and granted for the respective cohorts: ADYS (Regional Ethical Review Board in Lund Dnr: 2012/762), MPP (EPN Lund LU 244-02 and 2009/633), MDC baseline (LU 51-90), MDC re-examination (LU 532-2006) and UKB (Swedish Ethical Review Authority ID: 2022-01085-01 and in compliance with the UKB Ethics Advisory Committee guidelines). All participants provided written informed consent before enrolling in the study. The author was not involved in the process of drafting or reviewing any of the ethical applications.

Main Results

In this section only the key results will be presented with the purpose to bridge to the takeaways and conclusions of the thesis. For the one interested in the complete picture, I kindly refer to the results section of each respective paper appended at the end. A combined cohort characteristics table and histogram illustrating relative distribution of ADM-concentration across the studied cohorts (ADYS, **Paper I**; MDC and MPP **Paper II**) can be seen in **Table 4** and **Figure 11**.

Table 4. Cohort characteristics at a glance.

Cohort	ADYS[1]	MDC[2]	MPP[2]	UKB[2]
General Characteristics				
Age, years	71 (17)	58 (8)	68 (6)	57 (8)
Sex, % Female	56%	60 %	37 %	53 %
DMt2, %	N/A	10 %	16%	9 %
BMI, kg/m2	N/A	25.8 (4)	27.2 (4)	27.5 (5)
ADM-system peptides				
ADM, pg/mL	28 (17-50)	18 (14 - 24)	14 (10 - 19)	N/A
MR-proADM, nmol/L	N/A	0.45 (0.38 - 0.52)	0.70 (0.61 - 0.84)	N/A
ADM-Gly, pg/mL	N/A	27 (11 - 63)	N/A	N/A
PAM AMA, µg/(L*h)	N/A	14.4 (12.5 - 16.5)	12.5 (10.8 - 14.4)	N/A

Abbreviations: ADYS, Acute Dyspnea at the Emergency Department. MDC, Malmö Diet and Cancer study. MPP, Malmö Preventive Project. UKB, UK Biobank. DMt2, diabetes mellitus type 2; BMI, body-mass index; ADM, adrenomedullin; MR-proADM, mid-regional pro-adrenomedullin; ADM-Gly, glycine-extended ADM; PAM AMA, peptidyl-glycine α-amidating monooxygenase amidating activity. [1]adapted from Paper I. [2]adapted from Paper III. General characteristics averages presented as mean (SD) and count as %. Peptide marker averages presented as median (IQR).

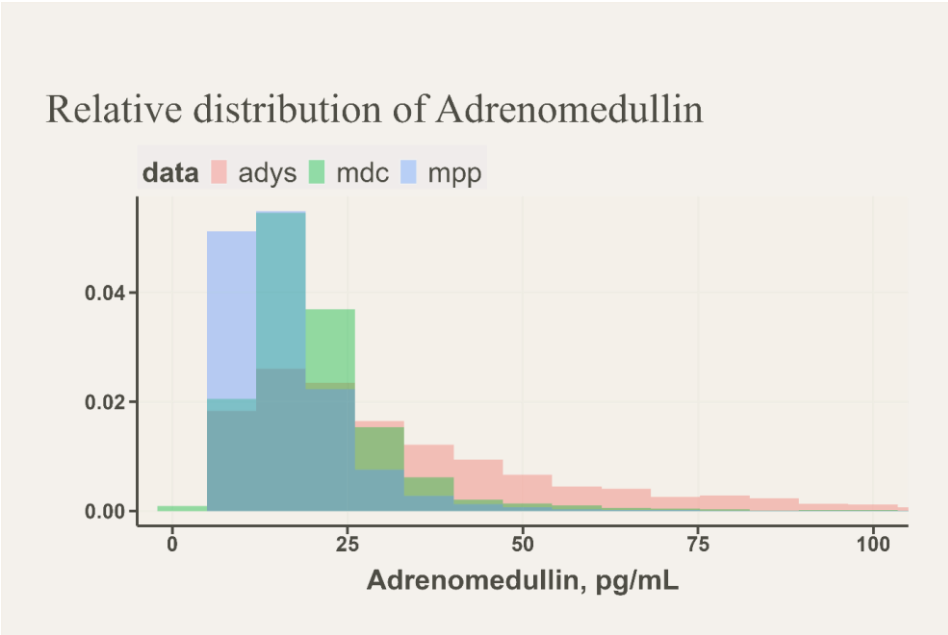

Figure 11. Combined histogram illustrating the relative distribution of adrenomedullin from the Acute dyspnea at the Emergency department (ADYS) cohort including acutely ill individuals to Malmö Diet and Cancer study (MDC) and Malmö Preventive Project (MPP) cohorts consisting of seemingly healthy middle-aged individuals. Adapted from Paper I and II[1, 2].

Paper I

Among the 1402 individuals included in the study, 179 individuals (13%) died within 90 days, with higher ADM being associated with higher risk of 90-day mortality (unadjusted OR: 2.1, 95% CI 1.7 to 2.6, $p < 0.001$), with the association remaining after also accounting for clinically established plasma biomarkers[1] (adjusted OR: 1.5, 95% CI 1.2 to 2.0, $p < 0.05$). The proportion of individuals passing away within 90-days of their ED visit was higher in those with higher ADM concentration at baseline, with 90-day mortality being 23% (81 individuals) in the top compared to 4% (15 individuals) in the lowest quartile (quartile comparison Q4 vs Q1 adj OR: 2.1, 95% CI 1.1 to 4.3, $p < 0.05$). The association between baseline ADM with 90-day mortality in minimally adjusted model (sex, age) remained in sub-group analysis of only admitted individuals (n = 822), both in those aged 65 or older (n = 1006) and younger than 65 (n = 396). Kaplan-Meier estimates for 90-day mortality stratified by ADM-quartile shown in Figure 12.

[1]CRP (mg/L), S-Creatinine (μmol/L), NT-proBNP (ng/L)

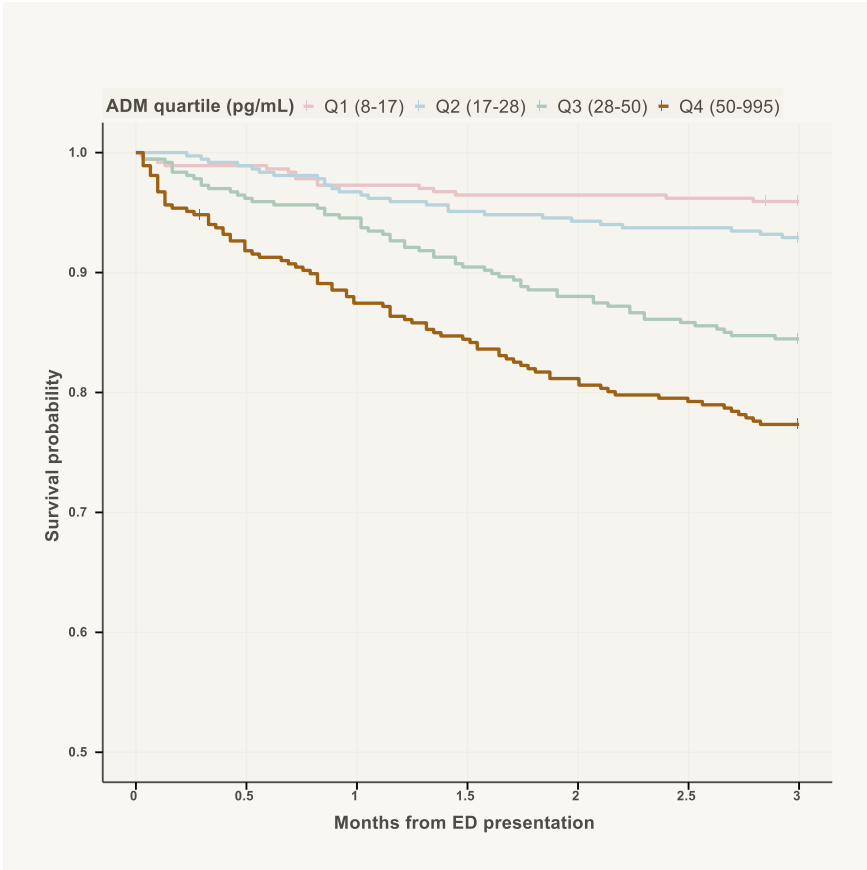

Figure 12. Kaplan-Meier estimates for 90-day mortality stratified by Adrenomedullin (ADM) quartile (Q1-4) in the ADYS cohort. Note cut Y-axis at 0,5. Adapted from Paper I[1].

Almost 60% (822/1402 participants) were admitted to hospital, with higher ADM associated with higher odds of hospitalization (adjusted OR: 1.5; 95% CI 1.2 to 1.8, $p < 0.001$). Similarly to 90-day all -cause mortality, the odds for hospitalization were higher in those with high compared to low ADM (adjusted OR: 2.8, 95% CI 1.8 to 4.3, $p < 0.001$).

Discriminatory capacity of ADM for 90-day mortality was evaluated by calculating areas under fitted ROC curves (AUC): (1) reference model2, (2) ADM alone and (3) a combined model of (1) and (2). The AUC for the reference model (1) was 0.79 (95% CI 0.76 to 0.82) and that of the combined model (3) was 0.80 (95% CI 0.76 to 0.83). Addition of ADM improved overall model fit compared with the base model, as indicated by the likelihood-ratio test ($\chi^2 = 9.8$, $df = 1$, $p = 0.002$). However,

[2]accounting for age, sex, CRP, S-Creatinine, NT-proBNP

the corresponding increase in AUC/C-statistic was small, suggesting limited incremental improvement in discrimination.

We also evaluated discriminatory capacity of ADM for hospitalization with similar results: AUC reference model (1) 0.80 (95% CI 0.78 to 0.83), combined model (3) 0.81 (95% CI 0.79 to 0.93) where overall fit was improved in the combined model ($\chi^2 = 14.1$, df = 1, p = 0.0002) with AUC difference indicating limited incremental improvement

Paper II

Of the 4809 individuals included from MPP, 768 were living with diabetes mellitus (16%), 1050 with obesity (22%) and 281 with both diabetes and obesity (6%). In MDC, the corresponding prevalence at the end of enrollment was 10% for DM (465 of 4878), 13% (612 individuals) for obesity and 6% (281 individuals) were living with both.

ADM cross-sectionally with diabetes and obesity

In a first step, the cross-sectional association between baseline ADM and DM and obesity in the cohorts was evaluated by fitting logistic regression models adjusted for sex and age. In both cohorts, baseline ADM was associated with diabetes (OR per 1 SD increment of log-transformed ADM in MPP: 1.55, 95% CI 1.44 to 1.67; in MDC: 1.22, 95% CI 1.11 to 1.33), obesity (OR in MPP: 2.17, 95% CI 2.01 to 2.34; in MDC: 1.64, 95% CI 1.52 to 1.78).

ADM and risk of developing diabetes

Baseline ADM levels were also associated with future diabetes risk with Cox regression models accounting for sex, age and metabolic risk factors3 Among MDC participants without known diabetes (Ncox = 4413), 705 individuals developed DM over a median follow-up period of 26 years, while 339 participants out of 4041 did so in MPP, with a median follow-up time of 9 years. In MDC, the association between ADM and future diabetes risk was weak (HR = 1.08, 95% CI: 1.00 to 1.16, p = 0.055). However, in MPP participants the association between baseline ADM and future DM was stronger than in MDC participants (MPP HR = 1.14, 95% CI: 1.01 to 1.29, p = 0.028). Kaplan-Meier estimates for diabetes incidence stratified by ADM-quartile in both cohorts is shown in Figure 13.

[3]categorical covariates: impaired fasting glucose, BMI, TG, HDL, CKD-stage and hypertension.

ADM-precursor peptides: MR-proADM, ADM-Gly and PAM

ADM-precursor peptides: MR-proADM, ADM-Gly and PAM AMA were also cross-sectionally associated with diabetes, obesity and future diabetes risk in the same manner as ADM.

MR-proADM was associated with both diabetes and obesity in both cohorts (MPP: diabetes OR per 1 SD increment of log-transformed MR-proADM = 1.40, 95% CI: 1.29 to 1.52, p < 0.001; obesity OR = 2.08, 95% CI: 1.91 to 2.26, p <0.001; MDC: diabetes OR = 1.49, 95% CI: 1.33 to 1.66, p < 0.001; obesity OR = 2.34, 95% CI: 2.09 to 2.61, p < 0.001; combined OR = 2.32, 95% CI: 1.93 to 2.79, p <0.001).

On the other hand, the overall association with increasing MR-proADM and future DM was difficult to pinpoint in both cohorts with confidence intervals indicating an imprecise point estimate (MDC: adjusted HR = 1.06, 95% CI: 0.97 to 1.15, p = 0.177 and MPP: adjusted HR = 1.15, 95% CI: 1.00 to 1.33, p = 0.053). However, in quartile comparison comparing participants with the highest MR-proADM values to those with the lowest were more likely to develop DM during follow-up (MDC Q4 vs Q1: adjusted HR = 1.34, 95% CI: 1.06 to 1.70, p =0.013 and MPP Q4 vs Q1: HR = 1.66, 95% CI: 1.14 to 1.41, p = 0.08).

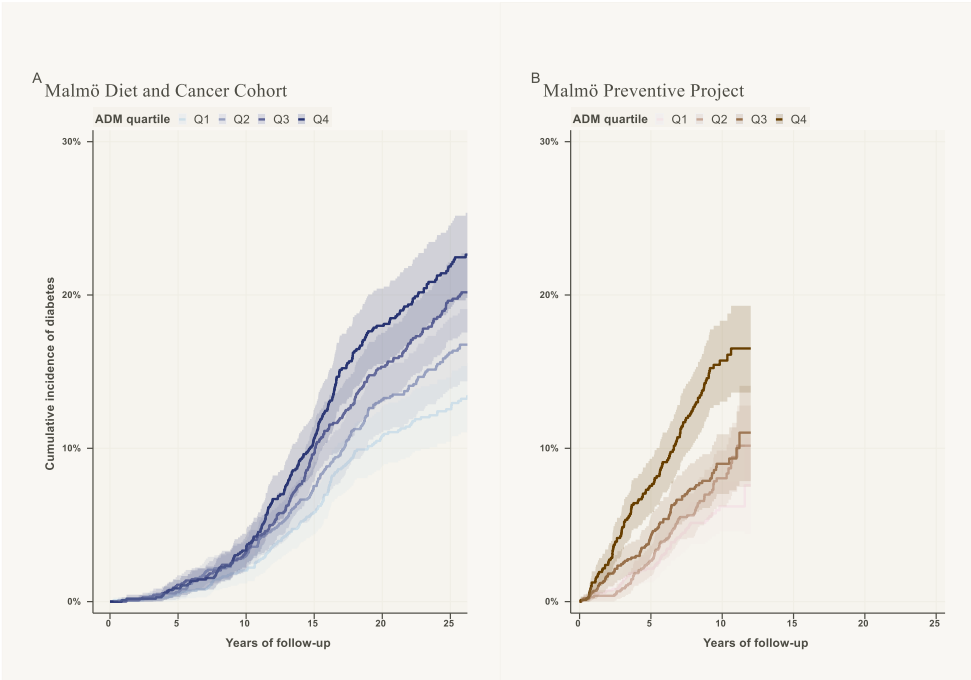

Figure 13. Kaplan-Meier estimates for incident diabetes mellitus in two large-scale population cohorts based in the Malmö region in Southern Sweden stratified by quartiles of ADM concentration. Note the difference in follow-up time. Adapted from Paper II[2].

ADM-Gly was only available for analysis in a sample from the MDC cohort (N = 4409). In contrast to ADM and MR-proADM, higher circulating ADM-Gly was associated with lower odds of both prevalent diabetes and obesity (per 1 SD increment of log-transformed ADM-Gly, diabetes OR: 0.87, 95% CI: 0.79 to 0.96, p = 0.008, obesity OR: 0.90, 95% CI 0.83 to 0.98, p = 0.021). Any concrete association between ADM-Gly concentration and future DM was not detected in this population (HR per 1 SD increment of ADM-Gly: 0.96, 95% CI 0.89 to 1.04, p = 0.33).

PAM AMA was measured in both MDC (N = 4878) and in a subset of the MPP sample (N = 4411). Notably, PAM AMA was universally inversely associated both diabetes and obesity outcomes in MDC (per 1 SD increment in PAM AMA diabetes OR: 0.81, 95% CI: 0.74 to 0.89, p < 0.001; obesity OR: 0.90, 95% CI: 0.82 to 0.97, p = 0.010). In MPP, higher PAM AMA was associated with lower odds of diabetes (MPP: OR: 0.88, 95% CI 0.81 to 0.96, p = 0.003), but the association with obesity was imprecise with the confidence interval compatible with decreased odds, no association, and increased odds, indicating a high degree of instability (OR: 1.04, 95% CI: 0.97 to 1.12, p = 0.3).

Increasing PAM activity was associated with a lower probability of future diabetes, even when accounting for multiple metabolic risk factors in the MDC sample (PAM AMA per 1 SD increment adjusted HR: 0.92, 95% CI: 0.85 to 0.99, p = 0.030). Whereas the effect was difficult to pinpoint in MPP even in the minimally adjusted model (HR = 1.06, 95% CI 0.94 to 1.20, p = 0.3).

Paper III

Variants of PAM gene associated with lower PAM AMA

Through exome wide sequence we identified two loss-of-function (LoF) variants in the PAM gene independently associated with decreased PAM AMA: (*a*) Ser5398Trp (rs78408340) and (*b*) Asp563Gly (rs35658696). LoF-variant carriers showed lower PAM AMA in both MDC-CC and MPP [expressed as 'per SD increment of log-transformed PAM AMA': in MDC-CC (*a*) -2.64, 95% CI: -2.38 to -2.41 and (*b*) -0.98, 95% CI -0.89 to -1.01; in MPP (*a*) -1.83, 95% CI -1.53 to -2.12 and (*b*) -0.82, 95% CI -0.70 to -0.94]. A weighted pooled aggregate score of (*a*) and (*b*) was then associated with diabetes and muscle mass-related measures where a one-unit increase in score corresponds to a 1 SD predicted change in PAM AMA from these variants in the MDC, MPP and UKB cohorts.

Association with diabetes and glucose homeostasis

Almost 1 in 10 were living with diabetes in the UKB cohort (~32,000/330,000). Individuals with a genotype associated with lower PAM AMA were more likely to have DM (OR: 1.18, 95% CI 1.15 to 1.20, $p = 9.17E^{-41}$), and other traits related to glucose homeostasis like fasting plasma glucose, HbA1c, Glucagon-like peptide-1 levels and reduced insulin concentration after. However, it was difficult to determine any directionality between PAM LoF genetic score and glucagon or gastric inhibitory polypeptide.

PAM loss-of-function alleles and sarcopenia

Among participants in UKB, 13,365 individuals (4%) showed a phenotype of sarcopenia with low lean muscle mass and grip strength. Higher PAM LoF genetic score was associated with higher probability of sarcopenic phenotype (OR per increase in PAM LoF genetic score: 1.08, 95% CI 1.04 to 1.13, p = 5.85E-5). However, the overall genetic score association was mainly driven by variant a in this sample (OR for presence of variant a: 1.31, 95% CI 1.16 to 1.47, p = 9.82E-6), while the association of variant b was imprecise (OR for presence of variant b: 1.03, 95 CI 0.97 to 1.09, p = 0.25). The association between the PAM LoF genetic score with a sarcopenic phenotype was not meaningfully modified neither DM (β of interaction between genetic score and DM: -0.008, SE 0.007, p = 0.265) or age (β of interaction between genetic score and age: 0.002, SE 0.003, p = 0.493), and was only detectable in the subset of participants without DM (N = 299,194; β of genetic score association with sarcopenia: 0.082, standard error 0.02, p = 7.60E-5).

Associations with other endocrine systems

PAM LoF genetic score was also further evaluated in relation to other hormones relevant to growth and metabolism. PAM LoF genetic score was associated with growth hormone (GH) in MDC-CC, insulin-like growth factor 1 (IGF-1) in UKB, which are also implicated in insulin resistance and DM.[149]

On the other hand, we could not discern any apparent directionality between PAM LoF genetic score and secretory granule peptides (NT-proBNP and MR-ANP) which were evaluated in MDC-CC (N=4653) and MPP (N=4769), noting very imprecise point estimates with wide confidence intervals.

Paper IV

The MDC sample (N=2674) analyzed was predominantly female (61%) and 56 years of age (IQR: 52 to 61). Study participants were followed for 16.5 years on average (IQR: 15.4-17.8).

Measurements of body composition and insulin resistance

Median body measurements among females were: BMI 24.6 kg/m2 (22.6 to 27.3 kg/m2); Waist-to-hip ratio (WHR) 0.78 (0.75 to 0.81), Waist-to-Height ratio (WHtR) 0.46 (0.43 to 0.50), waist circumference (WC) 75 cm (70 to 81 cm); and as a proxy for insulin resistance median HOMA-IR was 1.39 (0.91 to 1.97). Corresponding measurements among males were: BMI 25.7 kg/m2 (23.8 to 27.9 kg/m2), WHR 0.94 (0.90 to 0.97), WHtrR 0.52 (0.49 to 0.56), WC 91 cm (86 to 98), median HOMA-IR 1.66 (1.11 to 2.38).

Changes to body measurements during follow-up re-examination were on average generally small: ΔBMI 1.2 (-0.2 to 2.7), ΔWHR 0.04 (0.01 to 0.09). HOMA-IR increased on average between recruitment and follow-up examination (ΔHOMA-IR 0.49, IQR: -0.07 to 1.23).

ADM association with change in body composition and insulin resistance

In a linear regression model adjusted for sex, age and years of follow-up, ADM was associated with follow-up BMI (adjusted β per doubling: 1.11, 95% CI 0.83 to 1.39, $p < 0.001$), but the association was attenuated after also adjusting for baseline BMI (BMI-adjusted β per doubling: -0.121, 95% CI -0.27 to 0.03, $p = 0.106$).

The association between ADM with WHR differed between sexes[4]. In sex-stratified linear regression models adjusted for age and years of follow-up, ADM was associated with future WHR only in females, also when accounting for baseline BMI (BMI-adj β per doubling in females: 0.006, 95% CI 0.002 to 0.011, $p = 0.006$; and in males: -0.002, 95% CI -0.008 to 0.005, $p= 0.579$).

ADM was associated with HOMA-IR in a non-linear[5] fashion. Increase in HOMA-IR was concentrated between the first to the second quartile of ADM, with a flatter trajectory in the upper range of the distribution (Q2–Q4). In a minimally adjusted

[4]interaction term ADM and male sex, $p = 0.004$

[5]revealed by functional form analysis using restricted cubic spline modelling

model[6], the Q2 vs Q1 [15.9 pg/mL] contrast was (β: 0.059, 95% CI 0.026 to 0.092, p < 0.001), with Q3 [20.5pg/mL] (β: 0.087, 95% CI 0.042 to 0.132, p < 0.001) and Q4 [29.6pg/mL] (β: 0.102, 95% CI 0.049 to 0.155, p < 0.001). Additional adjustment for baseline BMI attenuated the Q3 and Q4 contrasts (Q3: β 0.032, 95% CI -0.002 to 0.065, p = 0.064; Q4: β 0.019, 95% CI -0.021 to 0.059, p = 0.347) to the point where the precision was insufficient to exclude null effect, while the Q2 contrast was slightly attenuated (β: 0.026, 95% CI 0.001 to 0.051, p = 0.039).

Individuals with an ADM level at the 75th percentile compared to the 25th percentile in the sample were more likely to have lower BMI at follow-up (ΔBMI at follow-up: -0.095 kg/m², 95% CI -0.210 to 0.020). The corresponding comparison for follow-up WHR yielded a change at follow-up of 0.0047 (95% CI 0.0013 to 0.0081) in women and -0.0017 (95% CI -0.0052 to 0.0017) in men, with the male estimate confidence interval being wide and indicating imprecision. For follow-up HOMA-IR, the Q3-versus-Q1 contrast was 1.2% increase in HOMA-IR (95% CI -1.0% to 3.4%).

ADM-system precursors and PAM AMA with changes in body composition and HOMA-IR

We further examined associations of ADM precursor peptides, MR-proADM and ADM-Gly, as well as PAM AMA using the same most-adjusted model specification[7] as for mature ADM. MR-proADM was associated with follow-up BMI, HOMA-IR and WHR (in females only) [BMI β per doubling: 0.471, 95% CI 0.195 to 0.746, p-adj = 0.003; log(HOMA-IR): 0.060, 95% CI 0.009 to 0.111, adj-p = 0.031; WHR in females: 0.010, 95% CI 0.003 to 0.018, adj-p = 0.021]. This association was robust to joint modelling with all other ADM-peptides (mature ADM, ADM-Gly, PAM) and to correction for multiple testing. The directionality of MR-proADM associations were consistent with those of mature ADM.

On the other hand, PAM showed inverse associations with follow-up HOMA-IR, and WHR in males only, with the HOMA-IR association also remaining after correction for multiple testing (log(HOMA-IR, β per doubling: -0.103, 95% CI -0.167 to -0.040, p = 0.001, p-adj = 0.004; WHR in males: -0.010, 95% CI -0.019 to -0.001, p = 0.030, p-adj = 0.089). Consistent directionality with higher PAM-activity being associated with protective effects for excess adiposity and insulin resistance, but not obesity [Excess adiposity in males OR per doubling: 0.38, 95% CI 0.18 to 0.78, p = 0.009, p-adj = 0.027]. The association of PAM with excess adiposity in females was modest in isolation [OR per doubling: 0.75, 95% CI 0.51 to 1.10, p = 0.139, p-adj = 0.209], but became more apparent in the jointly adjusted

[6]age, sex and baseline HOMA-IR

[7]sex, age, follow-up years for continuous outcomes only, and baseline measurements

model [OR per doubling: 0.67, 95% CI 0.45 to 1.00, p = 0.049, p-adj = 0.065], consistent with at least a partial suppression effect where including ADM-cascade markers as co-variates removed shared variance, isolating the independent PAM signal.

Confidence intervals for precursor peptide ADM-Gly associations were narrow and involved zero for all outcomes.

A summary of ADM and precursor peptide markers including PAM activity associations with anthropometric measures and HOMA-IR at follow-up can be seen in **Figure 14**.

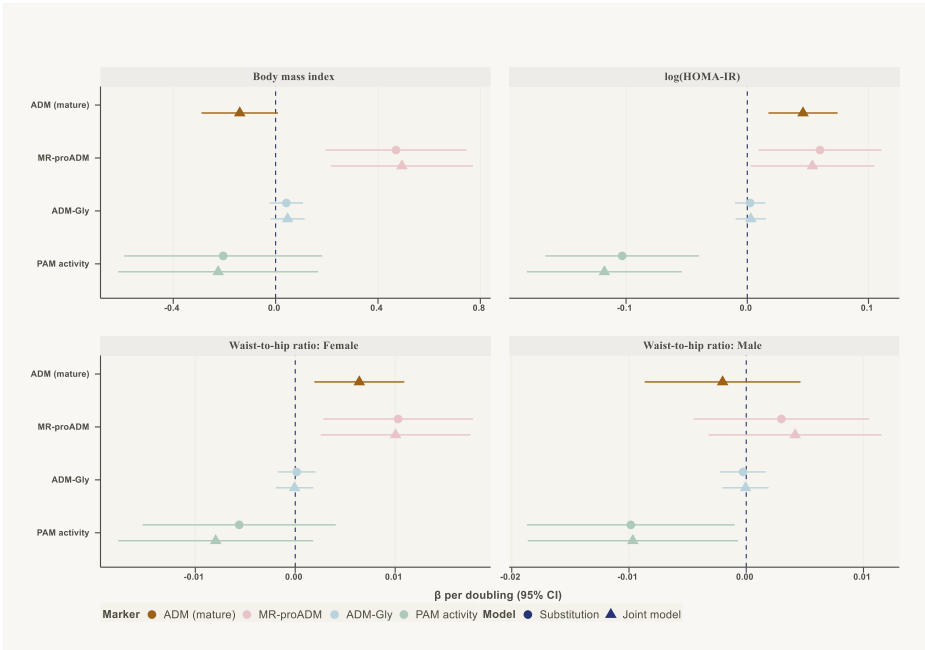

Figure 14. Forest plot showing Beta coefficients for body mass index, waist-to-hip ratio by sex and log-transformed HOMA-IR at follow-up per doubling of baseline ADM-cascade markers and PAM activity (Q3 vs Q1). Estimates are shown from both substitution analysis (circle) and joint modelling (triangle) are shown. Adapted from Paper IV[4].

Main Findings

A schematic summary of Adrenomedullin-system markers in each respective setting and the relevant outcomes is seen in **Figure 15.**

- ADM is associated with 90-day mortality and hospitalization in patients presenting with shortness of breath to the ED and is more closely associated with pulmonary congestion than other causes of dyspnea.
- ADM-measurement contributes unique information in a prognostication model of 90-day mortality on top of other clinically established risk-factors in patients with acute shortness of breath, but the overall gain is marginal and unlikely to be clinically significant.
- ADM and its stable prohormone fragment MR-proADM are cross-sectionally associated with DM and obesity at the population level, rather than predicting future changes in measures of adiposity, while we have insufficient understanding of how the precursor ADM-Gly associates with these conditions.
- People genetically predisposed to lower PAM AMA had higher risk of DM and lower muscle mass. PAM AMA is important for and serves as a signal for metabolic fitness independently from the ADM-system at the population level.

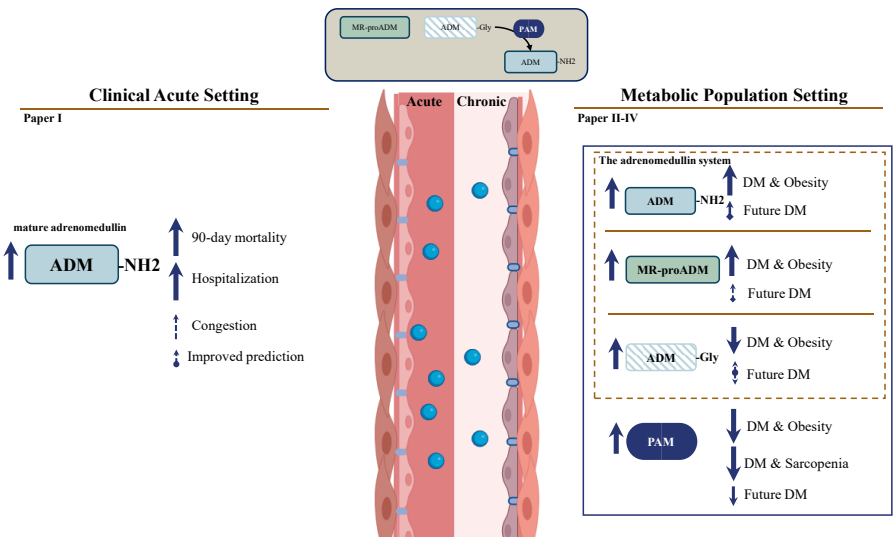

Figure 15. Schematic of summarized key results from included papers categorized by main setting and context. Bloodstream graphic in center created in BioRender. Figure created in Microsoft Power Point.**[AI[5]]**

Discussion and Synthesis

Vasculature as the playing field

The papers in this thesis approach ADM as a peptide signal of stress at the vascular endothelial wall. This signal is relevant in more than one clinical and biological setting. In the acute setting, ADM may reflect rapid vascular and interstitial fluid shifts during decompensated illness [72, 150].At the population level, the same peptide system may also index slower, chronic processes related to metabolic risk, adiposity, insulin resistance, and vascular dysfunction[30, 65, 151]. The unifying claim is thus not that ADM represents one pathological process or a single mode-of-action across all contexts. Rather, the thesis examines whether ADM-related biology can be understood as a recurring signal across distinct states of vascular and metabolic stress.

Paper I is situated at the acute end of this frame. It asks whether ADM has clinical utility in patients presenting with acute shortness of breath, where endothelial stress through fluid congestion, inflammation, and alterations in fluid distribution converge at the bedside. **Papers II** and **IV** move the question into the chronic population setting. They examine ADM in relation to metabolic traits and their development over time, shifting attention from acute clinical discrimination to longer-term associations with cardiometabolic risk. **Paper III** sits at a slight angle to this structure. It does not primarily address ADM as a circulating signal, but rather the PAM enzyme, required for the amidation and biological activation of a breadth of peptide hormones, including ADM. Its contribution is therefore indirect but conceptually relevant: it concerns the enzymatic machinery that may shape peptide bioactivity rather than the ADM signal itself.

This distinction matters because ADM can be read through a marker to mediator lens. As a marker, ADM may reflect underlying vascular, renal, inflammatory, or metabolic stress without necessarily being causally responsible for the observed outcomes. As a mediator, ADM may participate in the biological response to such stress, through effects on vascular tone, endothelial barrier function, natriuresis, inflammation, or tissue perfusion. ADM is plausibly both a sensor of physiological strain and an active peptide signal within compensatory or maladaptive responses. The purpose of the lens can be viewed in a pragmatic way: to provide a perspective to interpret what each paper can and cannot support.

Within this thesis, **Paper I** most clearly supports a marker interpretation, evaluating ADM as a bedside indicator in acute dyspnea. **Papers II** and **IV** occupy a bridging position, because longitudinal and population-based associations may suggest biological relevance but cannot, by themselves, distinguish causation from tracking, confounding, or reverse causation. **Paper III** contributes to the mediator side only indirectly, by focusing on PAM and peptide maturation rather than ADM concentrations or ADM-related outcomes directly. The stronger mediator argument for ADM thus rests mainly on external mechanistic and experimental literature, not on this thesis alone.

The thesis therefore positions ADM less as a disease-specific biomarker and more as a context-sensitive vascular stress signal, with potential relevance for both clinical risk assessment and mechanistic interpretation.

The role of ADM stretches from acute to chronic settings

In **Paper I**, addressing shortness of breath as a pathophysiological expression of acute vascular stress, we show that higher ADM in mildly-to-moderately dyspneic ED patients predicts 90-day mortality and hospitalization, and in extension worse prognosis. This finding is in line with previous studies evaluating ADM as a prognostic marker in the acute setting, however most of these studies have a scope focusing on more critically ill individuals, such as individuals with cardiogenic or septic shock[71, 152]. The paper contributes to the current understanding by extending that scope to a wider population of less-severely ill and undifferentiated population. However, it did not show any clear added value over other already established plasma biomarkers in the same setting (CRP, creatinine, NT-proBNP). It also explored an association with endpoints like use of diuretic therapy and heart-failure discharge diagnosis, which relate more definitively to vascular stress and containment failure, which could be interesting to look into more closely in future research.

In the setting of acute decompensation, the fact that a peptide hormone like ADM, closely related to vascular integrity, is elevated is hardly surprising. But, how about the same molecule being measurable, and carrying information, in individuals who are not acutely unwell at all: a middle-aged general population followed over many years, with no presenting complaint and no apparent health event to anchor the measurement. If ADM reflects only an acute episode, it should fall silent here. The following sections turn to that population, and defer until later the question of why a single molecule is relevant in both places.

Chronic metabolic stress and its association with ADM is the focus of **Papers II** and **IV**. **Paper II** shows that ADM is cross-sectionally associated with both DM and obesity, while also being associated with increased risk of future diabetes. In addition, it clarifies how the associations of precursors MR-proADM and ADM-Gly

as well as PAM are positioned in the chronic metabolic context. ADM as a marker of disrupted glucose metabolism and diabetes, has been proposed before[75, 77, 153], and the paper's primary contribution should be viewed as reproducibility attributed to the fact that the association was seen in both Malmö cohorts. Interestingly, the leading body of evidence regarding the ADM-system in a metabolic context is based on studies where MR-proADM is the exposure ADM-variable, rather than mature ADM[154-156]. There are multiple reasons why MR-proADM is an operationally preferable exposure ADM-marker in this setting, at least partly attributable to plasma stability with MR-proADM having a significantly longer half-life compared to mature ADM[87].

The cross-sectional picture from **Paper II** is therefore one of a peptide hormone which is reliably raised where metabolic strain is already present: associated with obesity and diabetes. A natural next expectation is that a marker raised alongside present disease could also predict its development, that a higher baseline concentration could indicate future weight gain and perhaps progressive insulin resistance.

The findings in **Paper IV** do not support this characteristic: baseline ADM concentration does not track subsequent overall weight change to any meaningful degree (**Paper IV**). The cross-sectional and longitudinal findings do not have to be seen as contradictory. A single ADM measurement may mainly reflect a person's current metabolic state, rather than predict future change. In that sense, the marker shows where an individual stands at the time of measurement, not where they are heading.

At the same time, **Paper IV** reveals a non-linear relationship between ADM and HOMA-IR, as a proxy for insulin resistance, but the attenuation pattern when also accounting for baseline BMI likely tells the tale that the flatter upper-end signal of the ADM-IR association is carried by adiposity, and as a result, ADM should not be viewed as a clean reflection of IR.

ADM giving notice on where the fat goes

Against the broadly null longitudinal picture, there is one standout finding worth discussing. Baseline ADM did not predict change in BMI (**Paper IV**), but it independently predicted an increase in WHR, albeit only in women. The effect reflected significant modification by sex rather than a uniform association (**Paper IV**). In other words, ADM did not provide any notice on how much adiposity an individual would gain but did so, in women only, on where it would be distributed.

These findings are consistent when read together. A cross-sectional signal whose insulin-resistance association is substantially adiposity-carried, and a longitudinal signal that predicts central redistribution rather than overall gain, are more naturally described as features of an adiposity-distribution marker than of an insulin-

resistance marker. The metabolic information mature ADM carries appears to primarily concern the distribution of adipose tissue, and its vascular demand, more than glucose dysregulation directly. The reading is compatible with an origin in richly vascularized visceral adipose tissue, though the present data establish the association, not the source.

On sex-specificity and fat redistribution

The sex-specificity of the WHR result invites an explanation that is taken up more fully among the limitations. Central fat redistribution is itself a characteristic feature of the menopausal transition, which is why an association between ADM and increasing WHR in women may in part track an age-related and hormonal process rather than ADM-driven one[157, 158]. The finding is reported here as a positive longitudinal signal; whether it survives that confounding is a question its current form cannot answer.

Inverse associations of ADM-Gly and PAM activity

Interpreting the ADM-Gly findings in **Paper II**, inverse association with prevalent diabetes and obesity in the MDC sample, requires first placing the peptide in the maturation cascade as the immediate substrate of the amidating step: PAM converts it to mature ADM. As a consequence, the concentration of the precursor measured at a single point reflects not only how much is produced but also how fast it is being depleted through conversion. Since the precursor is itself biologically inactive, its circulating level is best interpreted as a thermometer of ADM-pathway activity rather than as an independent signal.

Seen this way, the inverse cross-sectional association of ADM-Gly with DM and obesity (**Paper II**) makes sense. Under metabolic stress the ADM-system is activated and mature ADM, with its stable surrogate MR-proADM, are elevated (**Paper II**), ADM-Gly is drawn through the amidating step by this increased demand and its circulating level falls. As a result, the depletion is therefore best attributed to upstream activation of the system pulling substrate toward the mature peptide, rather than to any increase in PAM activity. A lower precursor concentration together with a higher mature peptide concentration is the expected outcome of this demand-driven switch, not a separate phenomenon requiring its own explanation.

Another finding in **Paper II** complicates this interpretation. PAM activity was also inversely associated with DM and obesity in the same sample ADM-Gly was measured (the MDC Cohort). Against the reasoning given above, it is undeniable that it gives rise to tension: if the enzyme that matures ADM is less active in the strained state, one might expect its substrate to accumulate rather than to deplete. The two precursor-side findings appear, on first inspection, to point in opposite directions.

It can be explained by PAM activity not functioning as a proxy of ADM-pathway activation in this situation. PAM amidates a wide variety of peptide substrates, of which ADM-Gly is only one[159]. Its activity is therefore better understood as a global indicator of amidation capacity. This perspective resolves the inverse association of PAM and the demand-driven depletion of ADM-Gly, since they do not measure the same reaction. This cross-sectional pattern together with the genetic findings of **Paper III**, considered later, is what makes the interpretation of PAM activity as an independent signal more than a convenience. The evidence regarding PAM activity as an independent entity is taken up, because what it establishes reaches beyond the ADM-system.

Context-dependence of the ADM-system

The ADM-related findings in **Papers I**, **II** and **IV** point toward a reconciled signal between the acute and chronic settings. In the acute setting, ADM is a strong response to vascular containment failure leading to fluid congestion, for example pulmonary edema and subsequent dyspnea, while it should be viewed as a contributor to signaling failure in a low-intensity chronic metabolic context, like in diabetes and fat redistribution.

This difference in magnitude between acute and chronic ADM-system activation is important to keep in mind. ADM has a circulating half-life of only 22 minutes, while the metabolic conditions like diabetes and obesity studied in **Papers II** and **IV** unfold over several years. The conventional concern dictates that such a short-lived molecule just captures a volatile, transient snapshot of physiology. But this view conflicts with two different situations. An acute crisis, like in acute dyspnea (**Paper I**) or septic shock (AdrenOSS-2) and a chronic disease state like diabetes or obesity (**Papers II** and **IV**). In acute conditions, like sepsis or heart failure, many tissues may be under severe strain simultaneously. In response to this stress, their vascular beds secrete an intense burst of ADM into the blood, and as a result, high ADM levels circulate in plasma.

Chronic disease is different, where affected tissue secretes ADM in smaller magnitude persistently. As the half-life is short, the elevated concentration may only be maintained if the peptide is secreted continuously. Which is why the elevated ADM-concentration in the chronic metabolic context cannot be interpreted as a temporary spike, but a sign of raised baseline secretion rate driven by chronically strained tissue. For example, in obesity, hypertrophied adipocytes may be battling chronic hypoxia and inflammation with the elevated ADM concentration may thus reflect this persistent stress signal.

This difference in ADM-pathway activation is also observed between the studied cohorts, where individuals in the ADYS cohort have generally higher ADM-values than individuals in the Malmö cohorts (**Papers I**, **II**, **IV**). In **Paper I**, a cutoff of

29pg/mL is proposed to be statistically optimal with regard to balancing sensitivity and specificity in that sample, however, it is unlikely to be transferable to the general population cohorts, given how ADM values are shifted in the non-acutely ill individual. Another example reinforcing the idea of proportionality is the design of a phase II trial (AdrenOSS-2)[160], which evaluated a non-neutralizing ADM-specific antibody which prolongs plasma-half life and intravascular ADM-signaling in critically ill patients with septic shock who became eligible when plasma ADM was higher than 70 pg/mL, a value far from those observed in both ADYS and Malmö cohorts.

ADM as part of a multi-purpose response

The findings and current evidence landscape points toward ADM being a multi-purpose peptide, like a biological swiss-army knife. One edge represented by acutely mobilized ADM as part of a response with the purpose of redirecting and restoring organ-level containment failure, and the other edge contributing to a detrimental metabolic spiral under chronic low-grade stress. An important limitation of the observational findings of **Papers II** and **IV** is that any causality cannot be established. Elevated ADM in individuals with obesity and insulin resistance is equally consistent with the marker reflecting an established metabolic state and with that state having led to further ADM release, and nothing in a cross-sectional or a tracking design can differentiate between them. This is usually presented as a limitation. Here, a plausible interpretation is not that one direction of the relationship is real, and the other is misleading but rather likely bidirectional where the processes may influence each other.

The relationship between adiposity and ADM is well supported at the tissue level. Expanding adipose tissue becomes hypoxic, and hypoxia drives adrenomedullin expression[161], adrenomedullin is correspondingly overexpressed in the adipose tissue of obese individuals [162]and is implicated in adipocyte lipid metabolism[163] and also in the lipid dysregulation of metabolically stressed adipose tissue[164]. Obesity, on this evidence, raises the signal rather than merely coinciding with it.

What makes the relationship self-reinforcing rather than a one-way response would be the consequences of ADM signaling within vascular beds of strained tissues. ADM is recognized to be important for new vessel formation[165] and a target pursued on that basis[166]. Adipose tissue cannot expand without a matching expansion of its vascular supply, so a signal secreted by hypoxic adipocytes that in turn promotes the vascularization permitting further expansion closes a feed-forward loop: more adipose mass, more local hypoxia, more adrenomedullin, more vascular support, and more mass. The biology behind the separate steps is well established. A more speculative claim is that these steps form a self-sustaining loop

that promotes fat tissue expansion over time. The presented data are consistent with that idea, but do not demonstrate it directly.

A second effect which runs parallel to and does not fit into the growth spiral has to do with insulin signaling. Independent of any effect on fat tissue, ADM has been reported to inhibit endothelial insulin signaling[86], a route by which chronically elevated ADM could dysregulate the metabolism directly rather than by enabling fat accumulation. The growth loop and the insulin-signaling consequence share ADM as a common denominator. Both are highly relevant when looking at ADM in the chronic context: a signal that helps sustain the tissue generating it and degrades the vascular insulin response. This mechanistic deduction cannot be established through data in this thesis and relies solely on external mechanistic studies.

PAM activity is an independent entity from the ADM-system

As mentioned briefly earlier, the findings regarding PAM activity and metabolic outcomes in **Paper II** being directionally opposite of those of ADM and MR-proADM indicate that it might not be the ADM-pathway activity proxy indicator at the population-level as was first hypothesized. Instead, perhaps the larger scale signal captured is the sum of all PAM-related functions, rather than merely ADM:s activating enzyme.

Among the studies in this thesis, the design of **Paper III** is the only approach that provides the only evidence that comes close to addressing causality regarding PAM activity and metabolic health outcomes. In **Paper III** the design relies on identified genetic variants which are combined into a score used as a proxy for lifelong differences in PAM activity. Because these genetic differences are fixed from birth, this design is less vulnerable to reverse causation[167]. As an example, diseases developing later in life cannot change an individual's inherited genotype.

The phenotype that emerged among carriers of genetic variants associated with lower PAM activity in **Paper III** was consistent with increased risk of diabetes and lower muscle mass. Now, it is highly unlikely that these findings are not exclusively ADM-related, given the wide span of possible peptide substrates amidated by PAM and may also involve the reported extra-catalytic function.

A major reason to study the ADM maturation cascade and analyze precursor peptides in addition to the mature ADM peptide was the potential implications of modulating the maturation step of the ADM-pathway. Yet lower PAM activity yields a sarcopenic, and not a beneficial, phenotype (**Paper III**).

Nevertheless, the choice of studying a phenotype related to muscle mass, and in extension sarcopenia, in **Paper III** different from that studied in **Papers II** and **IV**, which revolved around diabetes and obesity, might come across as opportunistic.

The rationale was that given PAM:s links to insulin and other anabolic hormones like growth hormone[168], the sarcopenic phenotype is biologically relevant. Low muscle mass is also a known important morbidity-increasing complication among individuals living with diabetes[169] Finally, the biological and clinical relevance combined with the availability of such data at scale in UKB which allows for statistically robust testing of the association, all align to motivate widening the scope beyond diabetes and obesity in **Paper III**.

Altogether, PAM activity emerges as a beneficial metabolic factor, its amidation capacity broadly supporting metabolic health, rather than through one peptide pathway, like ADM-activation.

Limitations

Association but not cause

A major limitation of this thesis is the risk of reverse causation. Because the thesis relies mainly on observational association data, it cannot fully establish whether the ADM-system peptides contribute to disease, or whether preclinical disease processes have already influenced what we measure in plasma. This is particularly relevant in the cross-sectional data, because the exposures were measured and registered either at the same time as the outcomes or only shortly before them. The possibility of subclinical disease having influenced the ADM-system marker levels is real. That is why the findings should not be read as direct causal evidence. Associations were found through statistical modelling, but they cannot prove the direction of influence between exposure and outcome or mechanism. Any causal interpretation in this thesis is based on evidence published by others involving study designs where claims of causality are more feasible, such as Mendelian randomization studies, pre-clinical mechanistic models, and intervention trials where the order of exposure and outcome is more clearly defined.

Effect size interpretability

Beyond the constraints of observational study design, another limitation lies in the clinical interpretability of reported effect sizes. Standardizing and log-transforming variables may be essential to satisfy statistical assumptions and mitigate biological skewness seen in peptide concentrations, as described previously in the Methodology chapter. But while this approach maximizes statistical power, the resulting effect estimates expressed as odds or hazard ratios "per 1 standard deviation (SD) increment in log-transformed peptide" lack intuitive clinical meaning. Transformation obscures the absolute biological scale, making it difficult

to understand how a 1 SD shift translates to a difference in the geometric mean or in units actually measured, like pg/mL. While these standardized estimates are valuable for identifying robust biological signals and comparing relative peptide strengths, they naturally limit the immediate translational utility and direct clinical application of the findings. Even if **Paper IV** shows intent of tackling this issue, choosing log2-scale for per doubling interpretation and predicted probability plots, the predominant form of reporting effect sizes throughout the papers in the thesis is based on dual transformation.

The alluring effect of large sample sizes

Yet another notable limitation is the modest absolute magnitude of the observed effect sizes and changes, perhaps most clearly appearing in **Paper IV**, where ADM-peptides and longitudinal changes in body measurement were modelled in linear regression models. Even if many peptide associations yielded statistical robustness, the individual effect sizes remain generally small. In the context of large-scale population cohorts, high statistical power can often surface minor signals that may not at all be clinically relevant in isolation. Even if this should not be too surprising when evaluating complex, multifactorial conditions like DM and obesity. A single circulating peptide, no matter how biologically crucial, does not operate in a vacuum. It interacts with redundant biological reality characterized by pleiotropic effects and receptor crosstalk with structurally similar peptides (such as ADM2 and CGRP). The signal from a single biomarker is often weakened by the many other metabolic pathways active at the same time, like crosstalk with ADM2 or CGRP which belong to same peptide-superfamily[52].

Clinical and therapeutic implications

From the perspective of bedside reality

Any Emergency physician or other colleague early in their career hoping to get a new reliable biomarker to help determine how to manage and prioritize the mildly dyspneic patient, ADM is unlikely to be of major benefit. Especially when comparing to progress in modalities other than blood sampling for bedside clinical decision-making, like the adoption of point-of-care ultrasound (POCUS). ADM:s relevance is likely higher in the setting of critical care, which is not included in any paper in this thesis.

A significant appeal of mature ADM is the fact that there is an assay available for bedside testing, in combination with a directed treatment in Enibarcimab, which appears safe but still without hard endpoint-data of any benefit[160]. Even if the chronic metabolic context dominates the contents of this thesis, while Enibarcimab is indicated in septic shock, it shows the ADM-system has therapeutic potential with

a plausible mechanism in the metabolic setting was brought to light by Cho and colleagues in 2025[86] where they show ADM inhibits endothelial insulin signaling.

Continuing within the chronic metabolic context, PAM has emerged as an interesting therapeutic target due to its unique role as the only known amidating enzyme and proposed beneficial metabolic effects [99, 170]. Amidst this scientific interest, a group led by Anna Gloyn recently published a landmark study which found that carriers of two specific genetic defects in the PAM-gene have GLP-1 resistance, where levels of the hormone glucagon-like peptide-1 (GLP-1) are higher, but less biologically effective at controlling blood glucose[171]. Their work also shows that the mechanism at work is related to downstream signaling as testing verified that the PAM defect does not impair receptor binding or direct receptor signaling. Further, they conducted a meta-analysis of three clinical trials of more than 1000 participants with DM where data demonstrated a clear disparity in treatment response after six months of GLP-1 receptor agonist therapy in individuals carrying these genetic variants. Interestingly, the findings were specific to GLP-1 receptor drugs, since carriers showed normal response to other standard, non-GLP-1 antidiabetic medications, including metformin, sulfonylureas, and DPP-4 inhibitors. The work by Anna Gloyn and colleagues is congruent with the interpretation of PAM activity in **Paper III**, where it is proposed that PAM ought to be seen as a marker of metabolic fitness at the population-level.

Conclusions and future perspectives

Weighing together the findings presented throughout the papers in this thesis and current knowledge on ADM, there is little doubt that it should be viewed as a context-dependent marker of metabolic stress at the vascular endothelial wall, and where the triggers and consequences of its biological activity vary by clinical situation and tissue type.

At the beginning of the journey in 2018, the hypothesis centered around mature ADM functioning as a generally protective compensatory response. Nevertheless, the evidence landscape has shifted toward a detrimental role of ADM in the metabolic context, while PAM has emerged as a promising candidate target where exact downstream pathways responsible are yet to be understood. A more complete understanding of cellular signaling pathways and mechanisms involved in, for example, GLP-1 resistance could pave the way for genetic screening to match patients with optimal therapies faster.

A complementary study to further improve our understanding of how it affects metabolism, an interesting interventional design would be to infuse ADM at various rates in individuals with different body composition, for example in lean, normal and overweight, and study the effects on glucose homeostasis including insulin-related measures. Alternatively, given the Mendelian randomization-like approach

in **Paper III**, proceeding with identifying genetic variants associated with skewed mature ADM concentration and associating these with metabolic outcomes and trajectories as an attempt to address the limitations inherent to observational study design.

Closing remarks

I began this scientific journey with a more certain, and probably more naïve, view than I now hold. I thought that if a biomarker could be measured precisely enough, it would eventually return a clear answer which in turn would extend to a clearcut action. Treat with this drug. Admit to this level of care. What this thesis has taught me instead is that a biomarker earns its place slowly. An observed association is only the beginning. Between that association and something that should change what a clinician does lies a long and arduous process, which only a few biomarkers survive.

ADM has been a fitting example through that lesson. It reads differently in the patient with acute decompensation than it does in the same person years earlier, apparently well and moving quietly with controlled chronic disease. Its meaning is not fixed by the assay, but by the clinical and biological context in which it is interpreted. In that sense, it resembles patients we meet at a daily basis at the department of Internal Medicine. Never defined by a single, but by several chronic diseases, and one or more acute disturbances, and a question that is never only what can be measured, but what should be done.

I am left more humbled about biomarker research than when I started, but I think that has made me a better clinician and a more careful researcher. The question has never been which test can answer everything. But rather which test, for this patient, in this situation, is worth asking and whether we are prepared to act on the result.

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