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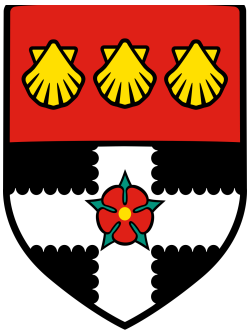

Translational Medicine for Cardiometabolic Disorders

A thesis submitted for the degree of Doctor of Philosophy

by

Andrew John Krentz MB ChB MD FRCP FRCPath FFPM FRSM FRSA

To my family.

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Acknowledgements

The original clinical research papers on which this thesis is based illustrate phases of my clinical research career as it progressed from its starting point in experimental medicine focused on the role of insulin in human disease to cardiometabolic epidemiology, early phase clinical trials, and real-world data studies using machine learning methodology.

The studies were performed in collaboration with many esteemed academic and life and data science colleagues at leading clinical research institutions in the UK and USA. I wish to express particular gratitude to the following individuals as mentors and colleagues: the late Elizabeth Barrett-Connor MD, Distinguished Professor at the University of California San Diego, USA; Chris Byrne PhD, inaugural Director of the Wellcome Trust Clinical Research Facility at the University of Southampton, UK; Marcus Hompesch MD, Founder and Chief Executive Officer, ProSciento, San Diego, USA; Richard Barker PhD OBE, Co-Founder of Metadvice and Visiting Professor at King's College London, UK; and Andre Jaun PhD, Co-Founder and Chief Technical Officer at Metadvice. Professor Barker's well-received book '*Bioscience: Lost in Translation? How precision medicine closes the innovation gap. Oxford University Press, 2016* was an important influence on the origins of this thesis. Dr Jaun merits special acknowledgement for his expert insights and expertise in machine learning which we have applied collaboratively to non-communicable cardiometabolic disorders. The list of valued collaborators would not be complete without acknowledging the support of Serge Umansky PhD, Co-Founder of Metadvice and Vasa Curcin PhD, Professor of Health Informatics and Head of Population Health Sciences at King's College London, UK. Numerous research fellows, including master's degree students from École Polytechnique Fédérale de Lausanne (EPFL) who I co-supervised with Dr André Jaun, contributed directly or indirectly to some of the studies. I also acted as PhD co-supervisor to Tianyi Liu at King's College London with whom I collaborated on machine learning studies cited in this thesis.

Jon Gibbins PhD, Professor of Cell Biology and Director of the Institute of Cardiovascular and Metabolic Research, University of Reading provided much appreciated expert supervision and unstinting support during the conception and execution of the thesis.

The financial support of the British Heart Foundation, the Wellcome Trust, Innovate UK and King's College London is gratefully acknowledged. Other financial support is documented where appropriate in the reprinted original research papers.

Declaration of Authorship

I confirm that this is my own work. Any use of material from other sources has been properly and fully acknowledged.

No generative artificial intelligence was employed in the writing of this thesis.

Signed: Andrew John Krentz
Date: 13th December 2023

Contributions to the published works

***Krentz AJ**, Von Muhlen D, Barrett-Connor E.*
Adipocytokine profiles in a putative postmenopausal polycystic ovary syndrome (polycystic ovary syndrome) phenotype parallel those in premenopausal polycystic ovary syndrome; The Rancho Bernardo Study
Metabolism 2012;61:1238-41.

In close collaboration with the late Professor Elizabeth Barrett-Connor (1935-2019), Distinguished Professor at the University of California San Diego, USA, I co-developed the original hypothesis that polycystic ovary syndrome could be identified in postmenopausal women (***Krentz AJ**, Von Muhlen D, Barrett-Connor E. Searching for polycystic ovary disease in post-menopausal women: evidence of a dose-response association. Menopause 2007;14:284-292*). Beyond the predicted associations with atherosclerotic cardiovascular disease, which were confirmed by our studies, additional validation of the phenotypic model – based on published clinical and biochemical criteria – was provided by observed associations with the adipocytokines leptin and adiponectin. This research at the University of California San Diego, USA was funded by the British Heart Foundation via an International Research Fellowship I was awarded in open competition based on the aforementioned original hypothesis. I was responsible for creating the epidemiological model using the database of the four decade-long longitudinal Rancho Bernardo Study of Healthy Aging. I analysed and interpreted the results, wrote the first and final drafts of the paper and presented the findings at major scientific conferences. In addition to the study presented in this thesis, papers aimed at providing validation for the model were generated: ***Krentz AJ**, Von Muhlen D, Barrett-Connor E. Associations between adipocytokines, insulin resistance and cardiovascular risk factors in older women: an exploratory factor analysis of the Rancho Bernardo Study. Horm Metab Res 2009;41:773-777 and **Krentz AJ**, Barrett-Connor E. Ghrelin levels in older women: relation to clinical and biochemical cardiovascular risk markers. Fertil Steril 2009;92:1753-4.*

***Krentz AJ**, Morrow L, Petersson M, Norjavaara E, Hompesch M*
The effect of exogenously administered glucagon versus spontaneous counter-regulatory responses on glycaemic recovery from insulin-induced hypoglycaemia in subjects with type 2 diabetes treated with a novel glucokinase activator AZD1656 and metformin
Diabetes Obes Metab 2014;11:1096-101.

As Senior Director for Scientific Services at the internationally renowned and disease-focused academic clinical research institute ProSciento, San Diego USA (www.prosciento.com)[1] I was responsible for assembling and interpreting the data of this study. The study was designed and executed using ProSciento's in-house knowledge and experience of clinical investigative techniques and their application to early phase cardiometabolic drug development, to which I contributed. I wrote the first draft of the paper and responded to expert peer review comments to create the final draft. The expanded institutional knowledge on which the study was based, and to which I contributed, was subsequently summarised in a state-of-the-art textbook on which I was the lead editor and author: ***Krentz AJ**, Heinemann L, Hompesch M (Eds). Translational research methods for diabetes, obesity & cardiometabolic drug development: A focus on early phase clinical studies. Springer 2015)*[2]. The phase 1b clinical study included in this thesis utilised ProSciento's fully automated Biostator® artificial pancreas technology which avoids operator bias in hyperinsulinaemic glucose clamp studies. While at ProSciento, and of relevance to the AZD1656 study methodology, I designed a series of novel studies exploring updated technology to create an updated second generation semi-automated version of the hyperinsulinaemic glucose clamp[3]. I also worked closely with senior academics at the University of California San Diego on innovative metabolic-imaging projects. In 2016, I was awarded Clinical Tutor status at UC San Diego.

[1] Formerly known as Profil Insititute for Clinical Research.
[2] Third edition in preparation (2025).
[3] Intellectual property and commercial considerations preclude inclusion of these studies in this thesis.

Clough GF, Turzniecka M, Walter L, ***Krentz AJ****, Wild S, Chipperfield A, Gamble J, Byrne CD. Muscle microvascular dysfunction in central obesity is related to muscle insulin insensitivity but is not reversed by high-dose statin treatment.*
*Diabetes 2009;58:1185-1191****.***

I was co-Principal Investigator (with Professor Christopher Byrne, University of Southampton, UK) on this six-month randomised placebo-controlled trial that I conceived and designed. The original hypothesis concerning the effects of statins on insulin sensitivity was an important aspect of my personal contribution; at that time, Prof Byrne was the Director of the Wellcome Trust Clinical Research Facility at the University of Southampton. I personally secured the external research funding for the study in the form of an unrestricted educational grant (Pfizer UK). The primary aim was to determine whether high-dose statin therapy was associated with changes in whole-body insulin sensitivity. The study represented an evolution of my interest in insulin resistance which had been developed, honed and presented as my Doctor of Medicine research thesis, University of Birmingham, UK (Krentz, 1991). The effect of statins on whole body insulin sensitivity was, and remains, of potential relevance to the reported increased risk of new-onset diabetes associated with agents in the class. This risk is especially apparent at higher doses of statins in subjects with features of the metabolic syndrome. Whether statins have vasculoprotective effects independent of their cholesterol-lowering actions was also of interest. The influence of metabolic factors on microvascular, i.e., small blood vessel, function was a theme for our collaborative group in Southampton. I was lead author for our research team on two preliminary papers that explored the interactions between large and small vessel disease in diabetes and the metabolic syndrome (***Krentz AJ****, Clough G, Byrne CD. Interactions between microvascular and macrovascular disease in diabetes. Diabetes Obes Metab 2007;9:781-791* and ***Krentz AJ****, Clough G, Byrne CD. Vascular disease in the metabolic syndrome: Do we need to target the microcirculation to treat large vessel disease? J Vasc Res 2009; 46:515-526*). From these literature views, it was apparent that the microvascular effects of statins had been under-researched. In addition to assessing insulin sensitivity, we therefore conducted additional studies of micro- and macrovascular function using validated non-invasive techniques. These studies – which employed state-of-the art clinical investigative methods – were performed by researchers at the University of Southampton and incorporated external expertise from the University of Birmingham, UK. I was involved in the study design, research ethics approval process, data analysis and interpretation, and the writing of the resulting

papers[4]. Related papers arising from this collaboration assessed the effect of experimental hyperinsulinaemia, generated by the glucose clamp, on aspects of cardiovascular function *Turzyniecka M, Wild S, **Krentz AJ**, Chipperfield A, Gamble J, Clough G, Byrne C. Skeletal muscle microvascular exchange capacity is independently associated with hyperglycaemia in non-diabetic subjects with central obesity Diabetic Med 2009;26:1112-9*; *Turzyniecka M, Wild S, **Krentz AJ**, Chipperfield A, Clough G, Byrne CD. Diastolic function is strongly and independently associated with cardio-respiratory fitness in central obesity. J App Physiol 2010;108:1568-74;* and *Clough G, L'Esperance V, Turzyniecka M, Walter L, Chipperfield A, Gamble J, **Krentz AJ**, Byrne CD. Functional dilator capacity is independently associated with insulin sensitivity and age in central obesity and is not improved by high dose statin treatment. Microcirculation 2011;18:74-84*. Thus, selection of the hyperinsulinaemic euglycaemic clamp technique, based on in-depth knowledge of methods for assessing insulin sensitivity, permitted a plurality of hypotheses concerning metabolic-vascular interactions to be rigorously tested. Relevant key references that detail methods for measuring insulin action include: ***Krentz AJ**, Weyer C, Hompesch M. Quantifying insulin action in human subjects (In: Krentz AJ, Weyer, Hompesch M (Eds). Translational research methods for diabetes, obesity & non-alcoholic fatty liver disease drug development: A focus on early phase clinical studies. 2ND Edition. Springer London 2019 pp 3-35* and ***Krentz AJ.** Classic metabolic actions of insulin: from physiology to disease and novel therapeutics. In: Krentz AJ (Ed). Insulin: deficiency, excess, and resistance in human disease. Elsevier. 2023 pp 25-60.*

[4] I also acted as co-supervisor to research fellow Dr. Magdalena Turzyniecka for her MD thesis which was based on the original studies presented in the relevant publications.

***Krentz AJ**, Haddon-Hill G, Père A, Pankova N, Jaun A.*
Machine learning applied to cholesterol-lowering pharmacotherapy: Proof-of-concept in high-risk patients treated in primary care
Metab Syndr Rel Dis 2023;21:453-9

In collaboration with senior data science colleagues at Metadvice[5], King's College London, I helped co-design this study. The study is positioned within a proprietary intellectual and scientific framework that aims to accelerate precision diagnostics and therapeutics in clinical practice and was informed by discussions with industry colleagues at Daiichi Sankyo UK. I was also closely involved in the data analysis and interpretation, wrote the first and final drafts, and responded to the comments of expert peer reviewers on behalf of my coauthors. As part of Metadvice's multi-disciplinary team of data scientists and clinicians I provided a perspective that bridged across (a) disease pathophysiology (b) pharmacotherapeutics (c) regulatory and national prescribing recommendations and (d) prescribing clinicians. My knowledge of the cardiometabolic medicine space has been integral to ensuring that our neural networks are appropriately aligned with current UK national clinical guidance. Our international perspective requires that I am also able to identify where national guidelines diverge. I have been responsible for ensuring that emerging new therapies, e.g., bempedoic acid for the treatment of hypercholesterolaemia, are appropriately integrated into existing management recommendations. I also serve as the lead within Metadvice with responsibility for ensuring the clinical validation of the neural networks in terms of safety and accuracy. In the broader context, as the founding Director for Cardiometabolic Disease[6] I work with academic colleagues whose expertise lies in machine learning, pharmacotherapeutics, and precision medicine. Together, we create and implement innovative clinical decision support and data analytics technology. The collective aim is to create a viable proprietary platform that brings personalised precision medicine to routine clinical care (the beta version of our clinical tool started roll out in UK general practices in Q42022). Our technology is designed to help optimise prescribing both of well-established and novel therapies for cardiometabolic disorders, help counter current disparities in healthcare, and reduce therapeutic inertia. This paper – which had joint Metadvice-King's College London authorship – was the first proof-of-concept output from our cardiometabolic medicine research programme. Our initial focus was on the comorbidity triad of type 2 diabetes, lipids, and hypertension. I was primarily instrumental in securing the funding for the study from Daiichi Sankyo, UK. While intellectual property considerations preclude

[5] www.metadvice.com
[6] Subsequently Chief Medical Officer.

divulgence of certain methodological details my role may be summarised as coordinating our interdisciplinary interface via my multifaceted professional perspective. I was also primarily responsible for the creation and then collaborative co-development of our well-received clinical user interface that displays individual patient status as a graphical perspective. It is the stated aim of Metadvice to (a) collaborate with academic and industry colleagues and (b) to disseminate our learning wherever possible within the public domain. We have supervised several students from the highly regarded EPFL higher education institution in Lausanne, Switzerland (https://www.epfl.ch/en/) who have completed their Master's degrees based on original research at Metadvice [7]. In the context of our life science-academic collaboration, I was a co-applicant on a successful Innovate UK Smart Grant bid with colleagues including Professor Vasa Curcin, Head of Department, Population Health Studies at King's College London, UK. In 2021, I was appointed to a visiting chair in Life Course & Population Health Science at King's College London. With Professor Curcin, Departmental Director, I co-supervised a PhD candidate at King's College London applying transfer learning techniques based on Metadvice's machine learning and expanding our research to national anonymised databases, i.e., the UK Clinical Practice Research Database (CPRD). Metadvice has also been collaborative discussions with the National Institute for Health and Care Excellence (NICE) concerning the transition to living guidelines.

[7] I co-supervised several of these students in collaboration with Dr André Jaun, Chief Technology Officer and Co-Founder of Metadvice.

Abstract

Personalised precision medicine, including sex-specific considerations, is gaining momentum in the management of complex cardiometabolic disorders. This thesis explores aspects of disease pathophysiology and the development and clinical implementation of relevant pharmacotherapies in men and women. A series of clinical studies published in peer reviewed journals is presented. Each study tested a specific hypothesis relevant to this overarching aim.

To investigate the life-long health implications of polycystic ovary syndrome a novel phenotype of the disorder was modelled in postmenopausal women. Dose-response associations were observed with (a) prevalent cardiovascular disease and (b) circulating adipocytokines – positive for leptin, negative for adiponectin – that mirrored the classic syndrome in younger women.

A novel glucose-lowering drug – the glucokinase activator AZD1656 – with theoretical potential to impair recovery from hypoglycaemia was studied in a phase 1b clinical trial in adults with type 2 diabetes receiving metformin. Counter-regulatory hormone responses to experimental hypoglycaemia were quantified using precision automated stepped hypoglycaemic clamps. Exogenous glucagon was effective in reversing hypoglycaemia during AZD1656 therapy.

New-onset diabetes is an adverse effect of statin therapy in which insulin resistance is implicated. In a phase 4 investigator-initiated study, non-classic effects of atorvastatin 40 mg daily were quantified in abdominally obese middle-aged adults. No effect was observed on whole-body insulin sensitivity as assessed using hyperinsulinaemic euglycaemic clamps. Microvascular function and adipocytokines were also unaffected by the high-intensity statin.

Statins are the most commonly prescribed cholesterol-lowering drugs. However, their use in clinical practice is frequently suboptimal. In a proof-of-concept real-world study novel machine learning methodology, trained on national clinical guidelines, was able to identify individuals failing to reach cholesterol goals and recommend personalised therapeutic options.

Collectively, the original papers illustrate steps along the translational medicine pathway. The studies also emphasise (a) metabolic-vascular disease intersections (b) the importance of detailed phenotyping and use of appropriate research methodologies and (c) novel strategies to promote personalised precision medicine. It is proposed that reversing the conventional translational pathway may usefully inform earlier stages of target identification and clinical drug development.

Executive Summary

Background

Multimorbid cardiometabolic disorders are characterised by the intersection of metabolic and haemodynamic risk factors and cardiovascular disease[8]. The rising global prevalence of these conditions, which include excess adiposity, type 2 diabetes, and dyslipidaemia, and the limitations of current therapies drives the search for safe and effective new therapies.

Methods

Examples from the candidate's original research activity are presented. These studies, each of which was published in a peer-reviewed scientific journal, test specific hypotheses in various cardiometabolic disorders. The selected papers encompass (i) epidemiological research and identification of potential new drug targets; (ii) early phase clinical and (iii) late phase post-approval drug development and (iv) personalised precision medicine in a real-world setting. The strengths and limitations of each study are critically appraised from the perspective of scientific knowledge at the time and also scientific knowledge that has subsequently become available. The importance of appropriate study designs, well-characterized populations, and the use of accurate and reproducible clinical research methods are emphasized throughout the thesis, as is the need to recognise sex-specific factors in cardiometabolic disease. Data derived from human studies are emphasised throughout the thesis.

Results

(i) *Epidemiological modelling of cardiometabolic risk in postmenopausal women with a putative polycystic ovary phenotype.* Polycystic ovary syndrome – defined as the presence of any two of the following: (1) clinical or biochemical hyperandrogenism, (2) evidence of oligo-anovulation, (3) polycystic appearing-ovarian morphology on ultrasound – is closely associated with adiposity, features of the metabolic syndrome, and type 2 diabetes. Excess adiposity, with attendant insulin resistance, is present in the majority of affected women. This adverse cardiometabolic risk profile would be expected to increase the risk of atherosclerosis. However, because women of reproductive age are at inherently low risk of atherosclerotic disease the

[8] The term cardiometabolic disease is used in this dissertation. It is recognised that renal and hepatic dysfunction often contribute to the disease burden in affected individuals.

implications of polycystic ovary syndrome for cardiovascular disease have proved difficult to determine. In order to clarify the long-term impact of polycystic ovary syndrome on cardiovascular disease a novel phenotype was sought in postmenopausal women. Hypotheses tested in this study included first, that the postmenopausal phenotype would be associated with prevalent cardiovascular disease; and second, that serum profiles of fat-derived adipocytokines would reflect those reported in premenopausal women with polycystic ovary syndrome thereby providing support for the validity of the model The study population was the well-characterised female participants of the longitudinal Rancho Bernardo Study of Healthy Aging in California, USA. Dose-response associations were observed between the phenotypic model, prevalent atherosclerotic cardiovascular disease, and also with the metabolically active adipocytokines leptin and adiponectin. Differential effects of standard measures of adiposity were observed on the adipocytokines with reduced adiponectin levels. Specifically, the association between leptin, but not adiponectin, and the putative postmenopausal polycystic ovary syndrome phenotype was eliminated by adjustment for waist circumference. This observation is consistent with the scientific literature suggesting that while leptin levels rise in proportion to overall fat mass, reduced adiponectin levels are modulated by central adiposity. Strengths of the study included the well-characterised cohort and the use of validated methods for ascertaining the presence of atherosclerotic cardiovascular disease. Potentially confounding effects of estrogen therapy and a history of oophorectomy served as exclusion criteria. While data on ovarian morphology was not available in the postmenopausal study cohort, polycystic ovaries do not have to be present to make the diagnosis. This paper was the first publication to define a polycystic ovary syndrome phenotype after the menopause. The observation that disordered adipocytokine profiles are sustained beyond the menopause supports the hypothesis that altered adiponectin and leptin physiology is implicated in the pathogenesis of polycystic ovary syndrome and associated cardiovascular risk. Additional evidence published in recent years provides support for pathogenic roles of these adipocytokines in polycystic ovary syndrome.

(ii) *Exploring cardiometabolic safety considerations of a novel glucose-lowering agent in subjects with type 2 diabetes***.** Safe and effective long-term control of glucose metabolism is a core aspect of the management of type 2 diabetes. Combinations of glucose-lowering medications with complementary modes of action are often required to achieve evidence-based glycaemic goals. Control of hyperglycaemia is of particular importance in mitigating the microvascular complications of diabetes. In turn, microvascular tissue damage, notably chronic kidney disease, has been reported to promote atherosclerosis. The advent of classes of

glucose-lowering agents with specific cardiovascular protective properties[9] has profoundly changed the pharmacotherapeutic approach to type 2 diabetes over the last decade. Nonetheless, effective and safe glucose control remains central to reducing the risk of microvascular complications of diabetes. Among unwanted effects of glucose-lowering medications, iatrogenic hypoglycaemia is especially hazardous in the presence of cardiovascular disease. Glucokinase, which acts as a glucose sensor in the insulin-producing islet β-cells of the pancreas, was identified as a novel target of a new class of glucose-lowering drugs – allosteric glucokinase activators. A study was conceived in response to theoretical concerns that agents in this class might impair recovery from iatrogenic hypoglycaemia in response to exogenous glucagon which mobilises glucose from hepatic glycogen. In a single centre phase 1b randomised, placebo-controlled, within-patient, crossover study counter-regulatory hormone responses to controlled experimental hypoglycaemia were quantified in metformin-treated patients (n=8) with type 2 diabetes receiving a novel glucokinase activator (AZD1656) using precision stepped hyperinsulinaemic hypoglycaemic clamp methodology. All participants were carefully screened to exclude clinical cardiovascular disease; blood pressure and heart rate were monitored continuously during hypoglycaemia. Only one participant was female since fertile women were excluded from participation[10]. The study demonstrated that intramuscular glucagon was effective in accelerating the time to recovery from insulin-induced hypoglycaemia during treatment with AZD1656. No adverse haemodynamic events were observed during the hypoglycaemic clamps. Strengths of the study included the cross-over study design and use of the automated hypoglycaemic clamp. A potential limitation was the absence of a comparator group that did not receive AZD1656. The results were interpreted as supporting the practical utility of exogenous glucagon as rescue therapy for iatrogenic hypoglycaemia in patients with type 2 diabetes treated with AZD1656 in combination with metformin. The emergence of safety and efficacy issues[11] subsequently prompted the development of safer dual-acting glucokinase activators to target the liver and pancreas, as well as hepato-selective agents. However, to date no glucokinase activators have been approved for use by western regulators.

(iii) *Nonclassic cardiometabolic effects of high-intensity statin therapy in insulin-resistant subjects with features of the metabolic syndrome.* Statins are potent cholesterol-lowering drugs supported by an extensive evidence base of randomised clinical trials. Statin-induced

[9] Glucagon-like hormone-1 receptor agonists, sodium-glucose co-transporter-2 inhibitors.
[10] This is standard practice in early-phase studies of new chemical entities.
[11] In addition to risk of hypoglycaemia, early examples of GKAs were associated with risks of hepatic steatosis and waning of glucose-lowering efficacy over time.

new-onset diabetes, which could potentially detract from cardiovascular benefits, was not recognized during regulatory phase 3 trials only becoming apparent after regulatory approval. Patients with features the metabolic syndrome are at highest risk. Initial experimental studies implicated drug-induced insulin resistance in the pathogenesis of statin-induced diabetes. In the light of the scientific literature available at the time, metabolic-vascular hypothesis was tested in a phase 4 investigator-initiated single centre randomised placebo-controlled clinical trial. Middle-aged adults (n=39) with ≥2 features of the metabolic syndrome who were at risk for atherosclerotic cardiovascular disease were randomised to receive high-intensity statin (atorvastatin 40 mg) daily or matching placebo for 6 months. Extensive cardiometabolic phenotyping included assessment of physical activity levels using an activity monitor, handgrip strength and cardiorespiratory fitness (VO_{2max}). Body composition was assessed using dual X-ray absorptiometry with magnetic resonance imaging to accurately quantify abdominal adiposity. Assessments of non-classic effects of statin therapy included whole-body insulin sensitivity using the hyperinsulinaemic euglycaemic clamp technique and second, skeletal microvascular function which was measured non-invasively using venous occlusion plethysmography. As predicted, circulating levels of cholesterol and inflammatory markers were significantly reduced by high-intensity statin therapy. In contrast, however, no statistically significant effects were observed in either whole-body insulin sensitivity or measures of skeletal microvascular function. Strengths of the study included the well-characterised study cohort and use of sensitive and reproducible investigative techniques. A potential limitation was that endogenous insulin secretion was assessed only in the basal, i.e., fasting, state. Clinical experimental studies published subsequently have all had methodological limitations that hinder elucidation of the molecular mechanisms responsible for statin-induced diabetes. Strategies to minimise the risk include using lower intensity statins regimens and combining statins with novel non-statin cholesterol-lowering agents that have neutral metabolic profiles.

(iv) *Quantifying and addressing translational gaps in real-world prescribing of statin and novel non-statin cholesterol-lowering medication using machine learning.* Statins remain the most widely used medications used to reduce cholesterol concentrations. However, statin monotherapy does not always achieve evidence-based cholesterol goals even at the highest recommended doses. Furthermore, statin-associated side effects, especially muscle symptoms, often limit the maximal doses that can be tolerated. Novel oral and injectable non-statin cholesterol-lowering drugs have become available in recent years. In the UK, however,

the use of potent injectable agents[12] has been restricted to relatively small numbers of individuals who are considered to be at highest risk of atherosclerotic events. Oral non-statin medications are used at relatively low levels in the UK and elsewhere. In a proof-of-concept study using novel machine learning methodology artificial neural networks were trained on UK national clinical prescribing guidelines and applied to real-world primary care electronic health records of patients receiving cholesterol-lowering medications (n=5,630). The majority of prescription (76%) were categorized as primary prevention, i.e., to avert the development of atherosclerotic cardiovascular disease. Statin monotherapy was the most common medication (82%). The neural networks confirmed that prescribed statin doses were frequently inadequate (71%) and not aligned with clinical guidance. Statin intolerance, necessitating lower doses, is a plausible explanation for this observation. However, the prevalence of statin intolerance in the study cohort was low (4%), possibly reflecting under-recording in the electronic health record. The machine learning model then provided personalised evidence-based therapeutic recommendations for patients not attaining therapeutic goals. These included increasing the statin dose for some individuals and identifying others as candidates for the novel oral non-statin cholesterol-lowering drug, bempedoic acid[13]. Reverse translational implications of this study include further exploration of how cholesterol goals can be attained using combinations of statin and novel non-statin agents that are well tolerated and cost-effective in the setting of low- to moderate risk of atherosclerotic cardiovascular disease.

Summary & conclusions

The papers presented in this thesis, each of which tested a specific hypothesis in a prevalent cardiometabolic disorder, collectively illustrate the translational medicine pathway of drug development. By convention, the process of translational medicine is a linear model that proceeds in steps from T0 to T5. This starts with the identification of unmet health needs and therapeutic targets (T0, T1), then progresses through preclinical and clinical studies (T2,T3) to conclude with licensed pharmacotherapeutics being deployed at scale in target populations (T4). Commencing with the epidemiology and pathophysiology of polycystic ovary syndrome, cardiovascular disease, adipocytokine profiles, T0 and T1) the thesis proceeds via experimental medicine and clinical trials (AZD1656, an experimental glucose-lowering drug for type 2 diabetes; non-classic effects of high intensity atorvastatin, T2 and T3) to post-approval real-

[12] Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors and inclisiran. High acquisition cost is the principal reason for the restrictions on use of these agents in the UK.
[13] Bempedoic acid was subject to prescribing restrictions in the UK at the time of the study.

world prescribing (statins and non-statin drugs, T4). The process of drug development – both de novo and drug repurposing – can take advantage of translational research as a bidirectional discipline wherein reverse translation (T5) incorporates insights from clinical studies to inform earlier stages of the pathway. The papers, each of which has particular strengths and limitations, provide an opportunity to explore the potential for reverse translational research. Each paper is appraised in the light of scientific knowledge at the time and considered in the context of scientific advances since publication. Insights with potential value for earlier stages of the translational pathway are identified and critically evaluated in the light of the scientific evidence that has since become available (T5). It is concluded that the papers provide prima facie support for reversing the translational medicine pathway to inform earlier stages in the development of cardiometabolic pharmacotherapeutics.

Keywords: translational medicine; cardiometabolic disease; insulin resistance; metabolic syndrome; polycystic ovary syndrome; type 2 diabetes; glucose clamp technique; vascular function; machine learning; deep learning

Chapter 1. Introduction

Global cardiometabolic disease burden; sex-specific considerations in men and women; pathophysiology of insulin resistance and metabolic syndrome; outline of thesis.

1.1 Cardiometabolic disorders: a modern pandemic

Over the course of the past century, a cluster of closely related cardiometabolic diseases have emerged as a global threat to health and longevity (Krentz, 2007, Noubiap et al., 2022). This timeframe coincides with the period since the discovery and first therapeutic use of insulin in 1921 and 1922, respectively. The role of impaired insulin action – insulin resistance – in the aetio-pathogenesis of prevalent non-communicable chronic cardiometabolic disorders, especially the metabolic syndrome and type 2 diabetes, has become increasingly clear over the intervening century (Ginsberg, 2000, Krentz, 2002a, Reaven, 1988). These disorders associate within a complex nexus or continuum of pathophysiology (Figure 1) (Krentz, 2023b). This metabolic continuum is characterised by intersections – often bidirectional – between vasculopathic metabolic derangements and dysfunctional haemodynamics (Kangas et al., 2019).

1.2 Sex differences in cardiometabolic disease

Atherosclerotic cardiovascular disease, principally ischaemic heart disease and stroke, are the leading cause of global mortality among men and women (Roth et al., 2020, Rethemiotaki, 2023). Recent data suggest that approximately 80% of cardiovascular deaths occur in low- and middle-income countries (Di Cesare et al., 2024). Although mortality rates from cardiovascular disease are generally lower in women than men, this is not the case in almost one-third of countries in the North Africa and Middle East and Sub-Saharan regions (Di Cesare et al., 2024)

Patients of both sexes with diabetes have rates of cardiovascular disease that are approximately 2-4-fold higher than their counterparts without diabetes (Dal Canto et al., 2019). It is noteworthy that women with diabetes have a greater risk of all-cause mortality, particularly from coronary heart disease, compared with men with diabetes (Wang et al., 2019). Since women of reproductive age are inherently more insulin sensitive than men, a difference largely attributed to endogenous estrogen (De Paoli et al., 2021), the development of type 2 diabetes implies greater relative impairment of insulin action in women. This hypothesis is supported by research showing higher mean body mass index, stronger correlations with android fat deposition, and more pronounced insulin resistance in women with type 2 diabetes than in men (Logue et al., 2011, Kwon, 2014, Kangas, 2019).

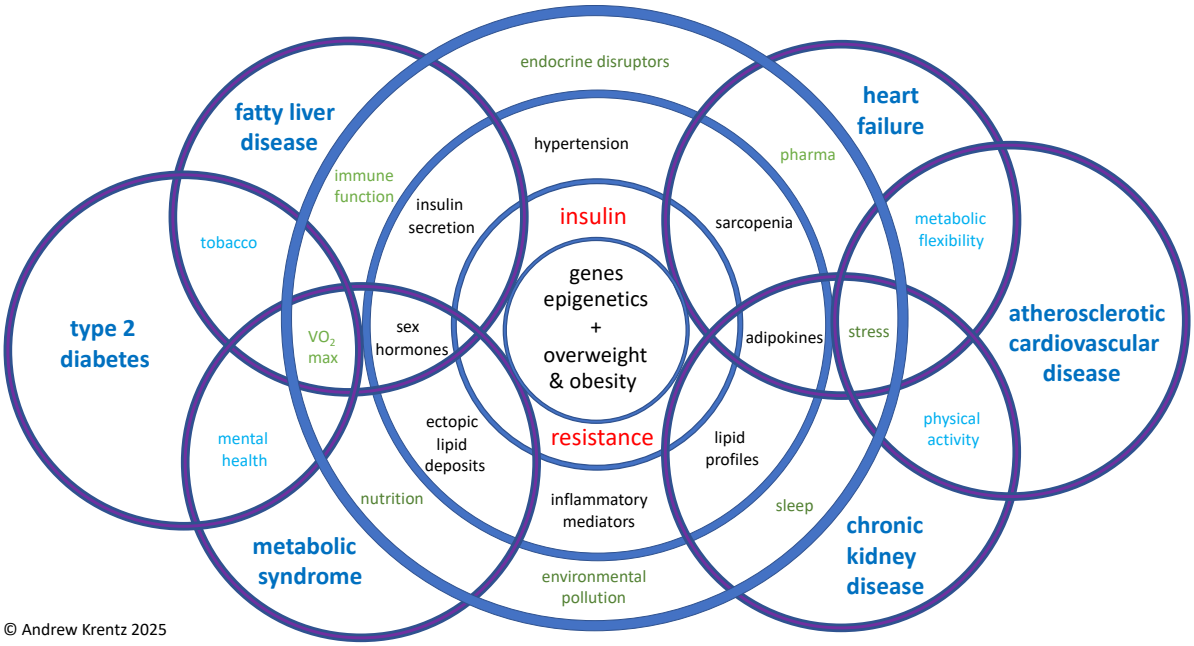
Insulin resistance associates with an adverse profile of risk factors for atherosclerosis with higher blood pressures and more adverse lipid profiles in women than in men[14] (Huxley et al., 2006). Treatment disparities that favour men vs women may be relevant to the higher relative risk for fatal coronary heart disease associated with diabetes observed in women compared to men (Huxley et al., 2006, Venditti et al., 2023). Furthermore, women in general are less likely to receive percutaneous coronary interventions for acute coronary syndromes (Sulaiman et al., 2021). Lower rates of adherence to and persistence with medications such as statins and glucose-lowering drugs may also be relevant to the discrepancies in outcomes between the sexes with women less likely to achieve therapy goals (Hambraeus et al., 2016). Statin-related side effects and patient perception of risks vs benefits have been identified as significant factors in the lower adherence rates to statins among women (Venditti et al., 2023). Treatment pharmacokinetics for women are different from that of men yet because of the underrepresentation of women in cardiovascular research results are often extrapolated to women (Rosano et al., 2015, Regitz-Zagrosek, 2006). This may lead women to more adverse effects of cardiovascular medications than their male counterparts (Santema et al., 2019).

1.3 Insulin resistance

In the decades-long quest to identify the initiators of polycystic ovary syndrome and type 2 diabetes, which incidentally often have an endocrine component, the concept of insulin resistance has been centre stage (Shulman, 2000). Insulin resistance may be usefully defined as existing wherever a normal amount of insulin elicits a subnormal biological response (Kahn, 1978). Epidemiological and experience evidence supports defective insulin action as a central – possibly fundamental – defect in prevalent cardiometabolic disorders including the metabolic syndrome and type 2 diabetes (Figure 1) (DeFronzo, 2010, Reaven, 2003, Kahn et al., 2014). Impaired insulin action is a characteristic feature of each individual component of the metabolic syndrome (see Section 1.2.1) (Di Pino and DeFronzo, 2019). Insulin resistance has been demonstrated to be closely associated with obesity and cardiovascular disease (Reaven et al., 2004). Impaired insulin action in skeletal muscle is the earliest identifiable risk factor for type diabetes (DeFronzo and Tripathy, 2009).

[14] Non-pregnant women of reproductive age tend to have lower levels of low-density lipoprotein cholesterol and higher levels of high-density lipoprotein cholesterol than men.

Figure 1. Nexus or continuum of cardiometabolic-endocrine pathologies.



Abbreviations: ED, endothelial dysfunction; OSA, obstructive sleep apnoea; PCOS, polycystic ovary syndrome; SNS, sympathetic nervous system; VO_2 max, maximal aerobic capacity.

Notes: Risk factors – some of which are included in the metabolic syndrome – and mechanistic features are not to scale nor are they intended to imply predefined pathways. The various factors – and some others omitted for clarity – contribute quantitatively to variable degrees resulting in unique individual phenotypes. Genetic predisposition or protection and behavioural or environmental factors affect cardiometabolic health via multiple mechanisms and levels from inter-organ communication to intracellular signaling, and epigenetic alterations of DNA. Adiposity and insulin resistance are positioned centrally in recognition of the extensive scientific literature supporting the fundamental relevance of these factors in aetiopathogenesis of cardiometabolic disorders. Nutrition includes macro- and micronutrient considerations. Risk factors and clinical disease phenotypes often intersect and may be modulated by ethnicity. For example, metabolic dysfunction-associated fatty liver disease (MASLD) is highly prevalent among individuals with obesity, especially when type 2 diabetes is also present. Moreover, some aspects of fatty liver disease may be more prevalent in non-White populations. Pharmacotherapy – including medications directed at attenuating risk factors or ameliorating cardiometabolic disease – may have neutral, positive, or negative effects on risk factor profiles and clinical disease. The term metabolic flexibility refers to ability to switch between glucose and lipid metabolism. Cardiorespiratory fitness correlates with aspects of glucose metabolism and insulin sensitivity.

Modified from: Krentz 2023b.

Rare inherited and acquired syndromes of severe insulin resistance have provided useful insights into the role of impaired insulin action in human disease (Semple et al., 2011, Melvin et al., 2018, Krentz, 2023c). Mutations of insulin receptors and genetic lipodystrophy syndromes produce extreme metabolic phenotypes (Semple et al., 2011) (Gong et al., 2024).

Of note, lipodystrophy syndromes[15] have features, e.g. hepatic steatosis progressing to steatohepatitis, dyslipidaemia, that are encountered in prevalent forms of obesity-associated insulin resistance (Akinci et al., 2019). The production of the adipocytokine leptin by lipodystrophic adipose tissue is decreased, particularly in generalised forms of lipodystrophy (Vigouroux et al., 2024). HAIR-AN (HyperAndrogenism, Insulin Resistance and Acanthosis Nigricans[16]) syndrome, is considered an uncommon sub-phenotype of polycystic ovary syndrome, is characterised by severe insulin resistance (Rager and Omar, 2006). While the cause of HAIR-AN remains undetermined the syndrome is considered to reflect ovarian androgen production by hyperinsulinaemia (Barbieri et al., 1988).

The degrees of insulin resistance in common cardiometabolic disorders such as type 2 diabetes are generally less pronounced and attributable to defects in insulin signalling distal to the binding of insulin to its cell membrane receptor (Kolterman et al., 1980). Factors such as the selection criteria for participants and control subjects, stage of natural history of disease, medications, and choice of investigative methods may influence insulin action (Zhao et al., 2023). These issues require consideration when designing clinical studies. For example, two groups of subjects may have similar total body fat as determined by body mass index but differing levels of visceral adiposity that is more closely associated with decreased whole-body insulin sensitivity (Despres et al., 2008). The limitations of body mass index are emphasised in a recent multinational commission that considered the definition and diagnostic criteria of clinical obesity[17] (Rubino et al., 2025). The commission called for excess body fat to be confirmed in individuals with a body mass index <40 kg/m^2 by additional clinical measures, e.g., waist circumference, waist-to-hip ratio, or waist-to-height ratio[18].

To take two examples, i.e., polycystic ovary syndrome and type 2 diabetes, reductions in insulin-mediated glucose disposal of ~35-40% and ~50%, respectively have been reported (Dunaif, 1997, DeFronzo and Tripathy, 2009). Prevalent non-genetic, i.e., acquired, forms of insulin resistance, such as that encountered in polycystic ovary syndrome and type 2 diabetes, can often be partially ameliorated by reducing excess adiposity or through the use of insulin sensitizing agents, e.g., thiazolidinediones (Yki-Jarvinen, 2004).

[15] Lipodystrophy syndromes may be partial or generalised and genetic or acquired.
[16] A hyperpigmented skin lesion closely associated with severe insulin resistance-hyperinsulinaemia.
[17] Clinical obesity is defined a chronic systemic illness characterised by alterations in the function of tissues, organs, the entire individual, or a combination thereof, due to excess adiposity.
[18] Only waist-to-height ratio does not have sex-specific cut-offs.

1.4 Methods for quantifying insulin action in human disease

The determination of insulin sensitivity requires that clinical investigative methodologies are safe, accurate, reproducible and appropriate to physiological metabolic dose-response relationships in humans (Rizza et al., 1981, Wallace and Matthews, 2002). Since the seminal studies of insulin action performed by Harold Himsworth in the 1930s (Himsworth, 1936), many methods have been developed[19]. These range from modelling of fasting plasma insulin and glucose concentrations[20] to the quantitative hyperinsulinaemic euglycaemic clamp technique (Dube et al., 2013). Each method is characterised by specific strengths and limitations (Wallace and Matthews, 2002, Krentz, 2019b).

1.5 Cardiometabolic continuum of disease

The rising prevalence of obesity is considered to be a major driver of the global multimorbidity burden of cardiometabolic disease (Sattar et al., 2023). The Global Burden of Disease investigators reported that high body mass index accounted for 4.0 million deaths in 2015, more than two-thirds of which were caused by cardiovascular disease (G.B.D. 2015 Obesity Collaborators. et al., 2017) after accounting for smoking and other causes of ill health (G.B.D. 2015 Obesity Collaborators. et al., 2017) .

The main cardiometabolic disorders recognised in clinical medicine include excess adiposity, the metabolic syndrome, type 2 diabetes, and atherosclerotic cardiovascular disease. In recent years, this list has expanded to include chronic kidney disease and metabolic dysfunction-associated fatty liver disease (Krentz, 2023b). The nomenclature describing this confluence continues to evolve. In 2023, for example, the American Heart Association coined the term cardiovascular-kidney-metabolic syndrome to highlight the interrelations between these disorders (Ndumele et al., 2023). The classic disease states recognised in clinical practice generally represent the late manifestations of underlying pathophysiological processes that have progressed subclinically for many years prior to diagnosis (Figure 1).

[19] Himsworth's studies, which involved injection of exogenous insulin with an oral glucose challenge, predated the development of the insulin radioimmunoassay that led to the recognition of hyperinsulinaemia as a hallmark of states of insulin resistance.
[20] Homeostasis model assessment (HOMA) developed at the University of Oxford https://www.rdm.ox.ac.uk/about/our-clinical-facilities-and-units/DTU/software/homa

1.6 Metabolic syndrome

The cluster of modifiable risk factors closely associated with insulin resistance is known as the metabolic syndrome (International Classification of Disease-10 E88.81). The core components of the metabolic syndrome which, by definition, cluster together in affected individuals, include visceral and ectopic adiposity, hyperglycaemia, systemic hypertension, hypertriglyceridaemia and reduced levels of high-density lipoprotein (HDL) cholesterol. (Table 1). The presence of the metabolic syndrome predicts the development of type 2 diabetes with a five-fold increased risk as well as atherosclerosis at a two-fold increased risk (Byrne, 2005). Degrees of hyperglycaemia below the diagnostic threshold for diabetes, i.e., impaired fasting glucose and impaired glucose tolerance, also increase the risk of atherosclerosis, cardiovascular mortality and total mortality in both sexes (Unwin et al., 2002).

Each of the diagnostic components of the metabolic syndrome is an independent risk factor for atherosclerotic cardiovascular disease (Table 1). The late Gerald Reaven MD of Stanford University is widely credited with bringing this constellation of risk factors to the attention of the medical community in what he tentatively termed the Syndrome X of insulin resistance (Reaven, 1988). In fact, risk factor clustering had been recognised for many years. Furthermore, Reaven's original description did not include abdominal adiposity. It should also be acknowledged that the insulin resistance-metabolic syndrome has been the subject of controversy. Some investigators maintain that the syndrome is of limited clinical value whereas others have found the concept useful in the quest to unravel the intersections between metabolic disease and atherosclerosis (Sattar, 2008, Eckel et al., 2005). Nonetheless, data continue to accrue demonstrating the prognostic value of the metabolic syndrome, e.g., following acute coronary syndromes (Ostadal et al., 2022).

Table 1. The metabolic syndrome: International Diabetes Federation definition.

Central obesity (defined as waist circumference* with ethnicity specific values) along with any two of the following four factors:

Raised plasma triglycerides

≥ 1.7 mmol/L
or specific treatment for this lipid abnormality

Reduced plasma HDL cholesterol

< 1.03 mmol/L in males
< 1.29 mmol/L in females
or specific treatment for this lipid abnormality

Raised blood pressure

Systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg
or treatment of previously diagnosed hypertension

Raised fasting plasma glucose

≥ 5.6 mmol/L
or previously diagnosed type 2 diabetes

* If body mass index is >30kg/m^2, central obesity may be assumed. For men the upper limit of waist circumference is 102 cm and for women is 88cm.

Source: www.idf.org

Of note, elevated levels of low-density lipoprotein (LDL) cholesterol are not included in the definition of the metabolic syndrome (Grundy, 2007). Glucose and insulin are relevant to aspects of cholesterol metabolism with glucose providing acetyl-Coenzyme A for cholesterol biosynthesis (Xiao et al., 2022). Insulin resistance promotes atherosclerosis via generation of small dense LDL-cholesterol particles that have enhanced atherogenicity (Toth, 2014). Insulin has an important role in the metabolism of the primary lipoprotein implicated in the pathogenesis of atherosclerosis, i.e. apolipoprotein B (Haas et al., 2013). Secretion and clearance of apo B are increased in the presence of insulin resistance (Haas et al., 2013). An extensive body of clinical trial data demonstrates that for any pretreatment LDL-cholesterol concentration the risk of cardiovascular events is reduced by medications – most commonly statins – that lower LDL-cholesterol (Cholesterol Treatment Trialists Collaborators et al., 2012, Naci et al., 2013). In the context of the metabolic syndrome, and beyond LDL-cholesterol, attention is increasingly

focused on the causal effects of triglycerides, triglyceride-rich lipoproteins, and remnant particles in the pathogenesis of atherosclerosis (Xu et al., 2024).

Reports of clustering of cardiometabolic risk factors can be traced back to the 1920s (Laakso and Kovanen, 2006). Since Reaven's description, which he termed Syndrome X[21], the metabolic syndrome has expanded to embrace nonclassic risk factors including low-grade inflammation, disordered adipocytokine physiology, and endothelial dysfunction (Alberti et al., 2006, Moller and Kaufman, 2005). Machine learning applied to metabolomic analyses reveals a complex and heterogeneous architecture of risk factors within the metabolic syndrome (Chen et al., 2023). The molecular genetic architecture of cardiometabolic disease has become more clearly delineated (Barroso and McCarthy, 2019). Factors including ethnicity, epigenetics, and sex steroid hormones modulate the phenotypic expression of cardiometabolic disorders over the life course (Krentz, 2023b).

Sexual dimorphism in cardiometabolic risk is well recognised. Epidemiological studies, including the Rancho Bernardo study and other datasets, women remain relatively protected from atherosclerosis prior to menopause (Barrett-Connor, 2013). As discussed in Chapter 2, differences between men and women are evident in aspects of cardiovascular pathophysiology. The relative under-representation of women in trials of cardiometabolic interventions and implications for therapy and clinical outcomes are also considered in Chapter 2.

1.7 Global trends in cardiometabolic disease

Cardiometabolic diseases are among the leading causes of death worldwide and are associated with excess healthcare costs globally (Roth et al., 2020). The management of individuals with long-term complex, multi-morbid cardiometabolic disorders such as polycystic ovary syndrome and type 2 diabetes is often suboptimal. In the United States, control of modifiable risk factors – glycaemia, blood pressure and cholesterol – has deteriorated in recent years[22]. Data from the National Health and Nutrition Examination Survey showed that between 2007-2010 and the 2015-2018, the percentage of adult participants with diabetes in whom glycaemic control (glycated haemoglobin level <53 mmol/mol) was achieved declined from 57.4% [95% confidence interval 52.9 to 61.8] to 50.5% [45.8 to 55.3]. From 2011-2014 to 2015-

[21] A multiplicity of names have been applied to the syndrome reflecting variable definitions that have been applied over the decades. Cardiac Syndrome X refers to microvascular angina pectoris, which mainly affects postmenopausal women.

[22] Primordial prevention aims to avert the emergence of risk factors in healthy subjects.

2018, the percentage in whom blood-pressure control (<140/90 mm Hg) was achieved decreased from 74.2% [70.7 to 77.4] to 70.4% [66.7 to 73.8]. Following major improvements in lipid control (non-high-density lipoprotein cholesterol level <3.4 mmol/L) in the early 2000s, minimal improvement was seen between 2007-2010. The percentage in whom all three targets were simultaneously achieved plateaued after 2010 and was 22.2% [17.9 to 27.3] in 2015-2018 (Fang et al., 2021).

These trends have major implications for public health systems (Gaede et al., 2008, Huang et al., 2023b). Even though an extensive evidence base guides the prevention and management of cardiometabolic disorders barriers to optimal care persist. These include high acquisition costs of new pharmacotherapeutics which are particularly relevant in low- and middle-income countries (Harrison et al., 2023, Barber et al., 2024). Aspects of safety data for widely used and less expensive medications remain incomplete. As discussed in Chapter 3, the effects of long-established drugs such as sulfonylureas and metformin on cardiovascular disease continue to be debated after many decades of clinical use (Krentz, 2002b, Li et al., 2021, Wang et al., 2023).

1.8 Precision cardiometabolic medicine

The final stages of the translational medicine pathway of drug development involve the integration of therapies into real-world clinical practice. Clinical guidelines for the management of cardiometabolic disorders have evolved in response to new clinical trial data. The benefits of sodium-glucose co-transporter (SGLT-2) inhibitors on heart failure and glucagon-like peptide (GLP)-1 receptor agonists on atherosclerosis have been integrated into recent guidance for the management of type 2 diabetes (Davies et al., 2022). It is now expected that new drugs will have beneficial effects not only on blood glucose but also on body weight, as well as cardiovascular and renal complications (Davies et al., 2022). However, while SGLT-2 inhibitors and GLP-1 receptor agonists target several multiple aspects of the cardiometabolic disease pathophysiology the management of major modifiable risk factors including hyperglycaemia, dyslipidaemia, and hypertension remains largely siloed within traditional clinical sub-specialties (Krentz and Jacob, 2019, Reiter-Brennan et al., 2021). Coincident with the emergence of cardio- and renoprotective glucose-lowering drugs – and indeed facilitated by the expanded range of pharmacotherapeutic options – the management of type 2 diabetes and related cardiometabolic disorders has embraced a precision approach that aims to deliver more personalised care (Dennis, 2020, Franks et al., 2023).

1.9 Aims and outline of thesis

The original research publications presented in chapters 2 to 5 consider aspects of the cardiometabolic nexus in more detail. Expanding the perspective beyond the insulin resistance and the metabolic syndrome, the chapters focus, respectively, on polycystic ovary syndrome (Chapter 2), type 2 diabetes (Chapter 3), and management of LDL-cholesterol (Chapters 4 and 5).

- In Chapter 2, a novel phenotype of polycystic ovary syndrome was modelled in postmenopausal women with the aim of providing insights into the life-time health implications of this prevalent disorder. This research provided support for a pathophysiological role of adipocytokines which has since been explored by the research community. The potential to target adipocytokines using novel and repurposed medications is discussed.

- In the quest for additional effective and safe glucose-lowering medications for type 2 diabetes, Chapter 3 provides an example of an early phase clinical study of an investigational glucokinase activator. This study illustrates the importance of a broad cardiometabolic perspective in the selection of participants and study methodology. The study, which was published shortly after GLP-1 agonists became available and before the advent of the SGLT-2 inhibitors, is considered (a) within the pharmacotherapeutic landscape of the time and (b) in the current era which has been transformed by drugs in the GLP-1 receptor agonists and SGLT-2 inhibitor classes.

- Chapter 4 examines the effects of high-intensity statin therapy on LDL-cholesterol and microvascular function while simultaneously quantifying the impact of the therapy on cardiometabolic profiles in high-risk non-diabetic, insulin-resistant individuals. This study was conceived in recognition of two clinical questions. First, does LDL-lowering improve microvascular function as well as decreasing the risk of atherosclerotic events? And secondly, is insulin resistance the mechanism responsible for new-onset diabetes in subjects with adverse cardiometabolic risk profiles? These hypotheses were testing in a state-of-the-art clinical investigation facility in healthy volunteers whose cardiometabolic profiles were characterised in detail using a range of biochemical and imaging methodologies. The results of the

study are appraised in the light of subsequent publications that have examined the effects of statin therapy on the microvasculature and insulin action.

- Chapter 5 explores the utility of a novel machine learning model on prescribing of cholesterol-lowering drugs in a UK primary care setting. The principles and broader implications of machine learning in the health and life sciences, including precision medicine and drug development, is reviewed along with the expanding role of in providing evidence that usefully complements clinical trials.

By intention, the published papers in this thesis employ a diverse range of methodologies appropriate to the hypotheses tested. These range from classical epidemiology to experimental clinical trials and advanced real-world data analytics. Details of the author's contributions to each study are provided together with acknowledgements of contributions from senior and junior academic and life science colleagues. Each of the papers is prefaced with a contemporary review of relevant pathophysiology and principles of management. In Chapter 6, each of the original papers is critically re-evaluated in the context of current scientific knowledge. This leads to a discussion in Chapter 6 of the translational research pathway for the development and deployment of new therapies for cardiometabolic disorders. The pathway is outlined and potential learning points from the papers presented in the thesis are considered in the context of forward and reverse translational research.

Throughout the dissertation, emphasis is placed on themes that may inform clinical research in cardiometabolic diseases. These include (a) the close and complex associations that exist between metabolic risk factors and cardiovascular disease and (b) shared pathogenic factors. For example, both polycystic ovary syndrome and type 2 diabetes are closely associated excess adiposity, insulin resistance, and features of the metabolic syndrome. Intersections between lipid and carbohydrate metabolism that may have implications for pathophysiology and pharmacotherapeutics are explored. Differences in risk and mechanisms of atherosclerotic cardiovascular disease between men and women are considered. The potential for pathophysiological interactions between microvascular and macrovascular disease are also reviewed. The importance of apposite study designs, detailed phenotyping of study participants, and the use of precise and safe quantitative research methods are highlighted. Where relevant, implications of the papers for personalised precision medicine are discussed.

Chapter 2. A putative postmenopausal polycystic ovary syndrome phenotype

Novel pathophysiological cardiometabolic insights between adipocytokines and cardiovascular disease in older women with a putative postmenopausal polycystic ovary phenotype.

2.1 Research summary

***Krentz AJ**, Von Muhlen D, Barrett-Connor E.*
Adipocytokine profiles in a putative postmenopausal polycystic ovary syndrome (polycystic ovary syndrome) phenotype parallel those in premenopausal polycystic ovary syndrome: The Rancho Bernardo Study.
Metabolism 2012;61:1238-41.

Background: polycystic ovary syndrome is the most common endocrine-metabolic disorder in women of reproductive age. While primarily characterised by hyperandrogenism and anovulation polycystic ovary syndrome is closely associated with abdominal obesity and insulin resistance (Joham et al., 2022). Adverse cardiometabolic risk factors include hyperglycaemia and hyperlipidaemia. Epidemiological studies have produced conflicting results concerning the risk of atherosclerosis in older women with a history of polycystic ovary syndrome (Papadakis et al., 2017). The complexity and heterogeneity of the phenotype has hampered epidemiological studies, especially as women transition to the post-menopausal state. As the recipient of a British Heart Foundation International Research Fellowship at the University of California San Diego[23] I tested the hypothesis that a polycystic ovary syndrome phenotype could be identified in postmenopausal women. The aim of my research was to generate a novel model that could help clarify the implications of polycystic ovary syndrome in the age group at highest risk of cardiovascular disease. The results were published in a series of original research papers in peer-reviewed journals.

Methods: The postmenopausal polycystic ovary syndrome model was based on the published literature from which clinical, biochemical, and endocrine criteria were identified (Krentz et al., 2007b)[24]. Female participants with intact ovaries were identified from the longitudinal Rancho Bernardo Community Study in California, USA[25]. The presence of ≥3 components of the phenotypic model was considered diagnostic of polycystic ovary syndrome; control women were defined as having ≤2 components. Serum levels of two cardiometabolic biomarkers of interest, i.e., leptin and adiponectin (Krentz et al., 2012b). The circulating levels of these regulatory adipocytokines, which are secreted by white adipocytes, are associated with multiple aspects of metabolic, vascular, and reproductive endocrine function .

[23] In the department of Professor Elizabeth Barrett-Connor, Distinguished Professor at the University of California San Diego, USA.
[24] The model diagnostic criteria included history of oligomenorrhoea, clinical or biochemical hyperandrogenism, infertility, abdominal adiposity, and/or insulin resistance (homeostasis model assessment, HOMA-IR). Polycystic ovary morphology is not required to substantiate a diagnosis of polycystic ovary syndrome.
[25] The Rancho Bernardo Study of cardiovascular risk was initiated in the 1970s under the directorship of Professor Barrett-Connor.

Results: The prevalence of the putative postmenopausal polycystic ovary syndrome phenotype (9.3%) mirrored the prevalence of polycystic ovary syndrome among women of reproductive age (Krentz et al., 2007b). The prevalence of atherosclerotic cardiovascular disease increased in line with an increasing number of components of the model. Linear dose-response associations were observed for each adipocytokine – positive for leptin and negative for adiponectin – with an increasing number of components of the model. The adipocytokine profiles paralleled those reported in classic premenopausal polycystic ovary syndrome (Krentz et al., 2012b). Using factor analysis, latent associations were identified between adipocytokines and cardiovascular risk factors in women with the postmenopausal polycystic ovary phenotype (Krentz et al., 2009b). Low levels of the multifaceted orexigenic hormone ghrelin, which are a reported feature of polycystic ovary syndrome in younger women, were also observed (Krentz and Barrett-Connor, 2009).

Interpretation: The observed dose-response associations between adipocytokines and the components of the putative phenotype provide support for the validity of the phenotypic model in postmenopausal women. It was concluded that the model could be of value in (a) future epidemiological studies addressing the risk of atherosclerosis associated with polycystic ovary syndrome and (b) informing the quest for novel pharmacotherapeutics, specifically by targeting altered adipocytokine profiles. The model might also be of value in addressing some of the challenges of managing polycystic ovary syndrome by quantifying the attributable cardiovascular health burden in older women.

2.2 Polycystic ovary syndrome

Polycystic ovary syndrome is a complex hormonal-metabolic disorder present in approximately 5-15% of women of reproductive age (Azziz et al., 2016, Adashi et al., 2023). In the majority of cases the syndrome is considered to result from interactions between polygenetic predisposition, environmental and behavioural factors (Dapas and Dunaif, 2022). Racial and ethnic differences are recognised with Hispanic women reportedly having the most severe phenotype in the US (Engmann et al., 2017). The syndrome is closely associated with excess adiposity (Barber et al., 2019). The global obesity epidemic and a rising global population predict that the prevalence of polycystic ovary syndrome will continue to increase (Barber and Franks, 2021).

Affected women may experience psychological symptoms (anxiety, depression, sleep and eating disorders) in concert with dermatological issues (hirsutism, acanthosis nigricans and acne) and reproductive difficulties (irregular menstrual cycles, infertility, endometrial cancer, and pregnancy complications). Well-documented adverse metabolic features include insulin resistance, metabolic syndrome, type 2 diabetes, cardiovascular risk factors and increased risk of cardiovascular disease (Teede et al., 2010, Boomsma et al., 2006, Apridonidze et al., 2005, Berni et al., 2021).

2.3 Pathophysiology and diagnosis

International guidelines for the assessment and management of polycystic ovary syndrome recommends use of the modified Rotterdam consensus criteria (Consensus Workshop Group., 2004, Teede et al., 2018). The diagnosis relies on the presence of any two of the following: (1) clinical or biochemical hyperandrogenism, (2) evidence of oligo-anovulation, (3) polycystic appearing-ovarian morphology on ultrasound, with exclusion of other relevant disorders[26]. Since no single diagnostic test is available the diagnosis of polycystic ovary syndrome is based on clinical criteria (Christ and Cedars, 2023). This results in four unique phenotypes: 1) complete, i.e., all three requirements are satisfied; 2) classic, i.e., anovulation and hyperandrogenism are present; 3) ovulatory, characterised by

[26] The differential diagnosis includes late-onset congenital adrenal hyperplasia, states of cortisol excess, hyperprolactinaemia, and adrenal and ovarian tumours.

polycystic ovarian shape and hyperandrogenism; and 4) non-androgenic, defined by the existence of polycystic ovarian morphology with anovulation (Broekmans et al., 2006). Cardiovascular disease risk associates with the three phenotypes that include hyperandrogenism (Rotterdam Eshre Asrm-Sponsored PCOS Consensus Workshop Group, 2004, Moran and Teede, 2009, El Hayek et al., 2016). The older National Institutes of Health criteria, which do not include polycystic ovarian morphology, are also still widely used (Chang and Dunaif, 2021, Consensus Workshop Group., 2004). It has been proposed that disease heterogeneity, allied to inconsistencies in diagnostic criteria between published clinical guidelines, leads to a high proportion of affected women remaining undiagnosed (Joo et al., 2020, Teede et al., 2023). Fluctuations in menstrual symptoms and metabolism during the menarche and menopausal transition further complicate diagnosis and impede the quest for precision personalised treatment (Joshi, 2024).

The aetiology of polycystic ovary syndrome has not been fully elucidated (Dapas and Dunaif, 2022). Familial clustering of cases indicates a genetic contribution to the syndrome. While rare Mendelian forms of polycystic ovary syndrome are associated with extreme phenotypes, a non-Mendelian pattern of inheritance is usual (Dapas and Dunaif, 2022). This is consistent with a complex genetic architecture, analogous to obesity and type 2 diabetes, that reflects the interaction of susceptibility genes and environmental factors (Dapas and Dunaif, 2022). Epigenetic changes have been described in ovarian granulosa cells (Sagvekar et al., 2019), adipocytes (Kokosar et al., 2016) and skeletal myocytes (Nilsson et al., 2018)

While polycystic ovary syndrome usually occurs in the presence of obesity, non-obese women may also be affected (Barrea et al., 2021). Androgen excess promotes visceral fat deposition (Pasquali and Oriolo, 2019) with putative bidirectional interactions between sex steroid hormones and adiposity traits (Loh et al., 2022). Dysfunctional adipose tissue may a role in the pathophysiology of polycystic ovary syndrome even in the absence of obesity (Chen et al., 2013, Bril et al., 2023). Along with genetic and environmental factors, epigenetic modulation via DNA methylation and microRNA regulation are implicated in dysregulated adipose tissue in polycystic ovary syndrome (Bril et al., 2023). Reported defects include impaired insulin signalling and glucose transport; dysregulation of lipolysis and

kinetics of non-esterified free fatty acids (NEFA), adipocytokine and cytokine dysregulation, subacute inflammation, mitochondrial dysfunction and oxidative stress (Bril et al., 2023). Preadipocytes and mature adipocytes express androgen receptors (Dieudonne et al., 1998). A proteomic study reported that the abundance in adipose tissue depots of proteins involved in metabolism were similar in women with androgen excess and in men suggesting that androgens masculinize the function of female adipose tissue (Montes-Nieto et al., 2013). Women with polycystic ovary syndrome have derangements of adipocyte products, characterised by higher levels of leptin and lower levels of adiponectin (De Medeiros et al., 2021). Pharmacological suppression of androgen concentrations in obese hyperandrogenic women with polycystic ovary syndrome on reduces visceral fat depots, and on metabolic abnormalities (Pasquali, 2006). In thermogenic brown adipose tissue, androgens reduce expression of uncoupling protein 1 (UCP1) leading to reduced biogenesis and mitochondrial respiration and a reduction in post-prandial thermogenesis (Lemaitre et al., 2023).

In parentheses, endometriosis, another prevalent disorder affecting women of reproductive age is also associated with an increased risk of atherosclerotic cardiovascular disease, namely ischaemic heart disease and cerebrovascular disease (Poeta Do Couto et al., 2023). Endometriosis is a benign, estrogen-dependent, chronic inflammatory disease the hallmark of which is the presence of endometrial glands and stroma outside the uterine cavity which may cause chronic pelvic pain (Vercellini et al., 2014). The increase in atherosclerosis associated with endometriosis is considered to result from chronic inflammation with oxidative stress and endothelial dysfunction (Marchandot et al., 2022).

2.4 Cardiometabolic risk profiles

Polycystic ovary syndrome and the metabolic syndrome share clinical, metabolic, and hormonal features (Sam and Dunaif, 2003, Ehrmann, 2005). Multisystem cardiometabolic defects in polycystic ovary syndrome include insulin resistance and fatty liver disease (Lonardo et al., 2019, Cooney and Dokras, 2021). Haemostatic and fibrinolytic defects have been described in affected women (Targher et al., 2014) as have endothelial dysfunction and chronic inflammatory activation (Gomez et al., 2022). Dysfunction of the single-cell monolayer that lines the entire vasculature reflects an imbalance between vasodilators, e.g., nitric oxide (NO) and vasodilators,

e.g., endothelin-1 (Little et al., 2021). Endothelial dysfunction, which has been proposed as an early subclinical predictor of atherosclerotic cardiovascular disease, can be quantified experimentally in response to mechanical or chemical stimuli (Little et al., 2021). Compared with controls, women with polycystic ovary syndrome have impaired endothelial function at the level of the microvasculature and in large vessels (Sprung et al., 2013). Microvascular dysfunction in polycystic ovary syndrome, which is independent of body mass index and age, has been attributed to hyperandrogenaemia (Moreau and Dubose, 2019).

Polycystic ovary syndrome is associated with an elevated risk of developing glucose intolerance and type 2 diabetes (Conway et al., 2014) (Azziz, 2018, Kakoly et al., 2019). Studies of whole-body insulin action using the glucose clamp technique have demonstrated decreases in insulin-mediated glucose disposal of approximately 35%-40% in women with polycystic ovary syndrome which are independent of obesity and body fat topography (Dunaif et al., 1989). Impaired insulin action is associated with the classic form of the syndrome that includes menstrual disturbances (Diamanti-Kandarakis and Dunaif, 2012). Compensatory hyperinsulinaemia is implicated in the pathogenesis of anovulation in women with polycystic ovary syndrome (Franks, 1995). Of note, hirsute women with hyperandrogenaemia and polycystic ovaries whose menstrual cycles are regular have serum insulin concentrations that are indistinguishable from those in weight-matched normal subjects (Franks, 1995).

Whether glucose intolerance, type 2 diabetes, coronary artery disease and stroke are direct consequences of polycystic ovary syndrome or arise via shared risk factors has been unclear until recently (Zhu and Goodarzi, 2022). Mendelian randomisation studies suggest that these cardiometabolic pathologies are not directly associated with polycystic ovary syndrome (Zhu and Goodarzi, 2022). Non-invasive assessments have provided evidence of subclinical atherosclerosis in women with polycystic ovary syndrome (Legro, 2003). However, since premenopausal women are intrinsically at low risk for cardiovascular events clinically evident atherosclerosis is rarely encountered in this population (Cooney and Dokras, 2021). The scientific literature relating to cardiovascular risk in women with polycystic ovary syndrome is discussed in more detail in Section 2.6.

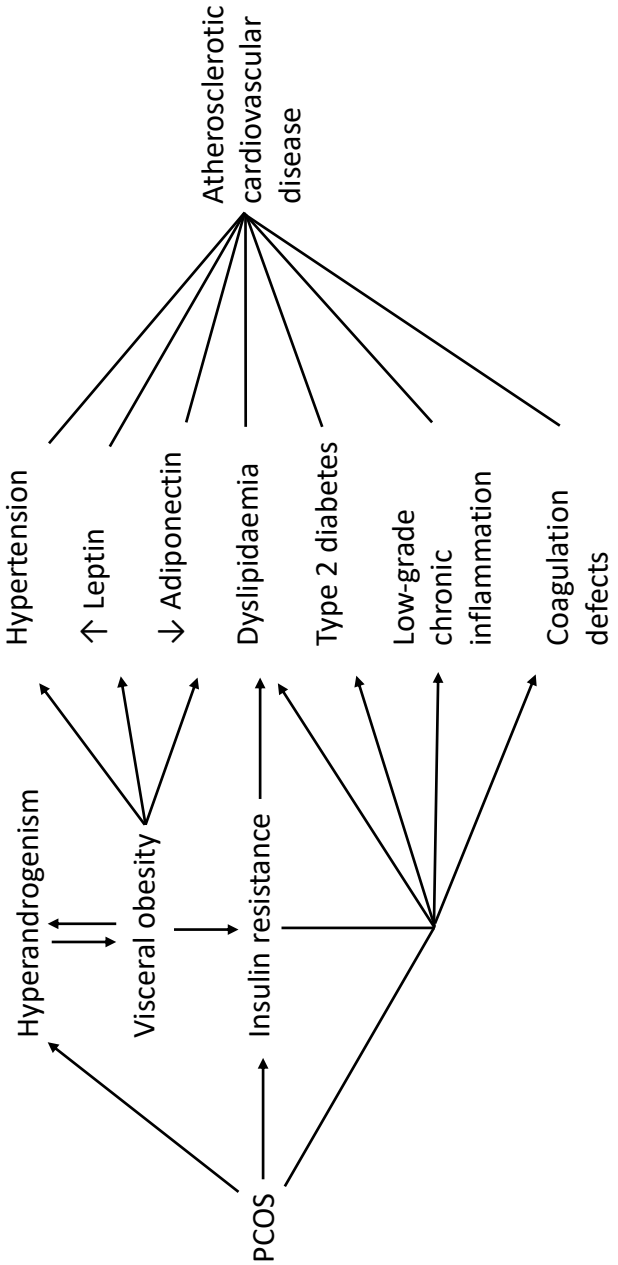
A male equivalent of polycystic ovary syndrome associated with early onset androgenic alopecia and adverse cardiometabolic risk profiles has been postulated in relatives of affected women (Di Guardo et al., 2020).

2.5 Adipocytokines

Adipocytokines, the best characterised of which are leptin and adiponectin, are pleiotropic signaling molecules secreted by white or brown adipose tissue that play a central role in regulating energy metabolism (Schuler-Toprak et al., 2022). Leptin and adiponectin affect metabolic and endocrine signalling in polycystic ovary syndrome (Schuler-Toprak et al., 2022). Inflammation within dysfunctional adipose tissue induces changes in adipocytokine levels (Piya et al., 2010). In turn, these alterations in adipocytokine physiology are considered to exert adverse effects on insulin sensitivity (Piya et al., 2010). Altered levels of leptin and adiponectin are implicated in the pathophysiology of the cardiometabolic risk profile associated with polycystic ovary syndrome (Figure 2) (Orio et al., 2016).

Circulating levels of leptin, a 167 amino acid 16-kilodalton protein encoded by the *ob* gene, increase in proportion with body fat mass (Mantzoros, 1999). The hormone, which acts via a transmembrane receptor, defends against low body weight by signalling to the hypothalamus to stimulate energy consumption (Considine, 2005). Leptin is a key hormone in energy homeostasis and neuroendocrine function, including reproduction (Chou and Mantzoros, 2014). Hyperleptinaemia is hypothesised to play a role in obesity-associated hypogonadism associated with obesity, polycystic ovarian syndrome, and type 2 diabetes (Chou and Mantzoros, 2014). In these conditions of chronic energy excess, mechanisms of reproductive dysfunction include hypothalamic leptin resistance as well as direct effects at the gonadal level (Karamouti et al., 2009). Aspects of the role of leptin on the cardiovascular system are complex and context dependent. Leptin is considered to have direct and potentially deleterious atherogenic, thrombotic, and angiogenic actions (Koh et al., 2008). Specifically, leptin stimulates vascular inflammation, oxidative stress and vascular smooth muscle hypertrophy potentially contributing to the pathogenesis of hypertension and atherosclerosis (Koh et al., 2008). Clinical studies have demonstrated that high leptin levels predict acute cardiovascular events, restenosis after coronary angioplasty and cerebral stroke independently of traditional risk factors (Beltowski, 2006). Elevated leptin concentrations are implicated in the pathogenesis of heart failure . Mechanistically, leptin promotes myocardial hypertrophy with circulating leptin levels correlating with left ventricular mass, even after accounting for body mass index (Hall et al., 2015). Leptin also stimulates myocardial fibrosis and endothelial dysfunction, effects that may be mediated in part via increased production of aldosterone by the adipocytokine (Huby et al., 2015).

Figure 2. Pathogenesis of cardiovascular disease in polycystic ovary syndrome.



Multiple risk factors for cardiovascular disease associated with polycystic ovary syndrome (PCOS): visceral adiposity, insulin resistance, type 2 diabetes, dyslipidaemia, arterial hypertension, low grade chronic inflammation, increased serum leptin levels, decreased adiponectin levels, and coagulation defects including platelet hyperreactivity and decreased plasma fibrinolytic activity.

Modified and expanded from: Orio et al., 2016.

Conversely, leptin positively regulates cardiac metabolism in supporting oxidation of glucose and fatty acids thereby protecting against cardiac lipotoxicity (Unger, 2005). Beyond its role in cardiometabolic diseases, leptin is also implicated in the pathogenesis of endometrial cancer, the incidence of which is increased several-fold in women with polycystic ovary syndrome [27] (Harris and Terry, 2016). The premalignant condition of endometrial hyperplasia is attributed to chronic exposure of the endometrium to oestrogen unopposed by progesterone (Harris and Terry, 2016). Leptin, the actions of which are modulated by oestrogens, participates in neoplastic phenomena including cellular proliferation and angiogenesis (Ray et al., 2018).

In contrast to states of excess adiposity, rare syndromes of lipodystrophy are characterised by leptin deficiency for which leptin analogues are used therapeutically (Meehan et al., 2016). Three decades after the discovery of leptin, however, major gaps in scientific knowledge persist concerning the role of leptin in the pathogenesis of obesity [28] (Flier and Ahima, 2024). In humans, leptin resistance has been described most clearly in subjects with obesity where circulating leptin levels are elevated and treatment with exogenous leptin does not reduce adiposity (Martin et al., 2008). Studies in animals and humans show that high dietary intake of fat and carbohydrates or low consumption of protein are drivers of leptin resistance (Mendoza-Herrera et al., 2021). The relative contributions of hyperleptinaemia vs. leptin resistance to dysmetabolic and pro-inflammatory effects remain largely unresolved (Martin et al., 2008). In contrast to polycystic ovary syndrome in which levels of luteinising hormone[29] are generally raised, obesity with increased leptin levels can induce hypothalamic leptin resistance and a reduction in gonadotrophin releasing hormone levels and pulsatility (Eng et al., 2024). Candidate leptin-sensitising drugs have been tested in preclinical models (Liu et al., 2015).

Adiponectin is a collagen-like protein produced by white and brown adipose tissue that circulates in the blood at high concentrations compared with other adipocytokines (Whitehead et al., 2006). Levels of adiponectin are normally higher in women than men (Chandran et al., 2003). This adipocytokine, which circulates in several molecular forms, exerts direct actions in liver, skeletal muscle and the vasculature (Pajvani et al., 2003,

[27] The association between polycystic ovary syndrome with ovarian cancer is less clear with no consistent association with breast cancer.
[28] Limited availability of leptin for human clinical investigations has been cited as a plausible reason for this evidence deficit.
[29] Luteinising hormone stimulates androgen production from ovarian theca cells.

Whitehead et al., 2006). Acting as a potent insulin sensitiser, adiponectin is considered to have favourable metabolic actions, notably protecting against type 2 diabetes (Spranger et al., 2003). Adiponectin also has anti-inflammatory, anti-atherosclerotic, and vasculoprotective effects (Lara-Castro et al., 2007, Siasos et al., 2012). In contrast to the situation pertaining to most other adipocytokines[30], adiponectin levels are reduced in obesity, type 2 diabetes, and other states of insulin resistance (Matsuzawa et al., 2004, Ryo et al., 2004, Toulis et al., 2009, Schuler-Toprak et al., 2022). A cross-sectional study that explored the relationship between adiponectin levels and diastolic dysfunction in women and men without diabetes reported a nonlinear relationship between adiponectin and left ventricular mass only in women suggesting a sex-specific interaction between adiponectin and cardiac remodeling (Norvik et al., 2017). With respect to heart failure, adiponectin generally offers protective effects (Theodorakis et al., 2024). However, a paradox exists, wherein elevated levels of adiponectin correlate with worse clinical outcomes (Bai et al., 2019). Whether this reflects a compensatory response to cardiac dysfunction or a maladaptive state of adiponectin resistance is unclear (Theodorakis et al., 2024).

Circulating adiponectin concentrations are independently and inversely associated with insulin resistance In women with polycystic ovary syndrome, (Spranger et al., 2004). However, while this association is well documented, the published literature contains some inconsistencies. A meta-analysis of 30 studies with a total of 2565 samples found significantly lower levels of adiponectin in nonobese women with polycystic ovary syndrome when compared to controls, albeit with heterogeneity among studies (Lin et al., 2020). There is evidence that aspects of adiponectin physiology are disrupted in women with polycystic ovary syndrome. For example, adiponectin secretion by adipocytes in response to cytokines/chemokines and to coculturing with adipose tissue macrophages favours greater suppression of adiponectin in polycystic ovary syndrome than in controls (Bril et al., 2023). In addition to alterations in circulating adiponectin levels, adiponectin receptor levels also appear to be distinctively regulated in adipocytes of women with polycystic ovary syndrome. Sex steroids appear to upregulate adiponectin receptors 1 and 2 (AdipoR1 and AdipoR2) in adipose tissue (Tan et al., 2006). Hyperinsulinaemia in non-obese women with polycystic ovary syndrome reportedly downregulates adiponectin receptors to induce a state of adiponectin resistance (Seow et al., 2009). In summary, current evidence suggests that adiponectin secretion by adipocytes in response to cytokines and chemokines, and in response to coculturing with adipose tissue macrophages, differs between women with

[30] For example, leptin, tumour necrosis factor-α, resistin, and interleukin-6.

polycystic ovary syndrome and controls, favoring greater suppression of adiponectin in polycystic ovary syndrome. Whether dysregulation of adiponectin is inherent to polycystic ovary syndrome or results from other factors, such as excess adiposity or insulin resistance, remains unclear (Bril et al., 2023).

2.6 Cardiovascular disease in women over the life course

Adverse trends in the prevalence of traditional risk factors for atherosclerotic cardiovascular disease, including diabetes mellitus, obesity and hypertension have been reported among women younger than 55 years (Vikulova et al., 2019). The diagnosis of cardiovascular disease is often delayed, and treatment is less optimal for women in general compared with men (Johnson et al., 2021). Differences in clinical presentation and underlying pathology, e.g., spontaneous coronary artery dissection, microvascular cardiac disease, may also be relevant to these disparities (Garcia et al., 2016, Jones et al., 2023). Sex hormones, adverse pregnancy outcomes and other reproductive factors may explain the preponderance of heart failure with preserved ejection fraction among women[31] (Kaur and Lau, 2022). While reports in the literature concerning risk of heart failure in women with polycystic ovary syndrome are sparse, concentric left ventricular hypertrophy has been reported in normotensive women with polycystic ovary syndrome that is not attributable to obesity (De Jong et al., 2022).

Premenopausal women in general are relatively protected against atherosclerosis compared with men (El Khoudary et al., 2020). The inherently low prevalence of clinical cardiovascular disease during the reproductive years has implications ascertainment of cardiovascular risk among women with polycystic ovary syndrome (Talbott et al., 2004). The use of differing diagnostic criteria between studies may also be relevant to epidemiological studies. Thus, women diagnosed according to the National Institutes of Health criteria, tend to display more metabolic abnormalities, associated with greater risk of cardiovascular disease, than women diagnosed according to the Rotterdam criteria (Anaforoglu et al., 2011). Premenopausal women with polycystic ovary syndrome have a higher prevalence of subclinical atherosclerosis as determined by non-invasive methods such as carotid intima media thickness (Talbott et al., 2000). In contrast, epidemiological studies of cardiovascular disease risk in women with polycystic ovary syndrome have yielded inconsistent results (Azziz, 2018) (Papadakis et al., 2017) (Kiconco et al., 2021). Recent real-world studies have provided clarification. First, a retrospective analysis of UK electronic health record data reported

[31] Defined as heart failure with a left ventricular ejection fraction >40%.

increased risks of incident myocardial infarction, angina, and revascularisation in younger women with polycystic ovary syndrome (Berni et al., 2021). Second, a population-based birth cohort study from Finland also concluded that polycystic ovary syndrome was a significant risk factor for cardiovascular events (Ollila et al., 2023). Significantly higher prevalence rates of myocardial infarction using the Rotterdam or National Institutes for Health diagnostic criteria (Ollila et al., 2023). Third, a UK Biobank study, which included/focused on non-obese women with polycystic ovary syndrome reported a more than two-fold excess risk of coronary artery disease and myocardial infarction among affected women (Zhao et al., 2024). The increased risk of cardiovascular disease was independent of polygenetic risk scores. The study also identified potential proteomics biomarkers for cardiovascular disease in women with polycystic ovary syndrome. Use of medications, including antiandrogens and metformin, used to treat polycystic ovary syndrome did not improve cardiovascular outcomes (Zhao et al., 2024). In addition, a meta-analysis across five databases indicated that the risk of incident cardiovascular disease was increased approximately 1.5-fold in women with polycystic ovary syndrome (Wan et al., 2024). The burden of cardiovascular disease associated with polycystic ovary syndrome varied by region: East Asia and Pacific regions had the highest rates of new cases of cardiovascular disease and South Asia experienced the highest increase in age-standardised incidence rates (Wan et al., 2024). These studies reinforce the need to direct attention to cardiometabolic risk factors in women with polycystic ovary syndrome (Gersh et al., 2025).

Transition from the premenopausal to the post-menopausal state is often accompanied by weight gain, visceral adiposity, greater insulin resistance, and less favourable profiles of cardiometabolic biomarkers (Anagnostis et al., 2022). Accordingly, beyond the menopause, the risk of cardiovascular disease is increased (Barrett-Connor, 2013, Anagnostis et al., 2022). It is increasingly appreciated that cardiometabolic risk in women should be considered over the life course and in the context of comorbidities (Welt and Carmina, 2013, Lobo et al., 2014, Jeong and Park, 2022). Whether adverse cardiometabolic risk profiles associated with polycystic ovary syndrome persist, deteriorate or improve at the menopause and beyond is presently unclear from the literature (Krentz et al., 2007b) (Helvaci and Yildiz, 2022).

Other reproductive disorders in women increase the long-term risk of cardiometabolic disease. These include hypertensive disorders of pregnancy and gestational diabetes (Appelman et al., 2015). Pregnancy is characterised by temporary insulin resistance which may unmask a predisposition to cardiovascular disease (Seely and Solomon, 2003, Barbour et al.,

2007). Premature menopause is also associated with elevated risk of cardiometabolic disease (Nappi et al., 2022).

2.7 Management

The management of polycystic ovary syndrome during reproductive years is anchored in non-pharmacological measures directed at achieving and maintaining weight loss sufficient to reduce insulin resistance, hyperinsulinaemia, and hyperandrogenism (Barber et al., 2019, Dong and Rees, 2023, Scragg et al., 2024). This includes attention to modifiable cardiometabolic risk factors, i.e., obesity, diabetes, and dyslipidaemia (Cooney and Dokras, 2021) (Cignarella et al., 2020). Non-pharmacological measures are supplemented where indicated by metformin, oral contraceptives, selective oestrogen receptor modulators, e.g., clomiphene, aromatase inhibitors, e.g., letrozole[32], anti-androgens, and assisted fertility measures (Hoeger et al., 2021, Teede et al., 2018). Insulin-sensitizing thiazolidinediones reduce triglycerides and fasting plasma insulin when compared with placebo in women with polycystic ovary syndrome (Abdalla et al., 2024). Thiazolidinediones improve anovulation, hyperandrogenaemia, hirsutism, and infertility, reinforcing the importance of adipose tissue dysfunction in the pathophysiology of polycystic ovary syndrome[33] (Bril et al., 2023). Metabolic and reproductive improvements with thiazolidinediones are also observed in lean patients with polycystic ovary syndrome (Ibanez et al., 2007). However, these drugs, which act on the nuclear peroxisome proliferator–activated receptor-gamma (PPAR-γ) receptors in adipocytes, cause significant increases in body weight when compared with metformin or placebo[34] (Abdalla et al., 2024). Myo-inositol, the actions of which are mediated via effects on insulin signalling, may improve glucose metabolism and reduce hyperinsulinaemia in women with polycystic ovary syndrome (Teede et al., 2023). Data on the effects of myo-inositol on menstrual cycles and fertility, however, remain scant (Teede et al., 2023).

Data on the efficacy and safety on GLP-1 receptor agonists and SGLT-2 inhibitors in the management of polycystic ovary syndrome are presently limited (Szczesnowicz et al., 2023, Tong et al., 2024, Roy et al., 2024). Surgical wedge resection of the ovaries, once a frequently performed procedure, is generally reserved for cases where pharmacological measures have proved unsuccessful (Della Corte et al., 2023). Investigators point to the need to manage polycystic ovary syndrome with greater personalisation and precision

[32] Use of letrozole for polycystic ovary syndrome is off label in the UK.
[33] Insulin-sensitizing drugs may restore ovulation with a risk of unplanned pregnancy.
[34] Thiazolidinediones cross the placenta and are contraindicated in pregnancy which limits their use in fertility-seeking women with polycystic ovary syndrome.

(Francone et al., 2023, Joshi, 2024). For example, the presence of insulin resistance, which cannot be readily quantified in routine clinical practice, provides a logical basis for insulin-sensitising medications to improve metabolic and endocrine profiles (Xing et al., 2020). In recognition of the heterogeneity of clinical phenotypes, recommendations have been published to guide epidemiological studies of polycystic ovary syndrome to the validity, integrity and comparability of data (Azziz et al., 2019). New biomarkers have been identified that could inform clinical management of the syndrome (Walford et al., 2024). Relevant biomarkers that are already routinely available include sex hormone-binding globulin, total and free testosterone, systolic and diastolic blood pressure, C-reactive protein, fibrinogen, oral glucose tolerance test, fasting insulin, homeostasis model assessment-insulin resistance index, total cholesterol and cholesterol subfractions, triglycerides, and lipoprotein(a) (Walford et al., 2024). It has been hypothesised that machine learning analytics applied to clinical data and multiple omics profiles could further improve stratification of this complex and heterogenous syndrome thereby facilitating more personalised therapy (Joshi, 2024).

Some features of polycystic ovary syndrome may resolve as women transition into menopause whereas others persist (Sharma and Mahajan, 2021, Millan-De-Meer et al., 2023, Hirschberg, 2023b). Menopausal hormone therapy has been proposed as a strategy for cardiovascular risk factor reduction. However, clinical trials have not consistently shown cardiovascular benefit (El Khoudary et al., 2020) (Prabakaran et al., 2021) (Gersh et al., 2021). That said, studies have shown a beneficial effect on cardiovascular morbidity and mortality if initiated <10 years since the final menstrual period or before the age of 60 years (Hale and Shufelt, 2015) (Anagnostis et al., 2019). It is recommended that decisions about menopausal hormone therapy [35] should be individualised based on assessment of vasomotor symptoms, risks, and benefits. Menopausal hormone therapy is not currently recommended for the sole purpose of cardiovascular disease prevention for women in general (Anagnostis et al., 2022). There remains a paucity of research on health implications of the menopause for women with polycystic ovary syndrome (Helvaci and Yildiz, 2022).

2.8 Adipocytokines: a therapeutic target in polycystic ovary syndrome?

It recognised that new therapeutic options are required to address the high prevalence of cardiometabolic risk factors in women with polycystic ovary syndrome (Yanes Cardozo and Romero, 2021). The cardiometabolic effects of leptin and adiponectin suggest that these

[35] Oestrogen ± progestogen ± testosterone, personalised to the individual. www.menopause.org.

adipocytokines might represent novel therapeutic targets (Figure 2.1) (Kim et al., 2022). Several drug classes – including well-established and novel agents – used in the management of cardiometabolic disorders raise adiponectin levels or lower leptin levels (Table 2) (Simonis, 2005, Chrusciel et al., 2016, Sahebkar et al., 2016, Katsiki et al., 2018, Siamashvili and Davis, 2021, Schuler-Toprak et al., 2022, Xu et al., 2022, Fontes et al., 2023, Youssef et al., 2023, Wang and Xia, 2022, Ryo et al., 2004).

Table 2. Cardiometabolic pharmacotherapeutics with actions on adipocytokines.

Drugs that raise adiponectin levels:

Metformin
Omega-3 fatty acids
Thiazolidinediones
SGLT-2 inhibitors
HMG-CoA reductase inhibitors (statins)

Drugs that reduce leptin levels:

Metformin[#]
DPP-4 inhibitors
GLP-1 receptor agonists[#]
SGLT-2 inhibitors[#]
Thiazolidinediones

Actions reported in human studies.

[#] These agents consistently reduce adiposity which in turn lowers circulating leptin levels.

HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A. See main text for details of other abbreviations and references.

The hypothesis that restoring normal adipocytokine levels confers reproductive and cardiometabolic health benefits in women with polycystic ovary syndrome is testable in clinical trials using appropriate methods and endpoints (Legro and Myers, 2004, Villa and Pratley, 2011, Spritzer et al., 2015, Xu et al., 2022). With the exception of dipeptidyl peptidase (DPP)-4 inhibitors[36], drugs with documented leptin-lowering actions also reduce adiposity (Table 2). There is currently no evidence that targeting hyperleptinaemia *per se* confers health benefits in polycystic ovary syndrome beyond reducing adiposity. A meta-analysis of the effects of pioglitazone on leptin in subjects with type 2 diabetes concluded

[36] DPP-4 inhibitors are licensed as adjuncts to diet and exercise in individuals with type 2 diabetes who require additional glucose-lowering therapy.

that a paucity of randomised controlled trials allied to statistical heterogeneity precluded robust conclusions (Ida et al., 2018). The potential for sexual dimorphism in responses to certain pharmacotherapies merits more detailed investigation. For example, pioglitazone reduces leptin levels in men but not women (Esteghamati et al., 2013). The potential of adiponectin as a biomarker of insulin resistance and a therapeutic target in polycystic ovary syndrome is considered in more detail in Section 6.2. The process of repurposing existing drugs for new clinical indications is discussed in Section 6.7.

As discussed in Chapter 6, GLP-1 receptor agonists have shown encouraging results in polycystic ovary syndrome (Han et al., 2019, Cena et al., 2020, Gersh et al., 2025). In addition, limited evidence suggests that statins may offer benefits mediated by reducing hyperandrogenism (Shawish et al., 2021). However, potential adverse metabolic effects of statins in insulin-resistant states need to be considered (see Chapter 4). Experimental therapies that have not progressed from preclinical to clinical development include modulation of thermogenic brown adipose tissue and soluble receptors to reduce levels of the multifunctional adipocyte-derived cytokine tumour necrosis factor (TNF)-α (Bril et al., 2023).

2.9 A novel postmenopausal polycystic ovary syndrome phenotype

By the early part of the 21st century it was considered that the cardiometabolic risk profiles associated with polycystic ovary syndrome would raise the lifetime risk of atherosclerosis. However, the cardiovascular implications of the syndrome had proved difficult to quantify in observational studies (Krentz et al., 2007b). Aspects of the pathophysiological associations between adiposity, adipocytokine profiles, and atherosclerosis in affected women remained uncertain. In order to help clarify the impact of polycystic ovary syndrome on cardiovascular health and provide mechanistic insights my colleagues and I studied postmenopausal women using a novel diagnostic approach based on the scientific literature. Participants who, by definition were at higher risk of cardiovascular than their premenopausal counterparts, were identified from the longitudinal Rancho Bernardo Study of Healthy Aging in California, USA. Hypotheses tested included: a postmenopausal polycystic ovary phenotype is associated with prevalent cardiovascular disease; serum profiles of the fat-derived adipocytokines leptin and adiponectin (a) reflect those in premenopausal women with the syndrome (Krentz et al., 2012b) and (b) are associated with prevalent cardiometabolic risk factors (Krentz et al., 2009b).

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Adipocytokine profiles in a putative novel postmenopausal polycystic ovary syndrome (PCOS) phenotype parallel those in premenopausal PCOS: the Rancho Bernardo Study

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ABSTRACT

The objective was to investigate whether the associations between leptin, adiponectin, and adiposity reported in classic polycystic ovary syndrome (PCOS) are also observed in elderly women with a novel putative postmenopausal PCOS phenotype. We studied 713 postmenopausal community-dwelling women. Diagnosis of the novel phenotype required the presence of ≥3 diagnostic features including: 1) a personal history of oligomenorrhea; 2) history of infertility or miscarriage; 3) current or past clinical or hormonal evidence of hyperandrogenism; 4) central obesity; 5) biochemical evidence of insulin resistance. Women in the control group had ≤2 of these components. Mean age (±SD) was 74±8 years for the study cohort. Sixty-six women (9.3%) had the putative PCOS phenotype. Serum leptin was higher (mean 25.70±15.67 vs 14.94+9.89 ng/mL, $P<.01$) and adiponectin lower (mean 11.72±4.80 vs 17.31±7.45 μg/mL, $P<.01$) in cases vs controls. Leptin was positively, and adiponectin inversely, associated with an increasing number of phenotype features ($P<.01$ for linearity). In age-adjusted regression analysis, adjustment for waist circumference eliminated the association between leptin and the PCOS phenotype, but not the association between adiponectin and the PCOS phenotype. In this novel postmenopausal PCOS phenotype, adipocytokine profiles and their associations with adiposity parallel those reported in younger women with classic PCOS. These results support our hypothesis that a putative phenotype analogous to PCOS can be identified in postmenopausal women using clinical and biochemical criteria. Use of this novel phenotype could provide a basis for studies of the delayed consequences of PCOS in older women.

1. Introduction

Leptin and adiponectin are adipocyte-derived hormones implicated in reproductive physiology [1-3]. Altered circulating levels have been reported in polycystic ovary syndrome (PCOS). Similar to women without PCOS, elevated serum leptin levels in PCOS appear to reflect body fat mass [4,5], independently of its anatomical location [6]. The

Abbreviations: PCOS, polycystic ovary syndrome; BMI, Body mass index.

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association between reduced adiponectin levels and fat mass in women with PCOS is more complex [7,8]. A recent meta-analysis concluded that adiponectin levels were lower in women with PCOS compared to healthy controls even after controlling for obesity [9]. The lowest adiponectin concentrations are seen in women with the classic clinical and biochemical features of PCOS, i.e., amenorrhea with hyperandrogenism [7].

In a novel approach, we have described a putative PCOS phenotype in older postmenopausal women based on characteristic clinical, hormonal, and biochemical components of PCOS [10]. In this study we tested the hypothesis that the associations of leptin and adiponectin with adiposity and other features of our putative phenotype mirror those seen in PCOS during reproductive years.

2. Methods

2.1. Study cohort

Among the participants in the 1984–87 Rancho Bernardo Study research clinic visit, we identified 713 women ≥50 years who (a) had intact ovaries, (b) were not taking estrogen therapy, (c) were at least one year past their last menstrual period, and (d) had serum estradiol concentrations <100 pmol/L. Height, weight, waist circumference, and hip circumference were measured with participants wearing lightweight clothing without shoes. Body mass index (BMI) was calculated as weight (kg)/height (m^2).

2.2. Definition of the putative PCOS phenotype

The criteria we selected and methods used to define the postmenopause PCOS phenotype have been described in detail [10]. In the absence of a universally agreed definition of PCOS [11,12] we used a novel definition based on the literature to identify the main clinical, endocrine, and metabolic features that characterize PCOS. Our definition required the presence of ≥3 of the following diagnostic features: 1) a history of oligomenorrhea; 2) infertility or miscarriage; 3) current or past clinical or biochemical evidence of hyperandrogenism; 4) central obesity; 5) insulin resistance (i.e., homeostasis model assessment value in highest quintile or fasting serum glucose ≥6.1 mmol/L). The control group comprised women with ≤2 of these diagnostic components.

2.3. Laboratory methods

Hormones were measured by radioimmunoassay after solvent extraction and Celite column chromatography using first-thawed samples in the University of California San Diego endocrine research laboratory of SSC Yen. Details of the assays have been reported elsewhere [10,13]. Adipocytokines were measured in the same serum samples at Linco Diagnostics Laboratory (St. Louis, MO) [14]. The sensitivity and the intra- and interassay coefficients of variation, respectively, were 0.8 mg/L, 6%, and 7% for adiponectin, 0.5 ng/mL, 4% and 5% for leptin, 0.2 mg/L. The laboratory reports no problem with the assay during two freeze-thaw cycles and the adiponectin levels in our report are similar to levels reported in the literature using the same assay [14].

2.4. Statistical analysis

Data were analyzed using SPSS version 11.5 (SPSS, Chicago, IL). One-way analysis of variance was used to compare groups and linearity was sought for trends in adipocytokines. Non-normally distributed data were analysed after logarithmic transformation. Differences between transformed and non-transformed data were minimal and the data are presented in the untransformed state.

2.5. Ethics approval

Informed written consent was obtained from all participants. The study was approved by the UCSD Human Subjects Protection Board.

3. Results

The mean (±SD) age of the 713 women was 74±8 years (range 51–89). The overall prevalence of the putative PCOS phenotype was 9.3%. Table 1 shows the mean age, body mass index, and waist circumference by PCOS phenotype; there were no statistically significant differences for these variables by PCOS status (Table 1). Mean time since menopause was 27.0±10.1 years (range 1 to 60) for women with the PCOS phenotype vs 24.2+12.4 years (range 1–59) forthe controls. Serum leptin was higher (mean 25.70±15.67 vs 14.94+9.89 ng/mL, $P<.01$; Fig. 1A) and adiponectin was lower (mean 11.72±4.80 vs 17.31±7.45 μg/mL, $P<.01$) in women ($n=66$) with the phenotype compared with the 647 without the phenotype (Fig. 1B). Leptin (Fig. 2A) was positively, and adiponectin (Fig. 2B) was inversely, associated with an increasing number of components of the phenotype ($P<.01$ for trends for both variables). A dose–effect association was evident, with the highest leptin and lowest adiponectin levels being seen in the women with the greatest number (i.e., 4 out of a possible total of 5) of diagnostic features of the phenotype. In age-adjusted multiple regression analysis, adjustment for waist circumference eliminated the association between leptin and the PCOS phenotype. The association between adiponectin and the PCOS phenotype was independent of either waist circumference or BMI.

Table 1 – Clinical characteristics of the study population (mean ± standard deviation).

	PCOS ($n=66$)	Controls ($n=647$)	P
Age (years)	72±9	74±8	<.05
Body mass index (kg/m^2)	28.0±4.8	23.9±3.3	<.001
Waist circumference (cm)	89.5±12.2	78.5±8.9	<.001

PCOS = Women with the putative polycystic ovary phenotype.

4. Discussion

Adipocytokine profiles in women with a putative postmenopausal PCOS phenotype paralleled those reported in premenopausal women with classic PCOS. First we confirmed that mean serum leptin was elevated compared with the control group, whereas adiponectin was reduced [15]. Second, the concentration of each adipocytokine was linearly associated with the number of diagnostic features of the phenotype–leptin positively and adiponectin negatively. Third, a dose–effect association was evident for both adipocytokines: a gradient, more pronounced for leptin, was observed in the adipocytokines as a function of an increasing number of the pre-specified features that define our phenotype. Fourth, the association between leptin, but not adiponectin, and the putative phenotype was eliminated by adjustment for waist circumference.

Our findings are consistent with studies in younger women with PCOS that have shown no increase in leptin beyond that expected for fat mass regardless of its anatomical location [5]. Our proposed postmenopausal PCOS phenotype is based upon the principal features of PCOS described in the literature [16,17]. We have published data showing our phenotype has demographic, metabolic, and hormonal characteristics that closely resemble the PCOS syndrome in reproductive age women [10,18]. PCOS is strongly associated with multiple metabolic defects [19] that are closely intertwined with leptin and adiponectin levels [20]. Thus, the finding of the dose–effect association provides additional support for our approach to identifying a novel PCOS phenotype in elderly women [19].

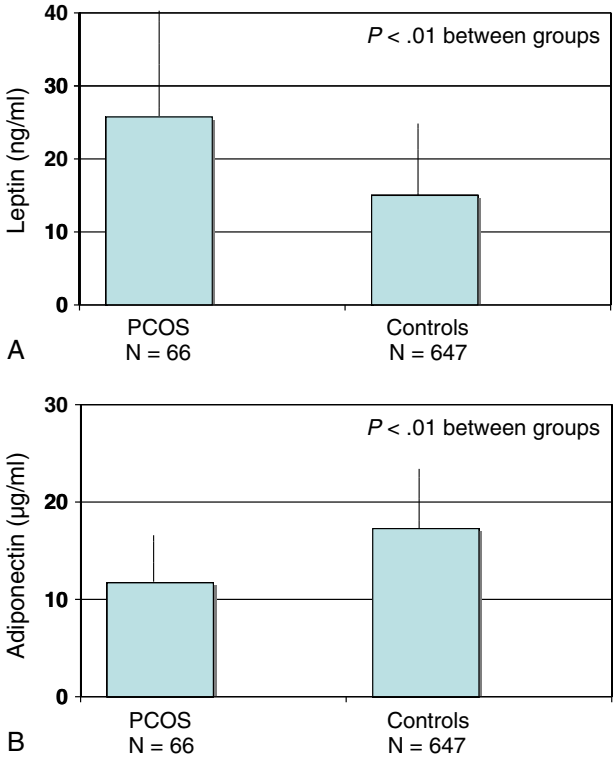

Fig. 1 – A. Mean (±SD) serum leptin concentrations in women with the PCOS phenotype vs controls. B. Mean (±SD) serum adiponectin concentrations in women with the PCOS phenotype vs controls.

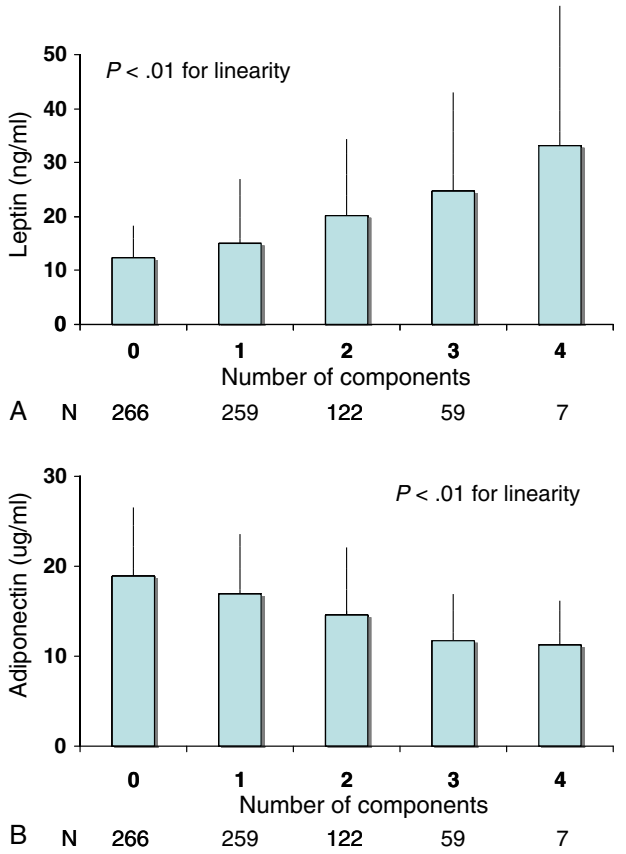

Fig. 2 – A. Association between mean serum leptin concentrations vs number of components of the postmenopausal PCOS phenotype. B. Association between mean serum adiponectin concentrations vs number of components of the postmenopausal PCOS phenotype.

Scientific interest in the clinical consequences of PCOS in later life is gathering pace [21,22]. Our unique approach has been to identify a constellation of clinical and biochemical variables in postmenopausal women that are characteristic of the syndrome during their reproductive years. This necessitates certain assumptions that in part reflect the challenges of diagnosing PCOS in younger women [12]. Strengths of our study include the large community-based sample of well-characterized women. We excluded women using menopausal hormone therapy, thereby eliminating their confounding effect on sex hormones and adipocytokines [10]. Potential limitations include the Caucasian composition of women in the Rancho Bernardo Study and a mean BMI of the cohort that is lower than that for the general U.S. population. Generic issues such as recall bias and the potential for misclassification are inherent in a retrospective study. We measured levels of total adiponectin, a protein that circulates in several forms. Recent data suggest that the high molecular weight oligomeric form of adiponectin may be preferentially reduced in women with PCOS [8].

In summary, adipocytokine profiles and their metabolic associations within a novel postmenopausal PCOS phenotype parallel those seen in classic PCOS. These findings provide additional support for our hypothesis that a putative phenotype analogous to PCOS can be identified in postmenopausal women. We suggest that this novel phenotype could provide a basis for studies of the delayed consequences of PCOS in older women.

Author contributions

AJK, DvM, and EB-C contributed to the design and conduct of the study, data collection and analysis, and data interpretation; AJK and EB-C drafted and revised the manuscript.

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Conflicts of interest

None to declare.

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Chapter 3. Early-phase clinical study of a novel glucose-lowering drug

A phase 1b clinical trial of a novel glucokinase activator in adults with metformin-treated type 2 diabetes.

3.1 Research summary

***Krentz AJ**, Morrow L, Petersson M, Norjavaara E, Hompesch M.*
The effect of exogenously administered glucagon versus spontaneous counter-regulatory responses on glycaemic recovery from insulin-induced hypoglycaemia in subjects with type 2 diabetes treated with a novel glucokinase activator AZD1656 and metformin.
Diabetes, Obesity & Metabolism 2014;11:1096-101.

Background: Management of type 2 diabetes usually requires a multiplicity of glucose-lowering drugs used in combination. The elevated risk of atherosclerotic cardiovascular disease in individuals with type 2 diabetes requires that new diabetes drugs must have demonstrable cardiovascular safety. Novel glucose-lowering agents in the glucokinase activator class have potential to cause hypoglycaemia (Sharma et al., 2022). In turn, hypoglycaemia can induce adverse cardiovascular events in vulnerable patients (Sanon et al., 2014). Intramuscular glucagon[37] is a well-established emergency rescue therapy for iatrogenic hypoglycaemia caused by glucose-lowering medications (Porcellati et al., 2021). Glucagon rapidly mobilises glucose from hepatic glycogen stores. Glucokinase is expressed in the liver as well as the islet ß-cells (Matschinsky and Wilson, 2019, Matschinsky and Porte, 2010). Since glucokinase plays a role in regulating the glycogen content of the liver AZD1656 carries a theoretical risk of impairing the glucose-mobilizing action of glucagon (Krentz et al., 2014). This randomised, two-way crossover phase 1b experimental study in metformin-treated adults with type 2 diabetes tested the hypothesis that recovery from insulin-induced hypoglycaemia would not be impaired by a novel glucokinase activator, AZD1656.

Methods: The stepped hyperinsulinaemic hypoglycaemic clamp technique was applied in a randomised, placebo-controlled, within-patient crossover study involving carefully characterised participants with type 2 diabetes treated with metformin. The study was performed at the ProSciento clinical research institute in San Diego, USA[38]. Experimental hypoglycaemia was progressively induced to pre-determined target glucose levels using state-of-the-art automated methodology[39] to avoid operator bias (Krentz, 2019b). The response of plasma glucose to a standard dose of glucagon were assessed in participants during therapy with AZD1656 or placebo.

[37] Glucagon antagonises the actions of insulin to mobilise glucose from tissue glycogen stores.
[38] I was Senior Director of Scientific Services at ProSciento 2011-2016.
[39] ProSciento is recognised internationally for its expertise in the use of precision Biostator® technology for glucose clamp studies.

Results: Intramuscular glucagon was effective in accelerating the time to recovery from experimental insulin-induced hypoglycaemia during treatment with AZD1656. The responses of other counter-regulatory hormones including catecholamines and cortisol to hypoglycaemia were not impaired by AZD1656. No clinically relevant changes or trends in blood pressure or heart rate were observed during the trial period.

Interpretation: It was concluded that clinical use of exogenous glucagon as rescue therapy for the treatment of iatrogenic hypoglycaemia would be expected to be successful in patients with type 2 diabetes receiving AZD1656 in combination with metformin.

3.2 Type 2 diabetes

Type 2 diabetes is a complex and heterogeneous cardiometabolic disorder that accounts for approximately 90-95% of all cases of diabetes. At the global level, type 2 diabetes is one of the most rapidly growing non-communicable diseases (Chatterjee et al., 2017). Type 2 diabetes is considered to be a polygenic disease characterised by numerous associated gene variants (Hattersley and Patel, 2017, Mahajan et al., 2018, Udler et al., 2019, Alimi et al., 2021). However, emerging evidence suggests greater levels of complexity with monogenic, oligogenic, and polygenic contributions (Bonnefond et al., 2025).

Gene-environment interactions and associated epigenetic markers are implicated in the aetio-pathogenesis of type 2 diabetes (Fraszczyk et al., 2022). The thrifty genotype hypothesis originally posited in the 1960s speculates that ancestral genetic predisposition to obesity and diabetes confers a survival advantageous in times of food scarcity, as it would promote efficient retention of energy stores, but would become disadvantageous in times of relative food abundance and low energy expenditure (Neel, 1999). The prevalence of type 2 diabetes in the 21st century is lowest in rural areas of developing countries, generally intermediate in developed countries, and highest in certain ethnic groups who have adopted western lifestyle patterns (Wareham et al., 2002). In the US and UK, it is suggested that the disproportionate impact of type 2 diabetes in minority ethnic groups may be compounded by interactions with social deprivation (Cheng et al., 2019, Nagar et al., 2021). The rising prevalence and incidence of youth-onset type 2 diabetes in the US and globally is a public health concern that predominantly affects non-white populations (Perng et al., 2023).

The PPARs, identified three decades ago, were prominent candidate molecular mechanisms for the thrifty genotype. PPARs are lipid-activated nuclear receptors necessary for multiple physiological processes relating to energy balance (Wagner and Wagner, 2023). By the early 2000s, it had been established that common variants in the *PPARG* and other genes influence susceptibility to type 2 diabetes (McCarthy, 2004). PPARs have also been implicated the fetal origins of adult disease hypothesis of David Barker. The hypothesis postulates that early developmental events, notably intrauterine growth retardation, predispose to type 2 diabetes and related cardiometabolic disease and other disease later in life (Barker, 2007, Guo et al., 2022). The human epigenome includes DNA methylation, histone modifications, and RNA-mediated processes (Ling and Ronn, 2019). It is hypothesised that disruption of the epigenome adipose tissue, skeletal muscle, the pancreatic islets, and liver may contribute to the development of obesity and type 2 diabetes (Ling and Ronn, 2019).

3.3 Pathophysiology

The principal biochemical defects of type 2 diabetes are variable degrees of relative insulin deficiency in association with resistance to the actions of insulin in target tissues (Kahn et al., 2014) (Galicia-Garcia et al., 2020a). Insulin resistance in muscle and liver contributes to the development of hyperglycaemia and heightens the risk of atherosclerosis through direct and indirect pathways (Abdul-Ghani et al., 2019). At the molecular genetic level, alleles associated with risk of type 2 diabetes reportedly have differential associations with cardiometabolic outcomes (Dicorpo et al., 2022). Reported subtypes of type 2 diabetes may reflect aetiological diversity with variable risks of long-term vascular complications (Ahlqvist et al., 2018). Recent data suggest a high prevalence of undiagnosed hypercortisolism among people with type 2 diabetes in whom glycaemic control is difficult to attain with multiple glucose-lowering medications (Buse et al., 2025).

It is recognised that the clinical classification of diabetes subtypes may be challenging (American Diabetes Association Professional Practice Committee, 2025). Type 2 diabetes is essentially a diagnosis of exclusion[40] (Udler, 2019). Insulin resistance and insulin deficiency are continuous and inter-related variables that may alter over the natural history of the development and progression of type 2 diabetes (Weyer et al., 1999). Accurate classification of diabetes at diagnosis is crucial for personalised precision disease management (Bowman et al., 2018). Meticulous phenotyping of diabetes, including microvascular and macrovascular complications, is also required for clinical trials (Sharma et al., 2021). However, this is increasingly problematic due to overlaps in characteristics between the common diabetes types, especially in younger patients (Tosur et al., 2024). As discussed in Section 3.5, many individuals with type 2 diabetes develop degrees of insulin deficiency that require exogenous insulin therapy to achieve glycaemic control (Evans and Krentz, 1999). Conversely, type 1 diabetes[41] is increasingly accompanied by features of the metabolic syndrome that have traditionally been associated with type 2 diabetes (Lee et al., 2021).

[40] The differential diagnosis includes monogenic and syndromic forms of diabetes, pancreatic injury, and latent autoimmune diabetes in adults (LADA).
[41] Type 1 diabetes is characterised by profound insulin deficiency that mandates lifelong insulin therapy.

3.4 Vascular complications: microvascular and macrovascular

Diabetes mellitus is defined as the degree of chronic hyperglycaemia that confers a risk of microvascular complications, specifically retinopathy[42] (Krentz and Bailey, 2005). The cumulative effects of hyperglycaemia, dyslipidaemia and hypertension – often present in combination – define the pathophysiology of diabetic kidney disease, diabetic retinopathy and diabetic neuropathy (Eid et al., 2019).

Intracellular biochemical mechanisms mediating tissue damage include activation of the polyol pathway (Garg and Gupta, 2022), formation of advanced glycation end-products (Goldin et al., 2006), protein kinase C activation (Meier and King, 2000), inflammation (Navarro and Mora, 2005), oxidative stress (Caturano et al., 2023), and insulin resistance (Brownlee, 2005). Hyperglycaemia leads to long lasting epigenetic modifications with self-perpetuating upregulation of proinflammatory and profibrotic genes. Systemic and tissue-specific protective factors, e.g., antioxidant enzymes, serve to partially counter these risk factors (Yu et al., 2024). The risk of vascular complications that may lead to premature mortality is greatest when type 2 diabetes is diagnosed in youth or early adulthood (Today Study Group et al., 2022, Lin et al., 2024). The main microvascular and macrovascular complications of diabetes are presented in Table 3.

Microvascular endothelial cells are vulnerable to hyperglycaemic because glucose transport rate cannot be downregulated in the presence of hyperglycaemia (Brownlee, 2005). This is thought to induce microvascular endothelial dysfunction, e.g., decreased availability of (Forbes and Cooper, 2013) oxide (NO), increased cellular permeability, increased leukocyte adhesion, and increased procoagulant activity, initiated by mitochondrial overproduction of reactive oxygen species (Brownlee, 2005). Microvascular dysfunction, including reduced capillary density, lowers insulin-mediated glucose disposal in skeletal muscle thereby contributing to hyperglycaemia (Jonk et al., 2007, Stehouwer, 2018).

[42] The microvasculature denotes vessels <150 μm in diameter.

Table 3. Microvascular and macrovascular complications of diabetes.

Microvascular disease

- Retinopathy
  - Non-proliferative – angiogenesis not evident; includes maculopathy
  - Proliferative – neovascularisation with risk of vitreous haemorrhage
  - Advanced diabetic eye disease – includes fibrosis, retinal detachment
- Nephropathy
  - Declining glomerular filtration rate – risk marker of accelerated atherosclerosis
  - Proteinuria – marker of all cause and cardiovascular mortality
  - Hypertension – promotes retinopathy and macrovascular disease
- Neuropathy
  - Distal symmetrical polyneuropathy – a major factor in diabetic foot disease
  - Autonomic neuropathy – may contribute to elevated atherosclerosis risk
  - Mononeuropathies – may affect multiple peripheral nerves

Macrovascular disease*

- Coronary artery disease – often more diffuse and severe in presence of diabetes
- Cerebrovascular disease – two- to four-fold increase in diabetes
- Peripheral arterial disease – risk increased two-fold in individuals with diabetes

Notes:

The elevated risk of heart failure associated with diabetes reflects a higher prevalence of coronary artery disease, which is often more diffuse in the presence of diabetes, and myocardial metabolic, functional and structural defects, i.e., diabetic cardiomyopathy. In addition to vascular complications, non-traditional concordant morbidities of type 2 diabetes, e.g., metabolic liver disease, share common risk factors and pathophysiological pathways.

The risk of atherosclerotic cardiovascular disease is increased even with levels of hyperglycaemia below the threshold for diabetes. Complex interactions may operate between microvascular complications, i.e., nephropathy, autonomic neuropathy, and macrovascular disease. See main text for details.

* Primary prevention of cardiovascular disease denotes measures to avert the first manifestation of atherosclerosis whereas secondary prevention aims to prevent recurrent events.

Large vessel disease, i.e., atherosclerosis, is the leading cause of premature mortality in patients with type 2 diabetes (Laakso, 2010, Shah et al., 2015, Mosenzon et al., 2021). Approximately one-third of people with type 2 diabetes have cardiovascular disease, including coronary artery disease, heart failure, and stroke (Einarson et al., 2018, Mosenzon et al., 2021). Cardiovascular disease may be recurrent or exacerbated by ageing chronic hyperglycaemia, dyslipidaemia, and impaired kidney function (Wilson, 1992). Cardiovascular mortality in general has declined in the US and other Western countries in recent years. However, cardiovascular mortality remains higher in individuals with diabetes compared to nondiabetic controls (Joseph et al., 2022). Bidirectional associations between atherosclerosis and type 2 diabetes may reflect a multiplicity of shared aetiopathophysiological factors (Fernandes Silva et al., 2021) (Krentz, 2002c) (Walker et al., 2022). The risk of atherosclerosis is also increased by degrees of dysglycaemia below the diagnostic threshold for diabetes, i.e., impaired fasting glucose and impaired glucose tolerance (Coutinho et al., 1999). It has also been suggested that degrees of dysglycaemia below the diagnostic threshold for diabetes and hypertriglyceridaemia may also be risk factors for microvascular dysfunction in the retina and peripheral nerves (Krentz et al., 2009a, Iqbal et al., 2021).

Mendelian randomisation data support the hypothesis that glucose levels that lie within the normoglycaemic range or higher are a causal risk factor for microvascular disease (Emanuelsson et al., 2020). The clinical potential of leveraging the overlap between microvascular and macrovascular disease is illustrated by a study of UK Biobank participants of whom >95% did not have diabetes (Yusufu et al., 2025). Using a deep-learning algorithm for automated segmentation and quantification of retinal vascular networks, abnormalities in retinal vasculature, including vessel diameter, fractal dimension and tortuosity identified on fundus photography improved the prediction of stroke events (Yusufu et al., 2025). Relevant to cardiovascular risk stratification in subjects with type 2 diabetes is the question whether microvascular disease increases the risk for macrovascular complications via direct or indirect mechanisms. To address this hypothesis, a population-based cohort study of 49,027 patients with type 2 diabetes examined the association between the combined endpoint consisting of cardiovascular death, nonfatal myocardial infarction, stroke and the cumulative presence of retinal disease, nephropathy and peripheral neuropathy over 5.5 years (Brownrigg et al., 2016). Each type of microvascular end-organ damage was associated with an increase in cardiovascular risk of approximately 35–40%. Relative cardiovascular risk increased in the presence of one, two or three microvascular diseases increased by 32%,

62% and 99%, respectively. Similar trends were seen for cardiovascular death, all-cause mortality and hospitalisation for heart failure (Brownrigg et al., 2016). Microvascular disease may promote the development and progression of atherosclerotic disease via mechanisms specific to affected organs or systems. For example, diabetic autonomic neuropathy may exacerbate coronary heart disease and raise the risk of sudden death (Agashe and Petak, 2018). Declining kidney function with proteinuria is associated with increasing cardiovascular mortality (Matsushita et al., 2015). Chronic kidney disease is also associated with an elevated risk of heart failure, i.e., the cardio-renal-metabolic syndrome (Whaley-Connell and Sowers, 2014). Diabetic cardiomyopathy, which contributes to risk of heart failure, is characterised in its early stages by impaired diastolic relaxation and later by heart failure in the absence of dyslipidaemia, hypertension or coronary artery disease (Jia et al., 2018).

Shared modifiable risk factors promote the development and progression of both macrovascular and microvascular complications of diabetes (Krentz et al., 2007a). Molecular and cellular mechanisms include inappropriate activation of the renin angiotensin–aldosterone system, mitochondrial dysfunction, excessive oxidative stress, inflammation, dyslipidaemia, and thrombosis (Jia et al., 2024). Chronic hyperglycaemia and acute glucose fluctuations induce oxidative stress while endothelial dysfunction arises from impaired insulin-mediated activation of the phosphoinositide 3-kinase/Akt/endothelial NO synthase pathway in endothelial cells resulting in decreased production of NO and increased endothelin-1 (Maruhashi and Higashi, 2021). Microvascular dysfunction contributes to hyperglycaemia and elevated blood pressure, and in the long term to the development of cardiometabolic disease. In the Framingham Heart Study, type 2 diabetes conferred a 2- to 4-fold increased risk of hypertension, peripheral arterial disease, and myocardial infarction (Fox, 2010). Experimental studies demonstrate that approximately 50% of individuals with hypertension have insulin resistance that could contribute to the development of diabetic vasculopathy (Swislocki et al., 1989). It is hypothesised that capillary dysfunction in skeletal muscle contributes to whole-body insulin resistance thereby promoting the development of the metabolic syndrome (Stehouwer, 2018). Microvascular endothelial dysfunction impairs insulin-mediated glucose disposal (Clark et al., 2003). A vicious cycle is constituted wherein hyperglycaemia along with the cardiovascular risk factors that comprise the metabolic syndrome further impair microvascular function (Stehouwer, 2018, Jia et al., 2024). Adipocytokines have direct pathogenic roles in microvascular dysfunction**.** Leptin activates human platelets and can limit trans-endothelial cell diffusion whereas adiponectin protects

endothelial cells and has anti-inflammatory actions (Elbatarny et al., 2007, El Husseny et al., 2017).

3.5 Pharmacotherapeutics

Once diagnosed, the majority of patients with type 2 diabetes require pharmacotherapy in the pursuit of evidence-based glycaemic goals. Traditionally, this approach involved the stepwise addition of oral glucose-lowering agents with different and complementary mechanisms of action, e.g., stimulating endogenous insulin secretion with a sulfonylurea while reducing inappropriate rates of hepatic glucose production using metformin (Kahn et al., 2014, Lamoia and Shulman, 2021). Certain oral glucose-lowering drugs, e.g., sulfonylureas, metformin, depend for their action on sufficient reserves of endogenous insulin secretion (Bailey, 2017b). A substantial proportion of patients are ultimately treated with exogenous insulin when islet β-cell function has deteriorated beyond the point of compensating for the prevailing degree of insulin resistance (Evans and Krentz, 1999).

3.5.1 Benefit-risk considerations of glucose-lowering pharmacotherapy

Certain classes of glucose-lowering agents are associated with unwanted cardiometabolic effects (Krentz, 2002b, Bailey & Krentz, 2017, Krentz, 2018, Kahn et al., 2014, Lamoia and Shulman, 2021). For example, sulfonylureas and insulin have long been recognized to carry risks of weight gain and hypoglycaemia which may detract from the benefits of these agents on glycaemia (Gehlaut et al., 2015, Apovian et al., 2019). Together with the biguanide metformin, sulfonylureas and insulin were the mainstays of pharmacotherapy for type 2 diabetes from the 1950s to the 1980s. During these decades, there was debate within the scientific community as to whether long-term glycaemic control would reduce the risk of vascular complications of type 2 diabetes. The 25-year observational study of 4,398 by Jean Pirart in Brussels, unprecedented in its size and duration, was influential in showing an association between poor glycaemic control[43] and the risk of retinopathy, nephropathy and neuropathy (Pirart, 1977c, Pirart, 1977a, Pirart, 1977b). In contrast, the degree of glycaemic control seemed to have little influence on other vascular manifestations such as atherosclerosis in the coronary and peripheral circulations. Pirart acknowledged that the non-randomised design of his study could not prove the existence of a causal link between long-term hyperglycaemia and the microvascular complications of type 2 diabetes. His

[43] Assessed by degree of glycosuria and fasting and postprandial blood concentrations.

observations generated a testable hypothesis, i.e., that sustained intensive glycaemic control would mitigate the development of the long-term vascular complications of type 2 diabetes. Randomised clinical trials, while providing important insights with implications for clinical practice, generated additional uncertainties about risk-benefit considerations of intensified glycaemic control using the pharmacotherapeutic options available to investigators at the time. The first drugs of three new classes of orally active glucose-lowering medications became available during the years that these trials were designed and conducted. These included the α-glucosidase inhibitor acarbose which slows intestinal carbohydrate digestion thereby targeting postprandial hyperglycaemia (approved in 1995) (Bischoff, 1995); the insulin-sensitizing thiazolidinediones[44] (Wise, 1997); and the DPP-4 inhibitors to enhance the endogenous incretin system (sitagliptin approved in 2006) (Ahren, 2007). In clinical practice, these newer agents were often usefully combined with each other and/or with metformin, sulfonylureas, and insulin. Incidentally, a clinical trial (STOP-NIDDM) reported a delay in the development of diabetes, hypertension, and cardiovascular complications in a high-risk population with impaired glucose tolerance (Chiasson et al., 2002, Chiasson et al., 2003). Criticisms of aspects of the study design led the investigators to publish a justification of the validity of their results (Chiasson et al., 2004). A subsequent trial conducted in Chinese patients[45] with coronary heart disease and impaired glucose tolerance failed to show any cardiovascular benefits of acarbose (Holman et al., 2017b).

3.5.2 Randomised clinical trials of intensified glucose control

In 1998, the landmark United Kingdom Prospective Diabetes Study (UKPDS) was published (UK Prospective Diabetes Study Group., 1998). UKPDS provided data on the benefits and risks in adults with newly diagnosed type 2 diabetes of an intensified glycaemic control policy based on a sulfonylurea or insulin vs. conventional treatment, i.e., diet (UK Prospective Diabetes Study Group., 1998). The early use of insulin in UKPDS was at variance with prevailing national clinical practice; exogenous insulin was generally reserved for patients in whom combination oral therapy could not provide adequate glycaemic control (Evans and Krentz, 1999). Other glucose-lowering drugs including metformin and the α-glucosidase inhibitor acarbose[46] were used in addition to sulfonylureas and insulin in response to

[44] Troglitazone was approved in 1997 and subsequently withdrawn from the UK the same year due to reports of unexpected hepatotoxicity, including fatalities.
[45] Acarbose has proved popular in East and South Asia.
[46] Acarbose was compared with placebo across the intensive and conventional therapy groups. At 3 years, a lower proportion of patients were taking acarbose compared with placebo (39 vs. 58%, $P < 0.0001$) primarily because of gastrointestinal side-effects.

progressive hyperglycaemic during the study (Holman et al., 1999). Over ten years, separation between the groups was maintained with median [range] haemoglobin A_{1c} being 11% lower at 7.0% [6.2-8.2] with intensive treatment compared with 7.9% [6.9-8.8] in the conventional therapy group. Most of the risk reduction in an aggregate diabetes-related endpoint was attributable to a 25% risk reduction ($p<0.001$) in microvascular endpoints, including the need for retinal photocoagulation Intensified glucose control was associated with a 16% reduction in myocardial infarction that did not attain statistical significance ($p=0.052$). The rates of major hypoglycaemic episodes were higher with sulfonylurea- and insulin-based therapy. Weight gain was higher in the intensive group than in the conventional group ($p<0.001$) with patients assigned to insulin experiencing greater gains than those assigned to sulfonylureas. In turn, the UKPDS stimulated a series of large, randomised, multicentre interventional trials focused on glucose-lowering in type 2 diabetes ((Patel et al., 2008, Gerstein et al., 2008, Duckworth et al., 2009). These comprised: the glucose control arm of the Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation trial (ADVANCE); Action to Control Cardiovascular Risk in Diabetes (ACCORD); and the Veterans Affairs Diabetes Trial (VADT). The trials tested the hypothesis that intensified glycaemic control achieved through pharmacotherapeutics, and assessed by glycated haemoglobin, would reduce morbidity and mortality attributable to long-term vascular complications of type 2 diabetes (UK Prospective Diabetes Study Group., 1998, Patel et al., 2008, Gerstein et al., 2008, Duckworth et al., 2009). None of these trials, while providing useful insights into therapeutic strategies, demonstrated reductions in cardiovascular mortality (Table 4).

Table 4. Trials of intensified glycaemic control in type 2 diabetes.

Study Name	*Therapeutic strategy*	*Glycaemic target for intensive therapy groups*	*Attained mean HbA_{1c} (%) for intensive vs control groups*	*Effects on macrovascular complications*	*Effects on microvascular complications*	*Comments*
UKPDS United Kingdom Prospective Diabetes Study	Sulfonylurea- or insulin-based therapy vs. diet*	Fasting plasma glucose <6 mmol/L	7.0 vs 7.9 over 10 years	Diabetes-related complications reduced No reduction in macrovascular events	25% reduction of (pooled) microvascular complications	Blood pressure control tested in embedded subgroup No systematic use of ACE inhibitors Pre-statin era
ADVANCE Action in *Diabetes* and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation	Glycaemic control based on modified release gliclazide (plus additional therapy if required)	HbA_{1c} $\leq$6.5%	6.3 vs 7.0	Combined micro and macro vascular endpoint reduced No reduction in macrovascular disease	Incident nephropathy -21% as measured by albuminuria progression, no change in eGFR	4X4 factorial design: when ACE-inhibition on board - no additional effect of glucose-lowering
ACCORD Action to Control Cardiovascular Risk in Diabetes	Combination use of all available classes of glucose-lowering drugs as necessary	HbA_{1c} <6.0%	6.4 vs 7.5	Increased mortality	Delayed onset of albuminuria, Reduced rate of progression of retinopathy	Increased cardiovascular mortality in intensive glucose control group
VADT Veterans Affairs Diabetes Trial	Combinations of glucose-lowering therapies as per baseline clinical criteria	Absolute reduction of HbA_{1c} by 1.5%	6.9 vs 8.4	No reduction in combined MACE, 3 point MACE + surgery for complication, heart failure, amputation for gangrene	Better: any increase in albuminuria, no improvement of deline in eGFR, nor retinopathy or neuropathy endpoints	Largest HbA_{1c} difference among type 2 diabetes intervention trials

Notes

With the exception of UKPDS which was conducted in participants with newly diagnosed type 2 diabetes, all the trials included adults with established type 2 diabetes and additional risk factors for cardiovascular disease.

* Glycaemic targets in the so-called conventional (=diet) treatment group were fasting glucose <15 mmol/L and the absence of osmotic symptoms. UKPDS 34 was a substudy in overweight or obese subjects with newly diagnosed type 2 diabetes who were randomly allocated to metformin as first-line pharmacotherapy or diet. Other glucose-lowering agents were added as necessary to control symptoms and excessive hyperglycaemia. See main text for further details.

HbA_{1c}, haemoglobin A_{1c}; MACE, major adverse cardiovascular events; ACE, angiotensin converting inhibitors; eGFR, estimated glomerular filtration rate.

Based on Jacob et al, 2021.

Moreover, ACCORD – the primary endpoint of which was a composite of nonfatal myocardial infarction, nonfatal stroke, or death from cardiovascular causes – reported higher total and cardiovascular mortality with intensive glucose-lowering therapy (Gerstein et al., 2008). This finding led to a discontinuation of intensive therapy after a mean of 3.5 years of follow-up. The unexpected results of ACCORD were subsequently debated at length (Lachin et al., 2014, Riddle, 2010). ACCORD focused on the levels of glycaemia achieved using a variety of strategies, not on the specific therapies used (Gerstein et al., 2007). Hypotheses that were advanced to explain the excess death rates with intensified glucose-lowering therapy included iatrogenic hypoglycaemia and weight gain resulting from drug combinations that included the use of sulfonylureas, insulin, and thiazolidinediones (Gerstein et al., 2008). Prior episodes of severe hypoglycaemia were a risk factor for mortality and occurred more often in the intensive treatment group. However, the excess of deaths was not accounted for by those subjects who had experienced earlier episodes of severe hypoglycaemia (Genuth and Ismail-Beigi, 2012). Post-hoc analyses of ACCORD, while generating testable hypotheses, did not provide a clear mechanistic explanation for the excess mortality with intensified glycaemic control (Chiasson and Le Lorier, 2014).

With the exception of the UKPDS, participants in ADVANCE, ACCORD and VADT had established type diabetes with a high prevalence of vascular complications. The routine use of non-glycaemic cardioprotective medication, e.g., statins, antihypertensive agents, may have reduced the probability of demonstrating a benefit of improved glycaemic control (Jacob et al., 2021). A meta-analysis of the trials showed that intensive glycaemic control resulted in a statistically significant 17% reduction in events of non-fatal myocardial infarction (odds ratio 0.83, 95% confidence interval 0.75-0.93), and a 15% reduction in events of coronary heart

disease (0.85, 0.77-0.93) (Ray et al., 2009). While UKPDS demonstrated protective effects on microvascular complications, principally diabetic retinopathy, from intensified glycaemic control treatment effects were inconsistent (UK Prospective Diabetes Study Group., 1998, Rodriguez-Gutierrez and Montori, 2016). In UKPDS, a subgroup of patients >120% their ideal bodyweight who were treated initially with metformin had statistically significant risk reductions for any diabetes-related endpoint , diabetes-related death, and all-cause mortality compared with less intensive therapy. This substudy was published as UKPDS 34 (UK Prospective Diabetes Study Group, 1998). These findings, which were not attributable to improved glycaemic control, positioned metformin as first-line therapy for type 2 diabetes for two decades (Cosentino et al., 2020). It is noteworthy that the mechanisms of action of metformin remain uncertain after many decades of clinical use. Metformin is considered to target hepatic mitochondria via adenosine monophosphate (AMP)-activated protein kinase (AMPK)-dependent and AMPK-independent actions (Foretz et al., 2023). In parentheses, the putative clinical benefits of metformin have since been extended beyond type 2 diabetes. While definitive clinical trial data are not available, it is proposed that metformin may have beneficial effects on aging and healthspan mediated via anti-hyperglycaemic actions, enhanced insulin sensitivity, reduced oxidative stress and protective effects on the endothelium and vascular function (Mohammed et al., 2021). Reported effects of metformin on cellular aging, oxidative stress, gut microbiota and epigenetics may also be relevant (Soukas et al., 2019).

It is hypothesised that the impact of intensive glucose-lowering strategies may persist beyond the period of therapeutic intervention. In a follow-up non-randomised observational study of the UKPDS participants for 10 years beyond the end of the main trial, statistically significant differences emerged favouring prior intensified glycaemic control on myocardial infarction and all-cause mortality (Holman et al., 2008). These observations suggest that the effects of intensive glucose-lowering might require longer timeframes to demonstrate benefit – the so-called legacy effect. There are caveats in the UKPDS 10-year follow up data since due to financial constraints during the final five years events could not be externally validated and adjudicated. A subsequent longer follow-up suggested that early intensive glycaemic control with sulfonylurea or insulin or metformin appeared to confer durable reduced risks of death and myocardial infarction compared with conventional glycaemic control, as defined in UKPDS (Adler et al., 2024). In contrast to UKPDS, no legacy effects were observed after the end of the randomised therapy period in ADVANCE, ACCORD and VADT (Zoungas et al., 2014, Accord Study Group, 2016, Hayward et al., 2015). However, with additional caveats

that recognise the different pathophysiology of type 1 and type 2 diabetes, the Diabetes Control and Complications Trial (DCCT) in patients with type 1 diabetes demonstrated a clear protective effect of intensive glycaemic control on microvascular complications with no effect on macrovascular events over a mean of 6.5 years (The Diabetes Control and Complications Trial Research Group, 1993). A protective effect on macrovascular disease was, however, subsequently observed in a post-trial follow up study (Nathan et al., 2005). Extending the consideration of putative legacy effects of pharmacotherapy beyond glucose control to lipids, durable benefits have also been reported for statin therapy in hypertensive participants in the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) (Sever et al., 2025).

3.5.3 Cardiovascular outcome trials of novel glucose-lowering drugs

In the presence of cardiovascular disease, insulin-induced hypoglycaemia in patients with type 2 diabetes is associated with risks of acute cardiovascular events (International Hypoglycaemia Study, 2019). Thus, avoidance of hypoglycaemia has been a major consideration in the development of new classes of glucose-lowering medications beyond those used in the trials of intensified glucose control for type 2 diabetes[47]. Moreover, following the controversy centred on potential adverse cardiovascular effects of the insulin sensitising agent rosiglitazone cardiovascular outcome trials were mandated by regulators to establish the safety of new glucose-lowering drugs (Nissen and Wolski, 2007, Krentz, 2011, Krentz, 2014). These trials were primarily powered to demonstrate prespecified non-inferiority relative to placebo in terms of cardiovascular safety (Krentz, 2014). This stipulation resulted in an unprecedented evidence base for the GLP-1 receptor agonist and SGLT-2 inhibitor classes, which proved to have unexpected cardioprotective properties (Schnell et al., 2020). These two classes, which have transformed the therapeutic landscape for type 2 diabetes, will be considered in more detail in terms of their mode of action, clinical benefits and limitations. Of note, there was no use of GLP-1 receptor agonists or the SGLT-2 inhibitors in UKPDS or ADVANCE since these trials were performed prior to the approval of these agents. Exenatide[48], the first GLP-1 receptor agonist to be approved, was used

[47] Any licensed glucose-lowering medication was acceptable in ACCORD and VADT; insulin was used by 35% of participants at entry in ACCORD.

[48] Exenatide – approved for clinical use in 2005 – is a synthetic version of exendin-4 which was discovered in the saliva of Heloderma suspectum, a lizard native to the Southwestern United States. Exendin-4 is a 39 amino acid peptide that has 53% homology with human GLP-1 and a longer circulating half-life conferred by resistance to degradation by dipeptidyl peptidase-4.

infrequently in ACCORD as part of multi-drug regimen (Bloomgarden, 2008). In response to the rosiglitazone cardiovascular safety controversy, the drug was subject to restricted use in the US and was withdrawn in 2010 in Europe (Nissen, 2010). With pioglitazone remaining as the sole agent in this class in many countries the use of thiazolidinediones – the only drugs for type 2 diabetes to specifically reduce insulin resistance – declined (Lebovitz, 2019). As mentioned, metformin has long been regarded as first-line monotherapy as an adjunct to diet and exercise for patients with type 2 diabetes in the absence of contraindications. The 2019 European Society of Cardiology guidelines on diabetes, pre-diabetes and cardiovascular diseases[49] generated controversy when metformin was no longer recommended as first-line pharmacotherapy for patients at high risk of cardiovascular disease (Cosentino et al., 2020, Jorsal et al., 2020). The 2019 guideline update reflected the unequivocal evidence of the cardioprotective properties of GLP-1 receptor agonists and SGLT-2 inhibitors that had become available since the previous update. In the light of the findings of these trials the 2019 recommendations stated that metformin could be considered as first-line therapy in overweight patients with type 2 diabetes without evidence of cardiovascular disease and SGLT-2 inhibitors or GLP-1 agonists should be first-line in diabetes patients at high or very high risk (Cosentino et al., 2020). However, cardiovascular outcome trials of GLP-1 receptor agonists and SGLT-2 inhibitors were generally performed in participants who were receiving metformin. Defenders of metformin pointed out that the European Society of Cardiology recommendations were based on data from subgroup analyses with an assumption that metformin is without additive cardiovascular benefit in combination with the newer glucose-lowering drugs (Jorsal et al., 2020). While it was acknowledged that no large-scale cardiovascular outcome trial with metformin has been conducted it was argued that metformin therapy should be viewed in an appropriate historical context (Jorsal et al., 2020).

It should be noted that, in contrast to the intensive glycaemic control studies (UKPDS, ADVANCE, ACCORD, the cardiovascular outcome trials of GLP-1 receptor agonists and SGLT-2 inhibitors did not aim to generate different levels of glycaemic control between the intervention and placebo groups. The intention of the UKPDS, ADVANCE, ACCORD and VADT investigators was to attain and subsequently maintain glycaemic separation between the treatment groups, i.e., intensive vs. less intensive control, and to maintain this separation over the time course of the trial. In the cardiovascular outcome trials of GLP-1 receptor agonists and SGLT-2 inhibitors the regulatory mandate required placebo-controlled cardiovascular safety to be assessed in the context of standard of care. The intention here

[49] Developed in collaboration with the European Association for the Study of Diabetes.

was to achieve similar levels of glycaemic control in the active and placebo treatment groups. During the trials – and contrary to the stated aim of glycaemic equipoise – glycated haemoglobin levels in the active treatment arms tended to be lower than for placebo. Differences of approximately 0.5% in glycated haemoglobin were observed between active and placebo treatment groups in the EMPA-REG OUTCOME trial of empagliflozin and the LEADER study of liraglutide, as discussed below. Based on the existing literature, these differences in glycaemia were considered insufficient to explain the observed cardiovascular benefits of these drugs (McGuire et al., 2021a). Thus, by design, glucose-lowering efficacy in the cardiovascular outcome trials of GLP-1 receptor agonists or SGLT-2 inhibitors was generally underestimated. In these trials, additional glucose-lowering drugs from other classes were permitted[50] in order maintain glycaemic control. Glycaemic goals were aligned to contemporary clinical guidelines with individualisation of targets as appropriate. During the LEADER trial, for example, more patients in the placebo group than in the liraglutide group had an intensification of glucose-lowering treatment, including greater use of insulin (Marso et al., 2016b). Even so, glycaemic control was superior in the liraglutide group.

The selection criteria for the cardiovascular outcome trials ensured recruitment of patients at high risk of cardiovascular disease to ensure accrual of sufficient numbers of clinical events to adequately test the cardiovascular safety hypothesis. Cardiovascular outcome trials were also mandated for agents in the DPP-4 inhibitor class of glucose-lowering agents[51]. These drugs, which offer weight neutrality and a negligible intrinsic risk of hypoglycaemia, proved a popular alternative to sulfonylureas, albeit with lower glucose-lowering potency (Mishriky et al., 2015). Alogliptin and sitagliptin, while demonstrating cardiovascular safety, did not confer significant cardiovascular benefits (White et al., 2013, Green et al., 2015). Saxagliptin did not increase or decrease the rate of ischaemic events but increased the rates hospitalisation for heart failure (Scirica et al., 2013).

3.5.4 Glucagon-like peptide-1 receptor agonists

GLP-1 receptor agonists are mimetics of the endogenous incretin system with origins in studies dating back to the 1960s (Creutzfeldt, 2005). Human GLP-1(7-37)amide, discovered in 1986 by Jens Juul Holst and Joel Habener, is a 30 amino acid peptide derived from pre-

[50] Dose reduction of non-study drugs was also permitted, as required. For patients who did not meet the recommended target for glycaemia, use of other GLP-1–receptor agonists, DPP-4 inhibitors, or pramlintide was not permitted.
[51] DPP-4 inhibitors reduce the degradation of endogenous GLP-1.

proglucagon and released from enteroendocrine cells in the distal ileum in response to ingested nutrients. GLP-1 augments insulin production and inhibits glucagon secretion (Orskov et al., 1986, Mojsov et al., 1986, Drucker et al., 2017). In subjects with type 2 diabetes, the incretin effect is either impaired or absent (Vilsboll and Holst, 2004). Relative insulin deficiency is accompanied by glucagon levels that are inappropriately non-suppressed in the presence of chronic hyperglycaemia (Unger and Orci, 1975) (Lund et al., 2014). Developed initially as glucose-lowering drugs for the treatment of type 2 diabetes the advantages of injectable GLP-1 receptor agonists, which are either derivatives of exendin-4[52] (lixisenatide, exenatide) or long-acting analogues of the native hormone (liraglutide, albiglutide, dulaglutide, semaglutide), over exogenous insulin were rapidly appreciated by patients and clinicians (Madsbad, 2016). While individual drugs differ with respect to pharmacokinetic properties, duration of action, and clinical effectiveness, GLP-1 receptor agonists as a class reduce hyperglycaemia and carry a low intrinsic risk of iatrogenic hypoglycaemia[53] (Trujillo et al., 2021). Moreover, the anti-hyperglycaemic actions are usually accompanied by weight loss compared with promotion of weight gain, as often occurs with sulfonylureas, thiazolidinediones, and insulin treatment (Nauck et al., 2021a). GLP-1 also inhibits gastric emptying and food intake, actions that maximise nutrient absorption while limiting excess weight gain (Drucker, 2018). In healthy human volunteers, GLP-1 infusion increased satiety and reduced solid food intake (Flint et al., 1998). GLP-1 agonists and dual-hormone successors have assumed centre-stage in medical management of obesity after decades of agents characterised by inefficient weight loss and unacceptable tolerability or safety issues (Krentz et al., 2016, Grandl et al., 2024). Ad libitum energy intake is lowered by GLP-1 agonists such with semaglutide consequent on reduced appetite and food cravings, better control of eating and lower relative preference for fatty, energy-dense foods (Blundell et al., 2017). Since 2005, the GLP-1 receptor monoagonists approved in the US and the UK for the treatment of type 2 diabetes are exenatide, lixisenatide, liraglutide, dulaglutide, and semaglutide which are administered via subcutaneous injection[54,55]. The placebo-controlled LEADER (Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results) trial in participants with type 2 diabetes at high risk of cardiovascular events was the first to show benefits of a GLP-1 receptor agonist – liraglutide

[52] Exendin-4, which has 53% homology with GLP-1, was discovered in the saliva of Heloderma Suspectum, a lizard native to the Southwestern United States and northern Mexico, in 1990. Exendin-4 is relatively resistant to degradation by DPP-4 compared with GLP-1.
[53] Hypoglycaemia risk may be increased when GLP-1 receptor agonists are combined with certain glucose-lowering drugs such as sulfonylureas or insulin.
[54] Exenatide extended release, dulaglutide, and semaglutide are administered once weekly.
[55] Semaglutide is also available as an oral formulation for the management of type 2 diabetes.

– on major adverse cardiovascular events along with a reduction in cardiovascular mortality and all-cause mortality in patients with type 2 diabetes (Marso et al., 2016b). Cardiovascular benefits were also confirmed for semaglutide, albiglutide[56], and dulaglutide (Marso et al., 2016a, Hernandez et al., 2018, Gerstein et al., 2019). The short-acting GLP-1 receptor agonists lixisenatide and exenatide-extended release confirmed non-inferiority in terms of cardiovascular safety but did not demonstrate superiority over placebo (Pfeffer et al., 2015, Holman et al., 2017a). Differences between studies in entry criteria, the conduct of the trials, and the fact that lixisenatide and exenatide are both derived from exendin-4 may be relevant to these observations (Nielsen et al., 2004, Melo et al., 2021). However, another GLP-1 receptor agonist based on exendin-4 – efpeglenatide – reduced cardiovascular endpoints in a high risk patients with either cardiovascular disease, renal disease, or both (Gerstein et al., 2021).

A subsequent meta-analysis of eight cardiovascular outcome trials of injectable GLP-1 receptor agonists reported a 14% reduction in the primary outcome of the three-component major adverse cardiovascular events, i.e., cardiovascular death, nonfatal myocardial infarction, and nonfatal stroke with heterogeneity between studies (Sattar et al., 2021). An oral formulation of semaglutide was approved by the Food & Drug Administration for glucose control in people with type 2 diabetes in 2019. In SOUL (Semaglutide cardiOvascular oUtcomes triaL) oral semaglutide reduced the risk of major adverse cardiovascular events compared with placebo in participants with type 2 diabetes and established cardiovascular disease, chronic kidney disease, or both (McGuire et al., 2025). In another landmark trial – SELECT (Semaglutide Effects on Cardiovascular Outcomes in People with Overweight or Obesity) – the cardioprotective benefits of semaglutide were shown to extend to the secondary prevention of cardiovascular disease in overweight and obese patients without type 2 diabetes (Lincoff et al., 2023)[57]. A meta-analysis that included ten randomised controlled trials of seven different GLP-1 receptor agonists in 67 769 participants with type 2 diabetes including SELECT showed a 19% reduction (hazard ratio 0·81, 95% confidence interval 0·72–0·92; I^2 =23·11%) with GLP-1 receptor agonists in a clinical composite kidney outcome (Badve et al., 2025). The study also confirmed a 14% reduction in major adverse cardiovascular events (hazard ratio 0·86, 0·80–0·92; I^2 =48·9%). GLP-1 receptor agonists also reduced the risks of hospitalisation for heart failure and death from any cause. Treatment effects were consistent regardless of diabetes status and across subgroups of

[56] Albiglutide was withdrawn by the manufacturer for commercial reasons in 2017.
[57] Liraglutide and semaglutide are licensed for the treatment of obesity.

age, sex, body mass index, and presence or absence of cardiovascular disease. In addition, the safety of GLP-1 receptor agonists on safety outcomes such as acute pancreatitis, pancreatic cancer, and medullary thyroid cancer was confirmed (Badve et al., 2025). Longer-term clinical considerations, notably the risk of weight regain on discontinuation of these agents, are under active investigation (Wilding et al., 2022).

The cardioprotection provided by GLP-1 receptor agonists is a prominent example of successful bench to bedside cardiometabolic translational research (Ravassa et al., 2012, Drucker et al., 2017). Integral to this translation from bench to clinical use, the cardiovascular biology of GLP-1 has been extensively documented (Drucker, 2016). GLP-1 acts via GLP-1 receptors in multiple tissues to impact cardiovascular function in health and disease (Helmstadter et al., 2022). While the mechanisms responsible for the clinical benefits observed in the cardiovascular outcome trials remain uncertain, reduced inflammation (Mehdi et al., 2023), lower levels of oxidative stress (Cai et al., 2018), improvements in endothelial function (Koska et al., 2015), improved left ventricular function (Sokos et al., 2006), promotion of atheromatous plaque stability (Wang et al., 2024), and decreased platelet aggregation (Jia et al., 2016) are reported effects of GLP-1 and/or GLP-1 receptor agonists (Drucker, 2016, Bray et al., 2021).

In addition to glucose control (Drucker, 2024), cardioprotection (Marx et al., 2022) and renoprotection[58] (Perkovic et al., 2024, Badve et al., 2025), incretin mimetics are being evaluated in other diseases within the cardiometabolic nexus including neurodegenerative diseases (Kopp et al., 2022) and steatotic liver disease (Newsome et al., 2021). Thus, GLP-1 receptor agonists improve health and quality of life, reduce mortality, and have potential to lower the risk of several leading causes of mortality and disability. No other medication class to date has shown such substantial and wide-ranging benefits (Sumithran and Ard, 2025).

The success of GLP-1-based medicines spurred the development of new and more potent molecular entities and combinations with unique pharmacokinetic and pharmacodynamic profiles (Drucker, 2024). Tirzepatide, is a potent glucose-dependent insulinotropic polypeptide (GIP) + GLP-1 receptor co-agonist which demonstrated superiority over semaglutide in terms of weight reduction and glycaemic control (Frias et al., 2021,

[58] In January 2025, semaglutide was approved by the Food and Drug Administration to reduce the risk of worsening kidney failure, end-stage kidney failure, and death in patients with type 2 diabetes and chronic kidney disease.

Nauck and D'Alessio, 2022, Rodriguez et al., 2024). Tirzepatide and semaglutide were compared in the SURPASS 2 trial (Frias et al., 2021). After 40 weeks follow-up the reductions in HbA_{1c} from a baseline of 8.3% with 5 mg, 10 mg, 15 mg tirzepatide and 1 mg semaglutide were 2.01%, 2.24%, 2.30% and 1.86%, respectively. The corresponding changes in body weight were 7.6%, 9.3%, 11.2% and 5.7%, respectively. After 40 weeks participants treated with 10 mg and 15 mg tirzepatide were still losing weight (Frias et al., 2021). The weight reduction achieved is considered a milestone in the pharmacological management of obesity with efficacy approaching that of bariatric surgery (Gou and Schwartz, 2023).

In the UK, tirzepatide was approved for the treatment of type 2 diabetes in 2022 and obesity in 2024. As in the cases of liraglutide and semaglutide, the National Institute for Health and Clinical Excellence (NICE)[59] placed certain restrictions on the use of tirzepatide, prescribing of which will not be restricted to specialist obesity clinics (Wilkinson, 2024). Tirzepatide is also being studied in other cardiometabolic disorders. In a phase 2 trial involving participants with metabolic dysfunction-associated steatohepatitis (MASH) and moderate or severe fibrosis, tirzepatide was more effective than placebo in providing resolution of steatohepatitis without worsening fibrosis (Loomba et al., 2024). In the SUMMIT trial in patients with heart failure with preserved ejection fraction and obesity, tirzepatide reduced the risk of a composite of death from cardiovascular causes or worsening heart failure vs. placebo and improved health status (Packer et al., 2024). A prespecified meta-analysis of tirzepatide clinical trial data showed no increase in the risk of major cardiovascular events in participants with type 2 diabetes (Sattar et al., 2022). A cardiovascular outcomes study of tirzepatide – SURPASS CVOT – is fully recruited and in progress. The trial includes an active comparator, the GLP-1 receptor agonist dulaglutide, in patients with type 2 diabetes (Nicholls et al., 2024).

The once-weekly triple GIP + GLP-1 + glucagon receptor agonist retatrutide is currently the most potent weight-reducing pharmacotherapeutic (Jastreboff et al., 2023). In a systematic review of clinical trials in healthy non-diabetic adults with overweight or obesity, the highest mean reductions in body weight were achieved with retatrutide; tirzepatide was placed second in efficacy, followed by semaglutide (Moiz et al., 2025).

[59] https://www.nice.org.uk/guidance/ta1026.

It is recognised, however, that current GLP-1 receptor agonists have limitations. These agents often have gastrointestinal adverse effects, especially at initiation and at high doses (Trujillo et al., 2021). Gastrointestinal symptoms[60] and non-response in terms of weight reduction or glycaemia may lead to discontinuation of therapy (Sikirica et al., 2017). Real-world evidence from the UK suggests that weight loss benefits may be lower than observed in clinical trials (Weiss et al., 2022). In a study from the US, adult new users with obesity or type 2 diabetes the overall prevalence of GLP-1 receptor agonist discontinuation was 26.2%, 30.8%, and 36.5% at 3, 6, and 12 months, respectively (Do et al., 2024). At 12 months, patients had significantly higher odds of discontinuation if they were Black or Hispanic, male, were enrolled in Medicare or Medicaid; lived in areas with very high levels of social needs; had obesity only, heart failure, or other cardiovascular conditions at baseline, and had new gastrointestinal adverse effects at follow-up (Do et al., 2024). Data on safety issues including reduced muscle strength, bone density and fractures, effects on exercise capacity, gastrointestinal motility, retained gastric contents and anaesthesia risk, pancreatic and biliary tract disorders, and cancer risk, remain incomplete (Drucker, 2024). An analysis of large real-world Veterans Administration databases that compared use of GLP-1 receptor agonists against conventional glucose-lowering drug in adults with type 2 diabetes suggested potential benefits, e.g., lower risk of substance misuse, reduced neurocognitive disorders, and risks, e.g., increased arthritic disorders, of the GLP-1 receptor agonist class (Xie et al., 2025). The authors acknowledged the possibility of possibility of residual confounding or misclassification bias inherent in the non-randomised study design (Xie et al., 2025). While reductions in major adverse cardiovascular events appear to be predominantly driven by direct tissue effects of GLP-1 receptor agonists, associated weight loss effects are considered to be relevant to variable degrees for the clinical benefits observed in other major outcomes, e.g., in metabolic liver disease (Sattar and Lee, 2025).

GLP-1 receptor agonists have generated intense interest beyond biomedical boundaries, primarily due to their impressive weight reducing effects (Sumithran and Ard, 2025). Public enthusiasm for these drugs has led to shortages of liraglutide and semaglutide and a debate about equity of access to these expensive medications (Sumithran et al., 2024).

[60] Nausea, vomiting, abdominal pain, diarrhoea.

3.5.5 Sodium-glucose co-transporter-2 inhibitors

In the kidney, SGLT-2 protein is highly expressed in the first segment of the proximal tubules where it is responsible for the reabsorption of approximately 90% of glucose in the glomerular filtrate (Wright et al., 2011). Partial inhibition of SGLT-2 provides a non-insulin-dependent mechanism to reduce glucose reabsorption and eliminate excess glucose in the urine; this leads to reduction in hyperglycaemia (Wright et al., 2011). SGLT-2 is a high-capacity secondary active co-transporter that transfers one sodium ion with one glucose molecule down an electrochemical gradient for sodium, generated by, for example, the activity of an Na^+-K^+ ATPase pump[61]. Translational research involving chemical modifications of the progenitor experimental SGLT-2 inhibitor phlorizin[62] led to a class of selective SGLT-2 inhibitors that include canagliflozin, dapagliflozin, empagliflozin and ertugliflozin (Mudaliar et al., 2016, Ghezzi et al., 2018, Scheen, 2020). SGLT-2 inhibitors competitively inhibit the transporter, reducing glucose reabsorption to lower the renal threshold for glucosuria. This enables 20–30% of filtered glucose (about 50–100 g glucose/day) to be eliminated in the urine of individuals with type 2 diabetes (DeFronzo et al., 2013, Abdul-Ghani et al., 2015). Since the amount of filtered glucose and glucosuria declines as circulating glucose concentrations are lowered effect of SGLT-2 inhibition in the euglycaemic range is minimised thereby avoiding hypoglycaemia (Abdul-Ghani et al., 2015). Increased glucagon secretion with stimulation of hepatic glucose production also protects against hypoglycaemia (Merovci et al., 2014). Since SGLT-2 inhibition is independent of insulin, therapeutic effects are possible in the presence of the insulin resistance and partial β-cell failure that characterises type 2 diabetes (Hsia et al., 2017). However, SGLT-2 inhibitors require adequate kidney function to filter sufficient glucose to create enough glucosuria and so reduce hyperglycaemia. The glucosuria-mediated calorie loss induced by SGLT-2 inhibition promotes weight reduction (Mudaliar et al., 2015). A minor osmotic diuresis contributes to reductions in blood pressure of approximately 4–10 mmHg (Oliva and Bakris, 2014). The principal side-effects of SGLT-2 inhibitors include an increased incidence of mycotic genital infections and diabetic ketoacidosis in vulnerable patients with more pronounced degrees of insulin deficiency that is characterised by relatively low degrees of hyperglycaemia (Mascolo et al., 2022, Peters et al., 2015). SGLT-2 inhibitor-associated Fournier's gangrene, a urologic emergency characterised by necrotising infection of the external genitalia, perineum, and perianal region, is rare (Bersoff-Matcha et al., 2019).

[61] ATP, adenosine triphosphate.
[62] A glucoside found in the root bark of fruit trees.

SGLT-2 inhibitors have been shown to reduce total and cardiovascular mortality principally in high-risk patients with type 2 diabetes, principally by reducing risk of heart failure (Ferro et al., 2021, Kumowski et al., 2021, Packer, 2021). In September 2015, the attendees at the Congress of the European Association for the Study of Diabetes in Stockholm[63] were astounded by the presentation of the first randomised investigation of a sodium–glucose co-transporter-2 (SGLT2) inhibitor, empagliflozin - the EMPA-REG OUTCOME trial (Ryden and Norhammar, 2024). The placebo-controlled EMPA-REG OUTCOME (Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes) trial was a breakthrough for clinical trials in diabetes with the demonstration of cardiovascular protection from empagliflozin (Zinman et al., 2015). In a hierarchical-testing strategy, having satisfied the non-inferiority criteria, testing for superiority over placebo confirmed that empagliflozin significantly reduced the primary major adverse cardiac event endpoint – defined as cardiovascular death, nonfatal myocardial infarction and nonfatal stroke. Compared with placebo, empagliflozin resulted in a significantly lower risk of death from cardiovascular causes (hazard ratio, 0.62; 95% confidence interval, 0.49–0.77, $p<0.001$), death from any cause (0.68; [0.57–0.82], $p<0.001$); and hospitalization for heart failure (0.65 [0.50–0.85], $p=0.002$). No significant decrease in non-fatal myocardial infarction or stroke was observed. Empagliflozin reduced hospitalization for heart failure by 35% (Zinman et al., 2015). EMPA-REG OUTCOME was the first demonstration that a glucose-lowering drug class could reduce heart failure hospitalisations and cardiovascular death. The EMPA-REG OUTCOME trial was soon followed by randomised trials of other SGLT-2 inhibitors which demonstrated similar cardiovascular and renoprotective effects (McGuire et al., 2021b). These trials were mainly event-driven and powered to study one primary outcome, i.e., a composite of major cardiovascular events, including heart failure. Support for a class cardioprotective effect of SGLT-2 inhibitors was provided by CANVAS (Canagliflozin Cardiovascular Assessment Study) which integrated data from two trials of canagliflozin to demonstrate a reduction in major adverse cardiac events and heart failure hospitalization risk (Neal et al., 2017). An increased risk of lower limb amputation, primarily at the level of the toe or metatarsal, was not confirmed in subsequent trials of canagliflozin (Arnott et al., 2022). Evidence of renoprotective effects of canagliflozin, based on a composite endpoint, were also reported (Perkovic et al., 2018). The DECLARE-TIMI 58 study (Dapagliflozin Effect on Cardiovascular Events) demonstrated a lower rate of cardiovascular death and hospitalisation for heart failure (Wiviott et al., 2019).

[63] The candidate was in attendance for this historic event.

The cardiovascular and renal benefits of SGLT-2 inhibitors in patients with type 2 diabetes, along with adverse effects, have been confirmed in several meta-analyses (McGuire et al., 2021b, Qiu et al., 2021, Marilly et al., 2022). The clinical benefits of SGLT-2 inhibitors extend to other high-risk groups independent of diabetes status (Patel et al., 2024). Cardioprotective effects of SGLT-2 inhibitors have been confirmed non-diabetic patients with heart failure (Teo et al., 2021). Benefits have been observed in subjects with reduced and preserved ejection fraction (McDonagh et al., 2021). In 2024, based on a database of primary and secondary outcome trials involving 100 952 patients, Usman et al conducted a high-quality systematic review and meta-analysis of the effect of SGLT-2 inhibitors on cardiovascular outcomes in patients with heart failure, type 2 diabetes, chronic kidney disease, and atherosclerotic cardiovascular disease, including acute myocardial infarction (Usman et al., 2024). SGLT2 inhibition reduced the risk of first hospitalisation for heart failure by 29% in patients with heart failure (hazard ratio 0·71 [95% confidence interval 0·67–0·77]), by 28% in patients with type 2 diabetes (0·72 [0·67–0·77]), by 32% in patients with chronic kidney disease (0·68 [0·61–0·77]), and by 28% in patients with atherosclerotic cardiovascular disease (0·72 [0·66–0·79]). Moreover, SGLT2 inhibition reduced cardiovascular mortality in patients with heart failure by 14% (0·86 [0·79–0·93]), by 15% in type 2 diabetes (0·85 [0·79–0·91]), by 11% in chronic kidney disease (0·89 [0·82–0·96]), and by 13% if the patients with atherosclerotic vascular disease (0·87 [0·78–0·97]). The authors concluded that the findings, which were consistent across demographic subgroups, support widespread adoption of SGLT-2 inhibitors to decrease morbidity, mortality and health expenditure associated with cardiometabolic diseases (Usman et al., 2024).

Real-world data have provided additional support for the clinical utility of SGLT-2 inhibitors. A Danish registry confirmed that empagliflozin and dapagliflozin reduce mortality in patients with reduced ejection fraction heart failure irrespective of the presence or absence of type 2 diabetes (Svanstrom et al., 2024). A 25% relative risk reduction for all-cause mortality and a 23% reduction in cardiovascular mortality was observed with patients using SGLT-2 inhibitors compared with non-users. The magnitude of association with reduction in mortality was consistent across subgroups. It is well recognised, however, that observational data on treatment effectiveness is subject to confounded founding factors; cautious interpretation of real-world data is appropriate (Manja and Heidenreich, 2024).

In addition to demonstrating the cardioprotective effects of SGLT-2 inhibitors, these trials served to highlight the elevated risk of heart failure among patients with type 2 diabetes (Lehrke and Marx, 2017). The Framingham Heart Study showed that heart failure was twice as common in men with diabetes and five times as common in women with diabetes than in age-matched control subjects (Kannel and Mcgee, 1979). Heart failure, which is increasing in prevalence and is a major cause of morbidity and mortality worldwide, has a prevalence ranging from approximately 1% to 3% of the general adult population in high income countries (Savarese et al., 2023). Drivers of this risk include ischaemic heart disease and metabolic factors including insulin resistance, glucose toxicity and lipotoxicity (Lehrke and Marx, 2017, Nakamura et al., 2022). However, current evidence indicates that SGLT-2 inhibitors are still underused in the management of heart failure (Chakrala et al., 2023). Among clinical practitioners, the potential for so-called euglycaemic diabetic ketoacidosis, the multi-morbid complexity of patients, and high drug costs may lead to hesitancy in prescribing SGLT-2 inhibitors for patients with type 2 diabetes (Teng et al., 2024). It is instructive to note that in a large clinical series that predated the introduction of SGLT-2 inhibitors, diabetic ketoacidosis with normal blood glucose concentrations was uncommon and encountered predominantly in people with type 1 diabetes (Jenkins et al., 1993).

Experimental evidence suggests that SGLT-2 inhibitors exert vasculoprotective effects via mechanisms independently of improved glycaemic control (Preda et al., 2024). For example, the cardiovascular benefits of empagliflozin in EMPA-REG OUTCOME were not altered when adjusted for glycated haemoglobin levels both at baseline and by analysis of time-updated glycaemia achieved during the trial (Inzucchi et al., 2018). Of note, the SGLT-1/2 inhibitor sotagliflozin, which more effectively reduces postprandial glucose excursions via inhibition of intestinal SGLT-1, was associated with a 23% reduced risk of major cardiovascular events with reductions observed in total myocardial and stroke compared with placebo in a prespecified post hoc analysis of SCORED (Sotagliflozin Cardiovascular and Renal Events in patients with Diabetes) (Aggarwal et al., 2025). A reduction in both myocardial infarction and stroke had not been observed with other SGLT inhibitors leading to renewed interest in the potential benefits of targeting postprandial hyperglycaemia.

The benefits of SGLT-2 inhibitors may usefully be considered along four major intersecting pathophysiological axes, i.e., systemic, vascular, cardiac, and renal levels

(Preda et al., 2024). Of these, cardiorenal effects appear to contribute to the clinical benefits of SGLT-2 inhibitors most prominently (Preda et al., 2024). By counteracting the effects of leptin, SGLT-2 inhibitors help reverse leptin-driven renal sodium and glucose retention (Packer, 2018). Postulated mechanisms of the clinical effects of SGLT-2 inhibitors also include reduced systemic inflammation (Scisciola et al., 2022), alleviated oxidative stress (Scisciola et al., 2022), and improved cellular energy metabolism (Verma et al., 2018).

3.5.6 Current pharmacotherapeutic landscape for type 2 diabetes

Based on effects observed in clinical trials, the cardiovascular benefits of GLP-1 receptor agonists and SGLT-2 inhibitors are considered to be largely independent of their glucose-lowering effects (Davies et al., 2022). Incretin-based therapies and SGLT-2 inhibitors are regarded as disease modifying drugs on the basis of direct organ protection and evidence from clinical trials and real-world studies (Mathieu, 2024). Several SGLT-2 inhibitors have been shown to reduce total and cardiovascular mortality in high-risk patients with type 2 diabetes, principally by reducing the risk of heart failure (Ferro et al., 2021, Kumowski et al., 2021, Packer, 2021).

Evidence suggesting benefit of SGLT-2 inhibitors in the prevention of recurrent cardiovascular events has been provided by a real-world study from Taiwan of electronic health records. Long-term use of SGLT-2 inhibitors vs DPP-4 inhibitors was associated with an 18% decrease in total cardiovascular disease risks for the overall cohort. The higher absolute risk associated with existing cardiovascular disease, i.e., secondary prevention, was considered to account for the greater benefit of treatment with up to 30% reduction in risk (Su et al., 2024). Of note, this real-world data study, which used the technique of propensity score matching to achieve comparable baseline characteristics, was able to assess the impact of the two classes of medication at baseline levels of hyperglycaemia (mean haemoglobin A_{1c}, 8.6%, 71 mmol/mol) that would preclude a head-to-head randomised controlled trial on ethical considerations. To explain, a contemporary interventional clinical trial would require that participants be treated to more intensive glycaemic goals in recognition of proven clinical benefits. However, in common with observational studies in general, residual unmeasured confounding is a recognised limitation of real-world data studies (Norgaard et al., 2017).

Clinical management of type 2 diabetes has progressively moved beyond the focus on glycaemia to multifactorial risk reduction and, more recently, use of agents with proven cardioprotective and renoprotective agents in high-risk patients (Jacob et al., 2021). It is noteworthy that SGLT-2 inhibitors also reduce the incidence of new-onset diabetes in individuals with pre-existing cardiovascular or kidney disease (Ostrominski et al., 2024). Hyperglycaemia is an undisputed epidemiological risk factor for microvascular complications in diabetes and is integral within the causal pathways leading to these long-term complications (Barrett et al., 2017). Alternative pharmaceutical approaches directed towards ameliorating the intracellular downstream consequences of hyperglycaemia, e.g., aldose reductase inhibition[64], have not successfully translated from animal models to clinical practice (Krentz et al., 1992, Bernardoni et al., 2024). Long-term control of hyperglycaemia is therefore the cornerstone of delaying the development and progression of the chronic microvascular complications of diabetes (Valensi et al., 2019). This is not to dispute the role of hypertension as a modifiable risk factor for both microvascular and macrovascular complications of diabetes[65] (Adler et al., 2000, Stratton et al., 2006, Patel et al., 2007, Zoungas et al., 2009). As discussed, beyond the proven impact on microvascular disease, limited evidence suggests that lower levels of glycated haemoglobin are associated with reduced rates of macrovascular complications over a longer timespan (Holman et al., 2008). Notwithstanding overall reductions in the incidence of diabetes-associated complications, a population-level burden persists as a consequence of an increasing incidence of diabetes coupled to longer lifetime exposure to hyperglycaemia because of younger diagnosis and increased life expectancy (Templer et al., 2024). Effective long-term glycaemic control is especially relevant for patients who develop type 2 diabetes in childhood or early adulthood who are at particularly high risk of developing microvascular complications that carry an adverse long-term prognosis (Misra et al., 2023).

Recent data suggest that less than half of people with type 2 diabetes achieve their individualised glycaemic goals (Lautsch et al., 2022). In terms of glucose-lowering effects, a hierarchy of efficacy is evident among medications for type 2 diabetes. If factors such as degree of pre-treatment hyperglycaemia and endogenous insulin reserve are matched then among oral agents, sulfonylureas are generally more effective at controlling hyperglycaemia

[64] The main action of aldose reductase inhibitors is to inhibit the conversion of glucose to sorbitol thereby reducing intracellular osmotic stress.
[65] UKPDS, ADVANCE and ACCORD assessed the impact of blood pressure-lowering strategies in addition to glucose control on microvascular and/or macrovascular complications.

than DDP-4 inhibitors or SGLT-2 inhibitors[66] (Scheen, 2021). This difference is recognised in clinical management guidelines in which sulfonylureas are positioned as rescue therapy for patients with greater degrees of hyperglycaemia (Moran et al., 2022). Nonetheless, use of sulfonylureas has declined in many countries reflecting concerns about hypoglycaemia risk and cardiovascular safety (Krentz, 2002b, Scheen, 2021). Among injectable therapies, exogenous insulin remains unrivalled in its glucose-lowering effects (Davies et al., 2022). Since glycaemic goals are rarely achieved using monotherapy the risks of combining agents from different classes in intensive glucose-lowering strategies, e.g., in ACCORD, merit reiteration (Chiasson and Le Lorier, 2014). Care is required in the selection of combinations of glucose-lowering medications to maximise long-term control of hyperglycaemia, favourable effects on cardiometabolic biomarkers, and cardio-renoprotection. A simultaneous aim of rational combination therapy is to minimise the potential for inducing hypoglycaemia or weight gain. The optimal combination of glucose-lowering drugs has not been determined and ideally should be based on individual phenotypic profiles (Khunti et al., 2025). As demonstrated in UKPDS, type 2 diabetes, as conventionally treated with lifestyle measures and glucose-lowering pharmacotherapy, is a progressive disease (UK Prospective Diabetes Study Group., 1998). However, bariatric surgery studies and the clinical research programme of Roy Taylor and colleagues have challenged this notion by demonstrating that diet-induced weight loss of sufficient magnitude can induce remission in a substantial proportion of subjects with type 2 diabetes (Ferrannini and Mingrone, 2009, Taylor, 2024).

3.5.7 Personalised precision medicine

While the expansion in the range of oral and injectable glucose-lowering drugs offers the potential for more personalised therapy additional clinical data are required to guide prescribers in their use (Wharton et al., 2023, Melson et al., 2024). Clinical trials such as GRADE (Glycemia Reduction Approaches in Type 2 Diabetes: A Comparative Effectiveness) have provided partial answers to the question of which drug combinations are most effective (Grade Study Research Group et al., 2022a). In GRADE, metformin-treated participants with type 2 diabetes were randomly assigned to receive insulin glargine U-100, the sulfonylurea glimepiride, the GLP-1 receptor agonist liraglutide, or sitagliptin, a DPP-4 inhibitor. All four medications improved glycaemia with glargine and liraglutide being modestly more effective in achieving and maintaining target glycated haemoglobin levels

[66] The glucose-lowering effects of SGLT-2 inhibitors are dependent on renal function, becoming less efficacious for glycaemic control once estimated glomerular filtration rate (eGFR) drops below 60ml/min/1.73m^2.

(Grade Study Research Group et al., 2022a). Severe hypoglycaemia while uncommon, was more frequent in the insulin and glimepiride groups. Over a mean treatment period of five years, the incidence of microvascular complications and mortality in GRADE were not different between the four treatment groups (Grade Study Research Group et al., 2022b). However, the trial was underpowered to detect differences in albuminuria and retinopathy was not assessed. In terms of the incidence of cardiovascular disease, a comparison of the liraglutide group with the other three groups combined showed a hazard ratio of 0.71 (95% confidence interval, 0.56 to 0.90). In interpreting the results, the investigators pointed to the low inherent risk of cardiovascular events in the study cohort as an explanation for the absence of an effect of liraglutide on cardiovascular mortality (Grade Study Research Group et al., 2022b). A major limitation of GRADE is the absence of an SGLT-2 inhibitor treatment group (Ryden and Standl, 2022). Even with more widespread use of SGLT-2 inhibitors and GLP-1 receptor agonists, the current evidence base for combining these agents is presently limited to trials of surrogate endpoints and post-hoc exploratory analyses (Scheen, 2024).

The heterogeneity of type 2 diabetes requires more personalised and accessible treatments (Dahlen et al., 2021). In parallel, continuous glucose monitoring and flash glucose monitoring technologies provide real-time and actionable data that can facilitate improvements in glycaemic control (Jancev et al., 2024). Global collaborations such as the Precision Medicine in Diabetes Initiative acknowledge the limitations of traditional definitions that do not fully capture underlying pathophysiology (Chung et al., 2020). As discussed, a diagnosis of type 2 diabetes requires exclusion of autoimmunity, single-gene mutations, and pancreatic disease as causes of hyperglycaemia (Florez, 2024). Treatment algorithms seldom consider the pathogenic pathways that may lead to hyperglycaemia, e.g., relative insulin deficiency vs insulin resistance, and therapeutic choices are not tailored to the individual (Florez, 2024). Focusing primarily on effects on attainment of glycaemic control, investigators in the MASTERMIND consortium used state-of-the-art Bayesian methods to develop and validate an individualised treatment selection algorithm for SGLT-2 inhibitors and GLP-1 receptor agonists in English and Scottish clinical datasets and data from clinical trials (Cardoso et al., 2020). Women were found to be markedly more responsive to GLP-1 receptor agonists compared to men. Targeting SGLT-2 inhibitors and GLP-1 receptor agonists based on predicted glycaemic response was associated with improvements in short-term tolerability and long-term risk of developing microvascular complications. In contrast, limited evidence was found for heterogeneity in other clinical outcomes, with

equipoise between the two therapies for major cardiovascular events. A benefit of SGLT-2 inhibitors over GLP-1 receptor agonists was evident for new-onset heart failure and adverse kidney outcomes independent of differences in glycaemic efficacy, which in turn reflected differences in the phenotypic characteristics of individual patients (Cardoso et al., 2020). More recently, the MASTERMIND research group developed a five-drug class model based on routine clinical data to identify optimal glucose-lowering therapies for people with type 2 diabetes. The most common model-predicted optimal therapy was GLP-1 receptor agonists followed by SGLT-2 inhibitors, sulfonylureas, thiazolidinediones, and DPP-4 inhibitors (Dennis et al., 2025).

The anticipated differentiation of newer incretin-based agents for specified subsets of metabolic disorders is expected to provide greater opportunities for personalised medicine approaches for people living with cardiometabolic disorders (Drucker, 2024). An extensive drug development pipeline includes a range of novel multi-agonists with greater organ selectivity and an extended range of oral and injectable therapies (Drucker, 2024). Glucose- and weight-lowering drugs with cardioprotective and renoprotective effects, i.e., GLP-1 receptor agonists and SLGT-2 inhibitors, have become well-established the search.

Disease-modifying glucose-lowering drugs, i.e., GLP-1 receptor agonists and SGLT-2 inhibitors, present an unprecedented challenge for new entrants to what is already a complex and competitive market. Nonetheless, the search continues for additional safe, effective and well-tolerated new pharmacotherapeutics that that provide long-term glycaemic control (Nauck et al., 2021b). Not all individuals with type 2 diabetes require disease-modifying medications. Approximately one-third of people living with type 2 diabetes are exposed to the highest risk of future cardiovascular events conferred by pre-existing cardiovascular disease, renal disease, or heart failure (Einarson et al., 2018, Hart et al., 2023). Disease-modifying glucose-lowering drugs are recommended for these patients (Davies et al., 2022). The cost-effectiveness of GLP1-receptor agonists and SGLT-2 inhibitors treatment in patients without established cardiovascular disease has been questioned (Jorsal et al., 2020). For the two-thirds of patients without compelling indications for disease-modifying glucose-lowering drugs, considerations including body weight, risk of hypoglycaemia, and drug acquisition cost inform the choice of pharmacotherapy (Davies et al., 2018). In these individuals, the legacy effect of early and optimal glycaemic control with attention to other modifiable risk factors reduces the risk of long-term vascular complications (Khunti et al., 2025). Changing thresholds of risk for initiating pharmacological primary prevention allied to

the underrepresentation of high-risk groups in clinical trials imply greater numbers of eligible patients with cost implications for healthcare systems (Young et al., 2023).

Primary and secondary cardiovascular prevention in patients with type 2 diabetes requires a multi-factorial approach to modifiable risk factors (Gaede et al., 2008). This includes control of glycaemia, blood pressure, and lipids according to evidence-based goals (Newman et al., 2017, Wright et al., 2020). The cardiovascular outcome trials of GLP-1 receptor agonists and SGLT-2 inhibitors were performed in participants who were already receiving standard of care therapy for these risk factors. However, only a small proportion of patients achieve satisfactory control of all modifiable risk factors and even so a residual elevation in cardiovascular risk is evident compared to people without diabetes (Bakke et al., 2020, Wright et al., 2020).

At the time the original research study presented in this chapter was conceived most of the classes of glucose-lowering drugs discussed above were already in clinical use; the notable exception was SGLT-2 inhibitors. Canagliflozin was the first SGLT-2 inhibitor to be licensed in 2013 (Elkinson and Scott, 2013). Moreover, at that time the cardioprotective benefits of GLP-1 receptor agonists had not been identified. Metformin was generally recommended as first-line pharmacotherapy and this practice was incorporated into the study design (Krentz et al., 2014).

3.6 Early-phase evaluation of a novel oral glucose-lowering drug

In the early part of the second decade the 21st century, prior to publication of the cardiovascular outcome trials that demonstrated the cardio-renoprotective properties of GLP-1 receptor agonists and SGLT-2 inhibitors, small molecule activators of glucokinase were being developed as a novel class of glucose-lowering medications for type 2 diabetes. Glucokinase is expressed in the islet ß-cells where it acts as the glucose sensor for the precision regulation of insulin secretion (Matschinsky and Wilson, 2019, Gersing et al., 2025). The original research paper presented in Chapter 3 is a single centre, randomised, open, two-way crossover phase 1 safety evaluation of a novel glucose-lowering drug using state-of-the-art precision glucose clamp methodology. This tested whether the glucokinase activator AZD165 could impede recovery from hypoglycaemia in metformin-treated patients with type 2 diabetes. In human studies, glucokinase activators, while effective glucose-lowering agents, were known to carry a risk of inducing hypoglycaemia (Hale et al., 2015,

Nakamura and Terauchi, 2015). Glucokinase is also expressed in hepatocytes (Matschinsky and Porte, 2010). Exogenous glucagon, administered by intramuscular injection, is used to treat acute severe iatrogenic hypoglycaemia when oral glucose administration is not possible (Porcellati et al., 2021). Glucagon rapidly mobilises glucose from hepatic glycogen stores. Since glucokinase plays a role in regulating the glycogen content of the liver it was considered that AZD1656 carried a theoretical risk of impairing the action of glucagon to mobilise hepatic glycogen (Krentz et al., 2014). Since hypoglycaemia could be hazardous to patients, especially those with cardiovascular disease, assessment of the utility of exogenous glucagon in this clinical scenario was considered to be essential to the drug development programme. In view of the potential risk of inducing acute cardiovascular events during experimental hypoglycaemia – even under the carefully controlled conditions of a state-of-the-art clinical research institute – all study participants were screened for cardiovascular disease prior to inclusion in the study. This included a detailed physical examination, including measurement of blood pressure, and a resting 12-lead electrocardiogram[67]. Continuous electrocardiographic monitoring was performed during hypoglycaemia and the subsequent recovery period.

[67] Clinical Study Report. A randomised, open, two-way cross-over, phase I study to evaluate the response to glucagon versus the spontaneous counter-regulatory response in type 2 diabetes patients treated with AZD1656 and metformin during hypoglycemia. Confidential. AstraZeneca, 2009.

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ORIGINAL ARTICLE

Effect of exogenously administered glucagon versus spontaneous endogenous counter-regulation on glycaemic recovery from insulin-induced hypoglycaemia in patients with type 2 diabetes treated with a novel glucokinase activator, AZD1656, and metformin

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Aims: To study the effect of exogenous i.m. glucagon on recovery from controlled insulin-induced hypoglycaemia in patients with type 2 diabetes treated with the novel glucokinase activator AZD1656, in combination with metformin.

Methods: This was a single-centre randomized, open, two-way crossover phase I, automated glucose clamp (Biostator®; Life Science Instruments, Elkhart, MD, USA) study (NCT00817271) in eight patients (seven men and one woman, mean age 58.6 years, body mass index 28.1 kg/m^2). All patients received a stable dose of metformin twice daily, ranging from 1000 to 2250 mg. A 2-day titration phase commenced with 40 mg AZD1656 twice daily, escalating to 80 mg twice daily if tolerated. This was followed by a single dose of 80 or 160 mg AZD1656, administered on days 5 and 8 when metabolic studies were performed. After an overnight fast on days 5 and 8, controlled hypoglycaemia was induced using an exogenous i.v. infusion of insulin. Plasma glucose was lowered in a stepwise fashion over 3 h to attain a target nadir of 2.7 mmol/l. This was sustained for 30 min, at the end of which the hypoglycaemic clamp was released. In random sequence, patients either received an i.m. injection of 1 mg glucagon or were allowed to recover from hypoglycaemia by endogenous counter-regulation. To avoid prolonged hypoglycaemia, a reverse glucose clamp was applied from 4 to 6 h post-dose.

Results: Three patients received 40 mg AZD1656 twice daily and five patients 80 mg twice daily. Mean plasma glucose at 20 min after release of the hypoglycaemic clamp was significantly lower (3.1 ± 0.3 mmol/l) for AZD1656 alone than for AZD1656 + glucagon (4.9 ± 0.8 mmol/l; $p < 0.001$ between the groups). Catecholamine and cortisol responses were similar on the AZD1656 + glucagon and AZD alone study days. Growth hormone response was 18% lower for AZD1656 alone ($p = 0.01$), consistent with the effect of a pharmacological dose of glucagon on growth hormone secretion. No safety or tolerability concerns were observed during treatment with AZ1656.

Conclusions: Exogenous glucagon was effective as a rescue treatment for hypoglycaemia induced during treatment with AZD1656, given in combination with metformin in patients with type 2 diabetes.

Keywords: antidiabetic drug, phase I–II study, type 2 diabetes

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Introduction

Glucokinase is present in hepatocytes and the β-cells of the pancreatic islets where it catalyses the conversion of glucose to glucose 6-phosphate. The enzyme is rate-limiting for glucose uptake and utilization in β-cells, where it plays a major role in regulating insulin secretion and is regarded as a physiological glucose sensor. Glucokinase is also present in liver parenchymal cells where it regulates hepatic glucose extraction and utilization [1]. Defects in these two processes contribute to the development of hyperglycaemia in type 2 diabetes [2]. Accordingly, pharmacological activation of glucokinase has emerged as an attractive therapeutic option for type 2 diabetes. Glucokinase activators (GKAs) stimulate glucose-dependent insulin secretion, increase postprandial hepatic glucose uptake and reduce hepatic gluconeogenesis [2]. AZ1656 is a potent GKA that activates rat and human glucokinase *in vitro*. Clinical studies in healthy volunteers [3] and in patients with type 2 diabetes [4] showed that AZD1656 was well tolerated with a dose-dependent glucose-lowering effect; however, a study conducted in people with type 2 diabetes found that the glucose-lowering efficacy of AZD1656 diminished over time [5].

Hypoglycaemia has been reported with other GKAs, although the risk is lower than that for sulphonylureas [6]. Theoretically, treatment with a GKA could interfere with the glucose-mobilizing effect of emergency treatment with parenteral glucagon. In a previous study we found preliminary evidence for reduced endogenous glucagon responses to experimental hypoglycaemia in healthy men who received either

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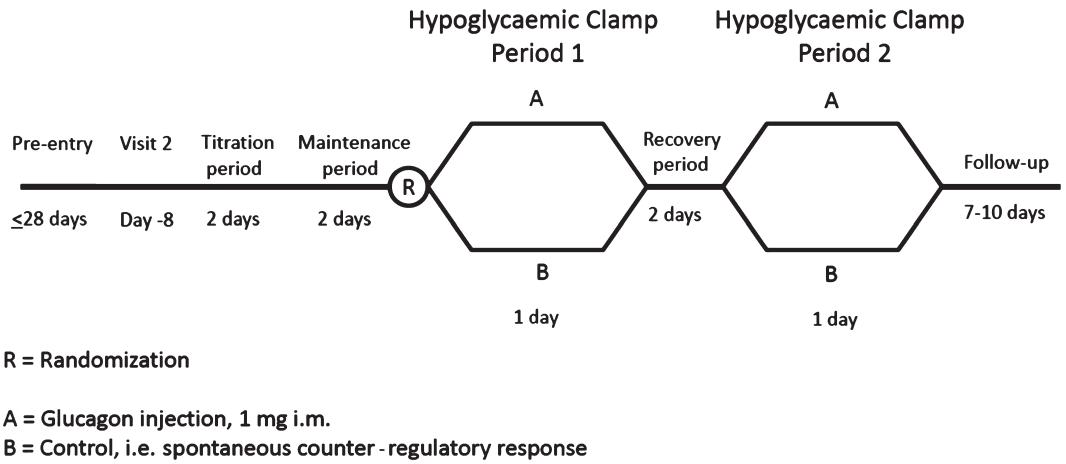

Figure 1. Flow chart of study design.

a single dose of AZD1656 or another GKA [7]. Glucagon, which converts stored glycogen to glucose, by injection can be a critical option for the treatment of severe hypoglycaemia outside the hospital setting. The primary objective of the present study was to compare the effect of exogenous glucagon on recovery from insulin-induced hypoglycaemia in a fasting state after a single oral dose of AZD1656 in patients with type 2 diabetes treated with metformin. Secondary objectives included evaluation of the safety and tolerability of AZD1656, the pharmacokinetic properties of AZD1656, and the response of glucose, C-peptide and insulin during hypoglycaemia. AZD1656 was added to metformin therapy in anticipation of the likely use of this combination were AZD1656 to reach clinical practice.

Methods

Inclusion and Exclusion Criteria

Men and non-fertile women with type 2 diabetes treated with metformin participated in the study, and approximately 10 patients were estimated to be randomized to achieve the goal of eight patients with evaluable data. The key criteria for inclusion were: age 18–75 years, gender male or female (but females of non-childbearing potential only), body mass index >19 and <40 kg/m^2, type 2 diabetes treated with a stable dose (defined as no change within the 3 months before enrolment) of metformin as monotherapy at a dose of >1000 mg/day, serum fasting C-peptide >0.3 nmol/l and negative glutamic acid decarboxylase antibodies at screening (visit 1).

Study Design

This was a randomized, open, two-way crossover phase I study, conducted in a single clinical research centre (Figure 1). Eight days before the start of treatment with AZD1656 (at visit 2) the enrolled patients were transferred to metformin tablets provided by the clinical research institute but maintained their previous treatment dosage regimen. During the residential period (visit 3) AZD1656 was administered as oral suspension (25 mg/ml) for 8 consecutive days. On day 1 of the in-house period, the patients entered a 2-day titration phase (guided by seven-point plasma glucose monitoring) starting with 40 mg AZD1656 twice daily on the first and 80 mg twice daily, if tolerated, on the second day. Thereafter, the tolerated dose of AZ1656 (40 or 80 mg twice daily), was maintained. On day 5, each patient was allocated to one of the treatment sequences AB or BA in random order and under conditions of controlled hypoglycaemia, induced by means of a glucose clamp (see below). The total daily AZD1656 dose (80 or 160 mg) for each patient was administered as a single dose in the morning after an overnight fast. At 3 h post-dose, the patients either received an i.m. injection of 1 mg glucagon (treatment A) or were allowed to recover from hypoglycaemia by endogenous counter-regulation (treatment B). After a recovery phase of 2 days (day 6 and day 7), during which AZD1656 was maintained at the same twice daily dose as on days 3 and 4, the patients again received a single oral dose of 80 or 160 mg AZD1656 under hypoglycaemic clamp conditions, followed by the alternate recovery treatment strategy. The study, which was conducted at a single centre (Profil Institute for Clinical Research, Chula Vista, CA, USA), was approved by an independent ethics review board and performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Hypoglycaemic Clamps

Controlled hypoglycaemia was achieved by means of a hypoglycaemic clamp using a glucose-controlled insulin infusion system (Biostator®; Life Science Instruments, Elkhart, MD, USA). In brief, plasma glucose was controlled at decreasing levels in a stepwise fashion: 5 mmol/l (for 60 min), 4 mmol/l (for 30 min) and 3.2 mmol/l (for 60 min) were maintained by i.v. insulin and/or glucose infusion. Plasma glucose was then clamped to a target nadir of 2.7 mmol/l (for 30 min) and released at 3 h post-dose (i.e. the insulin/glucose infusion was stopped; Figure 2). Then, 90 min after release of the hypoglycaemic clamp, the patients were served a meal with food *ad libitum*. To avoid prolonged hypoglycaemia, a reversed clamp was applied at 4 h after dosing with AZD1656, establishing increasing minimum plasma glucose levels of 3.2 mmol/l (for

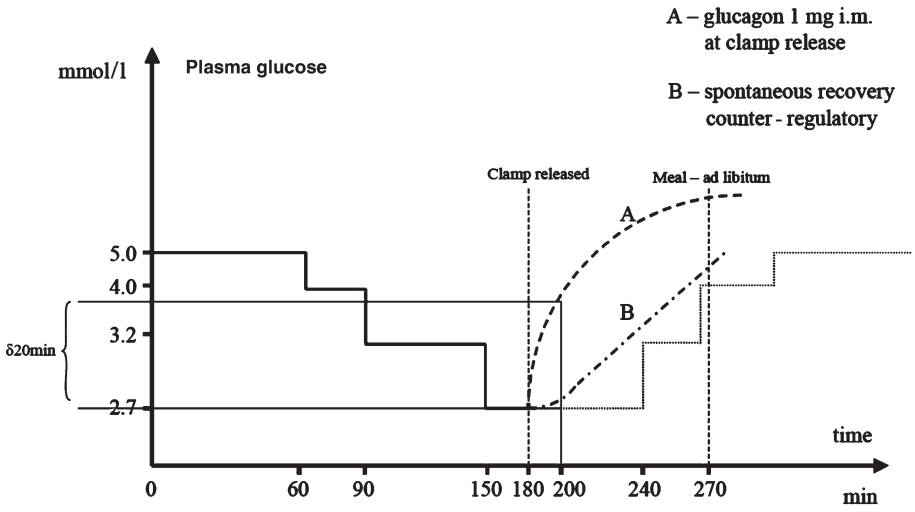

Figure 2. Overview of stepped hypoglycaemic clamp.

30 min), of 4.0 mmol/l (for 30 min) and of ≥5.0 mmol/l (for 30 min). At 6 h post-dose the Biostator was disconnected, provided that two consecutive plasma glucose values of at least 5.0 mmol/l were measured without ongoing glucose infusion. Glucose (measured using a YSI device; YSI, Yellow Springs, OH, USA), C-peptide, insulin and counter-regulatory hormones were collected for 6 h after AZD1656 dosing.

Main Outcome Assessments

The primary pharmacodynamic variables of the study were: (i) plasma glucose concentration 20 min after release of the hypoglycaemic clamp; (ii) time to two consecutive plasma glucose levels >3.5 mmol/l ($t_{3.5mmol/l}$); and (iii) time to two consecutive plasma glucose levels >5.0 mmol/l ($t_{5.0mmol/l}$). Secondary pharmacodynamic variables included: area under curve (AUC_{0-6}) of serum C-peptide, insulin and plasma glucose. Exploratory pharmacodynamic variables included AUC_{0-6} of norepinephrine, epinephrine, glucagon, cortisol and growth hormone. Pharmacokinetic variables included: AZD1656 and metabolite AZ12555623 concentration in plasma; AUC_{0-24}, C_{max}, t_{max}, $t_{1/2}$ (AZD1656 only); and oral clearance of drug from plasma for AZD1656 only. All patients who received at least one dose of AZ1656 (n = 11) were included in the safety analysis. Main variables included: adverse events, safety laboratory tests, seven-point plasma glucose profiles, physical examination, electrocardiogram, vital signs and body weight.

Hormone and Metabolite Assays

Cortisol, C-peptide and insulin were determined by electrochemiluminescence immunoassays using Cobas electrochemiluminescence immunoassay test kits and E170 analysers (both Roche Diagnostics GmbH, Mannheim, Germany), glucagon levels were determined by competitive radioimmunoassay (double antibody glucagon kit, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA), and epinephrine and norepinephrine levels were determined by the alumina extraction method followed by high-performance liquid chromatography with electrochemical detection [8]. Growth hormone levels were determined by an immunochemiluminometric assay using a monoclonal antihuman growth hormone antibody [an immunochemiluminometric assay test kit and Immulite 2000 assay system (both Siemens Medical Solutions Diagnostics AG, Zürich, Switzerland)]. Plasma concentrations of AZD1656 and its active metabolite AZD5658 were determined using validated methods consisting of solid-phase extraction and liquid chromatography followed by mass spectrometric detection as previously described [7].

Statistical Analysis

The sample size was based on experience gained from previous studies of AZD1656. The pharmacodynamic and pharmacokinetic analysis set included all evaluable data appropriate for the analysis of interest. The safety analysis set included all patients who received at least one dose of AZD1656 and for whom post-dose data were available. All analysis sets were based on an as-treated approach. Primary and secondary variables were analysed after log-transformation with a mixed-effects ANOVA model, where treatment, period and sequence were taken as fixed effects and patient within sequence as a random effect. An exception was the analysis of the primary variable plasma glucose 20 min post-clamp release, where no log-transformation was made. Within the model, least square-means per treatment and the difference between the means were determined and analysed using Fisher's least significance difference test. The individual differences between the two treatments for $t_{3.5mmol/l}$ and $t_{5.0mmol/l}$, respectively, were analysed using a two-sided pairwise Wilcoxon's signed-rank test. In addition, a Hodges–Lehmann shift estimate was derived for the differences, together with 95% confidence interval (CI). Pharmacokinetic variables were analysed in the same ANOVA model as used in the pharmacodynamic analysis, and for differences in t_{max} the same non-parametric method was used. All statistical tests were two-sided with the significance level 0.05. No α-adjustment was made. In all

pharmacodynamic analyses, AUCs were standardized to 1 h by dividing each AUC by the respective hours of the time interval (i.e. for AUC_{0-6} the value was divided by 6). The i.m. injection of glucagon resulted in markedly elevated levels of the hormone that exceeded the upper limit of detection for the assay. Accordingly, no formal statistical comparison was made with glucagon AUC for AZD1656 alone. For evaluation of AUC_{0-24} and C_{max} of AZD1656 and metabolite AZ12555623, the doses were normalized to 100 mg (AUC_{0-24}) and 50 mg (C_{max}). AUCs were calculated using the trapezoidal method and actual sampling time points. For calculation of pharmacodynamic AUCs the linear trapezoidal rule was used. For calculation of pharmacokinetic AUCs the linear trapezoidal rule was used up to the time of C_{max} and thereafter, calculations were made by means of the logarithmic trapezoidal rule. Descriptive statistics were provided for all safety variables; no statistical comparison was performed.

Results

Patients

A total of 42 patients were enrolled. Of these, 11 started AZD1656 treatment before randomization, and three discontinued the study before randomization on day 5, as they did not meet eligibility criteria with regard to titration. Patient demographics and baseline characteristics are shown in Table 1.

Plasma Glucose Concentrations During Recovery from Hypoglycaemia

Recovery of plasma glucose from hypoglycaemia was faster after the glucagon injection compared with the spontaneous recovery (Figure 3). Recovery of plasma glucose was significantly delayed for AZD1656 alone compared with AZD1656 + glucagon. This was reflected in the primary pharmacodynamic variables in which $t_{3.5mmol/l}$ and $t_{5.0mmol/l}$ were both significantly longer for AZ1656 alone (Table 2). Mean (+s.d.) plasma glucose at 20 min after release of the hypoglycaemic clamp was lower (−37%) for AZD1656 alone than for the AZD1656 + glucagon treatment (3.1 + 0.3 vs. 4.9 + 0.8 mmol/l; $p < 0.001$). All differences in primary variables between the two treatments were statistically significant (Table 2). The geometric mean plasma glucose (measured using the YSI device) AUC_{0-6} was smaller (−22%) for AZD1656 than for AZD1656 + glucagon because of the higher glucose concentrations during the 3 h after clamp release and glucagon injection. Five patients received i.v. glucose during recovery on the control clamp day, all during the spontaneous glucose recovery study.

Table 1. Patient demographics and baseline characteristics.

Characteristic	Value
Enrolled/Started study*/Randomized/Completers, n	42/11/8/8
Sex (male/female), n	7/1
Mean (s.d.) age, years	58.6 (11.7)
Mean (s.d.) body mass index, kg/m^2	28.1 (3.8)
Mean (s.d.) time since diagnosis of diabetes, years	8.0 (6.3)
Race, n	
White	5
Black	2
Hispanic	1
Mean (s.d.) glycated haemoglobin, %	7.4 (4.9)
Mean (s.d.) fasting plasma glucose, mmol/l	8.0 (1.9)
Mean (s.d.) metformin dose, mg/day	1656 (591)

*Patients received at least one dose of AZD1656 before randomization.

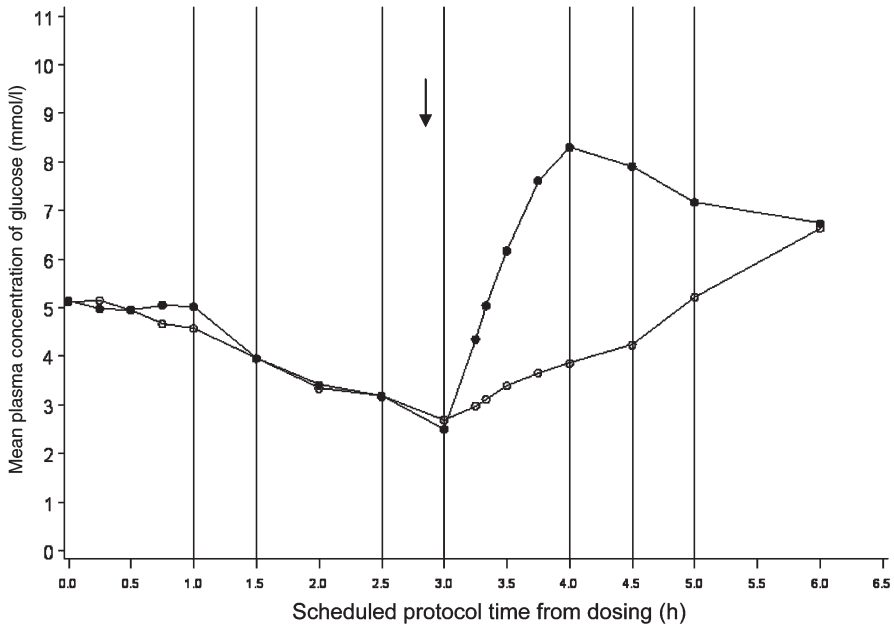

Figure 3. Mean plasma glucose concentration for the AZD1656 (open circles) and AZD1656 + glucagon studies (solid circles). The hypoglycaemic clamp was released at 3 h (arrow). On the AZD1656 + glucagon study day glucagon 1 mg was given at 3 h by i.m. injection. The vertical lines indicate the time periods for the preset target plasma glucose levels of the hypoglycaemic clamp and of the subsequent reversed clamp.

Table 2. Primary pharmacodynamic endpoints.

20 min glucose, mmol/l	Estimated mean (95% CI)	p
AZD1656 alone	3.1 (2.6–3.7)	
AZD1656 + glucagon	4.9 (4.4–5.5)	
	Versus AZD1656 + glucagon estimate (95% CI)	
	−37% (−49; −25)	<0.001
$t_{3.5mmol/l}$, min	Median	p
AZD1656 alone	37	
AZD1656 + glucagon	10	
	Versus AZD1656 + glucagon shift estimate (95% CI)	
	23 (11; 41)	0.008
$t_{5.0mmol/l}$, min	Median	p
AZD1656 alone	122	
AZD1656 + glucagon	23	
	Versus AZD1656 + glucagon shift estimate (95% CI)	
	95 (80; 106)	0.008

CI, confidence interval; $t_{3.5mmol/l}$, time to two consecutive plasma glucose levels >3.5 mmol/l; $t_{5.0mmol/l}$, time to two consecutive plasma glucose levels >5.0 mmol/l.

Counter-regulatory Hormones

The increase in glucagon concentrations after i.m. injection of 1 mg glucagon exceeded the upper limit of detection of the assay (Table 3). The geometric mean AUC_{0-6} values for norepinephrine, epinephrine and cortisol (Table 3) were similar (p = nonsignificant for each) for both AZD1656 + glucagon and for spontaneous counter-regulation studies. The geometric mean growth hormone AUC_{0-6} was moderately smaller (Table 3, p = 0.10) for AZD1656 alone than for the AZD1656 + glucagon treatment. For both the glucagon and the growth hormone AUC, the difference between the interventions was significant (p < 0.001 and p = 0.01, respectively).

C-peptide and Insulin

The geometric mean AUC_{0-6} values for C-peptide and insulin were smaller (−16 and −19%, respectively) for AZD1656 alone compared with the AZD1656 + glucagon treatment, reflecting the earlier onset of endogenous insulin production resulting from the faster recovery of plasma glucose after glucagon injection (data not shown).

Pharmacokinetics

AZD1656 was rapidly absorbed with a median t_{max} of 1 h and a terminal elimination half-life of approximately 4 h (Table 4).

Safety and Tolerability

There were no clinically relevant changes or trends in any laboratory safety variables or seven-point glucose profiles during the study. AZD1656 was generally well tolerated. Headache was the most frequently reported adverse event, reported by four patients.

Table 3. Comparison of AZD1656 versus AZD1656 + glucagon for counter-regulatory hormone AUC_{0-6}.

	AZD1656	AZD1656 + glucagon
Glucagon, pmol/l		
Estimated geometric mean (95% CI)	25.13 (20.31 to 31.09)	N/A*
Versus AZD1656 + glucagon, estimate (95% CI)	N/A*	
p	N/A*	
Norepinephrine, nmol/l		
Estimated geometric mean (95% CI)	1.97 (1.12 to 3.47)	2.08 (1.18 to 3.65)
Versus AZD1656 + glucagon, estimate (95% CI)	−5% (−18 to 11)	
p	0.452	
Epinephrine, nmol/l		
Estimated geometric mean (95% CI)	0.78 (0.49 to 1.24)	0.81 (0.51 to 1.29)
Versus AZD1656 + glucagon, estimate (95% CI)	−4% (−30 to 31)	
p	0.75	
Cortisol, µmol/l		
Estimated geometric mean (95% CI)	0.46 (0.37 to 0.58)	0.45 (0.36 to 0.57)
Versus AZD1656 + glucagon, estimate (95% CI)	1% (−22 to 33)	
p	0.899	
Growth hormone, ng/ml		
Estimated geometric mean (95% CI)	2.96 (1.19 to 7.38)	3.62 (1.45 to 9.03)
Versus AZD1656 + glucagon, estimate (95% CI)	−18% (−29 to −7)	
p	0.010	

AUCs were standardized to 1 h. n = 8 for all groups. AUC_{0-6}, area under curve; CI, confidence interval.
*N/A, not applicable because of the marked increase in glucagon levels after the i.m. glucagon injection resulting in glucagon levels above the detection limit for the method.

Discussion

The present study shows that i.m. glucagon can be used successfully in the treatment of hypoglycaemia in people with type 2 diabetes treated with AZD1656 in combination with metformin. All patients responded adequately to exogenous glucagon in the standard dose used for treatment of hypoglycaemia. Recovery from hypoglycaemia was significantly faster, with normal glucose levels (>5 mmol/l) being reached more rapidly with AZD1656 + glucagon than with AZD1656 alone.

During hypoglycaemia, the increase in counter-regulatory hormones norepinephrine, epinephrine, cortisol and growth hormone was similar for the AZD1656 + glucagon and AZD1656 alone studies. With the exception of growth hormone, the AUCs for the counter-regulatory hormones were not significantly different. The higher AUC for growth hormone observed with AZD1656 + glucagon is consistent with the effect of pharmacological doses of glucagon on growth hormone secretion [9]. No reliable conclusions about insulin and C-peptide could be reached, as patients received different amounts of insulin at different times in order to reach the

Table 4. Pharmacokinetic data.

	AUC_{0-24} (μmol*h/l)	C_{max} (μmol/l)	t_{max} (h)	$t_{1/2}$ (h)	CL/F (l/h)
AZD165	23.5 (15.1–45.4)	2.50 (0.49–3.34)	1.0 (0.5–12.0)	3.71 (3.02–5.04)	8.95 (4.61–13.8)
AZD12555623	4.44 (1.53–7.52)	0.26 (0.07–0.38)	1.50 (0.50–12.0)	—	—

AUC, area under the curve; C_{max}, maximum concentration; t_{max}, time to reach C_{max}; $t_{1/2}$, terminal elimination half-life; CL/F, oral clearance of drug from plasma.

hypoglycaemic plateau. No safety or tolerability concerns attributable to AZD1656 were identified in the present study.

A potential limitation of the study is the absence of a metformin monotherapy arm to assess the effect of exogenous glucagon in these patients under identical conditions of experimental hypoglycaemia in the absence of AZD1656. This would be necessary to determine whether treatment with AZD1656 may have had an effect on glucose recovery from hypoglycaemia in the presence of exogenous glucagon.

In summary, i.m. glucagon was effective in accelerating the time to recovery from experimental insulin-induced hypoglycaemia in patients with type 2 diabetes treated with a combination of AZD1656 and metformin. We interpret the results as indicating that clinical use of glucagon would be expected to be successful for the treatment of hypoglycaemia in patients receiving AZD1656.

Acknowledgements

The study was supported by AstraZeneca.

Conflict of Interest

Author contributions were as follows: A. J. K.: analysis and writing; L. M.: study design and conduct, data collection, analysis and writing; M. P.: study design, analysis and writing; E. N.: study design, analysis and writing; M. H.: study design and conduct, analysis and writing. M. P. and E. N. are employees of AstraZeneca.

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Chapter 4. Nonclassic cardiometabolic effects of statin therapy

Phase 4 investigator-initiated studies of the effects of high-intensity statin therapy on whole-body insulin sensitivity, glucose and lipid metabolism, and microvascular function in adults with features of the metabolic syndrome

4.1 Research summary

Clough GF, Turzniecka M, Walter L, ***Krentz AJ****, Wild S, Chipperfield A, Gamble J, Byrne CD. Muscle microvascular dysfunction in central obesity is related to muscle insulin insensitivity but is not reversed by high-dose statin treatment.*
Diabetes 2009;58:1185-1191

Background: 3-Hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase inhibitors (statins) are the pharmacological mainstay of atherosclerosis prevention (Cholesterol Treatment Trialists Collaboration et al., 2010). Statins have a generally excellent safety record. However, new-onset diabetes in individuals with features of the metabolic syndrome has been reported (Sattar and Taskinen, 2012). Insulin has direct vascular effects including increased microvascular perfusion in skeletal muscle (Coggins et al., 2001). Impairment of insulin-induced microvascular dilator responses observed in animal models of insulin resistance is hypothesised to reduce insulin-stimulated muscle glucose uptake (Clark et al., 2003). The effects of high intensity statins on microvascular function remain incompletely delineated. The hypothesis that microvascular dysfunction contributes to obesity-associated insulin resistance in the context of statin therapy was tested at a UK University Clinical Research Facility.

Methods: An investigator-initiated phase 4 study was designed to test the hypothesis that high-intensity statin therapy was associated with changes in (a) whole-body insulin sensitivity and (b) microvascular function[68]. All the study participants were offered statin therapy following conclusion of the study. The central study design was a double-blind placebo-controlled trial of high-intensity atorvastatin (40 mg daily) for six months in non-diabetic individuals (n=39) with central obesity in conjunction with one or more additional features of the metabolic syndrome. Calculated ten-year risk of atherosclerotic cardiovascular events >20% served as an exclusion since statin therapy would be mandated by clinical guidelines[69]. Glucose metabolism was assessed at baseline using 75 g oral glucose tolerance tests[70] and before and after statin therapy by haemoglobin A_{1c} levels. Insulin sensitivity in glucose and fatty acid metabolism was assessed using the two-step hyperinsulinaemic euglycaemic clamp technique, generally considered to be the reference method for the quantification of insulin action in humans. Other measures of insulin action included homeostasis model assessment and quantitative insulin sensitivity check index

[68] I co-designed the central clinical trial to determine the effects of atorvastatin on insulin sensitivity when Honorary Senior Lecturer in Medicine at the University of Southampton, UK.
[69] Assessed using the Framingham cardiovascular risk equation.
[70] Individuals with diabetes were excluded from the study.

(Turzyniecka, 2011). Participants were comprehensively phenotyped using state-of-the-art methods including magnetic resonance imaging to quantify abdominal adiposity. Skeletal microvascular function was assessed using venous occlusion plethysmography.

Results: In response to atorvastatin, plasma LDL-cholesterol levels declined whereas no change in LDL-cholesterol was observed in the placebo treatment group. Plasma C-reactive protein also declined in response to atorvastatin. At baseline, muscle microvascular exchange capacity was negatively associated with visceral fat mass and HbA_{1c} and positively with insulin-mediated glucose uptake in skeletal muscle.[71] For the placebo and treatment arms there was no statistically significant change in the main insulin action outcome after six months of statin therapy.[72] No changes were observed in HbA_{1C}, fasting plasma glucose, fasting plasma insulin, or in other measures of insulin action including effects on fatty acid metabolism. Muscle microvascular function was not improved by atorvastatin. No statin-associated changes were observed in leptin or adiponectin.

Interpretation: This interdisciplinary study demonstrated that, while lowering LDL-cholesterol and inflammatory markers as expected, high-intensity statin therapy did not significantly alter whole-body insulin sensitivity or skeletal microvascular function. No significant changes in glucose metabolism, adipocytokines, or body fat were observed with atorvastatin.

[71] M/I = The mean of glucose disposal divided by plasma insulin level during hyperinsulinaemia. Glucose disposal under these conditions principally reflects skeletal muscle glucose uptake.
[72] P=0.054.

4.2 Glucose clamp methodology in cardiometabolic research

The selection of precise, safe, and well-tolerated research methods is an important consideration in human metabolic studies (Krentz, 2019b). Chapters 3 and 4 employ variants of the hyperinsulinaemic glucose clamp, which provides an example of these requirements. As discussed in Chapter 1, it is hypothesised that impaired insulin action is a central metabolic defect in the pathogenesis and management of prevalent cardiometabolic diseases including polycystic ovary syndrome and type 2 diabetes. The glucose clamp technique, which involves infusions of exogenous insulin and variable-rate glucose, has been widely used by clinical investigators in metabolic studies requiring quantification of insulin action (Krentz, 2019b). Variants of the glucose clamp are also employed in the assessment of the pharmacodynamics of exogenous insulin formulations and in studies requiring states of controlled experimental hypoglycaemia (Swinnen et al., 2008, Boyle, 1994, Fabricius et al., 2021).

The method used in the study presented in Chapter 3 – the automated hyperinsulinaemic hypoglycaemic clamp – permits plasma glucose concentrations to be precisely manipulated within the hypoglycaemic range. This technique may be used to assess counterregulatory hormone responses[73] under conditions of standardised experimental hypoglycaemia (Norjavaara et al., 2012, Krentz et al., 2014, Fabricius et al., 2021). An intravenous insulin infusion is titrated to achieve a pre-defined glycaemic target in the hypoglycaemic range, or if designed as a stepwise hypoglycaemic clamp, to achieve and maintain multiple sequential targets. Arterialised blood is sampled at frequent intervals and a variable rate infusion of glucose is adjusted by a computer algorithm to maintain the target glucose concentration. At each plasma glucose level, blood samples to assess variables of interest, e.g., counterregulatory hormones, incretins, can be collected. At the end of the clamp the intravenous insulin infusion is terminated; time to spontaneous recovery to euglycaemia can be captured as an additional measure. In terms of recognised limitations, the predetermined and accurate square-wave decrements in glucose levels deviate from typical clinical scenarios in which the time course and pattern of hypoglycaemia are variable (Boyle, 1994).

[73] The classic counterregulatory hormones include glucagon, catecholamines, and growth hormone.

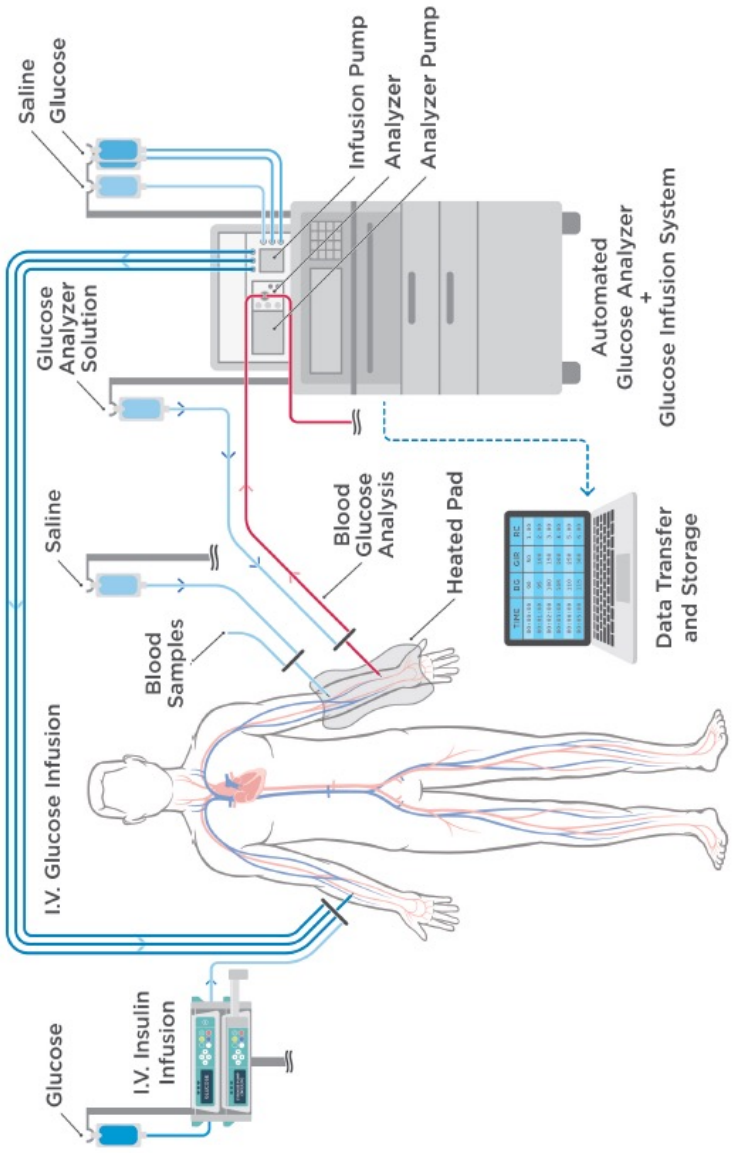
4.2.1 Automated vs. manual glucose clamps

The Biostator® was originally developed in the 1970s as a closed-loop artificial pancreas but did not fulfil its clinical promise (Heinemann and Ampudia-Blasco, 1994). The technology was subsequently repurposed from a glucose-controlled insulin infusion system to a glucose-controlled glucose infusion system (Fogt et al., 1978) (Figure 3). Compared with manual operator clamps in which the glucose infusion is controlled by clinical research personnel, the Biostator® closed-loop computerised algorithm provides an additional level of safety during hyperinsulinaemic clamps. Other advantages include low deviation from target glucose concentrations and avoidance of potential investigator bias (Heinemann and Ampudia-Blasco, 1994). Few clinical research institutions besides ProSciento in San Diego, USA[74] have expertise in automated glucose clamp technology using the Biostator®. The versatility of the technique extends to the assessment of pharmacokinetic and pharmacodynamics, i.e., insulin time-action profiles, of novel insulins (Krentz, 2019a).

Practical issues specific to the Biostator® include maintaining the patency of the double-lumen cannula which continuously samples whole-blood for measurement using a glucose oxidase method (Heinemann and Ampudia-Blasco, 1994).

[74] www.prosciento.com.

Figure 3. Biostator® automated glucose clamp.



The subject's left hand is heated by a pad maintained at a temperature of ~55°C to open arterio-venous channels. Arterialised blood supplemented with a heparin solution is continuously pumped from the hand vein to the glucose analyser (Biostator®). Venous samples for pharmacokinetics and other pharmacodynamic determinations are drawn from a cannula in a cubital vein of the left arm. The intravenous (I.V.) glucose infusion delivered into the forearm of the contra-lateral arm is continuously adjusted automatically to maintain target blood glucose concentration according to a predetermined algorithm. Saline solution is continuously infused at a low flow rate to keep venous access patent.

Source: Krentz 2019b.

4.3 Hyperinsulinaemic euglycaemic clamp

The two-stage hyperinsulinaemic euglycaemic clamp was used to quantify whole-body insulin sensitivity in a study of high-intensity statin therapy in the Wellcome Trust Clinical Research Facility at the University of Southampton[75]. The Southampton clamp study utilised a manual version of the clamp in which the variable rate glucose infusion rate was determined by a doctoral clinical research fellow based on the methodology described by DeFronzo and colleagues (DeFronzo et al., 1979). The study combined the clamp with measures of skeletal microvascular function. As presented in Table 5, hyperinsulinaemic euglycaemic clamps may be combined with various non-invasive assessments of metabolic-vascular function or invasive research methods such as tissue biopsy (Krentz, 2019b).

In an example of rapid translational research, shortly after the completion of proof-of-concept studies in healthy volunteers using the hyperinsulinaemic euglycaemic clamp (Boyle et al., 1993, Krentz et al., 1993) it was demonstrated in clinical practice that octreotide, a potent analogue of somatostatin, is an effective adjunctive therapy for severe sulfonylurea-induced hypoglycaemia (Krentz et al., 1993). Octreotide has since become standard of care for this life-threatening medical emergency (Glatstein et al., 2012).

4.4 Variants of the glucose clamp technique

The pancreatic – or islet cell – clamp which utilises octreotide as an alternative to native somatostatin, further attests to the versatility of the glucose clamp technique (Krentz et al., 1994). Octreotide suppresses endogenous hormone secretion; insulin, glucagon and growth hormone are by replaced by intravenous infusion to prespecified circulating concentrations (Krentz et al., 1994). In an innovative study, the octreotide islet cell clamp was combined with stepped hyperglycaemia under Biostator® control to characterise the effects of the SGLT-2 inhibitor dapagliflozin on the renal reabsorption of glucose (DeFronzo et al., 2013).

Glucose clamps are also used in the assessment of endogenous insulin section. In the hyperglycaemic clamp sustained hyperglycaemia, achieved by intravenous infusion of glucose, characteristically generates a biphasic insulin response with an early burst of insulin release followed by a gradually progressive increase in plasma insulin concentration (DeFronzo et al., 1979). Loss of the first-phase response, reflecting islet β-cell dysfunction, is an early (Davies et

[75] Insulin sensitivity may be considered the inverse of insulin resistance.

al., 1994) feature of states of glucose intolerance. The graded glucose infusion, is used create dose-response curves between endogenous insulin secretion rates and progressively escalating plasma glucose concentrations (Byrne et al., 1995).

Table 5. Hyperinsulinaemic glucose clamp: complementary methods.

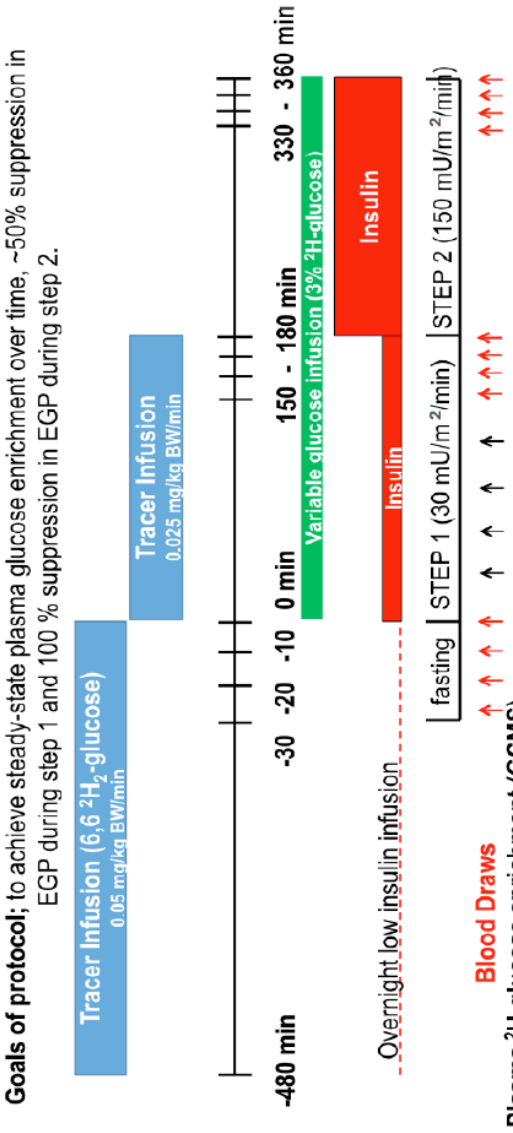
Method	Measurement of interest
Isotopic glucose tracer	Glucose turnover*
Functional imaging, e.g., PET/MRI	Regional tissue-specific insulin action
Indirect calorimetry	Substrate oxidation
Magnetic resonance spectroscopy	Intramyocellular lipid; hepatic lipid content
Positron emission tomography	Regional, e.g., brain, heart glucose metabolism
Venous occlusion plethysmography	Microvascular function
Isotopic glycerol tracer	Quantification of adipocyte lipolysis
Tissue biopsy of muscle, fat	Insulin-responsive enzyme expression
Microdialysis	Adipose tissue substrate metabolism

* Endogenous glucose production and glucose disposal (Ra_G, Rd_G, respectively).

PET, positron emission tomography; MRI, magnetic resonance imaging. Based on Krentz, 2019.

Figure 4 outlines the protocol for a two-step hyperinsulinaemic euglycaemic clamp. Isotopic determination of glucose turnover, i.e., rate of appearance and rate of disposal, are quantified using an infusion of di-deuterated [6,6-$^2H_2$] glucose tracer (Krentz, 2019b). Step 1 of the two-step variant of the hyperinsulinaemic euglycaemic clamp induces an elevation of circulating insulin concentrations suitable for determining the suppression of hepatic glucose production (Heise, 1998).

Figure 4. Two-step hyperinsulinaemic euglycaemic clamp.



Two-step hyperinsulinaemic euglycaemic clamp study with optional isotopically determined glucose turnover. An overnight variable rate insulin infusion may be required to achieve euglycaemia at the start of the clamp in subjects with diabetes. The hyperinsulinaemic clamp commences in the fasting state with a low dose insulin infusion at 30 mU/m^2/min for 180 min. The insulin infusion rate is then increased to 150 mU/m^2/min for a further 180 min. Euglycaemia is maintained by variable rate intravenous infusion of dextrose (20% v/v) determined by the Biostator® in response to frequent measurements of blood glucose. Blood draws for relevant metabolites and hormones are made during the final 30 min of each insulin infusion during quasi steady state.

EGP, endogenous glucose production.

Source: Hompesch M, Krentz AJ, Morrow L, Weyer C. Translational metabolic medicine: An early phase clinical research perspective. Keystone Symposium, La Jolla, California, 2016.

Step 2 attains levels of hyperinsulinaemia that ensure near maximal stimulation of glucose uptake, predominantly by skeletal muscle. Insulin sensitivity can be expressed in a variety of outputs using glucose clamp data (Table 6). The M value denotes whole body glucose metabolism at quasi steady state (Fig 1.5).

Table 6. Insulin sensitivity measures derived from hyperinsulinaemic glucose clamps.

M	Whole body glucose metabolism at steady-state	mg/kg/min*
MCR	Metabolic clearance rate of glucose	ml/kg/min
M/I	Glucose metabolism/mean steady-state insulin	(mg/kg/min)/pmol/L
SI_{clamp}	Insulin sensitivity index*	ml/(min x m^2) per pmol/L

* Calculated from two-step hyperinsulinaemic euglycaemic clamp

kg = fat free mass; results may also be normalised to body surface area or to alternative measures of metabolically active tissue, e.g., resting energy expenditure. The SI_{clamp} is the quotient of augmentation of M/I between the insulin infusion periods.

Source: Krentz AJ, 2019b

Dividing the M-value by the prevailing mean serum/plasma insulin concentration generates the M/I ratio, an index of tissue sensitivity to insulin (DeFronzo et al., 1979). M/I is discussed in more detail in Chapter 6.

4.5 Relevance of glucose clamp methodology to normal physiology

The standard one-step hyperinsulinaemic euglycaemic clamp focuses on insulin-mediated glucose disposal in skeletal muscle. The sustained hyperinsulinaemia generated by this method bears only a passing resemblance to normal physiology (Krentz, 2002a). Nonetheless, the hyperinsulinaemic euglycaemic clamp is the reference technique for determining whole-body insulin action and the effects of pharmacotherapeutics with putative effects on insulin sensitivity (DeFronzo et al., 1979, Muniyappa et al., 2008, Krentz, 2019b). Clinical research methods that attain circulating plasma insulin concentrations within the physiological range can provide insights into other important aspects of intermediary metabolism, i.e., hepatic glucose production and adipocyte lipolysis (Powrie et al., 1992, Krentz and Nattrass, 1996, Krentz, 2002a, Sondergaard et al., 2017). In this way, insulin

action can be appropriately partitioned according to the main metabolically active tissues, i.e., skeletal muscle, liver, and adipose tissue (Krentz, 2023a).

4.6 Nonclassic cardiometabolic effects of high-intensity statin therapy

The original research paper presented in Chapter 4 is a phase 4 investigator-initiated study of effects of high-intensity statin therapy on insulin sensitivity, glucose metabolism, and microvascular function in adults with central adiposity and features of the metabolic syndrome (Clough et al., 2009). In addition to quantifying the effects of 40 mg atorvastatin daily on lipid profiles, assessments of non-classic risk factors included whole-body insulin action on glucose and lipid metabolism and inflammatory markers including C-reactive protein and tumour necrosis factor-α. In addition, the response of the adipocytokines leptin, adiponectin, and resistin were examined. Microvascular function was assessed in skeletal muscle before and at the end of 6-months' therapy with atorvastatin using non-invasive methods.

Muscle Microvascular Dysfunction in Central Obesity Is Related to Muscle Insulin Insensitivity but Is Not Reversed by High-Dose Statin Treatment

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OBJECTIVE—To test the hypotheses that decreased insulin-mediated glucose disposal in muscle is associated with a reduced muscle microvascular exchange capacity (K_f) and that 6 months of high-dose statin therapy would improve microvascular function in people with central obesity.

RESEARCH DESIGN AND METHODS—We assessed skeletal muscle microvascular function, visceral fat mass, physical activity levels, fitness, and insulin sensitivity in skeletal muscle in 22 female and 17 male volunteers with central obesity whose age (mean ± SD) was 51 ± 9 years. We tested the effect of atorvastatin (40 mg daily) on muscle microvascular function in a randomized, double-blind, placebo-controlled trial lasting 6 months.

RESULTS—K_f was negatively associated with a measure of glycemia (A1C; $r = -0.44$, $P = 0.006$) and positively associated with insulin sensitivity (the ratio of insulin-stimulated glucose effectiveness, or *M* value, to the mean insulin concentration, or *I* value; $r = 0.39$, $P = 0.02$). In regression modeling, A1C, visceral fat mass, and *M*:*I* explained 38% of the variance in K_f (in a linear regression model with K_f as the outcome [$R^2 = 0.38$, $P = 0.005$]). *M*:*I* was associated with K_f independently of visceral fat mass (B coefficient 3.13 [95% CI 0.22–6.02], $P = 0.036$). Although 6 months' treatment with atorvastatin decreased LDL cholesterol by 51% ($P < 0.001$) and plasma high-sensitivity C-reactive protein by 75% ($P = 0.02$), microvascular function was unchanged.

CONCLUSIONS—Decreased insulin-mediated glucose uptake in skeletal muscle is associated with impaired muscle microvascular exchange capacity (K_f), independently of visceral fat mass. Muscle microvascular function is not improved by 6 months of high-dose statin treatment, despite marked statin-mediated improvements in lipid metabolism and decreased inflammation. ***Diabetes*** **58:1185–1191, 2009**

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Microvascular dysfunction is a cardinal long-term complication of type 2 diabetes. Reported microvascular defects in type 2 diabetes include impaired endothelium-dependent vasodilatation, reduced substrate delivery, and lower capillary density in insulin-sensitive tissues (1). Increased glycation of erythrocyte membrane proteins causing rigidity may result in an increased resistance to travel through the microcirculation (2), while concomitant alterations of the endothelial cell surface glycocalyx may modulate vascular permeability and exchange surface area (3). Obesity is the most important modifiable risk factor for the development of type 2 diabetes. Microvascular dysfunction has also been reported in obese subjects in the absence of diabetes, but it remains unclear which component(s) of obesity-linked pathophysiology contributes to microvascular dysfunction (1,4–6). Increased body fat mass is associated with molecular changes that contribute to altered vasodilatory responses, oxidative stress, abnormalities of vasoconstriction, and altered platelet adhesion; all of these defects could potentially influence solute delivery via the microvasculature (5). Insulin increases blood flow and microvascular perfusion in skin (7,8) and skeletal muscle (9,10), and impairment of insulin-induced microvascular dilator responses in skeletal muscle in animal models of insulin resistance, even at basal insulin concentrations, is believed to be a key factor in reduced glucose uptake (4,11). Thus, microvascular dysfunction might contribute to obesity-associated insulin resistance. Studies in insulin-resistant states in humans, such as obesity with or without the presence of type 2 diabetes (12), have shown impaired microvascular function where both insulin-mediated muscle microvascular perfusion and glucose uptake are reduced (5,13). The attenuation of insulin-stimulated muscle microvascular perfusion recruitment in obese humans is reminiscent of that reported in obese Zucker rats (6), being suggestive of common mechanistic pathways, including increased production of reactive oxygen species and reduced nitric oxide (NO) availability.

Based on results in animal models, it has been proposed that insulin acts to dilate the arterioles governing flow through capillary beds (14), thereby increasing substrate delivery (15). This occurs independently of, and appears to precede, increases in total blood flow and glucose disposal (14) resulting from dilatation of upstream arteriolar vessels (16). Entangled with this hypothesis is the concept of insulin-mediated redistribution of blood flow through the preferential perfusion of so-called nutritive vessels at the expense of nonnutritive routes (17). In healthy humans,

many, but not all, experimental studies have shown insulin to have dose- and time-dependent effects, increasing blood flow in a manner that parallels glucose disposal (18). However, whether the capacity of insulin to increase the number of patent capillaries (capillary recruitment) is impaired in insulin-resistant states, such as obesity, remains uncertain (19–21).

It is well accepted that treatment with statins decreases risk of macrovascular disease and that statins have pleiotropic actions. Statins are also effective treatments for targeting vascular risk in people with features of the metabolic syndrome (22). However, the extent to which statin therapy has beneficial effects on the microvasculature has been little studied and remains unclear. Within the macrovasculature, statins have been shown to improve endothelial function and attenuate endothelial dysfunction in the presence of atherosclerotic risk factors through upregulation of endothelial NO synthase and the increased production of NO, and such an effect has been shown to occur after 6 months of therapy (rev. in 23). However, the potential for statins to modulate endothelial function in smaller vessels has yet to be elucidated.

Although it is known that microvascular dysfunction occurs in obesity, the nature of the relationship between microvascular function in an insulin-sensitive tissue such as skeletal muscle and insulin-mediated glucose disposal in skeletal muscle is uncertain. The role of potential confounders, such as fitness and physical activity levels, in the relationship between skeletal muscle microvascular function and insulin-mediated glucose disposal in skeletal muscle have not been fully clarified. Moreover, it is not certain whether statin therapy confers any benefit on muscle microvascular function via hypothesized pleiotropic actions independently of an effect on circulating LDL cholesterol concentrations.

The aims of our study were to assess the relationship between insulin-mediated glucose disposal and measures of skeletal muscle microvascular function, including microvascular filtration capacity (K_f), a measure of microvascular integrity (isovolumetric pressure [P_{vi}]), and resting limb blood flow (Qa), and to test the effect of statins on these factors in individuals with central obesity. We hypothesized *1*) that decreased insulin-mediated glucose disposal in muscle is associated with a reduced muscle K_f and *2*) that 6 months' intensive high-dose statin treatment would reverse this microvascular dysfunction (potentially via the pleiotrophic actions of statins on NO production). We took care to assess potential confounders, such as physical inactivity and cardiorespiratory fitness, that are known to influence K_f (24).

RESEARCH DESIGN AND METHODS

The study was approved by the Southampton General Hospital research ethics committee (LREC05/Q1704/38) and conducted in accordance with the Declaration of Helsinki. All participants were unpaid volunteers and gave informed written consent. White European subjects aged 18–75 years were invited to participate in the study. Volunteers were eligible for the main study if they had central obesity and at least one other feature of the metabolic syndrome as assessed by International Diabetes Federation criteria (25). For ethical reasons, subjects were only included in the study if estimated cardiovascular risk was <20% over 10 years based on the equation derived from the Framingham Heart Study because national guidelines indicate that people at higher cardiovascular risk should receive statin treatment for primary prevention of cardiovascular disease. Exclusion criteria were known diabetes; renal, liver, or uncontrolled thyroid disease; uncontrolled hypertension (blood pressure >160/100 mmHg); treatment with lipid-modifying drugs; antihypertensive medication; corticosteroid therapy; or hormone replacement therapy. For more information on the subjects and methods, please refer to the supplemental appendix, available online at http://dx.doi.org/10.2337/db08-1688.

After completing the baseline tests, subjects were randomized in a double-blind placebo-controlled trial study design by an independent pharmacist to either 40 mg atorvastatin daily or to matched placebo for 26 weeks. The primary end point of the trial was a change in microvascular function. Previously, Charles et al. (26) studied 12 individuals in a 14-week training program during which lower limbs were trained for endurance exercise, and these authors showed a 79% improvement in K_f (from 2.4 ± 0.8 to 4.3 ± 0.9, $P < 0.05$). Brown et al. (24), using electrical stimulation for 4 weeks in five sedentary individuals (8 Hz, 3 × 20 min/day for 5 days per week), showed that K_f increased ~200%, from 3.38 ± 0.38 to 6.68 ± 0.62 ($P < 0.05$). We estimated that a sample size of $n = 40$ subjects would give us 99% power at the 5% significance level to detect a 1-SD increase in K_f and that, based on the changes shown with exercise and electrical stimulation studies, such a functional change would be physiologically relevant. Data are presented on 39 subjects because one person was unable to complete the study after suffering side effects of the prescribed trial medication.

Body composition, fat mass, and lean body mass were measured using a dual X-ray absorptiometry Delfia W 4500 instrument (coefficient of variation = 0.68%; Hologic, Bedford, MA) using a standard visual method to divide images into trunk, limb, and head. An abdominal magnetic resonance imaging scan was undertaken to assess visceral fat (27–29). An oral glucose tolerance test was performed with a 75-g glucose load with samples collected after 2 h.

The following tests were undertaken at baseline and at 26 weeks: microvascular function was assessed using a Filtrass venous occlusion plethysmographic system using a passive inductive transducer with an accuracy of ±5 μm (Compumedics DWL, Singen, Germany). Filtration (J_v in $ml \cdot min^{-1} \cdot 100\ ml^{-1}$) was measured from the slope of volume change in response to each pressure step over the last 2 min of its application, to allow for completion of vascular filling, and plotted against cuff pressure (P_{cuff}). The slope of this relationship, at pressures above those giving rise to net filtration, is a measure of microvascular filtration capacity (K_f) (30). Extrapolation of the relationship to its intercept on the P_{cuff} axis gives the isovolumetric venous pressure (P_{vi}) at which there is neither net filtration nor absorption (30,31). Muscle strength was assessed by measurement of handgrip strength using a Jamar dynamometer (Promedics, Blackburn, U.K.).

Hyperinsulinemic-euglycemic clamp. A hyperinsulinemic-euglycemic clamp was undertaken to assess whole-body glucose uptake (insulin-stimulated glucose effectiveness, or *M* value) during the steady state of the clamp (final 30 min of the clamp), both at baseline and after intervention while subjects were taking their trial medication (32).

Measurement of insulin sensitivity. Whole-body insulin sensitivity was measured as glucose uptake during the steady state of the clamp with an insulin infusion rate of 1.5 $mU \cdot kg^{-1} \cdot min^{-1}$. All individuals achieved euglycemia during the clamp with glucose concentrations clamped at 5.0 mmol/l. Whole-body glucose uptake (*M* value) was defined as the glucose infusion rate during the final 30 min of the test (in $mg \cdot kg^{-1} \cdot min^{-1}$) when steady-state insulin concentrations had been achieved. The ratio of *M* to the mean insulin concentration (*I* value) was used as an index of insulin sensitivity. *M*:*I* values were estimated by dividing the *M* value by the *I* value during the last 30 min of the clamp.

Cardiorespiratory fitness and physical activity energy expenditure. Cardiorespiratory fitness measured in terms of maximal oxygen uptake (VO_{2max}) was determined using a treadmill test and Cortex Metalyzer, and physical activity (physical activity energy expenditure and metabolic equivalents) was assessed using an activity monitor (Armband Sensewear Pro2) (33).

Statistical analyses. All statistical analyses were performed using SPSS for Windows version 16.0 (SPSS, Chicago, IL). Student's *t* test comparisons were undertaken to compare mean values of normally distributed data. Pearson correlation coefficients are presented for univariate analyses of normally distributed data. Where variables were not normally distributed, log transformation was undertaken to normalize the distribution. Multivariate linear regression models were used to describe factors that were independently associated with K_f as the dependent (outcome) variable. A *P* value of <0.05 was considered to be statistically significant for all analyses. Data are expressed as the mean ± SD and range unless otherwise stated. To test the effect of statin on measures of microvascular function, we analyzed microvascular function at the end of the trial, adjusting for randomization and baseline microvascular measures by factorial ANOVA.

RESULTS

Table 1 shows the baseline characteristics of subjects recruited to the study. The 39 healthy volunteers included 17 men and were aged 51 ± 9 years. Of the study subjects,

TABLE 1
Baseline characteristics of participants

	Mean ± SD	Range
Age (years)	51.4 ± 9.0	29.0–69.6
BMI (kg/m^2)	32.1 ± 4.6	26.0–47.9
Waist (cm)	105.3 ± 12.9	86.5–151.0
Total fat (%)	35.6 ± 7.4	21–48
Truncal fat (% of total)	52.2 ± 5.6	43–64
Systolic blood pressure (mmHg)	133 ± 14	93–155
Diastolic blood pressure (mmHg)	85 ± 9	64–104
Cardiovascular disease risk (% per 10 years)*	7.3 ± 5.1	0–17.3
Total cholesterol (mmol/l)	5.7 ± 1.1	3.2–9.3
LDL cholesterol (mmol/l)	3.7 ± 0.9	1.7–7.0
HDL cholesterol (mmol/l)	1.45 ± 0.36	0.92–2.45
Triglyceride (mmol/l)	1.4 ± 0.6	0.4–2.7
Glucose (mmol/l)	5.2 ± 0.7	4.0–7.4
A1C (%)	5.5 ± 0.3	4.9–6.3

% fat estimated by DEXA. *Estimated using Framingham risk score.

11 had two features, 18 had three features, 9 had four features, and 1 had all five features of the metabolic syndrome. Subjects were excluded if they had known diabetes at recruitment. On baseline testing one subject was found to have a fasting glucose of 7.4 mmol/l and therefore analyses were undertaken both including and excluding this person. Inclusion of data from this individual (who received no glucose-lowering medication during the study) did not change or affect the results, and the data are therefore presented for all 39 subjects who completed the 6-month trial. For more information on the results of this study, please refer to the supplementary material in the online appendix.

Figure 1 shows baseline K_f, Qa, and P_{vi} measurements. Mean values for K_f, Qa, and P_{vi} were $3.91 \pm 0.18 \times 10^{-3}$ ml · min^{-1} · 100 ml^{-1} · mmHg^{-1}, 4.01 ± 0.48 ml · min^{-1} · 100 ml^{-1}, and 20.5 ± 1.1 mmHg, respectively. There was considerable variability in all measures within the cohort with an approximately threefold difference in K_f levels between subjects.

We investigated the relationships between measures of microvascular function (K_f, Qa, and P_{vi}), measures of obesity and features of the metabolic syndrome, together with measures of physical activity and fitness. Table 2 shows correlation coefficients describing the relationships between K_f, age, features of the metabolic syndrome, Vo_{2max}, physical activity energy expenditure, and metabolic equivalents. Of the readily measurable features of the metabolic syndrome (waist circumference, blood pressure, glucose, HDL cholesterol, and triglyceride concentrations), K_f was statistically significantly associated with waist circumference ($r = -0.36$, $P = 0.025$). There was a borderline significant association with triglycerides ($r = -0.30$, $P = 0.07$), and there were no significant associations between K_f and age, blood pressure, glucose, or HDL cholesterol concentrations. Figure 2 shows the scatter plots for the relationships between K_f and visceral fat, A1C, and *M*:*I*. K_f was negatively associated with visceral fat ($r = -0.43$, $P = 0.015$) and A1C ($r = -0.44$, $P = 0.006$). K_f was also negatively associated with plasma high-sensitivity C-reactive protein (hsCRP) and soluble intracellular adhesion molecule (ICAM)-1 (both $P < 0.05$). K_f was positively associated with *M*:*I* ($r = 0.39$, $P = 0.02$). There were no significant associations of note with P_{vi}. Resting Qa was associated with respiratory exchange ratio ($r = 0.52$, $P = 0.002$). Neither P_{vi} nor Qa were associated with age ($r = -0.20$, $P = 0.24$, and $r = -0.12$, $P = 0.94$, respectively).

TABLE 2
Correlations between filtration capacity (K_f) and features of the metabolic syndrome, abdominal obesity, fitness, and physical activity

	r	*P*
Age (years)	0.02	0.89
Waist (cm)	−0.36	0.025
Visceral fat (kg)	−0.43	0.015
Subcutaneous fat (kg)	−0.28	0.12
Systolic blood pressure (mmHg)	−0.15	0.36
Diastolic blood pressure (mmHg)	0.08	0.63
Cardiovascular disease risk (% per 10 years)	−0.15	0.38
HDL cholesterol (mmol/l)	0.13	0.43
Triglyceride (mmol/l)	−0.30	0.07
Glucose (mmol/l)	−0.05	0.77
A1C (%)	−0.44	0.006
Steps (*n*)	0.35	0.04
Physical activity energy expenditure (metabolic equivalents)	0.20	0.24
Vo_{2max} (ml · min^{-1} · kg^{-1})	0.21	0.20
Glucose disposal (mg · kg^{-1} · min^{-1} · mIU^{-1} · l^{-1})	0.39	0.021

Multiple regression modeling was undertaken to further explore factors that were associated with K_f. In a regression model that included K_f as the outcome, 38% of the variance in K_f was explained by A1C, *M*:*I*, and visceral fat mass as the explanatory variables in the model ($R^2 = 0.38$, $P = 0.005$). To determine whether the association between *M*:*I* and K_f (observed in univariate analysis) (Table 2) was independent of visceral fat, we undertook regression modeling with K_f as the outcome variable and included *M*:*I* and visceral fat as explanatory variables. In this model, *M*:*I* and visceral fat explained 30% of the variance in K_f ($R^2 = 0.30$, $P = 0.008$). *M*:*I* was associated with K_f independently of visceral fat (B coefficient = 3.13, 95% CI 0.22–6.02, $P = 0.036$), whereas visceral fat was not associated with K_f independently of *M*:*I* (B coefficient = −0.09, 95% CI −0.40 to 0.22, $P = 0.55$). There was no effect of sex in our model.

Having observed associations with measures of insulin sensitivity and microvascular function in muscle, we investigated whether a functional measure of muscle performance (grip strength) was associated with measures of microvascular function. Only P_{vi} was associated with grip

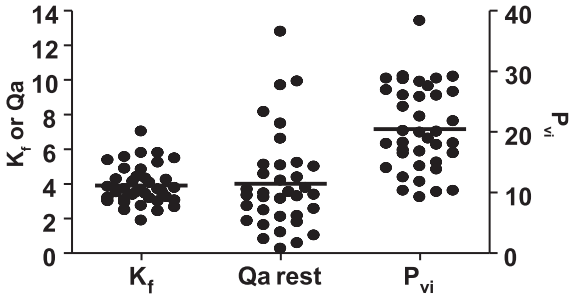

FIG. 1. Baseline measurements of filtration capacity (K_f), resting limb blood flow (Qa), and endothelial integrity (P_{vi}) from 39 individuals. All values were derived using venous congestion plethysmography from the raw data (see supplemental information in the online appendix). Qa = resting (ml · 100 ml^{-1} · min^{-1}); K_f = × 10^{-3} ml · min^{-1} · 100 ml^{-1} · mmHg^{-1}; P_{vi} = mmHg.

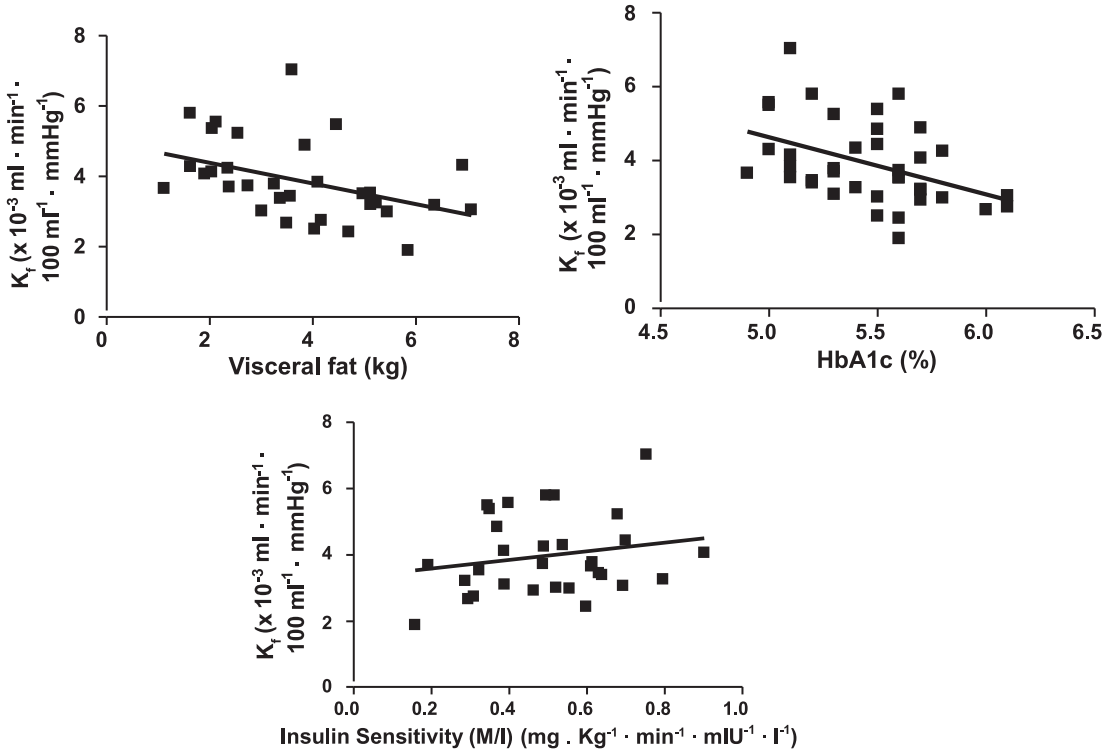

FIG. 2. Scatter plots for the relationships between K_f and visceral fat, A1C, and *M*:*I* from 39 individuals at baseline. K_f was negatively associated with visceral fat ($r = -0.43$, $P = 0.015$) and A1C ($r = -0.44$, $P = 0.006$) and positively associated with *M*:*I* ($r = 0.39$, $P = 0.02$).

strength {mean handgrip strength [(right + left)/2]} and P_{vi} ($r = -0.55$, $P < 0.001$). We observed similar results for the relationship between P_{vi} and left handgrip strength ($r = -59$, $P < 0.001$) and right handgrip strength ($r = -0.51$, $P = 0.001$).

We investigated the effect of 6 months of 40 mg/day of atorvastatin treatment in the randomized placebo controlled trial. In subjects in the placebo arm of the study ($n = 20$), mean baseline LDL cholesterol was 3.78 ± 1.05 mmol/l, and it was 3.70 ± 0.90 mmol/l at follow-up ($P = 0.51$). In contrast, in the treatment arm of the study ($n = 19$), LDL cholesterol fell from 3.53 ± 0.81 mmol/l at baseline to 1.73 ± 0.71 mmol/l at follow-up ($P < 0.001$). Subjects in the placebo arm of the study had a mean baseline triglyceride of 1.31 ± 0.68 mmol/l, and triglyceride was 1.19 ± 0.61 mmol/l at follow-up ($P = 0.34$). Mean baseline fasting triglyceride concentration in the treatment arm of the study was 1.41 ± 0.62 mmol/l, compared with 1.00 ± 0.58 mmol/l at follow-up ($P = 0.001$). Median baseline hsCRP in subjects in the placebo arm of the study was 2.0 mg/l (95% CI 1.09–10.47) compared with 3.0 mg/l (1.62–6.35) at follow-up ($P = 0.73$). Statins reduced hsCRP from a baseline value of 2.0 mg/l (95% CI 1.31–5.59) to 0.5 mg/l (0.35–4.65) at follow-up ($P = 0.02$). The change in LDL cholesterol concentration was positively correlated with the change in hsCRP ($r = 0.27$, $P < 0.002$). In both the placebo and treatment arms of the study, there was no change in body fat, *M*:*I*, A1C, or ICAM-1 measurements after 6 months of treatment (data not shown). Figure 3 shows K_f, P_{vi}, and Qa measurements before and after 6 months' treatment with atorvastatin. There was no change in K_f, P_{vi}, and Qa measurements with statin treatment, adjusting for baseline measures, age, and sex (K_f: $P = 0.99$; P_{vi}: $P = 0.28$; Qa: $P = 0.29$).

DISCUSSION

Our results show that in adults with central obesity, decreased insulin-mediated glucose uptake in skeletal muscle is associated with impaired muscle K_f independently of visceral fat mass. Our data demonstrate that the association between K_f and insulin-mediated glucose disposal is independent of visceral fat mass, with no confounding by physical inactivity or low fitness levels. Despite a wealth of evidence showing that statins confer a benefit in the macrovasculature, and despite a marked statin effect on LDL cholesterol levels (lowered by ~50%) and hsCRP (decreased by 75%), our results clearly show no effect of statin on measures of muscle microvascular function.

In our study, insulin-mediated glucose disposal (*M*:*I*) was positively associated with exchange capacity (K_f), suggesting that the more insulin-sensitive individuals have a greater exchange capacity, thereby facilitating muscle nutrient delivery. Although the range of values of K_f measured in our study group was considerable, they were similar to those reported previously for similarly aged individuals (26,34). The values of K_f are also within the range reported for individuals with pre-diabetes or diabetes without microvascular complications (35–38). We failed to show any association between age and any of the measures of microvascular function in our study. However, the mean age of our volunteers was 51.4 years with an SD of only 9 years. Thus, it is plausible that in our relatively small sample size of predominantly middle-aged subjects, we have failed to detect true associations between aging and measures of microvascular function. It is also possible that because we have not studied very aged individuals, we have not detected any obesity-independent, age-related change in microvascular function.

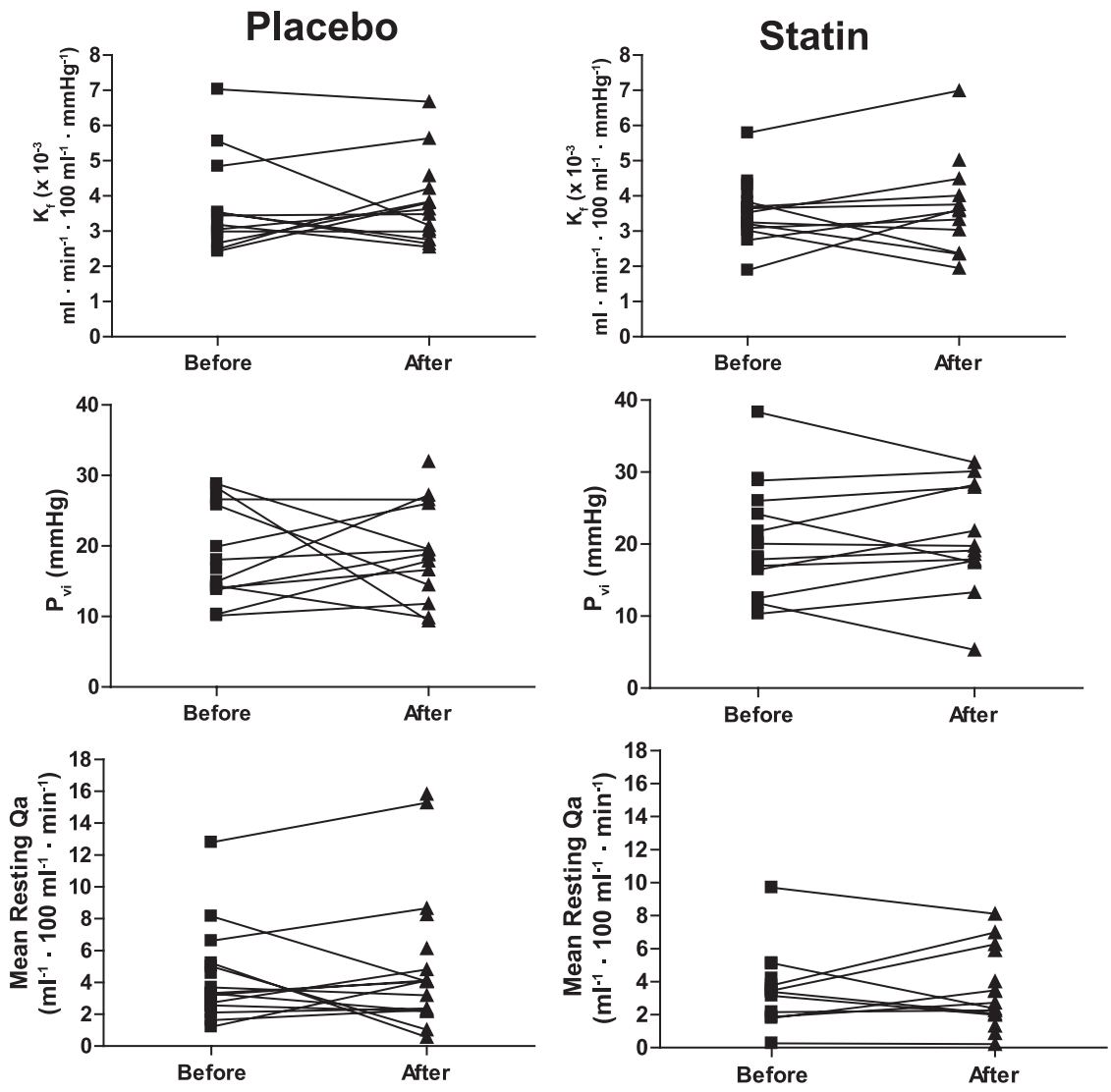

FIG. 3. K_f, isovolumetric pressure (P_{vi}), and resting limb blood flow (Qa) measured before and after 26 weeks' treatment with atorvastatin (40 mg once daily) or matched placebo.

Having shown associations between insulin-mediated glucose uptake in skeletal muscle and K_f, we also explored whether a functional measure of skeletal muscle performance (grip strength) was associated with measures of muscle microvascular function, since we have recently shown in a large cohort study that decreased grip strength was associated with metabolic syndrome (39) and type 2 diabetes (40). The mechanism underlying decreased handgrip strength in type 2 diabetes and metabolic syndrome is uncertain, but, interestingly, our results show that one measure of microvascular integrity (P_{vi}) was associated with decreased grip strength. In other studies, increases in P_{vi} have been associated with inflammatory disease (41), but whether vascular inflammation within skeletal muscle may contribute to loss of muscle strength in people with type 2 diabetes has yet to be explored.

Our results showed a marked effect of atorvastatin to decrease LDL cholesterol and CRP, but, in keeping with previous work (42) testing the effects of 12 weeks' treatment with atorvastatin, we showed no effect of atorvastatin on levels of inflammatory markers (e.g., ICAM-1, tumor necrosis factor-α, interleukin-6, endothelin 1, retinol binding protein 4, leptin, adiponectin, and resistin) (data not shown). Whereas statins have been shown to have a beneficial effect on endothelial function and blood flow within the macrovasculature (e.g., flow-mediated dilation), our data with high-dose atorvastatin for 6 months showed no effect of statin on any of the measured aspects of muscle microvascular function. Fegan et al. (43) showed, in individuals with type 2 diabetes, no improvement in cutaneous vascular response after 3 months' treatment with single or combined lipid-lowering therapy. Although no effect of 4 weeks' treatment with 20 mg/day atorvastatin was observed on vasomotor function by high-resolution ultrasound examination of the brachial artery (flow-mediated dilation and sublingual nitrate) (44), a beneficial effect of statins on aspects of endothelial function has been noted over 6 months (45). It is plausible that turnover of endothelial cells or neovascularization is needed to improve aspects of microvascular function as measured in our study. Six months' treatment with statins may be insufficient time for this to occur. It is possible that our failure to detect a difference in microvascular function with statin treatment represents a type 2 statistical error. However, our randomized placebo-controlled trial sample size gave us 97% power to detect a 1.0-SD change in K_f at

the 5% significance level, and a 1.0-SD change in K_f represents a ~1.1-unit change in K_f, or an increase in mean K_f from 3.9 to 5.0 $\times 10^{-3}$ ml · min^{-1} · 100 ml^{-1} · mmHg^{-1}. Our study was therefore powered to detect relatively modest changes in K_f, and more marked changes in K_f have been observed with electrical stimulation and with exercise. Electrical stimulation for 4 weeks increased K_f from 3.38 ± 0.38 to 6.68 ± 0.62 ($P < 0.05$) (24), and a 14-week training program during which lower limbs were trained for endurance caused a 79% improvement in K_f (from 2.4 ± 0.8 to 4.3 ± 0.9, $P < 0.05$) (26,35–38). Thus, these data suggest that our study was powered to detect physiologically relevant changes in K_f.

The methods available to investigate muscle microvasculature are limited, and those used to measure the whole tissue or insulin-mediated capillary perfusion and flow are very invasive, for example, radiolabeled imaging techniques (46), contrast enhanced ultrasound using albumin microbubbles (9), needle-inserted laser Doppler probes (8), or measurements of the distribution of blood flow by [^{15}O]H_2O as an index of flow heterogeneity (47). One way of assessing blood/muscle exchange, and hence to assess impaired muscle microvascular function, is to quantify the capacity of the microvascular bed to filter fluid. Plethysmography is a well-validated, noninvasive technique that uses small-step increases in venous occlusion pressure and measurement of the resultant changes in limb volume to provide a measure of microvascular filtration capacity (K_f) (48). K_f, which is measured predominantly in the muscle of the lower limb, has been shown to be differentially sensitive to increases in capillary surface area, as found in training schedules (26), as well as sensitive enough to detect increases in capillary perfusion, as in studies involving chronic electrical stimulation (24). K_f thus appears to be an important and sensitive measure to detect impairment of microvascular function, and the methodology used (plethysmography) does not affect the function of the vasculature under study (17). In the current study, a noninvasive measure was used that was acceptable to subjects returning for testing at the end of the study. We reasoned that the greater technical ease of the Filtrass system and its good reproducibility, together with its noninvasive nature, would result in better compliance in our nonpaid volunteers who were required to return for follow-up measurements at the end of the intensive 6-month clinical trial. Many of the techniques mentioned above used to assess changes in blood flow in muscle rely on visualization of erythrocyte movement or their particulate surrogates. Moreover, the movement of plasma, which determines bulk transfer and microvascular surface interchange of small solutes to sustain the optimal diffusion gradient, cannot be readily visualized. Plethysmographic assessment of K_f goes some way toward addressing this matter by measuring the rate of fluid exchange across the whole muscle microvascular bed. This enables evaluation of microvascular filtration parameters, by which significant differences due to pathophysiology and/or therapeutic interventions can be studied. Other more invasive techniques provide evidence of an insulin-mediated microvascular "recruitment" in human muscle through a selective action on precapillary arterioles (9–11,13,49) to redirect blood to "nutritive" vascular beds. The measurement of K_f or Qa by plethysmography does not allow us to distinguish between variations in muscle blood flow due to shifts, or redistribution, within microvascular networks and those in total blood flow, as determined by the resistance vessels supplying the muscle. It is possible that variation in muscle blood flow due to shifts, or redistribution, within microvascular networks could explain some of the wide variance in microvascular measurements (Fig. 1) that we observed across our cohort.

In summary, we have shown a strong association between skeletal muscle K_f and decreased insulin sensitivity in skeletal muscle in men and women with central obesity. Despite marked decreases in LDL cholesterol and hsCRP concentrations caused by 6 months' high-dose statin treatment, there was no improvement in any measure of skeletal muscle microvascular function, suggesting that these factors do not make an important contribution to control of microvascular function. Our data emphasize that further studies are now required to investigate the effects of insulin-sensitizing agents on microvascular function both in individuals at risk of type 2 diabetes and in those who have type 2 diabetes.

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SUPPLEMENTAL INFORMATION

Muscle Microvascular Dysfunction in Central Obesity is Related to Muscle Insulin Insensitivity but is not Reversed by High-Dose Statin Treatment. Clough et al. 2009.

MATERIALS AND METHODS

Identification of suitable participants was based on a screening visit. This consisted of a brief interview, eliciting previous relevant medical history, resting blood pressure, anthropometry (waist circumference, body mass index (BMI) and sampling of plasma glucose, lipid profile, thyroid, liver and renal function tests after a 12 hour fast.

Subjects were encouraged to maintain their normal diet and lifestyle throughout the duration of the study to avoid changes in body weight >5% from baseline body weight, thus to minimise the effects of potential confounding effects of changes in body weight or activity on the study results. Subjects were weighed in light clothing to the nearest 0.1 kilogram using a Seca electronic scale and height was measured using a Seca 220 stadiometer to the nearest 0.1 cm. BMI was calculated according to the formula (weight in kg)/([square of height in metres]). Anatomical waist circumference was measured over bare skin midway between the costal margin and the iliac crest. Blood pressure was measured for both screening assessment and the main study using the OMRON 705CP blood pressure monitor.

The forty subjects recruited to the study attended the Wellcome Trust Clinical Research Facility at Southampton University Hospitals Trust on approximately 4 different occasions for baseline phenotyping studies. Both active drug and placebo tablets were supplied by Pfizer as part of an Independent Research grant to the Investigators and both tablets were identical in appearance.

Plasma glucose from OGTTs was measured on samples collected in fluoride oxalate tubes by hexokinase method on Beckman Unicel DXC800 (Beckman Coulter, Fullerton, CA, USA) and during the clamp studies measured on samples collected in lithium heparin tubes by the glucose oxidase method using a YSI 2300 STAT glucose analyser (Yellow Springs, Ohio, USA). Total cholesterol, high-density lipoprotein (HDL) cholesterol and triacylglycerols were measured on fasting serum samples using enzymatic colorimetric methods on Beckman Unicel DXC800 automated analyzer (Beckman Coulter, Fullerton, CA, USA). HBA_{1c} was measured by HPLC using a cation exchange cartridge on Bio-Rad Variant II Turbo (Bio-Rad Laboratories, Irvine, CA, USA) . A two-point calibration of the calculated data was used to align the results with the DCCT standard.

C-reactive protein (hsCRP) and soluble ICAM-1 were measured in plasma at 0 and 26 weeks in all subjects.

Hyperinsulinaemic euglycaemic clamps. Clamps were undertaken in the morning after a 12 h overnight fast. All subjects were asked to abstain from alcohol and strenuous exercise for 48 hours prior to testing.

Two points of venous access were established in the same arm. The first was obtained by retrograde cannulation of a vein on the dorsum of the hand with the 20g cannula. This hand was warmed to 50°C in a hotbox to improve oxygenation of venous blood thus providing 'arterialised' venous blood samples [1]. The level of oxygenation was checked at 2-3 time points during the clamp by measurement of blood gases. The arterialised venous blood samples were used for glucose and insulin concentration measurement. Blood glucose measurements were recorded every 5 minutes (YSI 2300 STAT glucose analyser). Blood samples for insulin measurements were taken at baseline, 90, 120 minutes and at 10 minutes intervals during the last 30 minutes of insulin infusion, separated and the plasma frozen at -80°C for analysis. The second venous access was established in the antecubital fossa with the 18g gauge cannula for exogenous insulin infusion. Human insulin (Actrapid), Novo Nordisk) was given at a rate of 0.2 mIU/kg/min for one hour and then at the continuous rate of 1.5mIU/kg/min for 2 hours after 7-minutes of stepped priming infusion using a syringe driver (Syramed usp 6000). The insulin infusion was prepared in 0.9% NaCl to which 1ml of subject's blood was added to prevent adsorption of insulin to the plastic surface of the syringe walls. Blood glucose concentrations were maintained at a target of 5 mmol/l by adjusting the rate of 20% dextrose infusion, based on the plasma glucose measurements obtained at 5-minute intervals using a variable-speed infusion pump (Volumed uVP5005). Subjects were rested for 1 hour after the venous cannulation, before insulin infusion was begun. Intact insulin was analysed using the dissociation-enhanced lanthanide fluroimmunoassay (DELFIA) method and Wallac 1420 mutilabel counter. All standards, quality controls and samples were analysed in duplicate.

Microvascular function. Subjects lay supine in a quiet room at an ambient temperature of 20˚C. The studied calf was supported at heart level, with the mid-calf region free for the attachment of the plethysmographic sensor. The Filtrass strain gauge sensor unit, with its inelastic nylon measuring line, in its flexible guide-holder, was placed around the calf at the point of maximum circumference and attached to the Filtrass sensor itself. At the start of the protocol, the strain gauge automatically measured the calf circumference, adjusted its tension and then calibrated itself. A congestion cuff was wrapped around the ipsilateral thigh and coupled into the Filtrass unit for automatic inflation during the protocol. For these studies we used a specifically designed, set protocol. After applying the start pressure of 4.0 mmHg, three initial pressure steps of 60 mmHg were applied, each for 10 seconds, with an intervening rest phase of 20 seconds at 4.0 mmHg. These were to measure limb blood flow,

derived from the initial slope of the response. Small (~10 mm Hg) cumulative increases in congestion pressure, from zero to a pressure not exceeding subjects' diastolic blood pressure, were then applied. Each step was sustained for just over 4 minutes. Further assessments of blood flow were made at the end of each cumulative pressure step, by raising the cuff pressure to 80 mmHg, for 10 seconds, followed by 50 seconds rest at the preceding cumulative pressure. The strain gauge tension was automatically re-balanced, to the starting tension, at the end of each component of the pressure protocol; this procedure minimized pitting. The whole protocol, which took between 30 and 40 minutes, was saved, at the end of the study, for off-line, blinded analysis.

The values for blood flow at each cumulative pressure step were normalized by expressing them as a percentage of the resting Qa value. We used the Darcy equation, that governs blood flow through parallel circuits of the cardiovascular system, Qa = (Pa-Pv)/R, where R is the peripheral vascular resistance, Pa is the arteriolar and Pv the venous hydrostatic pressure, respectively [2] together with the values of venous congestion pressure, at each pressure step, to calculate the value of blood flow that would have been expected had the pre-capillary resistance remained constant throughout the procedure. Leg peripheral vascular resistance was calculated from blood flow and leg MABP less the venous pressure. These predicted values of blood flow were also expressed as a percentage of the initial values.

Previous studies have shown that in healthy individuals calf blood flow, expressed as a percentage of baseline values, does not change significantly as the venous congestion pressure was increased in small cumulative steps and remains significantly greater than that predicted from the Darcy equation [3].

Muscle strength. Muscle strength was assessed by measurement of handgrip strength using a Jamar dynamometer (Promedics, Blackburn UK). The subjects were seated with their arms rested on the arms of a chair, and the sides altered between each measurement. For the analysis, we used the best score out of the total of three measurements from each hand [4].

Cardiorespiratory fitness and physical activity energy expenditure. Cardiorespiratory fitness was quantified by measurement of maximal oxygen consumption during the incremental treadmill test. Volunteers were fitted with an air-tight facemask for analysis of expired air and breath-by-breath analysis of oxygen consumption and CO_2 production was made using a Cortex Metalyser instrument (Cortex Biophysik, Germany). The treadmill test was commenced at 1.3 m/s (3 miles/h) and 0% gradient. The gradient or speed increased alternately by 2% increments every 2 min (i.e., stage 1: 1.3 m/s, 0% gradient; stage 2: 1.3 m/s, 2% gradient; stage 3:1.55 m/s, 2% gradient.). Volunteers were asked to continue to exhaustion and until the respiratory exchange ratio (RER) was >1.1 unless they experienced chest pain or felt unwell.

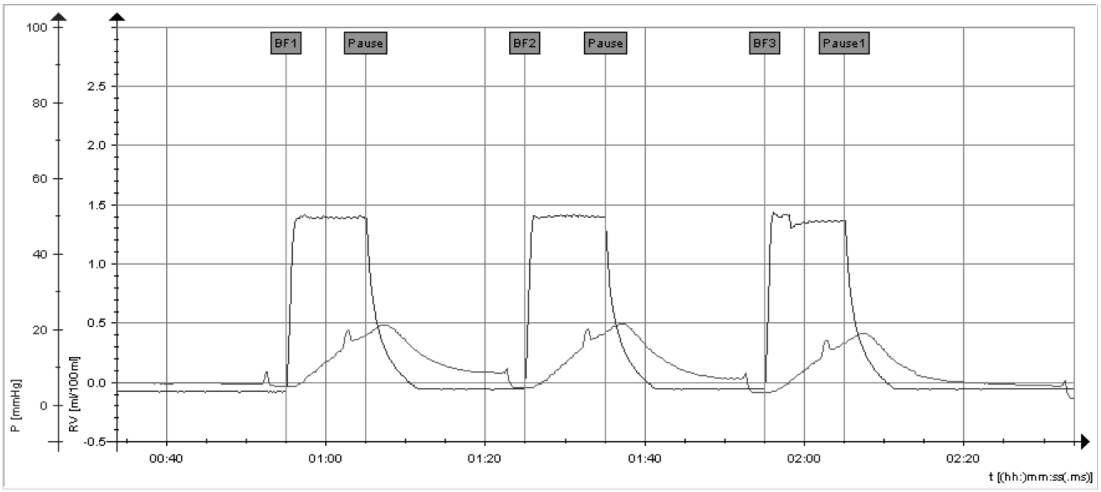
Physical activity. The activity monitor device, which was worn around the upper arm for 4 days, contains sensors for 2 plane accelerometery, near body temperature, skin temperature and the galvanic skin response. The stored output from the monitor was processed using the manufacturer's software.

Statistical analyses. The normality of the data was checked by examining the distribution of each result and checking kurtosis and skewness of the data.

RESULTS

Fig 1 shows a representative record of the raw microvascular data from one participant. The upper trace (a) depicts the responses to the three initial 50 mmHg pressure steps used for the assessment of Qa. The taller, rectilinear responses reflect the pressure steps. Below these the slower, curvilinear traces reflect the calf volume responses to the increase in pressure. The lower trace (b) shows the complete recording from this participant. On the extreme left are the 3 blood flow measurements (Qa) depicted in fig. 1a above. These are followed by the progressive rectilinear increases in pressure, representing the cumulative cuff pressure increases. We also see the pressure trace briefly increasing to 80 mmHg at the end of each cumulative pressure step, facilitating the assessment of Qa at each step. The slower, curvilinear trace reflects the calf volume response to each of these cumulative pressure steps. Fluid filtration (Jv) is measured from the slope of the last 2 minutes of the volume response. Kf is derived from the relationship between Jv and Pcuff. Pvi is derived by back-extrapolation of Kf to the abscissa, where Jv = zero [5].

Fig 1 (a)



(b)

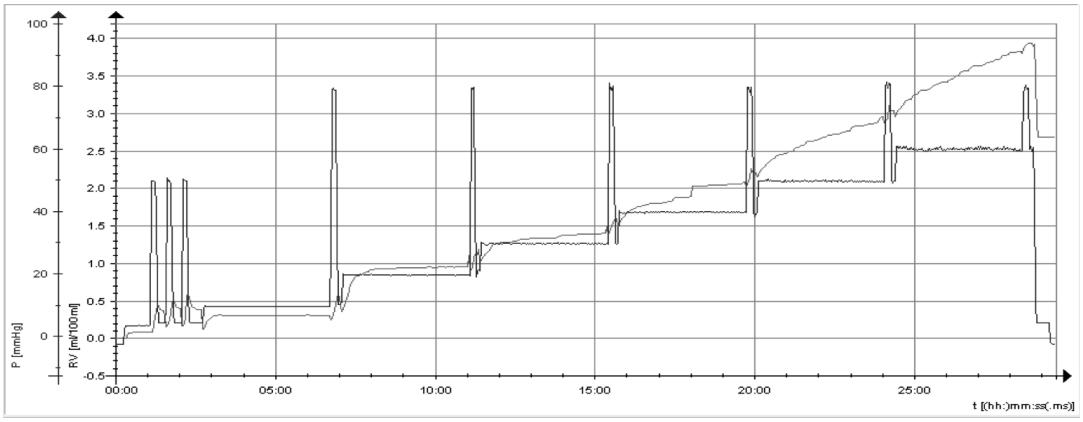


Fig 1 *Raw trace of (a) Qa measurement and (b) Kf small step plethysmography with added Qa measurement at each pressure step in lower limb using strain gauge plethysmography in a single individual. The upper trace (a) depicts the responses to the three initial 50 mmHg pressure steps used for the assessment of Qa. The taller, rectilinear responses reflect the pressure steps. Below these the slower, curvilinear traces reflect the calf volume responses to the increase in pressure. The lower trace (b) shows the complete recording from this study. On the extreme left are the 3 blood flow measurements (Qa) depicted in (a). These are followed by the progressive rectilinear increases in pressure, representing the cumulative cuff pressure increases and the brief increases in pressure to 80 mmHg at the end of each cumulative pressure step, for the assessment of Qa at each step. The slower, curvilinear trace reflects the calf volume response to each of these cumulative pressure steps.*

In healthy individuals it has been previously observed that blood flow (Qa) remained constant [6, 7] even as congestion pressures approached mean arterial pressure and significantly greater than that predicted by the Darcy equation, which they attributed to a progressive reduction in precapillary resistance due to retrograde transmission of vasodilatory signals via the endothelium. In patients with preeclampsia and marked endothelial dysfunction, blood flow was not sustained in the face of increasing venous congestion pressure. If we take the endothelial cell dysfunction of pre-eclampsia as a model [6], the difference that we see suggests that many of our study participants are unable to sustain an appropriate limb perfusion in the face of increasing venous pressure and may thus also have profound endothelial dysfunction.

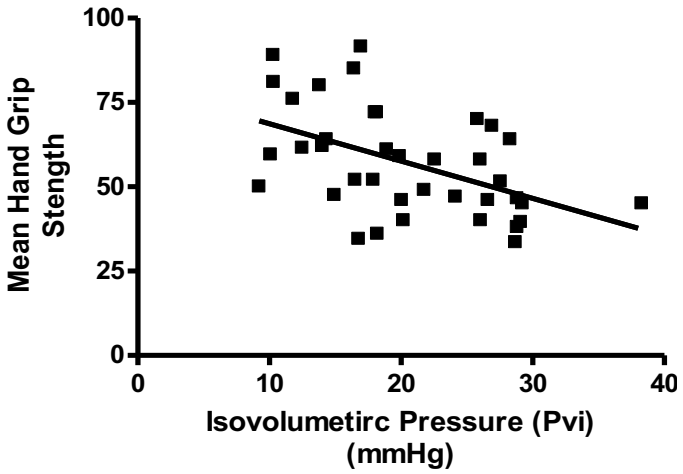


Fig 2 *Association between mean hand grip strength ([right + left] / 2) and isolumetric pressure (Pvi) from 39 participants at baseline (r=-0.55, p<0.001).*

DISCUSSION

Mean resting limb blood flow (Qa) measured using venous congestion plethysmography was higher in our patient cohort than that reported previously in young healthy [7] or in older overweight and obese individuals [8]. The lowest values of Qa were observed in the more active, fitter individuals with low blood pressure, greater insulin sensitivity and less evidence of inflammation. Whether the raised resting Qa in some of our cohort is evidence of chronic dilator state or a function of increased adiposity, and hence non nutrient blood flow to the tissue, remains to be elucidated. Resting muscle microvascular perfusion is influenced by local capacity for vasodilatation. In people with insulin resistance, it is plausible that metabolites released from excess lipid processing down-regulate the dilator pathway and up-regulate the endothelin-1 mediated vasoconstrictor pathway. This is supported by our finding that the ability to sustain blood flow to the muscle is severely compromised in our subjects. Previous studies have shown that in healthy individuals Qa remains constant at increasing congestion pressure (Pv) (see [5]) but that Qa is not maintained in individuals with generalized vascular dysfunction [2]. The authors argued that these observations were suggestive of a progressive reduction in pre-capillary resistance associated with endothelial dysfunction and an altered arteriovenous signaling mechanism. Examination of the relationship between Qa and Pv provides strong evidence for profound endothelial dysfunction in the healthy individuals with central obesity and decreased insulin sensitivity recruited into the current study. Together these data suggest that both muscle microvascular perfusion and surface area available for exchange are reduced in these individuals with consequent effects on important aspects of muscle function relating to nutrient handling.

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Chapter 5. Closing real-world translational gaps in cholesterol management

Real-world proof-of-concept study of machine learning applied to cholesterol-lowering therapy in a primary care setting.

5.1 Research summary

***Krentz AJ**, Père A, Pankova N, Jaun A.*
Optimizing cholesterol-lowering therapy for the prevention of cardiometabolic disease: an exploratory proof-of-concept neural network analysis in a UK primary care setting.
Metabolic Syndrome & Related Disorders. 2023;21:453-459.

Background: Expert consensus guidelines, e.g., those produced by NICE, aid clinicians in the appropriate use of lipid-lowering pharmacotherapy. Statin therapy is central to reducing the risk of atherosclerosis. Suboptimal lipid profiles are often compounded by other modifiable risk factors such as type 2 diabetes and hypertension (Krentz, 2023b). National audits in the UK and other countries suggest that less than half of patients at risk of atherosclerosis achieve recommended cholesterol goals (Akyea et al., 2019). The implication of this shortfall is that many patients experience potentially preventable cardiovascular events. Thus, a translational gap exists between the evidence base for lipid-lowering therapy and real-world clinical practice (Barker, 2016).

Methods: Artificial neural networks in the form of diagnostic and therapeutic decision trees were created based on lipid management guidance in place for England[76]. By design, the neural networks reproduced the guidance with >95% accuracy. Following data extraction natural language processing and therapy identification algorithms were applied to anonymised electronic records from six South London primary care practices. Patients with a recorded history of lipid lowering therapy were identified and classified as primary or secondary cardiovascular disease prevention cohorts[77]. Statin doses were classified as high, medium or intensity according to NICE criteria. Data were further analysed using neural networks trained to incorporate NICE recommendations for a novel non-statin medication, bempedoic acid. This drug, which had recently received marketing approval[78], was restricted by NICE for use in patients unable to tolerate statin therapy at any dose[79].

Results: The majority of patients (76%) were primary prevention with the remainder in the secondary prevention cohort. Major modifiable comorbidities included type 2 diabetes in 13% patients and hypertension in 32% patients; all three risk factors were present in another 28% of

[76] As Director of the Cardiometabolic Division at Metadvice and Visiting Professor at King's College London I supervised MSc and PhD students at EPFL Lausanne and KCL in the creation of the artificial neural networks.
[77] By definition, patients had a 10-year risk of cardiovascular event >10% calculating using the QRISK equation or belonged to a clinical category at high risk of atherosclerosis, e.g., chronic renal failure.
[78] Bempedoic acid received marketing authorisation valid throughout the European Union in 2020.
[79] At the time of the study the cardioprotective effects of bempedoic acid were being studied in clinical outcome trials.

patients. Among patients receiving statin monotherapy only 71% were receiving high-intensity treatment as per NICE guidance; rates of high-intensity statin use were similar for the primary and secondary prevention cohorts. The neural network recommended either increasing the statin dose or adding alternative lipid-lowering medications. Individuals were identified by the neural network as being suitable candidates for bempedoic acid therapy according to national guidance at the time.

Interpretation: These novel results support the utility of artificial intelligence data science analytics to (a) quantify suboptimal lipid-lowering prescribing in a primary care setting (b) identify high-risk individuals for whom more intensive lipid lowering is indicated and (c) recommend appropriate pharmacotherapeutic interventions among well-established and novel medications. Translating the results into clinical practice, the neural networks have been incorporated into commercial precision medicine technology that integrates with the primary care electronic medical record. The technology assists clinicians in (a) identifying patients requiring a change in therapy and (b) recommending individualised next-step therapy[80]. This proprietary clinical decision support system, which is in use in a number of primary care networks in England, helps counter therapeutic inertia while ensuring that patients receive evidence-based cholesterol-lowering therapy.

[80] www.metadvice.com.

5.2 Machine learning: emerging role in healthcare

Artificial intelligence applied to healthcare offers prospects for increased diagnostic accuracy, reduced costs, and time savings while reducing human errors (Alowais et al., 2023). Clinical and laboratory information, imaging data, biometrical data and large-scale data from patient registries, can all be processed. The resulting models can improve disease phenotyping, improved prognostication, and facilitate therapeutic decisions (Luscher et al., 2024). Accordingly, artificial intelligence has been hailed as having potential to revolutionise personalised medicine, optimise medication dosages, enhance population health management, establish clinical guidelines, provide virtual health assistants, support mental health, improve patient education, and positively influence patient-physician trust (Topol, 2019, Kresoja et al., 2023, Alowais et al., 2023).

Machine learning is a branch of artificial intelligence that, applied in the context of healthcare, can identify complex patterns in multi-modal data to facilitate disease prediction or classification (Maceachern and Forkert, 2021). By encompassing a plurality of algorithmic models and statistical methods machine learning can solve problems without specialised programming (Habehh and Gohel, 2021). Machine learning is a general-purpose artificial intelligence method that can learn without the *a priori* need for relationships within the data to be defined (Miotto et al., 2018). These characteristics make machine learning particularly well suited to extracting knowledge from large datasets.

5.3 Deep learning

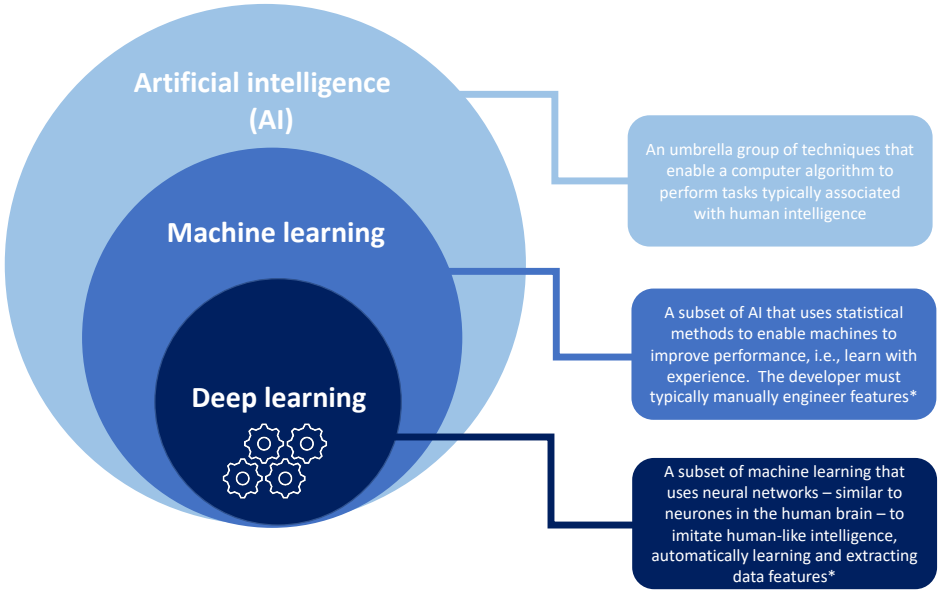
Deep learning, which can be used to integrate data sources to produce multi-modal insights, is a subfield of machine learning that utilises multi-layered artificial neural networks that require minimal human involvement (Figure 5) (Amal et al., 2022, Mackenzie et al., 2024). Deep learning is distinguished from traditional single-layer artificial neural networks by the number of hidden layers which confer additional analytic capabilities (Miotto et al., 2018)[81]. Using a web of connected nodes which emulate human neurons, deep learning is able to process data in a non-linear fashion.

To provide ground truths the neural network is first trained on a suitably pre-processed dataset. The data to be analysed enters via the input layer then passes through

[81] Creation of the first functioning perception to mimic neuronal function is credited to Frank Rosenblatt in 1958 (1921-1978).

hidden layers to the output layer. Investigators must be mindful of caveats such as the potential for bias, which may be inherent in a training dataset, as well as errors arising from underfitting and overfitting. In the former scenario, which is characterised by low bias and high variance, the neural network model has a lower number of free parameters, is more easily portable across different datasets, but yields larger unexplained differences between the model and the data. With overfitting the model fits the training data too closely with performance being degraded on test data. (Krittanawong et al., 2017). Factors including the length of training and model complexity are relevant to avoiding these issues; overfitting may result from excessive training and complex models whereas underfitting is a risk if the duration of training has been inadequate or insufficient input variables preclude determining a meaningful relationship between the input and output variables. These issues can be addressed by attention to hyperparameters of the model or modifications to the training set (Padmanabhan et al., 2021, Charilaou and Battat, 2022). Such optimisation steps help ensure that the model generalises well to new data and performs reliably.

Figure 5. Overview of artificial intelligence and its subcomponents.



* A feature refers to an individual and measurable characteristic of data and is typically numerical or categorical. Modified from Mackenzie et al, 2024.

5.4 Machine learning applied to cardiometabolic disorders

Machine learning has potential to assist in improving aspects of healthcare from disease prediction and diagnosis to practical translation of therapies into real-world clinical practice according to the principles of precision medicine. For example, colleagues at King's College London and I have demonstrated that machine learning methods can outperform traditional risk scores for prediction of cardiovascular disease (Liu, 2025). In this context, machine learning aims to identify relationships without being explicitly defined, enabling the processing of heterogeneous clinical data to identify hidden patterns. Caveats of this approach include heterogeneity of published studies, risks of bias, and limited reporting of methodological details by researchers.

Machine learning is increasingly being applied to extract inherent knowledge from complex datasets (Li et al., 2022). Detailed insights into disease phenotypes, e.g., risk of cardiovascular disease associated with obesity in the absence of traditional risk factors, creates a rationale for the development of novel biomarkers to aid disease prediction (Luo et al., 2024). The evolution of omics technologies – genomics, proteomics, and metabolomics – provides a means improve the ability of novel biomarkers to refine disease risk predictions (Tahir and Gerszten, 2020). Metabolomic data from the UK Biobank have been shown to predict the development of common cardiometabolic disorders including myocardial infarction, ischaemic stroke and type 2 diabetes[82] (Nightingale Health Biobank Collaborative, 2024). Of note, metabolomic scores were more strongly associated with disease onset than polygenic scores for most of the diseases. In a subset of participants in whom metabolomic biomarkers were measured at two time points different risk scores were associated with of disease. This suggests that repeat measurements may capture changes induced by treatment and lifestyle changes (Nightingale Health Biobank Collaborative, 2024).

A high value is placed on the need for trust in the context of clinical recommendations generated by machine learning. While artificial intelligence applied to medicine can outperform humans in certain analytical tasks concerns have been expressed about constrained levels of explainability (Amann et al., 2020). Explainable artificial intelligence (XAI) aims to improve decision systems by making predictions transparent (Westerlund et al., 2021). A model can be interpretable without being explainable, and vice versa

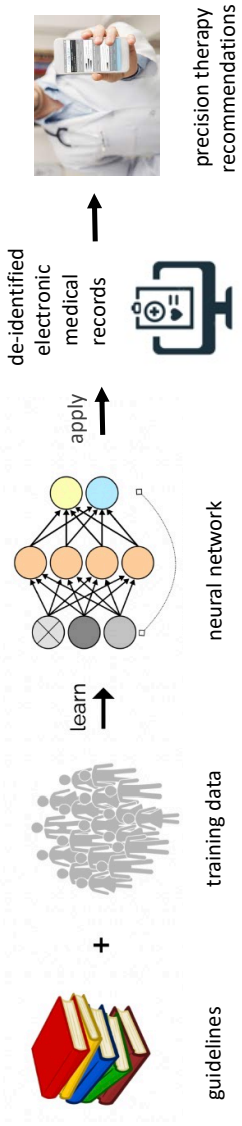
[82] Other non-communicable diseases predicted in this study included vascular and other dementia, depressive disorders, chronic obstructive pulmonary disease and certain cancers. Metabolomic data were generated by nuclear magnetic spectroscopy technology.

(Oikonomou and Khera, 2023). As discussed in Chapter 6, SHAP values (SHapley Additive exPlanations), based on game theory, provide an approach to explainable artificial intelligence based on the marginal contributions of each feature to model predictions (Oikonomou and Khera, 2023).

Figure 6 displays a scenario relevant to the original research paper presented in this Chapter wherein a neural network is first taught diagnostic criteria and treatment recommendations for cardiometabolic disorders to reproduce national clinical guidelines[83] to a predefined level of accuracy of approximately 95%. In a subsequent step, the neural network is trained on a sample of de-identified electronic medical records (eMRs) of patients extracted from primary care databases. Once trained on cardiometabolic clinical guidance, electronic healthcare data can be entered to generate precision therapy recommendations. Potential caveats of this approach should be considered. First, clinical guidelines produced by NICE have sometimes attracted criticism for recommendations that are not well-aligned with accepted clinical practice. A case in point is the release of a revised 2015 consultation on management of type 2 diabetes following pressure from healthcare professionals who objected in particular to the recommendation that a little-used insulin secretagogue, repaglinide, be offered as first-line pharmacotherapy for metformin-intolerant patients (O'Hare et al., 2015). A second problem with NICE guidance is ensuring timely updates (O'Hare et al., 2015). Here again, NICE guidance for type 2 diabetes serves as an example. A comprehensive update of the 2015 guidance of glucose-lowering pharmacotherapy was not released until 2022[84]. The update belatedly incorporated the landmark cardiovascular outcome trials of SGLT-2 inhibitors and GLP-1 receptor agonists. Even so, the update remained at variance with international counterparts not least with respect to the positioning of and indications for GLP-1 receptor agonists (Moran et al., 2022, Davies et al., 2022). The evidence reviewed did not demonstrate cost effectiveness for GLP-1 receptor agonists as a class and no change was made in their position as fourth line medications. The cardiovascular benefits of the GLP-1 receptor agonist class were not reviewed in depth for this update.

[83] www.nice.org.uk.
[84] https://www.nice.org.uk/guidance/ng28. Last updated June 2022.

Figure 6. Schematic of a neural network.



Notes: The neural network is trained on clinical guidance, with low inherent levels of bias, and representative electronic medical record datasets prior to analysing data of interest. Statistical significance testing which prevents (a) so-called machine learning hallucinations, i.e., incorrect or nonsensical outputs and (b) neural network extrapolations, and which is incorporated before recommendations are displayed to clinicians, is omitted for clarity.

Based on: Krentz AJ et al. A neural network model for the prediction of comorbid cardiometabolic diseases: proof of concept using UK primary care data. Presentation at 83rd Scientific Sessions of the American Diabetes Association, San Diego, USA, 2023. Diabetes 2023;72(suppl 1):1270P.

Other guideline committees base their recommendations primarily on evidence of efficacy and safety with less emphasis on pharmacoeconomics. Some clinical guidelines, e.g., those produced by the American Diabetes Association which are released annually, now incorporate new data as determined appropriate[85]. In response to delayed or incomplete clinical guidelines, once created and validated the accuracy and clinical relevance of neural networks is subsequently amenable to additional ad hoc training as new clinical trial data become available.

Machine learning has been used to identify gaps in translational delivery of care for cardiometabolic disorders and to offer personalised evidence-based recommendations (Krentz, 2023e). For example, in a proof-of-concept study a neural network was able to predict the development of first and subsequent cardiometabolic risk factors, i.e., hypertension in individuals with pre-existing type 2 diabetes and type 2 diabetes in patients with a prior diagnosis of hypertension (Krentz, 2023d). If confirmed in prospective studies, accurate prediction of future comorbidities could encourage a more proactive approach to cardiometabolic disease. Preferential use of specific medications might not only ameliorate current modifiable risk factors but also prevent or delay the development of predicted future co-morbidities. Of note in this context, SGLT-2 inhibitors and GLP-1 receptor agonists may prevent or induce remission of risk factors including hypertension and diabetes (Sawami et al., 2023, McGowan et al., 2024).

Digital twin technology[86] fuses physiological, environmental, and healthcare data into machine learning and generative phenotypic models to create real-time patient predictions that can model interactions with clinical environments and interventions to accelerate personalised care (Thangaraj et al., 2024).

5.5 Advantages and limitations of machine learning

In clinical research, machine-learning algorithms may offer advantages over traditional statistical regression methods for risk prediction by their ability to address multiple and correlated predictors, non-linear relationships, high-dimensionality data, and interactions between predictors and endpoints (Goldstein et al., 2017). Non-linear signal processing and machine learning guided analysis techniques have the potential to improve diagnosis and

[85] https://professional.diabetes.org/standards-of-care/living-standards-update
[86] A digital twin may be defined as a mathematical model that generates real-time data indistinguishable from its physical counterpart, system, or process.

treatment of diabetes and cardiovascular diseases (Colmenarejo, 2020, Agliata et al., 2023, Dierckx et al., 2023, Armoundas et al., 2024). For example, a recent analysis using Mendelian randomisation data from the UK Biobank indicated that body mass index, serving as a proxy for excess adiposity, may have non-linear causal relationships with cardiometabolic disease and risk factors (Mutie et al., 2023). Specifically, body mass index was associated with type 2 diabetes, hypertension, and coronary artery disease, but not chronic kidney disease or stroke. This study also reported differences between men and women with respect to the causal effect of body mass index on LDL-cholesterol with no detriment being evident in women. Moreover, although body mass index conferred an increased risk of coronary artery disease in men, it appeared not to be a strong risk factor in postmenopausal women (Mutie et al., 2023). Novel insights using machine learning are being reported concerning the association between diabetes and cardiovascular disease. In a proof-of-concept multicentre study conducted in South Korea, a deep learning model that used electrocardiographic data[87] was able to predict the future development of type 2 diabetes in a subgroup of non-diabetic adults (Kim et al., 2024).

At the level of healthcare delivery, artificial intelligence and machine learning present opportunities albeit presenting novel challenges in terms of integrating the technology into clinical practice (Haug and Drazen, 2023). Taking the example of clinical diabetes care, potential benefits of machine learning include improved patient self-management based on more effective use of self-monitoring data and clinician decision support systems that predict diabetes subtypes, the risk of long-term complications, and responses to pharmacotherapies (MacKenzie SC 2023). More generic benefits include more efficient use of time during clinical consultations that could facilitate clinician-patient collaboration with improved patient engagement in self-care (Topol, 2019). The applications of artificial intelligence in cardiovascular care have accelerated as a result of multimodal inputs and generative technology (Jain et al., 2024). Artificial intelligence is progressing rapidly in multiple domains, including healthcare (Bellini and Bignami, 2025). Large language models, a form of machine learning, is powered by sophisticated algorithms allied to immense datasets (Clusmann et al., 2023). The potential benefits of generative artificial intelligence[88] in delivering care in cardiometabolic clinical practice extend to automation of administrative tasks, supporting research, and enhancing patient self-management. Large language

[87] Primarily focused on the QRS duration of 12-lead electrocardiograms.
[88] Generative artificial intelligence differs from traditional artificial intelligence in that it can generate content, e.g., summaries of text and answers to questions, rather than just analysing existing data.

models[89] have potential to transform how medical information is processed, decisions are made, and care is delivered (Clusmann et al., 2023). These models can generate coherent and contextually relevant outputs from inputted keywords or queries (Bellini and Bignami, 2025). Taking the case use of the medical management of obesity using GLP-1 receptor agonists and related drugs it has been suggested that generative artificial intelligence, another type of machine learning, has the potential to offset the clinical and administrative demands thereby preventing overburdened health care provider workforces and health care delivery systems from becoming overwhelmed (Stevens et al., 2024). Agentic artificial intelligence is an advancement beyond classical reactive artificial intelligence. This is a new class of artificial intelligence, considered the third wave, which is designed to act with autonomy, proactively setting its own goals and taking autonomous steps to achieve them without direct human intervention (Sheeran, 2024). Agentic artificial intelligence can assist in creating personalised treatment plans for chronic diseases by analysing large amounts of patient data to predict disease progression and suggest tailored treatment plans (Sheeran, 2024). Another notable development is Edge AI, which brings immediate processing capability where time-sensitive tasks require prompt responses. This is achieved by processing data locally, i.e., on the device, reducing latency, enabling real-time decision-making and minimising data to be transmitted to central servers (Wu et al., 2024).

Two examples with relevance to cardiometabolic medicine highlight the challenges to deploying machine learning applications in routine clinical practice. First, a deep learning system for detecting diabetic retinopathy – a major cause of preventative visual loss – created by Google Health failed to meet performance expectations when tested in a real-world setting (Ruamviboonsuk et al., 2022). Despite high theoretical accuracy, the tool proved impractical in a challenging rural setting where a paucity of electronic medical records and slow internet connectivity detracted from optimal deployment of the technology (Ruamviboonsuk et al., 2022). The second example is the development of DeepMind's artificial intelligence for early prediction of acute kidney injury which failed to improve renal recovery rate or reduce admissions to intensive care relative to a control group (Connell et al., 2019).

[89] The best-known example of a large language model is Chat GPT (Generative Pre-trained Transformer). https://openai.com.

5.6. Emerging artificial intelligence capabilities

Generalist medical artificial intelligence models that can handle a wide range of medical data through self-supervision to generate outputs with advanced medical reasoning abilities (Moor et al., 2023). Challenges include ensuring high standards of privacy and data security, mitigating hallucinations, and the effective integration of these technologies into existing systems at scale (Bellini and Bignami, 2025). Practical implementation of artificial intelligence into clinical medicine requires customisation and adaptability. Artificial intelligence is required to adapt to and interact with individual patients and health-care professionals. Customisation is one of the requirements of algoethics, which handles the ethics of artificial intelligence from development to implementation (Montomoli et al., 2024).

It is recognised that clinicians may be wary of using machine learning to diagnose and manage disorders citing concerns about so-called black box models (Petch et al., 2022). This term denotes complex models that are not straightforwardly interpretable by humans (Petch et al., 2022). In response, the field of explainable machine learning is evolving rapidly. However, current explainability techniques have limitations that clinicians need to understand before applying them in a scientific or clinical setting (Petch et al., 2022). There is a lack of consensus on the definition of explainability as viewed by data science experts, regulators, and healthcare professionals (Arbelaez Ossa et al., 2022).

5.7 Machine learning to assess real-world cholesterol-lowering therapy

The original research paper presented in Chapter 5 (Krentz, 2023e) was a proof-of-concept study that employed novel machine learning methodology to identify deficiencies in prescribing of cholesterol-lowering medications in routine clinical practice Artificial neural networks, which were trained on current UK national clinical prescribing guidelines, were used to examine real-world primary care electronic health records of high-risk patients receiving cholesterol-lowering medications. The study, which was a collaboration between Metadvice, the creators of the proprietary neural networks, and the School of Life Course Sciences at King's College London, UK, was integral to the development of a clinical decision support system to support precision personalised prescribing of pharmacotherapeutics for people living with cardiometabolic disorders. In addition to identifying patients in whom cholesterol goals were not attained, the neural networks ascertained the reasons, e.g., suboptimal statin dosing, and recommended guideline-based therapy recommendations.

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Machine Learning Applied to Cholesterol-Lowering Pharmacotherapy: Proof-of-Concept in High-Risk Patients Treated in Primary Care

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Abstract

Objectives: Machine learning has potential to improve the management of lipid disorders. We explored the utility of machine learning in high-risk patients in primary care receiving cholesterol-lowering medications.
Methods: Machine learning algorithms were created based on lipid management guidelines for England [National Institute for Health and Care Excellence (NICE) CG181] to reproduce the guidance with >95% accuracy. Natural language processing and therapy identification algorithms were applied to anonymized electronic records from six South London primary care general practices to extract medication information from free text fields.
Results: Among a total of 48,226 adult patients, a subset of 5630 (mean ± standard deviation, age = 67 ± 13 years; male:female = 55:45) with a history of lipid-lowering therapy were identified. Additional major cardiometabolic comorbidities included type 2 diabetes in 13% ($n = 724$) and hypertension in 32% ($n = 1791$); all three risk factors were present in a further 28% ($n = 1552$). Of the 5630 patients, 4290 (76%) and 1349 (24%) were in primary and secondary cardiovascular disease prevention cohorts, respectively. Statin monotherapy was the most common current medication (82%, $n = 4632$). For patients receiving statin monotherapy, 71% ($n = 3269$) were on high-intensity therapy aligned with NICE guidance with rates being similar for the primary and secondary prevention cohorts. In the combined cohort, only 46% of patients who had been prescribed lipid-lowering therapy in the previous 12 months achieved the NICE treatment goal of >40% reduction in non–high-density lipoprotein cholesterol from baseline pretreatment levels. Based on the most recent data entry for patients not at goal the neural network recommended either increasing the dose of statin, adding complementary cholesterol-lowering medication, or obtaining an expert lipid opinion.
Conclusions: Machine learning can be of value in (a) quantifying suboptimal lipid-lowering prescribing patterns, (b) identifying high-risk patients who could benefit from more intensive therapy, and (c) suggesting evidence-based therapeutic options.

Keywords: machine learning, artificial intelligence, cholesterol, cardiovascular risk, statins, statin intolerance, bempedoic acid

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Introduction

CHOLESTEROL-LOWERING MEDICATIONS are extensively used to reduce the risk of atherosclerotic cardiovascular disease (ASCVD) as primary or secondary disease prevention.[1] However, real-world data demonstrate that implementation of these agents in clinical practice is often suboptimal.[2,3] In the United Kingdom >50% of patients fail to attain cholesterol goals recommended by the National Institute for Health and Care Excellence (NICE).[4] Escalation of lipid-lowering therapy is frequently not implemented.[5] Concerns about inadequate lipid-lowering and failure to achieve treatment goals is not confined to nonspecialist clinical practice. Recent national and international studies that include secondary care centers of excellence in Europe and the United States have shown that lipid-lowering pharmacotherapy for patients at high risk of ASCVD is not fully aligned with expert guidance.[6,7]

The therapeutic landscape has become more complicated in recent years. Novel cholesterol-lowering medications must be positioned within existing treatment algorithms. The limitations of statins to lower cholesterol as monotherapy and issues of tolerability have prompted the development of nonstatin cholesterol-lowering drugs.[8,9] Examples include the Niemann–Pick C1-Like 1 protein inhibitor ezetimibe[10] and the adenosine triphosphate citrate lyase inhibitor bempedoic acid.[11] In the United Kingdom, prescribing proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors have been restricted to secondary care specialist services.[12–14]

Precision or personalized medicine aims to provide best care by considering therapeutic options in the context of individual patient characteristics.[15,16] Machine learning technology offers opportunities to facilitate personalized medicine. Patients are stratified according to factors including disease subtype, risk, prognosis, and treatment response.[17,18] The limited impact of machine learning on clinical practice to date may in part reflect the challenges of interdisciplinary research to provide solutions in which clinicians have confidence.[18] Informed by the principles of precision medicine we explored the potential of machine learning to improve the management of cholesterol in a heterogeneous population of primary care patients with a high prevalence of cardiometabolic comorbidities.

Study Objectives

We sought to (a) identify adult patients at high risk of ASCVD and (b) recommend appropriate cholesterol-lowering therapy where therapeutic goals had not been attained. Our approach meshed classical field knowledge, i.e., clinical guidelines, with electronic medical records (eMRs) to provide evidence-based therapy recommendations. Additional focus was placed on the potential role of bempedoic acid, which at the time of the study was being integrated into UK clinical practice according to specific NICE guidance.

Methods

Algorithms

Neural networks were developed to reproduce the relevant NICE guidelines for lipid management guidance for England (CG 181)[12] using a simple feed-forward neural network (3 fully connected layers, 130 neurons in total) with rectified linear unit activations and a sigmoid function as the final layer. Binary cross-entropy was minimized with an equal probability for each therapy to achieve a 99.9% accuracy in predicting the right therapy (=sample accuracy) and 95.9% accuracy to both predict the right therapy and none of the alternatives (=patient accuracy). At the time of the study, the principal classes of cholesterol-lowering medications being employed by primary care clinicians were statins and ezetimibe. Inclisiran, an injectable small interfering RNA, which limits production of PCSK9, had not been introduced into UK clinical practice.[19] Nonetheless, because NICE had given provisional approval to use of inclisiran for secondary prevention of ASCVD this option was included in the neural networks alongside PCSK9 inhibitors.[20] National prescribing recommendations (TA694) for bempedoic acid were also incorporated.[21] According to prevailing NICE guidance at this time, bempedoic acid could be used as an adjunct to diet in adults only if statins were contraindicated or not tolerated and when ezetimibe alone was not adequate to attain therapeutic goals.[21] For the purposes of the study, the definition of statin intolerance applied by the neural network assumed that statins were not tolerated at any dose.

Data extraction and processing

Anonymized eMRs from six South London primary care general practices spanning 1988–2021 were extracted. Data from free text fields were analyzed using natural language processing, and therapy identification algorithms were applied to identify patients with a history of lipid-lowering medication. Patients were classified on the basis of Systematized Nomenclature of Medicine Clinical Terms (SNOMED CT) codes* into primary or secondary cardiovascular disease prevention cohorts based on the absence or presence of coded cardiovascular disease, respectively.[22] ASCVD was defined as coronary heart disease, stroke, and peripheral artery disease. Primary prevention patients either had a calculated 10-year risk of a cardiovascular event >10% or belonged to high-risk clinical categories for which lipid-lowering therapy is indicated by NICE.[12] Major comorbidities that often contribute to risk of ASCVD, that is, type 2 diabetes and hypertension,[7] were identified from SNOMED codes as were familial hypercholesterolemia and a coded history of statin intolerance.

Efficacy of cholesterol-lowering therapy

A reduction in non–high-density lipoprotein (HDL) cholesterol >40% from pretreatment level was considered the primary marker of therapeutic efficacy, in accordance with NICE guidance.[12] Cholesterol-lowering medication was subdivided into low, medium, and high-intensity statins, that is, specific doses for pravastatin, simvastatin, atorvastatin, and rosuvastatin.[23] Current cholesterol-lowering therapy was defined as active prescriptions within a 30-day range. Therapy recommendations were generated in accordance with current NICE guidance (Table 1).

*https://digital.nhs.uk/services/terminology-and-classifications/snomed-ct

Table 1. Recommendations Generated by the Neural Network

Main recommendations:
- Increase statin dose according to NICE guidance
- Add injectable therapy, *i.e.,* PCSK9 inhibitors or inclisiran
- Add ezetimibe

The remaining recommendations correspond to different scenarios:
- No therapy: patients who have never received cholesterol-lowering therapy and for whom no therapy is indicated, *i.e.,* primary prevention, calculated QRISK 10-year probability of ASCVD event <10%
- Start atorvastatin 20 milligrams daily: patients who have never received cholesterol-lowering medication and for whom high-intensity statin therapy is indicated for primary ASCVD prevention, *i.e.,* QRISK >10% or high-risk clinical category, *e.g.,* chronic kidney disease
- Start atorvastatin 80 milligrams daily: patients who have not received cholesterol-lowering therapy for whom high-intensity statin therapy for secondary ASCVD prevention is recommended by NICE
- Continue current treatment: patients who are currently receiving cholesterol-lowering medication for whom no change of cholesterol-lowering therapy is indicated, *i.e.,* cholesterol target attained
- Refer to specialist lipid service for expert advice: patients who are currently receiving cholesterol-lowering therapy for whom (a) a change of therapy is required since cholesterol target has not been attained and (b) no remaining standard therapeutic options are available according to NICE guidance
- Add bempedoic acid according to NICE TA694 guidance

ASCVD, atherosclerotic cardiovascular disease; NICE, National Institute for Health and Care Excellence; PCSK9, proprotein convertase subtilisin/kexin type 9.

Clinical safety validation

Safety considerations, for example, contraindications and major drug interactions, were included as integral elements of the neural network.[10,24,25] To ensure that recommendations made by the neural network were aligned with safe prescribing, two experienced clinicians independently performed a detailed review of the neural network recommendations for a randomly selected sample of patient records. The clinical safety validation exercise spanned patients across a range of ages at different stages in the natural history of cardiometabolic comorbidities. The duplicate results were cross-checked to reach a consensus between the clinicians for each record. Minor adjustments to aspects of data extraction and data processing by the neural network were subsequently implemented where indicated.

Data governance

All patient data were anonymized. Only data from living patients who had previously consented to share their data anonymously for research purposes were included in the analysis.

Results

Study population

The total general practice population studied was 48,226 patients. A subset of 5630 patients with a history of treatment with cholesterol-lowering medications was identified. Of these, 76% ($n=4290$) and 24% ($n=1340$) were in the primary and secondary cardiovascular disease prevention cohorts, respectively (Fig. 1). The mean ± standard deviation age of the 5630 patients was 67 ± 13 years with a male:female ratio of 55:45. Cholesterol levels requiring pharmacotherapy as an isolated risk factor was present in 1540 (27%) patients. Additional major modifiable comorbidities included type 2 diabetes in 13% ($n=724$) patients and hypertension in 32% ($n=1791$) patients; all three risk factors were present in a further 28% ($n=1552$) patients. A diagnosis of familial hypercholesterolemia was coded in 52 (1%) patients. A history of statin intolerance was coded in 230 (4%) patients; of these, 182 were in the primary prevention and 48 in the secondary prevention cohorts, respectively.

Cholesterol-lowering therapy

Prescribing cholesterol-lowering medications gradually increased over time from a low level in the early 1990s to reach a plateau from 2010 onward (data not shown). National prescribing data allied to our own observations from UK primary care settings show that use of more potent second- and third-generation statins—especially atorvastatin—increased between 2010 and 2020.[26] Statin monotherapy was the most common recorded medication at the most recent data entry point (82%, $n=4632$) with no difference in rates for the primary and secondary prevention cohorts at 82% ($n=3500$) and 84% ($n=1132$), respectively. Of the patients treated with lipid-lowering medications the majority (47%, $n=2655$) had been prescribed intensive statin therapy as initial pharmacotherapy; therapy had been escalated in a further 29% ($n=1614$) patients from a moderate intensity to a high-intensity statin at the time of the analysis.

Among statin-treated patients, 71% ($n=3269$) statin monotherapy was high-intensity treatment as per NICE guidance, that is, defined as statins at doses expected to reduce low-density lipoprotein (LDL) cholesterol by >40%.[12] Rates of high-intensity statin use were similar for the primary and secondary prevention cohorts at 70% (2433) and 74% (836), respectively. No current lipid-lowering medication was recorded for the most recent data point in 17% patients in the primary prevention cohort and 11%

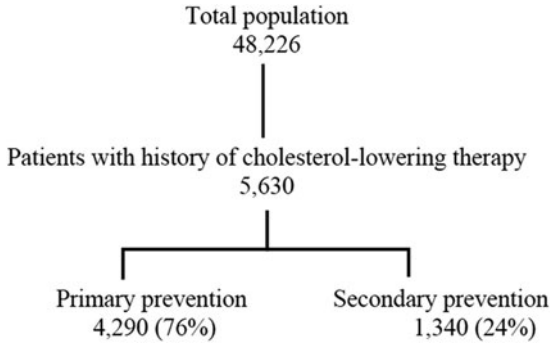

FIG. 1. Study population.

secondary prevention cohort. Ezetimibe was used either in combination with a statin ($n=184$) or as monotherapy ($n=73$) with no differences between the primary and secondary prevention cohorts. Among the 184 patients treated with combination statin+ezetimibe the intensity of statin was classed as high in 43 patients, moderate in 12 patients, and low in 129 patients. PCSK9 inhibitor therapy, specifically evolocumab, was identified in a single patient.

Therapeutic goal attainment

Among patients receiving cholesterol-lowering medications within the preceding 12 months 46% failed to achieve the recommended NICE goal of >40% reduction from baseline in non–HDL cholesterol levels. The distribution of goal attainment—as judged by percentage reduction in non–HDL cholesterol from pretreatment levels—was similar for the primary and secondary prevention cohorts (data not shown). This observation held when LDL cholesterol was substituted for non–HDL cholesterol.

Therapy recommendations

Based on the most recent data entry point current cholesterol-lowering therapy was endorsed by the neural network in 40% patients (Fig. 2). The neural network recommended increasing statin dose in 46% patients, adding ezetimibe in 3%, adding bempedoic acid in 1%, or escalating to a PCSK9 inhibitor in 1%. Referral to a specialist lipid clinic for expert clinical advice was recommended in 9% patients where NICE guidance did not provide clear recommendations. As per NICE TA694, that is, interpreting a diagnosis of statin intolerance as indicating no statin therapy at any dose, the neural network identified a total of 78 patients as potential candidates for bempedoic acid at some point during their therapeutic history; of these, 55 were in the primary prevention cohort and 23 in the secondary prevention cohort.

Discussion

This proof-of-concept study demonstrates the utility of a neural network to identify patients requiring therapy escalation and to recommend appropriate intensified pharmacotherapy to attain treatment goals in alignment with national management guidelines. We believe our results support the notion that machine learning can help facilitate the aims of precision medicine in managing cholesterol-lowering medication in high-risk patients.

The study demonstrates the value of machine learning to quantify misalignments between national clinical guidance and real-world prescribing of cholesterol-lowering medications. Statins were the most commonly used lipid-modifying pharmacotherapy in our study, as recommended by the guidelines. However, the intensity of statin therapy was suboptimal in approximately one-third patients. Greater use of high-intensity statins was advocated in the 2014 update of NICE guidance; this concept had become well established in UK clinical practice by the time of our study.[12] Failure to attain cholesterol goals in the era of high-intensity cholesterol-lowering therapy has been reported in other surveys of the UK primary care prescribing.[4,23] The similarity of the degree of non–HDL cholesterol lowering for primary and secondary prevention suggests similar therapeutic decisions and/or adherence to cholesterol-lowering therapy for both cohorts.

The algorithms uncovered not only widespread statin underdosing but also evidence of inadequate monitoring of therapeutic response (data not shown). According to NICE guidance operative at the time of the study, low- or medium-intensity statin therapy was restricted to individuals with statin intolerance, patient preference, or drug interactions.[12,27] We suspect that some cases of statin intolerance may not have been recorded in the eMR.[28] Statin intolerance is associated with suboptimal lipid-lowering and hence higher risks of ASCVD.[29,30] We consider that the 4% prevalence of recorded statin intolerance in our study may be an underestimate since under-reporting of statin intolerance has been documented in the United Kingdom and elsewhere. Some studies have estimated rates of ~10%–15%.[31] A recent international meta-analysis of randomized controlled trials (RCTs) and real-world data involving >4 million patients found an overall global prevalence of statin intolerance of 9.1%.[32] The prevalence of statin intolerance when diagnosed using defined clinical and laboratory criteria was higher in real-world cohorts than among participants in RCTs. While the Cholesterol Treatment Trialists' Collaboration concluded that statin therapy causes a small excess of mostly mild muscle pain, the clinical challenge of statin intolerance or reluctance remains a barrier to cholesterol goal attainment.[33]

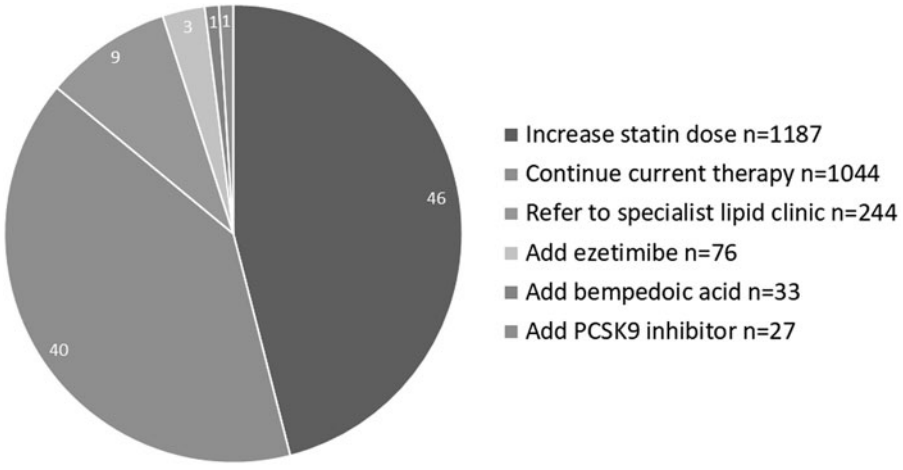

FIG. 2. Therapeutic recommendations delivered by the neural network for 2611 patients (%, *n*) receiving cholesterol-lowering medication based on the last available data point.

Patients receiving suboptimal pharmacotherapy are potential candidates for additional or alternative lipid-modifying therapy.[34,35] The algorithms trained on NICE guidance endorsed current cholesterol-lowering therapy in 40% of the patients. For the remaining patients, an increase in statin dose was the most frequent recommendation in nearly half of the patients requiring more intensive therapy. In parentheses, our neural networks have the capability to quantify the gap between the non–HDL cholesterol and therapeutic goal at the level of individual patients (data not shown). Such additional information could help prioritize care by focusing on patients who are furthest from target and hence who might benefit most from a change in cholesterol-lowering therapy. Moreover, the neural networks can identify and quantify therapeutic inertia in prescribing cholesterol-lowering medications (data not shown). We are developing a complementary ambient machine-learning solution that integrates with primary care eMR systems to support personalized medicine in the context of complex comorbid cardiometabolic diseases using our neural networks.

The addition of nonstatin medications, injectable therapies or referral to specialist lipid clinics in secondary care was proposed by the neural networks for some patients. Clinical guidelines for treating patients at high cardiovascular risk generally advocate maximally tolerated doses of statins and other drugs to achieve recommended lipid levels or relative reductions of LDL cholesterol or non–HDL cholesterol.[36] In accordance with NICE guidance that was in place at the time of the study, nonstatin oral therapies, ezetimibe and bempedoic acid, were recommended by the algorithms in 3% and 1% patients, respectively. Ezetimibe can be used in combination with statins or as monotherapy. Support for the efficacy of ezetimibe on ASCVD comes from clinical trial data showing reductions in major adverse cardiovascular events in high-risk groups.[10,37] Our results imply that ezetimibe was not being used in accordance with NICE guidance in all eligible patients, that is, added to statin if a 40% reduction in non-HDL cholesterol is not achieved. Bempedoic acid is an effective cholesterol-lowering drug with a novel mechanism of action.[38] Dose–response models predict that combining bempedoic acid with the lowest dose of commonly used statins would achieve a similar degree of LDL cholesterol lowering as quadrupling the statin dose.[39] The prescribing of bempedoic acid in England was subject to restrictions at the time of our study pending the results of cardiovascular outcome trials.[40] According to TA694 guidance, use of bempedoic acid was permissible only if statins were contraindicated or not tolerated and when ezetimibe alone did not adequately control LDL cholesterol.[21] Thus, under the most stringent interpretation of TA694 bempedoic acid either as monotherapy or in combination with statins at any dose was not sanctioned by NICE. For the purposes of our study the neural networks were aligned with this interpretation of the guidance. That said, it should be noted that at the time of our analysis there was a degree of uncertainty among lipid specialists whether the TA694 guidance could potentially be interpreted more liberally to include patients on suboptimal doses of statins.[†] The US National Lipid Association has defined complete statin intolerance as inability to take any lipid-lowering dose, whereas partial intolerance denotes maximal tolerated doses that fail to achieve therapeutic objectives.[34] A less restrictive definition of statin intolerance would have been expected to increase the number of potential candidates for bempedoic acid in our study because patients with partial statin intolerance would also have become eligible. The impact of changes to prescribing guidance can be readily assessed using the neural network.

The identification of a solitary patient being treated with a PCSK9 inhibitor reflects the generally low level of prescribing for these injectable cholesterol-lowering drugs in England. The constraint that prescribing of PCSK9 inhibitors was confined to secondary care cardiology and lipid clinics may be relevant to this observation.[36]

Strengths of our study include a low probability of prescribing bias reflecting NICE guidance and the highly structured primary care eMR system in England. However, caveats and limitations should be considered. First, owing to the UK data governance restrictions no information was available on deceased patients. This limitation applies to mortality from any cause and was not confined to deaths from cardiovascular disease. Whether patients who died from cardiovascular disease had clinical characteristics, for example, greater levels of comorbidity or responses to cholesterol-lowering therapy that were different to the participants, cannot be determined from our analysis. We aim to address these issues in future studies. Second, although our study was principally concerned with common forms of polygenic cholesterol disorders, it should be noted that recorded prevalence of familial hypercholesterolemia was low. In the absence of universally agreed diagnostic criteria underdiagnosis of familial hypercholesterolemia is well recognized in the United Kingdom and elsewhere.[41] Third, missing eMR data reduced the accuracy of some of aspects of the analyses.

To summarize, our results provide support for the utility of machine learning data-science analytics to (a) identify suboptimal cholesterol-lowering prescribing, (b) identify high-risk patients who might benefit from more intensive therapy, and (c) recommend evidence-based next-step therapeutic options aligned with national guidance. Our results are consistent with recent audits that have identified underuse of combination cholesterol-lowering therapies.[42] Our study also demonstrates the utility of machine learning to model specific prescribing scenarios.

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Authors' Contributions

A.J.K.: Conceptualization, methodology, validation, analysis, writing, visualization, supervision, funding acquisition.

[†]https://www.england.nhs.uk/aac/wp-content/uploads/sites/50/2020/08/Statin-intolerance-pathway-January-2022.pdf

G.H.-H.: Methodology, analysis, writing. X.Z.: Methodology, analysis, writing, visualization. N.P.: Analysis, writing, resources, data curation. A.J.: Conceptualization, methodology, validation, analysis, data curation, writing, resources, visualization, supervision.

Author Disclosure Statement

The authors report no other competing interests in this work.

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Chapter 6. Summary and conclusions

Prospects for precision cardiometabolic medicine; potential for bidirectionality of the translational medicine pathway.

6.1 Overview of the published papers

The papers presented in this dissertation illustrate associations between prevalent metabolic and cardiovascular disorders. They also serve to highlight the need for appropriate study designs, careful consideration of inclusion and exclusion criteria, and the used of clinical investigative methods that are sensitive, precise and of relevance to human physiology[90]. The papers bridge across academia and life sciences industries to demonstrate practical and complementary bidirectional relationships.

The studies examined a range of prevalent non-communicable cardiometabolic disorders. As discussed in Chapter 1, these share common aetiopathogenic features and often cluster together in complex multimorbid constellations. Interactions between polygenic predisposition and environmental and behavioural factors result in phenotypic expression of a range of highly prevalent diseases including the metabolic syndrome, polycystic ovary syndrome, and type 2 diabetes. Comorbid existence of two or more cardiometabolic disorders in affected individuals is increasingly common and is associated with additional increased risks of mortality (Canoy et al., 2021, Khunti et al., 2023). As noted in Chapter 1, excess adiposity and insulin resistance are considered central features of these disorders which may be considered within a nexus or continuum of risk factors which increase the risks of developing atherosclerotic cardiovascular disease (Krentz, 2023b). Interactions are often evident between carbohydrate and lipid metabolism and between microvascular dysfunction and macrovascular disease (DeFronzo, 2010, Stehouwer, 2018, Meir et al., 2024).

Chapter 2 provides an example of pathophysiological intersections in which metabolic and cardiovascular disease are entwined in the polycystic ovary syndrome, requiring careful selection of study participants through detailed phenotyping. Moving from epidemiology to clinical trials, Chapters 3 and 4 illustrate the utility of glucose clamp methodology in studies of cardiometabolic disorders. Chapters 3 and 4 are also reminders that pharmacotherapeutics developed to treat cardiometabolic disorders may sometimes generate unwanted metabolic effects. Such caveats may be predictable and testable, as in the case of the novel glucokinase activator studied in Chapter 3 which carried a known risk of hypoglycaemia (Matschinsky and Porte, 2010). Others, such as statin-associated new-onset diabetes, may not be foreseen, becoming apparent only in the post-marketing period (Preiss and Sattar, 2011). However, even when a class of medication is well established in clinical

[90] Outlined in Krentz AJ. MD research thesis, University of Birmingham, UK. 1991.

practice gaps between the evidence base and real-world prescribing may lead to shortfalls in benefits. Here again, statins provide a notable example (Toth et al., 2018, Cheeley et al., 2022). A major barrier to statin use is patient perception of clinical side-effects, principally in the guise of muscle symptoms (Banach et al., 2015). However, carefully designed and executed clinical research has demonstrated that symptoms are often attributable to a nocebo response (Howard et al., 2021). A more personalised approach to cholesterol-lowering pharmacotherapy that maximises clinical benefits while minimizing tolerability issues is increasingly being facilitated by the introduction of novel non-statin drugs (Averna and Cefalu, 2023). Non-statin oral and injectable cholesterol-lowering agents offer the prospect of avoiding or attenuating the diabetogenic effect of statins in vulnerable individuals (Pirillo and Catapano, 2022, Carugo et al., 2022). In Chapter 5 machine learning applied to anonymised data from primary care electronic medical records was able to identify patients prescribed cholesterol-lowering drugs who failed to achieve recommended treatment goals. The neural network then offered actionable therapeutic recommendations for patients requiring intensification of therapy.

It is apposite to consider the studies presented in this thesis in terms of their strengths and limitations and to appraise the results in the context of more recent research. It is also appropriate to reflect on the deficiencies in knowledge that persist and how these might be addressed in future research. To complete the cycle from community to bench the implications of the studies in the later stages of the translational pathway are discussed in more detail.

6.2 Polycystic ovary syndrome in postmenopausal women

As outlined in Chapter 2, polycystic ovary syndrome is the most common endocrine disorder in women of reproductive age. Health and quality of life may be adversely impacted across the life course in affected women, including post-menopausal years (Hirschberg, 2023a). Recently published Mendelian randomisation studies have suggested that factors including obesity, androgens, fasting serum insulin, and sex hormone-binding globulin may play a causal role in polycystic ovary syndrome (Zhu and Goodarzi, 2022). However, there remains a paucity of longitudinal studies conducted in older women in whom the syndrome was diagnosed during their reproductive years. Chapter 2 demonstrated how defining and validating a putative polycystic ovary syndrome phenotype in postmenopausal women provided insights into the long-term consequences of the syndrome (Krentz et al., 2007b). Insulin resistance was assessed using the HOMA method which, based on fasting glucose and insulin concentrations, is well suited to epidemiological studies but focuses on basal glucose metabolism which

primarily reflects hepatic insulin sensitivity (Wallace et al., 2004). The study was conceived in the light of accumulating evidence of cardiometabolic health considerations among women across the life course (Lobo et al., 2014, Gianos et al., 2021, D'ignazio et al., 2023). In the Rancho Bernardo dataset, the prevalence of atherosclerotic cardiovascular disease increased with the number of components that defined the postmenopausal polycystic ovary syndrome phenotype (Krentz et al., 2007b). Circulating levels of adiponectin and leptin were linearly associated with the components of the putative phenotype, positively for leptin and inversely for adiponectin. The adipocytokine profiles mirrored those observed in premenopausal women with classic polycystic ovary syndrome (Krentz et al., 2012b). The observation that adipocytokine profiles in postmenopausal with the putative polycystic ovarian phenotype mirror those reported in younger women provides support for the validity of the model. The association between leptin, but not adiponectin, and the putative postmenopausal polycystic ovary syndrome phenotype was eliminated by adjustment for waist circumference (Krentz et al., 2012b). Of note, leptin levels in women of reproductive age with polycystic ovary syndrome increase in proportion to the degree of obesity (Caro, 1997). In contrast, lower adiponectin levels in women with polycystic ovary syndrome are reportedly independent of weight and/or body mass index (Carmina et al., 2005, Ardawi and Rouzi, 2005, Aroda et al., 2008, Mirza et al., 2014, Prasad et al., 2023, O'Connor et al., 2010, Baldani et al., 2019). Women in the Rancho Bernardo cohort with the putative polycystic ovary syndrome had a mean ± standard deviation body mass index of 28.0 ± 4.8 kg/m^2 [91]. A meta-analysis that included thirty studies of nonobese women with polycystic ovary syndrome found significantly lower levels of adiponectin compared to controls (Lin et al., 2020).

Since low adiponectin levels are associated with reduced whole-body insulin sensitivity the adipocytokine may be a marker of insulin resistance in polycystic ovary syndrome even in the absence of obesity (Groth, 2010). The literature is not unanimous on this point; some studies have reported that adiponectin is inversely associated with body mass index in women with polycystic ovary syndrome (Chen et al., 2015, Polak et al., 2017, Cardoso et al., 2020). These discrepancies may reflect different study designs and diagnostic criteria for disease phenotypes. Another caveat lies in the measurement of total adiponectin, rather than the high molecular weight form, in most of these studies. As discussed in Chapter 2, it is hypothesised that high molecular weight adiponectin may be more relevant to cardiometabolic pathophysiology (Groth, 2010, Shirazi et al., 2021). Since

[91] Overweight is defined by the World Health Organization as a body mass index ranging from 25.0 to 29.9 kg/m^2. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.

adiponectin, particularly high molecular weight adiponectin, are negatively correlated with polycystic ovary syndrome and hyperandrogenism it has been suggested that raising adiponectin levels could have beneficial effects on insulin resistance as well as the regulation of hypothalamic gonadotrophin releasing hormone secretion, ovarian steroidogenesis, and dysregulated ovulation (Schuler-Toprak et al., 2022). The relationship of adiponectin with androgen levels suggests a unique relationship in polycystic ovary syndrome and it has been suggested that adiponectin could serve as a biomarker for the disorder (Groth, 2010). A lower adiponectin to leptin ratio, in concert with excess adiposity, has been observed in females who develop polycystic ovary syndrome during adolescence (Whooten et al., 2024). Translation of evidence of a protective effect of adiponectin in animal models of polycystic ovary syndrome into clinical practice might permit intervention with medications to raise adiponectin levels (Singh et al., 2017, Benrick et al., 2017). However, the observation in general populations that high levels of adiponectin are associated with higher cardiovascular and all-cause mortality suggests the need for caution (Menzaghi and Trischitta, 2018). No clear mechanistic explanation has been identified for these unexpected findings which seem to contradict the anti-atherogenic and insulin-sensitising actions of adiponectin (as discussed in Chapter 2). Confounding by the direct correlation that exists between adiponectin and natriuretic peptides, which are established predictors of mortality, has been proposed (Tsukamoto et al., 2009). Adiponectin is a novel target in adiposity-associated cardiometabolic disorders (Nauck et al., 2021b). It is unclear whether putative resistance to adiponectin may be of clinical relevance to adiponectin-directed therapeutic strategies in cardiometabolic disorders (Nauck et al., 2021b, Zhao et al., 2021).

The most promising new pharmacotherapeutic agents to emerge for polycystic ovary syndrome during the last decade are the GLP-1 receptor agonists (Szczesnowicz et al., 2023). These agents offer an opportunity to simultaneously address comorbidities and phenotypic features of polycystic ovary syndrome (Siamashvili and Davis, 2021). Current evident indicates that in obese women with polycystic ovary syndrome GLP-1 receptor agonists reduce body mass index, circulating triglycerides, waist circumference, and total testosterone levels (Austregesilo De Athayde De Hollanda Morais et al., 2024). Increased levels of sex-hormone binding globulin and adiponectin levels with increased ovulation/menstrual frequency and pregnancy rates during *in vitro* fertilisation have also been reported (Bril et al., 2023). It is hypothesised that the metabolic benefits of GLP-1 receptor agonists may be mediated in part by reductions in circulating leptin and increases in adiponectin levels (Frossing et al., 2018, Yaribeygi et al., 2021). Weight loss before pregnancy may restore ovulation and improve

maternal and fetal outcomes. However, GLP-1 receptor agonists are considered class C for use in pregnancy and are not recommended. Manufacturers of semaglutide recommend abstaining from the medication for eight weeks before pregnancy because of observed teratogenicity in animal studies (Goldberg and Boots, 2024)[92]. The 2023 international evidence-based guideline suggests that anti-obesity agents, including GLP-1 receptor agonists, may be considered for women with polycystic ovary syndrome according to guidance applicable to general populations (Teede et al., 2023). Long-term data on GLP-1 receptor agonists for obese women with polycystic ovary syndrome are not yet available (Goldberg and Boots, 2024). The potential for broader cardiometabolic effects of GLP-1 receptor agonists, i.e., improved whole body insulin sensitivity, reduced risk of atherosclerotic cardiovascular events, and improved hepatic steatosis also merit further study in affected women (Siamashvili and Davis, 2021).

SGLT-2 inhibitors also have beneficial effects in polycystic ovary syndrome with reductions in body mass index, waist and hip circumference and fat mass (Lempesis et al., 2023). Circulating insulin and androgens are also reduced along with blood pressure (Lempesis et al., 2023). As in the case of GLP-1 receptor agonists, some of the benefits of SGLT-2 inhibitors observed in insulin-resistant disorders may also be attributable to reduced leptin levels and increased adiponectin levels (Bonnet and Scheen, 2018). Adequately powered trials focused on relevant clinical and biochemical outcomes are needed to establish whether GLP-1 receptor agonists and SGLT-2 inhibitors may have a more prominent future role in the clinical management of polycystic ovary syndrome (Dong and Rees, 2023).

In the Rancho Bernardo Study, latent associations were observed between adipocytokines and a range of cardiovascular risk factors using factor analysis (Krentz et al., 2009b). The dose-response associations between the adipocytokines and the clinical, metabolic, and hormonal components of the putative postmenopausal polycystic ovary syndrome provide support for the validity of the phenotypic model. Additionally, parallels between the model and premenopausal polycystic ovary syndrome were evident in another aspect of nonclassic endocrine pathophysiology relevant to cardiometabolic risk (Pradhan et al., 2013). Specifically, low levels of the multi-faceted orexigenic hormone ghrelin were observed with an inverse linear dose-effect association with components of the phenotype (Krentz and Barrett-Connor, 2009). Ghrelin levels are decreased in premenopausal women with polycystic

[92] Because of the potential for impaired gastrointestinal absorption of oral contraceptives, women starting GLP-1 receptor agonists are advised to use alternative methods for 4 weeks while starting the medicine and for 4 weeks after each dose increase.

ovary syndrome and correlate with the degree of insulin resistance (Schofl et al., 2002). Limited data also indicate that low ghrelin levels are associated with the metabolic syndrome among middle-aged adults in both sexes (Ukkola et al., 2006).

Strengths of the research included, first, the detailed and comprehensive clinical, biochemical, and hormonal data collated over decades in the longitudinal Rancho Bernardo Study of Healthy Aging; second, the exclusion of estrogen therapy use by participants; and third, the exclusion of women with a history of oophorectomy. Subsequent observations in postmenopausal women from the Rancho Bernardo investigators at the University of California San Diego have confirmed that the ovary continues to be a major source of androgens throughout the lifespan of older women (Laughlin et al., 2000). Recent cross-sectional and longitudinal studies have demonstrated persistence of hyperandrogenism and associated abnormalities and cardiometabolic risk factors in post-menopausal women with polycystic ovary syndrome (Puurunen et al., 2011, Schmidt et al., 2011). Limitations include the continuing absence of a universal definition of polycystic ovary syndrome and the predominantly white Caucasian and generally non-obese participants in the Rancho Bernardo study. Hepatic steatosis, which is now recognised as a component of the cardiometabolic profile of polycystic ovary syndrome, was not included the model (Shahbaz et al., 2022). Data on ovarian morphology was not available in the Rancho Bernardo postmenopausal cohort. Of note, a diagnosis of polycystic ovary had not necessarily been made in affected women during their reproductive years. Indeed, estimates suggest that a high proportion of women are not aware that they have the syndrome or have a delayed diagnosis (Ding et al., 2016). As discussed in Chapter 2, anatomical polycystic ovary morphology is not required, nor does polycystic ovary morphology alone establish the diagnosis since this finding is present in 30-50% of normo-androgenic, ovulatory women (Christ and Cedars, 2023).

6.3 Cardiometabolic assessment of an investigational glucose-lowering drug

Since atherosclerosis is the leading cause of death among patients with type 2 diabetes it is imperative that glucose-lowering therapies do not add to the burden (Krentz, 2005a). Hitherto, no class of glucose-lowering medication was able to reduce the toll from cardiovascular disease. On the contrary, some widely used diabetes medications, notably sulfonylureas and the insulin sensitising agent rosiglitazone were tainted with the possibility that they might paradoxically increase the risk of myocardial infarction (Krentz, 2002b, Krentz, 2011). Within-class heterogeneity of cardiovascular risk-to-benefit considerations between specific drugs

added further layers of nuance and uncertainty (Riveline et al., 2003, Chiquette et al., 2004). Large-scale clinical trials testing the hypothesis that intensive glycaemic control *per se* would reduce the incidence of atherosclerosis failed to provide clarity (Kelly et al., 2009). Indeed, concerns were generated that combining glucose-lowering agents from different classes could increase all-cause mortality in high-risk patients (Gerstein et al., 2008). While iatrogenic hypoglycaemia was the prime suspect of elevated mortality the mechanisms of harm remain elusive (Genuth and Ismail-Beigi, 2012). Hypoglycaemia activates the sympatho-adrenal system potentially introducing haemodynamic instability and dangerous cardiac arrhythmias (Sanon et al., 2014).

Newer classes of glucose-lowering drugs with low intrinsic risk of hypoglycaemia include the dipeptidyl peptidase (DPP)-4 inhibitors, the GLP-1 receptor agonists, and the SGLT-2 inhibitors. In recent years these classes of pharmacotherapeutics have radically changed the therapeutic approach to managing type 2 diabetes with less use of sulfonylureas (Scheen, 2021). Beyond the expectations of the clinical research community, potent clinical benefits of these agents in the GLP-1 receptor agonist and SGLT-2 inhibitor classes became evident from clinical trials in which cardiovascular safety was the principal consideration. As discussed in Chapter 1, in response to the controversy concerning reported adverse cardiovascular outcomes with rosiglitazone the Food & Drug Administration mandated large-scale cardiovascular outcome trials for all new non-insulin glucose-lowering drugs (Krentz et al., 2012a). A revelation of these trials was the demonstration not only of safety – defined as prespecified non-inferiority to placebo – but of cardioprotection and renoprotection (Krentz, 2016, Jacob et al., 2021). Of note, these cardiometabolic benefits are not explained by effects on glucose control or other major risk factors for atherosclerosis (Zelniker et al., 2019). Prior to testing in large-scale trials, which are generally conducted during phase 3 of drug development, any new glucose-lowering agent must negotiate phase 1 and phase 2 studies. Chapter 3 details a single centre, randomised, open, two-way crossover phase 1 safety evaluation of a novel glucose-lowering drug using state-of-the-art precision methodology (T2). The hypothesis tested was the potential for AZD1656, a small molecule glucokinase activator, to impede recovery from hypoglycaemia in patients with type 2 diabetes. Glucokinase is expressed in the islet ß-cells where it is regarded as the glucose sensor for the precision regulation of insulin secretion (Matschinsky and Wilson, 2019, Gersing et al., 2025). In human studies, glucokinase activators effectively lower blood glucose but carry a risk of inducing hypoglycaemia (Hale et al., 2015, Nakamura and Terauchi, 2015). Glucokinase is also expressed in the liver (Matschinsky and Porte, 2010). Exogenous glucagon, conventionally administered by intramuscular injection,

has long served as a crucial rescue therapy for acute severe iatrogenic hypoglycaemia (Porcellati et al., 2021). Glucagon rapidly mobilises glucose from hepatic glycogen stores. Since glucokinase plays a role in regulating the glycogen content of the liver AZD1656 carried a theoretical risk of impairing the glucose-mobilizing action of glucagon (Krentz et al., 2014).

Several aspects of this study merit discussion. The two-fold increase in risk of atherosclerotic disease in patients with type 2 diabetes requires that care is taken to avoid exposing vulnerable patients to potentially hazardous cardiovascular events (Morrow, 2015). This includes clinical research methods that rely on exogenous insulin infusions which are inherently hazardous to study participants. In practice this involves careful exclusion of study subjects with known cardiovascular disease and screening with non-invasive methods to exclude subclinical atherosclerosis[93]. Only non-fertile women were eligible for participation in the AZD1656 study[94]. As consequence, only one of the participants was female. Women of childbearing potential are generally excluded from early phase clinical trials of new chemical entities because of fetal safety concerns[95] (Morrow, 2015). The under-representation of women in this study merits consideration as sexual dichotomy has been reported in counterregulatory responses to hypoglycaemia (Diamond et al., 1993). Specifically, it has been observed that women have lower responses that may be due in part to a lower glycaemic threshold for the activation of hormone secretion (Diamond et al., 1993).

Some insulin-based metabolic investigative techniques carry a higher risk of inducing hypoglycaemia than others. The insulin tolerance test, which involves an intravenous bolus of exogenous insulin with measurement of glucose responses, provides an example of a method which carries an unpredictable risk of hypoglycaemia (Krentz, 2019b). Beyond insulin-induced hypoglycaemia, degrees of cardiovascular risk are inherent in some methods, notably the insulin suppression test for the assessment of whole-body insulin sensitivity (Greenfield et al., 1981). The insulin suppression test comprises continuous infusions of high-dose insulin + epinephrine to suppress endogenous insulin secretion + propranolol to counter potentially cardiotoxic effects of the catecholamine (Krentz, 2019b). The resulting steady-state plasma glucose provides an indirect assessment of insulin sensitivity. The somatostatin analogue octreotide provides an alternative means of suppressing endogenous insulin secretion[96] in

[93] While acknowledging the limited sensitivity of the tests such as the resting 12-lead ECG to detect coronary heart disease.
[94] Clinical Study Report D1020C00018. AstraZeneca. On file.
[95] Women of reproductive potential are required to exercise extreme caution to avoid pregnancy.
[96] Along with inhibition of glucagon and growth hormone.

variants of the glucose clamp (Krentz et al., 1994). As discussed in Chapter 1, the automated hyperinsulinaemic hypoglycaemic clamp permits plasma glucose concentrations to be precisely manipulated with minimal risk to study participants (Krentz, 2019b). Other strengths of the study include continuous telemetric electrocardiographic safety monitoring throughout the hypoglycaemic clamp and the application of a reverse glucose clamp as required. With respect to cardiovascular safety, no clinically relevant changes or trends in blood pressure or heart rate were encountered during the AZD1656 treatment period. However, an isolated short self-limiting cardiac arrhythmia was observed in a single patient during the post-hypoglycaemia recovery period clamp[97]. Potential limitations of the AZD1656 study include the open design and lack of a placebo arm. However, these considerations are effectively negated by the use of the automated glucose clamp which avoids operator bias in the adjustment of the variable rate intravenous glucose infusion together with the randomised crossover study design (Heinemann and Ampudia-Blasco, 1994). The presence of a comparator group that did not receive AZD1656 would be required to quantify counterregulatory responses in the absence of the glucokinase activator.

The results of the study were interpreted as supporting the practical utility of exogenous glucagon as rescue therapy for iatrogenic hypoglycaemia in patients with type 2 diabetes treated with AZD1656 in combination with metformin. The development of AZD1656 was subsequently terminated in 2011 due to waning glucose-lowering efficacy over time (Hale et al., 2015). The reasons for the inability of some glucokinase activators to provide sustained glucose-lowering is unclear (Nakamura and Terauchi, 2015). The class remained an attractive therapeutic option with at least 15 pharmaceutical companies developing glucokinase activators (Matschinsky and Porte, 2010). This enthusiasm was evident even though moderate dose-dependent hypoglycaemia, elevations in serum triglyceride levels and hypertension were reported (Nakamura and Terauchi, 2015, Matschinsky, 2013). Safer dual acting agents targeting the liver and pancreas along with hepato-selective agents have been developed in response to these issues (Haddad et al., 2024). The range of adverse cardiometabolic and haemodynamic effects associated with glucokinase activators reinforces the principle of rigorous early-phase safety evaluation of experimental therapies for type 2 diabetes (Krentz, 2019b). As discussed in Chapters 1 and 2, the high burden of cardiovascular risk conferred by type 2 diabetes necessitates avoidance of additional risk inadvertently conferred by glucose-lowering medications (Krentz, 2014). In September 2022, dorzagliatin was licensed in China for

[97] Confidentiality considerations preclude a more detailed description. The study participant was offered a detailed cardiovascular assessment by a cardiologist to exclude undetected coronary artery disease.

adult patients with type 2 diabetes (Syed, 2022). To date, no glucokinase activators have been approved by regulators in Western countries.

6.4 Nonclassic effects of high-intensity statin therapy

The study presented in Chapter 4 also used glucose clamp technology. In this case, the hyperinsulinaemic euglycaemic clamp was used to measure insulin action and to explore vascular effects of a widely used statin. The clinical trial performed at the Wellcome Trust Clinical Research Facility at the University of Southampton showed that 6-months of treatment with high-intensity atorvastatin did not impair whole-body insulin sensitivity nor improved aspects of microvascular function (Clough et al., 2009). Statin therapy is the cornerstone of preventing atherosclerosis primarily by reducing LDL-cholesterol levels. The pivotal role of LDL-cholesterol in the development and progression of atherosclerosis has been confirmed (Ference et al., 2017). Mendelian randomisation studies have shown dose-dependent log-linear associations between the absolute magnitude of exposure to LDL-cholesterol and risk of atherosclerotic cardio-vascular disease (Ference et al., 2012). Several years after the widespread adoption of statins into clinical practice[98] a trial of rosuvastatin revealed a potential risk of deteriorating glucose metabolism (Ridker, 2009). Thus, statins might be reducing the impact of a major modifiable metabolic risk factor, i.e., LDL-cholesterol, while promoting the development of another, i.e., hyperglycaemia. As clinical trials and real-world data accumulated it appeared that the risk was greatest with higher doses of statins especially when used in insulin-resistant subjects with features of the metabolic syndrome (Sattar et al., 2014). An example of the potential for clinical implications of interactions between lipid and carbohydrate metabolism is exemplified by heterozygous familial hypercholesterolaemia[99]. This inherited dyslipidaemia, the most common monogenic disorder in humans[100], confers a high lifetime risk of atherosclerotic cardiovascular disease (Akioyamen et al., 2017, Kastelein et al., 2020). Long-term statin therapy is the mainstay to control elevated LDL-cholesterol concentrations (Pang et al., 2020). A low prevalence of type 2 diabetes has been reported in some familial hypercholesterolaemia cohorts, raising the question of whether these patients are protected against type 2 diabetes[101] (Besseling et al., 2015). However, a recent report from the European

[98] Registration clinical trials of statins did not generate a safety signal for new-onset diabetes.
[99] More than 80% of cases are due to pathogenic variants in the LDL receptor gene (*LDLR*) located on chromosome 19.
[100] UK prevalence is approximately 1 in 250. Reported prevalence rates are lower in Europe compared with North America and Australia that may reflect Founder Effects of population migrations.
[101] Heterozygous familial hypercholesterolaemia was hypothesised to reduce susceptibility to type 2 diabetes via a reduction in fully functioning LDL receptors required for cellular uptake of cholesterol affecting islet β-cell function.

Atherosclerosis Society Familial Hypercholesterolaemia Studies Collaboration registry, which provides data on every World Health Organization region, showed no protection against type 2 diabetes among individuals with heterozygous familial hypercholesterolaemia (European Atherosclerosis Society Familial Hypercholesterolaemia Studies, 2024). In the registry population, obesity was highly prevalent among individuals with heterozygous familial hypercholesterolaemia. Obesity, statin therapy, and advancing age were associated with a higher risk of type 2 diabetes, with obesity showing the strongest association (European Atherosclerosis Society Familial Hypercholesterolaemia Studies, 2024). The investigators concluded that known risk factors for type 2 diabetes in the general population are equally applicable to individuals with heterozygous familial hypercholesterolaemia (European Atherosclerosis Society Familial Hypercholesterolaemia Studies, 2024). Thus, shared decision making between physician and the patients with heterozygous familial hypercholesterolaemia should focus on obesity as by far the leading risk factor for type 2 diabetes; in comparison, the adverse effects of statins in this population was considered to be trivial (European Atherosclerosis Society Familial Hypercholesterolaemia Studies, 2024). It should be noted, however, that a cross-sectional study design cannot provide information on the cumulative effect of long-term statin therapy on diabetes risk.

As discussed in Chapter 2, polycystic ovary syndrome is characterised by insulin resistance and the metabolic syndrome. In a study from Finland, six months of high-intensity statin therapy (atorvastatin 20mg daily) lowered LDL-cholesterol levels but impaired dynamic measures of insulin sensitivity in women (n=28) with polycystic ovary syndrome (Puurunen et al., 2013). One woman in the atorvastatin and placebo groups respectively had impaired glucose tolerance at baseline. Specifically, a higher area under the curve (AUC) was observed for insulin in response to glucose challenge in the statin-treated women with no concomitant change in glucose AUC. This provides indirect evidence of impaired insulin action in the statin-treated women. Of note, however, there was an imbalance in glucose AUC at baseline which was higher in the women randomized to atorvastatin. In addition, body mass index was numerically higher in the statin-treated women at baseline (30.4 vs 26.7 kg/m^2). No changes were observed in testosterone levels between the groups (Puurunen et al., 2013).

Meta-analyses of randomised statin-dosing trials showed that higher-intensity statin therapy associated with increased risk of new-onset type 2 diabetes (Preiss et al., 2011). Variants of the 3-hydroxy-3-methyl-glutaryl coenzyme-A (HMG-CoA) reductase gene that reduce HMG-CoA reductase activity are associated with an increased risk of diabetes among

individuals with impaired fasting glucose levels (Ference et al., 2016). The literature suggests that diabetogenic effects may differ between statins (Ray, 2013). While some statins, e.g., atorvastatin are associated with increased HbA_{1c} levels in patients receiving intensive therapy, other statins, e.g., pitavastatin, have demonstrated neutral or favourable effects on glucose metabolism in patients with and without type 2 diabetes or the metabolic syndrome (Ray, 2013). However, in a recent trial conducted in patients with human immunodeficiency virus receiving antiretroviral therapy, a statistically significant higher incidence of new cases of diabetes occurred with pitavastatin compared with placebo in line with other statins (Grinspoon et al., 2023). Current evidence supports hierarchy of risk with some statins, e.g., simvastatin, rosuvastatin and atorvastatin, being more strongly associated with new-onset diabetes than others, e.g., pravastatin (Galicia-Garcia et al., 2020b). In terms of overall risks vs health benefits, the balance remains firmly in favour of benefits. Sattar and colleagues calculated that treatment with statins compared to placebo in 255 subjects over 4 years would generate one new case of diabetes while preventing 5.4 major cardiovascular events (Sattar et al., 2010). New onset diabetes appears to be a class effect for statins since no risk has been observed with non-statin cholesterol-lowering drugs (Brinton, 2021). As discussed below, the diabetogenic effect of statins has prompted consideration of therapeutic strategies using combinations of cholesterol-lowering drugs with the aim of reducing the risk of diabetes without compromising cholesterol goal attainment (Brinton, 2021).

It has been hypothesised that the diabetogenic effect of statins might be mediated via insulin resistance (Baker et al., 2010). Early studies with small sample sizes failed to provide clarity. However, a recent systematic review of the literature concluded that statins in general reduce insulin sensitivity (Dabhi et al., 2023). Modest statin-associated weight gain, as noted in randomised trials, and ß-cell toxicity have been proposed as diabetogenic factors (Sattar, 2023). A detailed experimental study in a multi-ethnic group of non-diabetic subjects at Stanford University[102] concluded that atorvastatin 40 mg for 10 weeks reduced whole-body insulin sensitivity by a median of 8% (Abbasi et al., 2021). Of 75 participants who were enrolled, 71 completed the study. No statistically significant relationship between change in LDL-cholesterol and the change in insulin resistance was observed. In this open study the investigators also measured insulin secretion using the graded-glucose infusion test and found a median increase of 9% over baseline. Minor increases in the area under the curve for glucose were observed in oral glucose tolerance tests along with an increase in fasting insulin concentrations (Abbasi et al., 2021). These results suggest that increased insulin

[102] Conducted by leading exponents of the insulin suppression test.

secretion in the presence of a modest increase in insulin resistance was not sufficient to maintain pre-treatment glucose tolerance (Reaven, 1988). Impairment of islet ß-cell function associated with statin therapy may explain this inability to fully compensate for insulin resistance (Galicia-Garcia et al., 2020b). Baseline insulin resistance was a significant predictor of the change in insulin resistance, i.e., lower baseline insulin resistance was associated with greater increase in insulin resistance in response to statin therapy (Abbasi et al., 2021). Insulin resistance did not increase significantly in more insulin resistant participants with features of the metabolic syndrome; however, an increase in insulin secretion was observed in this subgroup (Abbasi et al., 2021).

Both the insulin suppression test and the hyperinsulinaemic euglycaemic clamp induce a state of pharmacological sustained hyperinsulinaemia via infusion of exogenous insulin. Insulin resistance, measured directly in the insulin suppression test, correlates with the direct measure obtained using the hyperinsulinaemic euglycaemic clamp (Knowles et al., 2013). Both techniques are labour-intensive and require operator expertise to optimise accuracy and reproducibility. In the case of the hyperinsulinaemic euglycaemic clamp these considerations are counterbalanced by its status as the reference method for assessing whole-body insulin action (Krentz, 2019b, Gastaldelli, 2022). Both techniques assess insulin-mediated glucose disposal by skeletal muscle, which is the main determinant of insulin sensitivity (Krentz, 2019b). In the hyperinsulinaemic euglycaemic clamp whole-body glucose uptake (M value) is defined as the glucose infusion rate during the final 30 min of the test when steady-state insulin concentrations had been achieved. The ratio of M to the mean insulin concentration (I value) is used as an index of insulin sensitivity. Operator experience is a factor in manual clamps that is less important when using automated clamp technology. Intra-individual coefficients of variation of <10% have been reported for repeated hyperinsulinaemic euglycaemic clamps performed in healthy volunteers (Soop et al., 2000). Numerous factors, e.g., ethnicity, prior physical activity, sleep deprivation, may affect whole-body insulin sensitivity (Krentz, 2019b); these variables should be considered and, where appropriate, controlled as far as possible in glucose clamp studies. As discussed in Chapter 4, the two-step variant of the hyperinsulinaemic euglycaemic clamp has advantages over the one-step standard version. The lower insulin infusion during the first step permits assessment of insulin-mediated suppression of lipolysis at physiologically relevant insulin concentrations (Krentz, 2019b). Details of the clamp methodology, including insulin doses, are presented in Supplement 1 to the published paper (Clough et al., 2009).

Inhibition of HMG-CoA reductase activity by statins has emerged as a key mechanism in reducing insulin sensitivity (Carmena and Betteridge, 2019, Galicia-Garcia et al., 2020b). Thus, with respect to the most prevalent manifestations of statin toxicity, i.e., statin-associated muscle symptoms and new-onset diabetes, skeletal muscle is centre-stage (Ward et al., 2019). In the Southampton study, skeletal microvascular function was examined as a nonclassic vascular effect of atorvastatin. Dysfunction of both large and small blood vessels may be evident in cardiometabolic disorders often with intricate bidirectional pathophysiological associations (Krentz, 2023b). Whether statins have vasculoprotective effects beyond lowering LDL-cholesterol has been the subject of much debate. In this context, the effects of statin on microvascular function have been under-researched relative to their effects on macrovascular disease (Krentz et al., 2007a, Krentz et al., 2009a, Preiss, 2014). A meta-analysis that included twenty randomised controlled studies of the effect of high-intensity statin pretreatment on coronary microvascular dysfunction in patients with coronary heart disease undergoing percutaneous coronary intervention reported benefits on thrombolysis in myocardial infarction grading[103], myocardial blush grade[104], and index of microvascular resistance[105] (Huang et al., 2023a). A separate study investigating the pleotropic effects of simvastatin suggested benefits on endothelial epigenetics to protect endothelial function (Liu et al., 2023). In contrast, no effects of high-intensity statin therapy were observed in the Southampton study. Differences in anatomical sites, i.e., skeletal muscle vs myocardium, participant characteristics and/or measurement techniques, may account for the discrepancies between the reported studies.

Impairment of microvascular function is associated with cardiovascular and metabolic risk factors such as abdominal obesity and insulin resistance in the absence of diabetes (Clough et al., 2011). Insulin increases blood flow and microvascular perfusion in skeletal muscle (Coggins et al., 2001). Impairment of insulin-induced microvascular dilator responses in skeletal muscle observed in animal models of insulin resistance and in human studies is a factor in reduced muscle glucose uptake (Clark et al., 2003). Changes in microvascular flow, focused on capillary recruitment in muscle, indicate this to be a key site for early insulin action at physiological levels (Clark et al., 2003). Thus, microvascular function and glucose metabolism are closely interconnected. At baseline in the Southampton atorvastatin study, muscle microvascular exchange capacity was negatively associated with visceral fat mass and HbA_{1c} and positively with insulin-mediated glucose uptake in skeletal muscle (M/I).

[103] A measure of coronary artery perfusion.
[104] An angiographic measure of myocardial perfusion.
[105] A quantitative evaluation of coronary microcirculatory function.

As presented in Chapter 4, no statistically significant changes in either whole-body insulin action or microvascular function were observed in response to atorvastatin (Clough et al., 2009). In addition to the hyperinsulinaemic euglycaemic clamp, assessments of insulin action in the Southampton study included homeostasis model assessment and quantitative insulin sensitivity check index (Turzyniecka, 2011, Krentz, 2019b). Beyond the well-recognised implications on the regulation of carbohydrate metabolism insulin resistance affects all aspects of lipid and lipoprotein metabolism. Lipolysis of stored triglycerides within adipocytes liberates NEFA and glycerol into the circulation. Adipocyte lipolysis is highly sensitive to inhibition by insulin at low circulating concentrations via actions on hormone sensitive lipase. This action is impaired in states of insulin resistance and can be detected at low-physiological levels of insulin (Krentz, 2002a). The response of adipose tissue lipolysis to the low-dose insulin infusion was estimated by calculating the percentage of change between the mean plasma concentration of NEFA at baseline and after 60 minutes (Turzyniecka, 2011). In parentheses, although hepatic cholesterol synthesis is modulated by hepatic insulin action circulating cholesterol levels are not included in the definition of the metabolic syndrome. While insulin resistance is associated with changes in cholesterol metabolism the effects of insulin are mediated primarily via lipoprotein metabolism (Pihlajamaki et al., 2004). In brief, insulin resistance is associated with increased secretion of very low-density lipoproteins (VLDL) with increased plasma triglycerides (Choi and Ginsberg, 2011). Increased delivery of in NEFA stimulates the assembly and secretion of VLDL (Choi and Ginsberg, 2011). Insulin resistance is also characterised by higher numbers of small, dense LDL-cholesterol particles along with hypertriglyceridaemia and reduced levels of HDL-cholesterol (Berneis and Krauss, 2002). As discussed, small dense LDL particles, which are susceptible to oxidation and have a propensity to traverse the endothelium and enter the arterial wall, are considered highly atherogenic in the context of insulin resistance and the metabolic syndrome (Berneis and Krauss, 2002). No statistically significant changes were observed any measures of carbohydrate metabolism or insulin-regulated lipid metabolism in the University of Southampton study (Clough et al., 2009).

Strengths of the study included a robust study design, i.e., a six-month randomised placebo-controlled trial of a well-established high-intensity statin. The trial was conceived and conducted by an interdisciplinary team of investigators with expertise in assessing microvascular function and human metabolism. The study participants were carefully selected to ensure that the hypothesis was tested with rigour, i.e., centrally obese, non-

diabetic subjects with two or more features of the metabolic syndrome. Individual 10-year cardiovascular risk was calculated. The ratio of male to female participants was appropriate and balanced. Subjects who were found to be at sufficiently high risk of atherosclerotic disease to merit statin therapy according to prevailing national clinical guidance were excluded on ethical considerations. Extensive cardiometabolic phenotyping included assessment of physical activity levels using an activity monitor, handgrip strength and cardiorespiratory fitness (VO_{2max}). Body composition was assessed using dual X-ray absorptiometry. Visceral fat mass was determined using state-of-the-art magnetic resonance imaging. A range of inflammatory markers was measured together with adipocytokines including leptin, adiponectin, and resistin. Atorvastatin reduced LDL-cholesterol by approximately 50% and C-reactive protein by 75%, respectively. However, no statistically significant changes in body composition or adipocytokines were observed (Clough et al., 2009). Evidence in humans suggests that resistin is produced within adipose tissue by resident macrophages (Curat et al., 2006). While sharing structural similarities with adiponectin, resistin is associated with insulin resistance and pro-atherogenic action (Liu et al., 2022).

In summary, multiple aspects of insulin action were assessed in the University of Southampton study. These included basal, i.e., principally hepatic, and stimulated, i.e., skeletal muscle, glucose metabolism. Furthermore, insulin sensitivity in adipocyte metabolism was assessed in the low-dose insulin infusion of the two-step hyperinsulinaemic clamp. Potential limitations of the study include the homogeneity of the participants who were exclusively white European. A possibility exists that the hyperinsulinaemia induced during the glucose clamp may have obscured effects of statin therapy on hepatic insulin sensitivity. Glucose production by the liver is maximally regulated at insulin concentrations in the low physiological range (Krentz and Nattrass, 1996). Radiolabelled glucose tracer methodology would be required to rigorously test this hypothesis (Krentz, 1991). It is apposite to note here the relevance of the complementary measures of insulin action used in the Southampton study which are calculated from fasting insulin and glucose levels (Krentz, 2019b). Since fasting glucose concentrations are primarily determined by the restraining action of basal insulin levels on glucose production from hepatocytes these measures provide an indirect assessment of hepatic insulin sensitivity. Assessment of changes in glycaemia included fasting plasma glucose and HbA_{1c} with neither measure changing in response to atorvastatin. While glucose tolerance was assessed in all subjects at baseline and at six months using a 75 g oral glucose tolerance test; minor recategorisations of

glucose tolerance were noted for both the statin- and placebo-treated subjects with no clear pattern being observed. No dynamic assessment of endogenous insulin secretion was performed in the Southampton study. Since the methods used for assessing insulin sensitivity differed between the studies direct comparisons are not possible. Nonetheless, the sample sizes and study designs merit consideration. The possibility that the smaller sample size in the Southampton study may have resulted in a type 2 statistical error cannot be excluded (Armitage, 1987). The Southampton study was conceived as an interdisciplinary research project and was powered to detect a change in muscle microvascular exchange capacity rather than insulin sensitivity. Some other differences between the two studies are noteworthy. First, despite the smaller sample size (n=39 vs.71 completers) the Southampton trial employed a rigorous parallel group placebo-controlled design. This contrasts with the open-label study of Abbasi et al which did not include a placebo control group (Abbasi et al., 2021). Another difference between the studies is the fact that all participants in the Southampton study were statin-naïve whereas 38% of the Stanford subjects previously been exposed to statin therapy (Abbasi et al., 2021). A within-subject cross-over trial, in which participants received atorvastatin vs. placebo in random sequence, may have been the optimal design since this would have increased the statistical power of the Southampton study (Armitage, 1987). However, a within-subject crossover design may have generated issues of participant dropout, a recognised risk in trials with longer durations of observation. The Stanford and Southampton studies provide information on study design and the selection of investigative techniques that could aid the design of future mechanistic studies.

6.5 Machine learning to improve real-world cholesterol lowering therapy

Ensuring that individuals receive efficacious and appropriate therapy is an aim of precision or personalised medicine. Precision medicine aspires to be a safer and more efficacious, accessible, and equitable way of delivering care thereby helping to achieve optimal outcomes for the individual patient. The challenge of reducing the toll of atherosclerosis using cholesterol-lowering drugs is a pertinent example of how gaps between evidence and real-world practice may emerge. In the UK, less than 50% of patients achieve recommended goals for cholesterol levels (Akyea et al., 2019). This shortfall can, at least in part, be attributed to failure of compliance with guideline recommendations (Reynolds et al., 2021). Suboptimal adherence to therapy by many patients also contributes to the translational gap (Ferdinand et al., 2017, Karalis, 2023). Between 40 and 75% of patients discontinue statin therapy within one year of

initiation (Banach et al., 2016). Nonetheless, statins remain the foundation of cholesterol-lowering therapy; they are the most prescribed medications worldwide. Optimal use of statins – and avoidance of unwanted effects that detract from their efficacy – is therefore a major public health issue (Preiss and Sattar, 2011). In Chapter 5, a novel machine learning model trained on clinical guidelines was used to identify individuals at high risk of atherosclerosis who were receiving cholesterol-lowering medication. This analysis confirmed other published audits of care that have shown that statins are often not used at appropriate dose intensities, i.e., high-risk patients are often under-treated. Issues of therapeutic inertia and underuse of proven newer therapies are not confined to the UK or to the primary healthcare sector; even centres of excellence in the USA and Europe under-perform against clinical guidelines in terms of achieved lipid targets in patients at highest risk (Cannon et al., 2021, Ray et al., 2023a). Beyond identifying patients who did not achieve the recommended goal[106] the neural networks were able to identify individuals who were candidates for the novel non-statin lipid-lowering medication bempedoic acid (Tummala et al., 2022). The study was conceived and executed by the eHealth startup Metadvice in collaboration with the School of Life Course and Population Sciences at King's College London. Metadvice was founded by a group of scientists[107] in 2018 with the intention of improving outcomes for patients with complex long-term conditions through the application of artificial intelligence to healthcare. The primary objective of this study was to explore the utility of machine learning to promote precision medicine in cholesterol-lowering therapy. Since the study included proprietary technology, some methodological details are not within the public domain. Testing the hypothesis involved creating artificial neural networks that reproduced prevailing national clinical guidance for cholesterol-lowering with a prespecified high degree of accuracy. After training the neural networks on real-world data anonymised electronic health records from six primary care practices in South London were analysed. Among patients receiving statin monotherapy approximately 30% were not receiving high-intensity treatment as per NICE guidance; rates of high-intensity statin use were similar for the primary (70%) and secondary (74%) prevention cohorts, the latter group being at particularly high risk of further atherosclerotic events. Familial hypercholesterolaemia and statin intolerance were recorded in 1% and 4% of patients, respectively. It is considered that these figures are underestimates since the published literature suggests that both categories are underdiagnosed (Qureshi et al., 2021, Banach et al., 2015). The neural networks generated next-step therapy recommendations aligned with NICE guidance. The main recommendations included,

[106] Defined as >40% reduction from pre-treatment levels in non-high density (HDL) cholesterol.
[107] Founders: Richard Barker PhD, André Jaun PhD, Serge Umansky PhD.

increasing the statin dose, adding ezetimibe (Hammersley and Signy, 2017), and adding injectable cholesterol-lowering therapy (Krentz, 2023e). Prior neural network-based audits of primary care prescribing for cardiometabolic disorders conducted by Metadvice had revealed unduly long follow-up intervals between clinical consultations and failure to escalate or add therapy as indicated by clinical guidelines (data on file).[108] These deficiencies in the process of care were also identified in the study presented in Chapter 5. The shortfall implies that patients often do not derive the full benefit of proven therapy. Reducing therapeutic inertia using supportive machine learning technology could help ensure that patients derive the full benefit of proven therapies. In the UK, prescribing pharmacists and nurses are increasingly involved in delivery of care for long-term risk factors such as cholesterol. Prediction by the neural network of the next treatment step assists when allocating patients to the most appropriate healthcare professional. For example, a recommendation to start an expensive new cholesterol-lowering medication might be directed to a more experienced clinician. Achieving the objective of reduced therapeutic inertia could have implications for earlier stages of the translational medicine pathway (Fig 6.4) since better cholesterol-lowering could potentially be achieved without the need for novel drugs. That said, limitations in statin efficacy allied to well-recognised tolerability issues continue to drive the search for alternative cholesterol-lowering medications. Ezetimibe, which inhibits Niemann-Pick C1 Like 1 protein inhibitor to reduce cholesterol absorption, is a non-statin cholesterol-lowering drug devoid of muscular and metabolic side-effects (Hammersley and Signy, 2017). However, despite proven efficacy, very good tolerability, and low acquisition cost real world use of ezetimibe as an adjunct (or alternative) to statins remains low (De Backer et al., 2019). A more recently introduced non-statin cholesterol-lowering drug is the adenosine triphosphate citrate lyase (ACL) inhibitor bempedoic acid. The latter became available in the UK in 2020[109]. Of note, bempedoic acid is devoid of statin-associated muscular symptoms (Ballantyne et al., 2021). As a prodrug, bempedoic is converted to bempedoyl-CoA by very long-chain acyl-CoA synthetase 1. This enzyme is highly expressed in the liver but is undetectable in the skeletal muscle (Ballantyne et al., 2021). The absence of active drug generation in muscle is of relevance for many patients who report statin-associated muscle symptoms (Pirillo and Catapano, 2022, Nissen et al., 2023). Whether real or perceived, these symptoms are a major barrier to effective cholesterol-lowering on a population basis. Another attractive attribute of bempedoic acid is that new-onset diabetes has not been observed in trials of bempedoic acid to date (Pirillo and Catapano, 2022, Nissen et al., 2023). A placebo-controlled safety and efficacy trial in patients with heterozygous familial hypercholesterolaemia

[108] Data on file www.metadvice.com.
[109] With restrictions on use imposed by the National Institute for Health & Care Excellence (NICE).

reported a lower incidence of new-onset or worsening diabetes with bempedoic acid (Ray et al., 2019). A prespecified analysis of the CLEAR Outcomes trial of 13,970 patients[110] showed a neutral impact of bempedoic acid on glucose metabolism (Ray et al., 2023b). Specifically, among patients free from diabetes[111] at baseline, treatment with bempedoic acid over an average follow-up period of 3·4 years did not result in an increase in HbA_{1c} or glucose concentrations, nor in the incidence of new-onset diabetes. Moreover, bempedoic acid was associated with modest weight loss (Ray et al., 2023b). Of note, the absolute benefits in the diabetes subpopulation were almost double that observed in those without diabetes, an observation attributable to the higher baseline risk of participants with diabetes (Ray et al., 2023b) .

High-intensity statin regimens are often required to achieve cholesterol goals. High-intensity statin therapy carries a higher probability of both muscle symptoms and new-onset diabetes[112]. Lower doses of statin in combination with non-statin cholesterol-lowering agents may help avoid new-onset diabetes in selected patients at risk of developing statin-associated new-onset diabetes (Brinton, 2021). Real-world evidence demonstrating low rates of cholesterol goal attainment have generated calls for more extensive use of combination therapy, e.g., statins together with ezetimibe, bempedoic acid or proprotein convertase subtilisin/kexin type 9 serine protease (PCSK9) inhibitors (Faridi and Desai, 2023). Use of lower doses of statins and adding non-statin therapy offers the prospect of greater number of patients attaining recommended cholesterol goals while reducing the tolerability and adverse metabolic issues associated with high-intensity statin doses (Brinton, 2021, Faridi and Desai, 2023). It is hypothesised that this strategy may help address gaps in care, minimise health disparities and simplify treatment decisions (Faridi and Desai, 2023).

The Metadvice-King's College London proof-of-concept study evaluated a proprietary artificial intelligence platform that aims to brings personalised precision medicine into routine clinical care. Translating the observations of this real-world study back into clinical practice is the logical next step Using Metadvice population analytics, primary care practices can identify patients requiring changes to their cholesterol-lowering therapy. In parallel, since 2022 Metadvice has been deploying a proprietary clinical decision support tool that provides ambient artificial intelligence during clinical consultations. In brief, the neural network interrogates the

[110] Participants in CLEAR Outcomes were unwilling or unable to take guideline-recommended doses of statins.
[111] Either normoglycaemia or prediabetes.
[112] High-intensity statin therapy is defined as drugs at doses capable of reducing LDL-cholesterol by >40%.

electronic health record and provides evidence-based recommendations, e.g., for cholesterol-lowering, to the clinician (Figure 6). All therapy recommendations are aligned with NICE guidance and enriched with real world evidence outcomes. The rationale for prescribing decisions is provided by click-through links to the relevant national guidance and proportion testing to ensure statistical significance of the recommendation in the retrospective data. Ongoing safety and efficacy testing are integral to the deployment of the clinical decision support tool. Alignment of the neural networks with updates from NICE, or outside the UK, with guidelines of healthcare systems is readily achievable with appropriate retraining.

Strengths of the study presented in Chapter 5 include the inherently low bias of the guidelines-trained neural networks and the highly structured nature of primary care electronic records (Krentz, 2023e). The balance of male to female subjects was appropriate (55:45) and ethnic minorities reflected the UK population. Limitations include the potential for missing data in electronic health records and incomplete recording of clinical disorders. It should be noted, however, that the neural network is able to make partial use of incomplete records. Since the study was retrospective, the efficacy of the therapy recommendations will require confirmation in prospective studies.

Metadvice machine learning methodology is currently being developed to provide additional precision therapy recommendations where indicated. Non-linear relationships within health data may require such models to learn more complex relationships. For example, individuals for whom clinical guidance does not provide clearly defined treatment choices can be compared with cohorts within the electronic database who have similar features. SHAP values (defined in Chapter 1), a method developed in cooperative game theory and used to increase transparency and interpretability of machine learning models, can be used to provide statistical justification to adopt recommendations based on the observed outcomes of these cohorts. Ultimately, prescribing decisions reside with the clinician. In a follow-up Metadvice-King's College London study using the UK Clinical Practice Research Datalink[113] Krentz et al found that up to 20% of primary care patients could potentially achieve cholesterol-lowering superior to that achieved by NICE guidance at lower than recommended statin doses (Krentz et al., 2025). The latter study, which was independently conceived and conducted, replicated results of a machine learning study in a northern California healthcare database by researchers at Stanford University (Sarraju et al., 2022). The hypothesis that lower statin doses are associated with

[113] An anonymised dataset representative UK primary care, https://cprd.com.

improved concordance with therapy that results in more effective reductions in cholesterol, is testable in prospective studies.

The Metadvice-King's College London studies provide examples, albeit tentative and requiring confirmation, of how black box criticism of machine learning methods applied to healthcare might be averted (London, 2019, Molnar et al., 2020). Beyond supporting personalised precision medicine in prescribing for patients with cardiometabolic disease. As observed in Chapter 4, Metadvice artificial intelligence methodology can also identify individuals who are eligible for new medications that are being introduced into local or national clinical practice (Krentz, 2023e). Practical portability of the machine learning algorithms between clinical datasets has been demonstrated (Krentz et al., 2025).

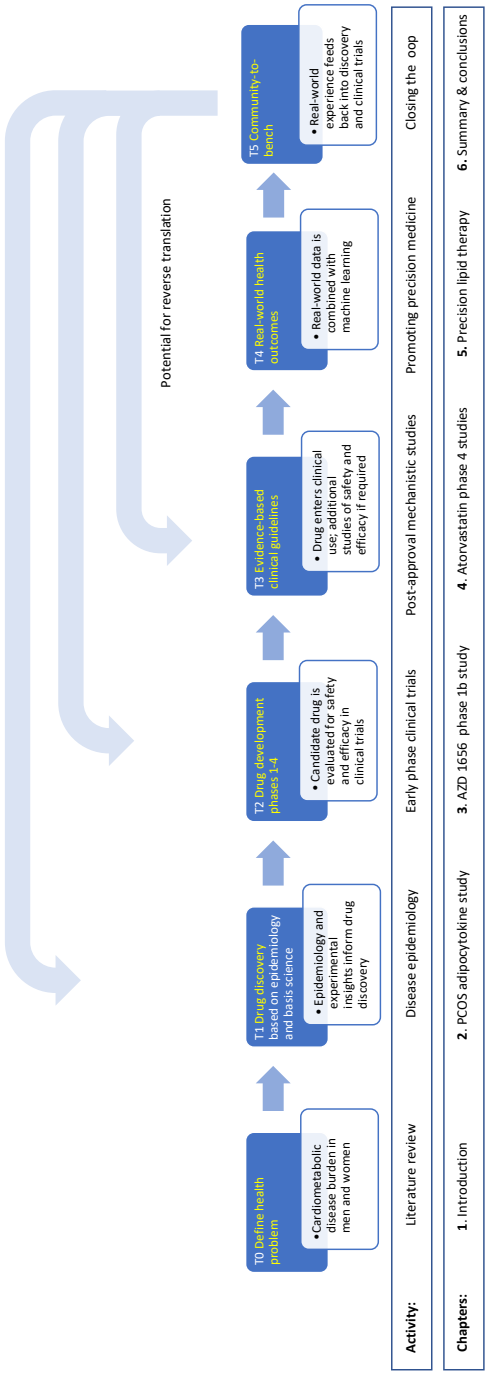
The Metadvice-King's College London study presented in Chapter 5 provides tentative support for the utility of novel machine learning-based data analytics in relation to cholesterol-lowering pharmacotherapy (Krentz, 2023e). Metadvice aims to expand the disease repertoire to provide a comprehensive multi-morbidity approach for complex cardiometabolic disorders using explainable artificial intelligence. In pursuit of this objective, Metadvice has generated proof-of-concept data suggesting that the development of comorbidities can be predicted from electronic medical records (Krentz, 2023d). It is also appropriate to consider the value of data beyond that routinely recorded in electronic health records. Precision medicine is often discussed in terms of the potential benefits offered by molecular genetics (Dainis and Ashley, 2018). Pharmacogenomic data that provide insights into how individuals absorb, transport, metabolise, or eliminate lipid-lowering drugs could enhance precision lipid-modifying therapy (Shatnawi et al., 2023). This principle extends to other major cardiometabolic disorders such as type 2 diabetes (Mannino et al., 2019). Accurate phenotyping, however, remains imperative. This principle is particularly relevant when applied to long-term cardiometabolic disorders that are overwhelmingly polygenic and heterogeneous. Typically, many genes, each with small effects, interact with environmental and behavioural factors to produce multiple disease phenotypes which may evolve over time via gene-gene and gene-environment interactions (Blackett and Sanghera, 2013). Accurate delineation of subgroups, ideally based on pathogenic criteria, is required if precision medicine is to advance.

6.6 The translational medical research pathway

Translational medicine has been described as a method and process of facilitating medical advances efficiently from scientists to clinicians. The process is often summarised in terms of moving discoveries from the laboratory bench to the bedside and from the bedside into the community. Each of the papers presented in this dissertation tested an original hypothesis positioned along the cardiometabolic translational medicine pathway (Figure 7). The individual studies may be considered to align with the translational research as follows: pathophysiological insights gleaned from epidemiology, specifically polycystic ovary syndrome (Chapter 2); clinical trials of novel (AZD1655, Chapter 3) and well-established (atorvastatin, Chapter 4) pharmacotherapeutics; population-level implementation of approved drugs, principally statins but including other cholesterol-lowering agents, in routine clinical practice (Chapter 5).

In the quest to develop new pharmacotherapies, the translational medicine pathway starts with defining health problems and proceeds to drug discovery, clinical trials, and post-approval implementation in clinical practice (Khoury et al., 2007) (Table 7). Several versions of the translational medicine pathway have been proposed by investigators active in specific fields of biomedicine. As reflected by the papers selected for inclusion in this thesis, translational medicine is an inherently multidisciplinary process that brings academia and the life sciences together within the rigorous regulatory framework of the drug approval process (Khoury et al., 2007). This collaborative approach increasingly requires interactions within multidisciplinary teams including clinicians, pharmaceutical physicians, data scientists, engineers, and regulatory experts.

Figure 7. Translational medicine pathway.



PCOS, polycystic ovary syndrome.

In the process of reverse translational research, knowledge gained from forward translation has potential to feed back to earlier stages of drug discovery and development.

The alignment of the original research papers in this dissertation to the pathway is indicated in the lower panel.

Table 7. Stages of the translational medicine pathway.

T0 Identification of opportunities and approaches to health problems.
T1 Moving basic discovery into a candidate health application.
T2 Assessing the value of application for health practice leading to the development of evidence-based guidelines.
T3 Moving evidence-based guidelines into health practice, through delivery, dissemination, and diffusion research.
T4 Evaluating real-world health outcomes of population health practice.
T5 Late translation addresses health disparities to inform discovery science and clinical trials; feedback of knowledge to earlier stages.

Modified from:

https://www.iths.org/investigators/definitions/translational-research/;
https://uofuhealth.utah.edu/research/news/2021/07/research-strategy-translational-research; Tossas et al., 2020.

6.6.1 Cardiometabolic translational medicine

An understanding of the epidemiology of disease allied to accurate disease phenotypes is required to inform inclusion and exclusion criteria for clinical trials. Stages T0 and T1 are essential early components of the translational pathway that define healthcare needs. The T1 and T2 stages may present hurdles to be successfully negotiated if an experimental medication is to reach the market based on efficacy, tolerability and safety considerations. Complex cardiometabolic syndromes present challenges to clinical investigators evaluating novel therapeutic interventions. Innovations such adaptive trial designs that adjust study protocols based on new information according to prespecified rules can help reduce the cost and duration of clinical trials (Pallmann et al., 2018). Acceleration of evidence translation through clinical trials powered by artificial intelligence may be achieved through improved eligibility screening, better matching of participants to trials, and data-driven predictive enrichment of trials (Khera et al., 2024). Adaptive phase 2 and 3 study designs, which may be enhanced by artificial intelligence, are becoming more popular in cardiology. Such trials can potentially be stopped early thereby reducing sample size and trial resources (Zhang and Saju, 2023).

The stages of translational medicine have also been defined in terms of blocks encountered in the pathway (Sanchez De La Nava et al., 2022, Barker, 2016). In translational cardiovascular research – as in other areas of medicine – these blocks include (a) the transfer of new laboratory knowledge of disease mechanisms into the development of new methods for diagnosis, therapy and prevention and (b) first-in-human testing with translation of results from clinical studies into everyday clinical practice and healthcare decisions (Lauer and Skarlatos, 2010).

Medications that successfully traverse the translational pathway (T2) of phase 1-3 clinical trials may still face potential challenges in the post-licensing period (T3 T4) of their life cycle. Even when a medication has become well-established in clinical practice, e.g., statins to reduce LDL cholesterol levels, questions about efficacy and safety may arise during post-marketing (phase 4) phase. Infrequent adverse drug effects may not be apparent from regulatory clinical trials which typically include thousands of participants filtered by inclusion and exclusion criteria that are not always representative of real-world clinical practice. Consequently, concerns about emerging adverse effects are typically addressed in post-marketing studies. Second, newly approved medications, which are generally more expensive than existing therapies and which may be subject to regulatory constraints that reflect incomplete information from regulatory clinical trials, must be integrated into existing treatment pathways (T4). Notable examples exist of innovative cardiometabolic drugs whose adoption in clinical practice has been slow in the UK and elsewhere[114]. The T5 stage extends beyond the public health model of care to the social health model that emphasises improvements in the wellness of populations by changing inefficient social structures.

Atherosclerotic cardiovascular disease remains the foremost global cause of death among non-communicable chronic diseases (Roth et al., 2020, Vaduganathan et al., 2022). While rates of cardiovascular disease have been declining, adverse trends in cardiovascular disease have been reported in young adults (Okoth et al., 2022). In the UK during 2000-2019, reductions in coronary heart disease benefitted those aged >60 years whereas no improvement was observed in the burden of cardiovascular in younger age groups (Conrad et al., 2024). Sex differences have been observed with coronary heart disease mortality stagnating in young women (Wilmot et al., 2015). These adverse trends have been observed as lipid-lowering medications have proliferated (Ferraro et al., 2022). Lipid

[114] These include the proprotein convertase subtilisin/kexin type 9 serine protease (PCSK9) inhibitors for hypercholesterolaemia.

management guidelines have become more convoluted with the approval of new non-statin cholesterol-lowering medications. Audits have confirmed that the use of newer pharmacotherapeutics with proven efficacy, e.g., potent non-statin cholesterol-lowering agents, is suboptimal even in specialised centres of excellence; therapeutic goals for modifiable cardiovascular risk factors such as LDL-cholesterol are often not achieved in part because therapy is not intensified according to clinical guidelines (Cannon et al., 2021). Under-use of newer agents partly explains why progressively more challenging therapeutic goals are often not achieved in high-risk patients (Ray et al., 2023a). The high acquisition cost of injectable LDL-cholesterol lowering drugs, i.e., PCSK9 inhibitors and inclisiran, have led regulators and health insurance companies to restrict patient access (Apostolou et al., 2022, Baum et al., 2017). Reluctance among clinicians to prescribe new therapies may be relevant in some instances[115].

The principal barrier to effective cholesterol-lowering at individual and population level, however, is the reluctance expressed by many patients about perceived or actual tolerability issues associated with statin therapy (Toth et al., 2018, Newman et al., 2019). This issue is centred around muscle symptoms, clinical trial data – including n-of-1 within-patient crossover studies – suggest are often attributable to a nocebo response (Wood et al., 2020, Apostolou et al., 2022). Women are less likely to adhere to statins and to meet target LDL-cholesterol goals than men (Goldstein et al., 2016). Gender-based disparities in statin adherence can be linked to provider-level, psychosocial, and medication intolerance factors (Goldstein et al., 2016). It should be acknowledged, however, that in the context of multi-morbidity, managing a plurality of cardiometabolic risk factors simultaneously usually requires polypharmacy which in itself is problematic (Gaede et al., 2008). In general, adherence reduces as a function of number of daily medications with negative implications for risk factor control, clinical outcomes, and health economics (Stewart et al., 2023).

6.6.2 Closing gaps in translational healthcare

The value that outputs of the translational medicine pathway may have for earlier stages in the process should not be disregarded. Real-world studies that utilise routinely collected data may be of value in pharmacovigilance and in optimising clinical trials (Dang, 2023). Clinical trials may be emulated to create virtual simulations that mimic real-world trials using real-world data and machine learning techniques. This approach can help optimise trial

[115] https://www.rcgp.org.uk/representing-you/policy-areas/inclisiran-position-statement.

design. Real-world data are tentatively being considered by regulatory authorities as part of the drug approval process (Rudrapatna and Butte, 2020). With caveats, integrating data from multiple sources may help fill gaps in knowledge generated by randomised controlled trials. Real-world studies, while having the potential to complement randomised controlled trials, have limitations and challenges (Liu and Panagiotakos, 2022). These issues – which range from data gathering to data quality control to decision making – exist in all stages of the real-world data life cycle (Table 8).

Table 8. Limitations and challenges of real-world health data.

Data quality Real-world data are often used for other purposes than what they are originally collected for and may lack requisite information.
Machine learning and statistical methods Analytics need to be appropriate to the task to be effectively deployed in the real world.
Explainability and interpretability Modern machine learning approaches are often employed as black boxes with a lack of understanding of relationships between input and output and causal effects.
Reproducibility and replicability These are major principles in scientific research, including real-world data.
Privacy Information in real-world data is often sensitive and may include medical histories, disease status, financial details, social behaviours.
Diversity, equity, algorithmic fairness, and transparency Certain types of real-world data may be biased and unbalanced toward a certain demographic or ethnic group, be limited in terms of diversity or inclusivity, and in some cases may exacerbate disparity.

Based on Liu and Panagiotakos, 2022.

For example, it is well-recognised that real-world evidence carries risks of residual confounding by factors relevant to observations or outcomes that may not have been identified in the design of the study (Norgaard et al., 2017). In large randomised controlled trials, distribution of relevant factors is balanced between treatment groups through the randomisation of participants (Samuel et al., 2020). In observational studies, treatment may be assigned based on systematic differences that influence outcomes, thus potentially reducing the required comparability between exposure groups to make causal inference. Strategies that have been proposed to address this issue range from pseudo-randomisation and use of active comparators to Mendelian randomisation [116] (Norgaard et al., 2017). The propensity score is an estimate of the probability of receiving treatment conditional on observed baseline covariates (Austin, 2011). The propensity score assists in the design and analysis of observational, i.e., non-randomised, studies to mimic some of the characteristics of a randomised controlled trial by balancing covariates (Austin, 2011). Propensity scores are used to reduce bias in observational studies (D'Agostino, 2007). The quality of the resulting data is dependent on the adequacy of the propensity score model and the analysis method. Integration of propensity scores into the design and analysis of an observational study can help mitigate confounding by indication and improve internal validity. However, propensity scores are not a substitute for the gold standard of participant randomisation (Lalani et al., 2020).

Even after a drug enters clinical practice obstacles may conspire to detract from effective implementation[117] (Barker, 2016). It has been suggested that enduring health outcome gaps between, for example racial and ethnic groups, stem in part from differences in upstream health determinants including policies with negative impacts (Tossas et al., 2020). It is argued that inconsistent integration of community input into existing research frameworks perpetuate health inequities across the research continuum (Tossas et al., 2020). Accordingly, it seems appropriate to take a broad perspective to help ensure, for example, that relevant high-risk ethnic groups are adequately represented in clinical trials. This consideration is pertinent to the predisposition that particular ethnic groups have to cardiometabolic disorders (Sattar and Gill, 2015, Lopez-Neyman et al., 2022). The under-representation of women in cardiovascular research is well-recognised (Jin et al., 2020). This asymmetry has generated considerable concern within the research community since cardiovascular disease is the leading cause of death in women, with women from minority

[116] Exploiting random allocation of genes to test hypotheses of causality.
[117] Termed the discovery-to-delivery disconnect.

groups experiencing the highest mortality rates (Tobb et al., 2022). Issues of enrolment of women in cardiovascular trials, which is attributable to multiple factors including socioeconomic and community factors[118], requires a multifaceted approach. This may involve action at the level of the patient and clinical care as well as societal and research study leadership (Tobb et al., 2022). To date, artificial intelligence tools have infrequently been applied within cardiovascular trials (Cunningham et al., 2024). While artificial intelligence holds promise for improving recruitment to clinical trials the effectiveness of this approach requires further study (Lu et al., 2024). There is concern that artificial intelligence could reinforce existing inequities compared with traditional approaches (Cunningham et al., 2024).

Insights gained from real-world experience can also be used to inform not only best clinical practice but discovery science. This approach, designated T5, reverses the pathway along which new therapies progress from the laboratory bench to the bedside and ultimately the community. Recognition of socioeconomic and ethnicity factors that may represent barriers to prevention and management of prevalent cardiometabolic disorders falls within the scope of T5. To take an example, in a UK study, Asian ethnicity was associated with the highest prevalence of type 2 diabetes, followed by the Black and White ethnic groups. Furthermore, socioeconomic deprivation was a relatively greater risk factor for type 2 diabetes among individuals of South Asian and African ancestry, compared to those with European ancestry (Nagar et al., 2021). Encouragingly, recent UK data suggest that efforts to reduce high rates of vascular complications among South Asians with type 2 diabetes have been, at least in part, successful (Chaturvedi and Fuller, 1996, Johns and Sattar, 2017). Speculatively, these reductions reflect earlier diagnosis of diabetes and more effective cardiovascular risk factor management (Johns and Sattar, 2017).

The implications of these results extend beyond the UK. Asian populations generally have higher prevalence rates of type 2 diabetes than white Europeans (Widyahening et al., 2019). Asia accounts for more than half of the global burden of diabetes mellitus (Widyahening et al., 2019). Asian populations not only have a lower average body mass index with higher total and central adiposity for a given body weight compared with matched white populations but also have greater susceptibility to metabolic diseases and vascular complications (Ramachandran et al., 2012). These epidemiological observations have stimulated clinical research aimed at identifying the pathophysiological basis for this

[118] Social determinants of disease.

susceptibility to diabetes. The burden of cardiometabolic diseases also differs between countries and ethnic subgroups of Asia (Zhou et al., 2016, N. C. D. Risk Factor Collaboration, 2021). Investigators based in Singapore recently reported that visceral adiposity, measured using dual-energy x-ray absorptiometry, is an independent risk factor for metabolic disease, accounting for a large fraction of type 2 diabetes cases in each of three ethnic groups studied, i.e., Chinese, Malay, and Indian (Zhou et al., 2016). While visceral adiposity accounted for most of the cardiometabolic disturbances in Malay compared with Chinese individuals, the high levels of insulin resistance and type 2 diabetes among Indian individuals were not fully explained by increased adiposity (Mina et al., 2024). Such results have implications for understanding of the aetiology of cardiometabolic disease, and for interventions to improve metabolic health in Asian populations.

6.7 Machine learning in drug discovery and drug repurposing

Experimental drugs fail during clinical development for two main reasons; first, insufficient efficacy and second, safety issues. Opportunities to apply machine learning exist all stages of drug discovery (Lipinski et al., 2019, Priya et al., 2022). The ability of deep learning methods to elicit insights from input data, coupled with nonlinear input-output capabilities, augment classical machine learning techniques which rely on traditional molecular descriptors relating to the structural or physicochemical attributes of a compound (Sarkar et al., 2023). Data mining of biomedical information, e.g., *in silico* screening of chemical libraries, has led to an increase in target identification. Computer-driven ligand discovery has additional potential to reduce barriers to entry while molecules can be generated and used to identify novel targets and explore understudied targets. Generative artificial intelligence is becoming an integral to modern drug discovery and development[119]. This technology is also supporting lead generation by enabling *in silico* synthesis of new chemical compounds (Vamathevan et al., 2019, Schneider et al., 2020). The Food and Drug Administration has received hundreds of regulatory submissions for drugs that have used artificial intelligence in their discovery and development (Warraich et al., 2025). Commenting on prevalent chronic diseases, including cardiovascular disease and diabetes, the Food and Drug Administration has identified a need for a research environment that facilitates the development and evaluation of reliable biomarkers and surrogate endpoints to address the failure of many candidate therapeutics that are promising in phase 2 trials but fail in phase 3 trials

[119] In drug discovery, generative artificial intelligence enables the design of small-molecule and biologic drug candidates.

(Warraich et al., 2024a). Regulatory authorities also recognise that digital health technologies hold promise in preventing or managing chronic diseases by supporting people with, or at risk of, cardiometabolic disease through continuous monitoring and feedback on physiological measures and relevant behaviours, e.g., diet, exercise, glucose levels, medication adherence (Warraich et al., 2024b).

In discussing the role of artificial intelligence in drug development, particular mention is merited of AlphaFold, an artificial intelligence solution that was designed to predict protein structure. AlphaFold, which was developed by Google DeepMind Technologies, uses a machine learning approach that incorporates novel neural network architectures and training procedures[120] (Jumper et al., 2021). AlphaFold was the first computational method capable of regularly predicting protein structures with atomic accuracy even in cases in which no similar structure is known (Jumper et al., 2021). AlphaFold 2 predicted the structure of almost all 200 million proteins (Burki, 2024). AlphaFold 3, the latest version, is regarded as an unprecedented advance to further drug discoveries, among other research applications (Desai et al., 2024). AlphaFold 3 is regarded as turning point in the field of computational biochemistry and drug development along with receptor modulation and biomolecular development . It is anticipated that AlphaFold 3 and similar models will provide unparalleled insights into the structural dynamics of proteins and their interactions to catalyse further innovations in biomedicine (Desai et al., 2024). AlphaFold, and related computational techniques, are set to become essential tools of modern biology (Jumper et al., 2021).

Off-label use of approved drugs for unlicensed indications is often employed in clinical practice. New indications for an existing medication requires specific supportive evidence. As discussed in Chapter 3, GLP-1 receptor agonists are being evaluated for as novel therapies for neurodegenerative and other disorders (Schubert et al., 2020, Kopp et al., 2022). Drug repurposing is also considered in Chapter 2 in the context of polycystic ovary syndrome. In contrast to *de novo* drug discovery, drug repositioning explores the potential efficacy of existing drugs in order to treat diseases outside their original or current therapeutic indication. The advantages of drug repositioning over traditional drug development include reduced development time and cost (Gil and Martinez, 2021). Since the reused drug has already been shown to be safe in humans, early phase clinical trials can be omitted. Traditionally, successes in drug repurposing have mainly resulted from

[120] In 2024, Demis Hassabis and John Jumper of Google DeepMind were jointly awarded one half of the Nobel Prize in Chemistry for developing AlphaFold.

opportunistic and serendipitous findings (Pushpakom et al., 2019). An oft-cited example pertinent to cardiometabolic medicine is the repositioning of the phosphodiesterase-5 (PDE-5) inhibitor sildenafil. The drug was originally developed as a potential anti-anginal agent but instead became the first oral therapy for erectile dysfunction[121] (Goldstein et al., 2019). Repurposing the well-established anti-Parkinson's dopamine D(2) receptor agonist drug bromocriptine[122] as a glucose-lowering agent for type 2 diabetes in the United States provides another example (Defronzo, 2011).

Innovations in two areas of personalised medicine – deep learning computational approaches to associate drug effects with diseases and predictive model systems to screen drugs for therapeutic effects – can provide a pipeline to systematically repurpose drugs to treat cardiovascular and metabolic diseases such as insulin resistance (Abdelsayed et al., 2022). Machine learning methods can rapidly identify drug candidates for diseases that can then be verified by in vivo or in vitro experiments or using databases, including real world clinical data (Cousins et al., 2024, Timmons et al., 2022). For example, Wu et al. used imputed human disease gene expression signatures, drug perturbation data, and clinical electronic health record data to identify candidate drugs to repurpose for the treatment of hyperlipidaemia and hypertension (Wu et al., 2022).

6.8 Reversing the translational research pathway

A reappraisal of the translational pathway invokes a community-to-bench model in which feedback from high-quality population-derived data informs discovery science and clinical trials (Tossas et al., 2020). In this model, drug discovery and development takes advantage of translational research as a bidirectional discipline. While forward translation is a one-way linear paradigm with a distinct beginning and end reverse translation can allow full advantage to be taken of clinical trial data from the most predictive model, i.e., humans (Stoch, 2025 In press). In this way, translational research becomes a feedback loop in which each step continuously influences others. Reverse translational research also encompasses drug repurposing, e.g., exploring pharmacotherapeutics that modulate adipocytokines (Shakhnovich, 2018).

[121] Erectile dysfunction is a harbinger of cardiovascular disease in men.
[122] As a Quick-Release formulation.

As discussed in Section 6.6, the original research papers presented in this dissertation may be considered in the context of the translational research pathway. This commenced with stages T0 T1 where epidemiological modelling was used to determine whether a polycystic ovary syndrome phenotype could be defined in post-menopausal women to (a) quantify risk of atherosclerotic cardiovascular disease and (b) provide mechanistic insights that might inform the quest for new therapeutics. Moving to stage T2, the potential benefits and adverse effects of cardiometabolic therapeutics were considered in a phase 1b study of a novel class of oral glucose-lowering medication, AZD1656. Extending the perspective to encompass both carbohydrate and lipid metabolism, post-marketing investigator-initiated phase 4 studies (T3) explored nonclassic vascular and metabolic actions of statins. These tested the hypothesis that drug-induced insulin resistance or alternations in microvascular function are implicated in statin-associated new-onset diabetes. T4 of the translational medicine pathway is illustrated by a proof-of-concept study evaluating the utility of machine learning to optimise the use of well-established and novel cholesterol-lowering medications for high-risk patients with cardiometabolic disease in real world clinical practice.

In extending translational research to the T5 stage, i.e. reverse translation, the studies presented in Chapter 4 and Chapter 5 have implications for more proximal stages of the translational medicine research pathway (Figure 7), i.e., taking research observations generated from the community back to the bedside. Statin-induced diabetes has stimulated experimental clinical research aimed at identifying causal mechanisms which is still ongoing but, as discussed, remains blighted by suboptimal study designs. The realisation that high-intensity statin therapy has limitations in terms of achieving current evidence-based cholesterol goals in high-risk individuals has generated an emerging consensus in favour of greater use of combination statin + non-statin therapy. Putative benefits of this approach include (a) bringing more patients to target (b) improving adherence with therapy and (c) reducing the risk of new-onset diabetes. As demonstrated in Chapter 5, machine learning can be used to ensure best practice in prescribing of cholesterol-lowering therapy. Since local populations may respond differently to medications compared with participants in clinical trials the use of machine learning could facilitate the evolution of precision cardiometabolic medicine (Coles et al., 2021).

6.9 Potential of precision medicine to improve healthcare outcomes

Innovative approaches are required to address aspects of inertia in the management of cardiometabolic disorders. Precision medicine is an emerging field that may help address gaps in the translational medicine pathway. According to the US Food and Drug Administration, precision medicine – also known as personalised medicine – is an innovative approach to tailoring disease prevention and treatment that considers individual differences in factors including genes, environments, and lifestyles. The goal of precision medicine is to target the right treatments to the right patients at the right time[123] (Food and Drug Adminstration., 2018). Improving risk prediction and efficacy of medications, reducing side-effects, minimising drug-drug interactions, and reducing health disparities are among potential benefits of precision medicine (Noyes et al., 2021, Ahmad et al., 2024, Pearson et al., 2024, Ingelman-Sundberg and Pirmohamed, 2024, Singh et al., 2024).

It is hypothesised that precision medicine could accelerate the pace of innovation and ensure more effective clinical implementation of new treatments at several stages of the translation process (Barker, 2016). Precision medicine spans diagnostics, prediction, prevention, prognosis, treatment, and monitoring (Chung et al., 2020, Franks et al., 2023)[124]. Novel insights into disease aetiology and pathophysiology facilitate translatable precision medicine in cardiometabolic and other disorders. The role of the incretin system in the pathogenesis of type 2 diabetes is a prominent example (Drucker and Nauck, 2006). However, some investigators have expressed scepticism about claims for precision medicine for complex diseases such as type 2 diabetes demanding that existing evidence thresholds be exceeded before implementation (Griffin, 2022). Precision medicine is most readily developed for monogenic diseases, e.g., sulfonylurea therapy as the treatment of choice for certain uncommon inherited forms diabetes caused by mutations in the hepatocyte nuclear factor 1α gene (Pearson et al., 2003). For complex polygenic diseases within the cardiometabolic nexus, identifying specific therapies according to molecular genetics approach is not feasible (Shields et al., 2023). Other strategies that could be more readily implemented, such as patient stratification based on clinical and routinely collected biochemical criteria, may be more appropriate. This approach has been successfully employed in a three-drug, three-period, randomised crossover trial to identify second- and third-line glucose-lowering drug classes most likely to deliver the greatest glycaemic

[123] Also known as P4 medicine: predictive, preventive, personalised, and participatory.
[124] While precision and personalised medicine may contain nuanced distinctions the term precision medicine is considered to capture the reality of the discipline more accurately.

reduction for individual patients with type 2 diabetes receiving metformin or metformin-sulfonylurea (Shields et al., 2023).

The potential utility of pharmacogenomics has attracted considerable attention in the context of precision medicine for metabolic and cardiovascular disorders (Li and Florez, 2022, Ghorbannezhad et al., 2024, Van Den Broek et al., 2024). While numerous studies provide evidence for the effect of specific genotypes on the outcomes of vascular drugs, to date the adoption of pharmacogenetic testing in clinical practice has been slow (Ross et al., 2023). An example of progress in this field, based on evidence of cost-effectiveness, is the recommendation by the National Institute for Health and Clinical Excellence to test for the CYP2C19 genotype in people with an acute ischaemic stroke or a transient ischaemic attack to determine whether clopidogrel is a suitable antiplatelet drug (National Institute for Health and Clinical Excellence, 2024). Among challenges to the clinical implementation of pharmacogenetic technologies more generally are the need to modify existing clinical pathways and to educate clinicians (Pirmohamed, 2023).

Datasets of increasing granularity are being provided by omics profiling technologies (Hu and Jia, 2021). Multi-omics relevant to the practice of precision medicine in cardiometabolic disorders include genomics, proteomics, and metabolomics (Tahir and Gerszten, 2020). The large volumes of complex data generated by high throughput omics are amenable to analysis using machine learning (Reel et al., 2021).

Personalised precision approaches to medical nutrition therapy are gaining momentum. Approximately 70% of the burden of cardiovascular disease is attributable to modifiable risk factors, such as high blood pressure, dietary habits, overweight or obesity, and tobacco use (Yusuf et al., 2020). Of these factors, nutrition is a major modifiable factor in the development and management of long-term cardiometabolic disorders (Yu et al., 2018). Current dietary recommendations – based primarily on studies of self-reported eating behaviours that are recognised to be of limited accuracy – do not consider individualised responses to macronutrients or timing of food consumption (Nunes et al., 2024). Emerging data indicate a role for personalised nutrition based on genetic, phenotypic, medical, behavioural, and/or lifestyle characteristics (Cross et al., 2024). A recent randomised controlled trial in 347 subjects reflective of the US adult population demonstrated that a personalised diet improved aspects of cardiometabolic health compared to standard dietary advice (Bermingham et al., 2024). Personalised dietary advice was based on an app-based

food characteristics, individual postprandial glucose and triglyceride responses to foods, microbiome data and health history. Machine learning was used to assess the link between microbiome compositions with dietary and metabolomic outcomes. After 18-weeks of diets based on personalised vs. standard US recommendations, significant reductions were observed in triglycerides with improvements in LDL-cholesterol concentrations in highly compliant participants. Variations in the relative abundance of microbiome species discriminated individuals based on changes in weight and hip circumference in the personalised intervention group only (Bermingham et al., 2024). The US National Institutes of Health Nutrition for Precision Health (NPH) study will recruit 10,000 adults from diverse backgrounds to gain insights into how individuals respond differently to food. Artificial intelligence methods will be used analyse information provided by participants and from electronic health records in order to develop algorithms that predict individual metabolic responses to dietary patterns (National Institutes of Health, 2024). The stated objective of the project is to offer customised nutritional advice to improve overall health.

Deployment of precision medicine in diagnostics, patient stratification and individualised therapeutics is dependent on the use of clinical research methods in the proximal stages of drug development that are safe, accurate and reproducible. It is noteworthy that, beyond discussions of strengths and limitations of individual published studies, this guiding principle currently receives relatively scant acknowledgement in the scientific literature.

6.10 Conclusions

The papers presented in this dissertation serve to illustrate stages of the translational medicine pathway of relevance to prevalent cardiometabolic disorders in men and women. This commenced by defining health problems and potential novel therapeutic targets (T0, T1) and proceeded over successive chapters via the T2 and T3 stages of drug development to optimising implementation of approved medications in real-world clinical practice (T4). The individual studies uphold the utility of the translational medicine pathway while pointing to refinements that could be incorporated in future research (T5). Critical evaluation of the studies also offers insights into strategies – traditional and emerging – that may help to address gaps in translational medicine. For example, adverse cardiometabolic effects of novel pharmacotherapeutics are not always predictable or evident from early-phase clinical trials (Chapters 3 and 4).

The original research papers were selected to demonstrate how the principles of precision medicine may be applied to multiple stages of the translational medicine pathway. Considerations apposite to scientific rigour in human cardiometabolic research include the need for appropriate and ethical study designs – with explicit recognition of specific strengths and limitations – along with comprehensive participant phenotyping that addresses the heterogeneity inherent in the disorders of interest. Use of clinical research methods that are safe, accurate, reproducible, and relevant to normal physiology has been emphasised throughout the dissertation. Chapter 4 (T2 T3) explored pathophysiological metabolic-vascular and microvascular-macrovascular intersections which merit further attention within the cardiometabolic disease nexus.

Collectively, the papers provide support for the contention that insights gained from later stages of the translational cardiometabolic medicine pathway have potential to usefully inform earlier stages including discovery science and clinical trials (T5). This hypothesis is testable within the ever-evolving disciplines of drug discovery and clinical development of novel therapies.

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