From Measurement to Management of Kidney Function: Population-Based Insights into Biomarker Discordance, Risk Factors, and Outcomes

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Abstract

Chronic kidney disease is a major risk factor for cardiovascular diseases and mortality, yet both the measurement of kidney function and the management of kidney-related risk in the general population require further exploration. This thesis aims to move from measurement to management of kidney function by examining modifiable behaviours, identifying patterns of kidney function and their associations with adverse outcomes, and evaluating treatment strategies and their potential cardiorenal benefits.

A total of five published manuscripts were included in this thesis to achieve the above aims. These studies utilized data from the UK Biobank and several Swedish national registers.

The first study in Chapter 4 presents a systematic review and meta-analysis of intervention studies in generally healthy populations, examining how changes in physical activity influence kidney-related biomarkers. Higher physical activity was consistently associated with higher serum creatinine, while other biomarkers showed little or no change. The second study in Chapter 5 uses UK Biobank data to examine how changes in physical activity relate to changes in kidney function in more than 11,000 participants free of chronic kidney disease at baseline. The findings differed by biomarker. When rapid decline was defined using creatinine-based estimated glomerular filtration rate (eGFR), lower physical activity appeared protective, whereas cystatin C-based eGFR showed the opposite pattern, with higher activity associated with lower risk. The creatinine-cystatin C based eGFR showed no clear association.

The following two studies in Chapters 6 and 7 focus on discordance between creatinine-based and cystatin C-based eGFR. Chapter 6 synthesises evidence from 18 studies and shows that individuals with lower cystatin C-based eGFR relative to creatinine-based eGFR have substantially higher risks of mortality and cardiorenal events, while those with higher cystatin C-based eGFR tend to have lower risks. Chapter 7 extends this work in a large UK Biobank cohort, showing that 15.5% of the study population has discordantly lower cystatin C-based eGFR and that this discordance is strongly associated with all-cause mortality, particularly in older (65 years and above) and obese (BMI≥30 kg/m^2) people, independent of established risk factors.

The last study in Chapter 8 shifts to pharmacological management in type 2 diabetes, comparing oral fixed-dose combination therapy with loose-dose combinations using Swedish national registers. In a propensity score matched cohort of 27,766 people, fixed-dose combinations were associated with a lower risk of heart failure, especially in older adults (65 years and above), with 47% of this benefit being mediated by improved medication adherence. No clear differences were observed for other cardiovascular or kidney outcomes.

In conclusion, this thesis provides population-based evidence that selection of kidney function biomarkers may play an important role in evaluating health outcomes, while intraindividual discrepancies in kidney function may reveal distinct physiological or pathological processes. In addition, pharmacological strategies may affect cardiorenal risk. Key limitations include reliance on estimated rather than measured GFR, potential residual confounding, the inability to establish causality inherent to observational analyses, and limited generalisability to more diverse populations.

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Publications as first author in the PhD period

1. **Liu Q**, Welsh P, Celis-Morales C, Lees JS, Mark PB, Pazzagli L*. Fixed-dose vs loose-dose combination antidiabetic therapy and cardiorenal outcomes in type 2 diabetes: a nationwide comparative effectiveness study. *Cardiovasc Diabetol*. 2025 Sep 23;24(1):365. doi: 10.1186/s12933-025-02936-w. (Q1/IF=10.6)
2. **Liu Q**, Celis-Morales C, Lees JS, Sattar N, Ho FK, Pell JP, Mark PB, Welsh P*. Discordance between Cystatin C-Based and Creatinine-Based Estimated Glomerular Filtration Rate and Mortality in the General Population. *Clin Chem.* 2025 Aug 1;71(8):858-869. doi: 10.1093/clinchem/hvaf063. (Q1/IF=6.3)
3. **Liu Q***, Welsh P, Celis-Morales C, Ho FK, Lees JS, Mark PB. Discordance between cystatin C-based and creatinine-based estimated glomerular filtration rate and health outcomes in adults: a systematic review and meta-analysis. *Clin Kidney J*. 2025 Jan 8;18(3):sfaf003. doi: 10.1093/ckj/sfaf003 (Q1/IF=3.9)
4. **Liu Q***, Celis-Morales C, Lees J, Mark P, Welsh P. Effect of exercise on kidney-relevant biomarkers in the general population: a systematic review and meta-analysis. *BMJ Open*. 2025 Jan 8;15(1):e093017. doi: 10.1136/bmjopen-2024-093017. (Q2/IF=2.4)
5. **Liu Q***, Celis-Morales C, Sattar N, Welsh P. Association of glycaemic control with intraocular pressure in a large general population: Results from the UK Biobank. *Diabetes Obes Metab*. 2024 Nov;26(11):5192-5201. doi: 10.1111/dom.15865.(Q1/IF=5.7)
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8. **Liu Q***, Yang M, Shen S, Gong C, Lan Z. Cardiac Abnormalities in Patients With Severe Fever With Thrombocytopenia Syndrome: A Systematic Review. *Open Forum Infect* Dis. 2023 Oct 13;10(11):ofad509. doi: 10.1093/ofid/ofad509. (Q2 /IF=3.8)

9. **Liu Q***, Gong C, Geng Y, You J. Elevated alanine transaminase is nonlinearly associated with in-hospital death in ICU-admitted diabetic ketoacidosis patients. *Diabetes Res Clin Pract.* 2023 Mar;197:110555. doi: 10.1016/j.diabres.2023.110555. (Q1/IF=5.1)
10. **Liu Q**, You J, Zhong M, Wu Z, Geng Y, Huang C*. Hemoglobin level is negatively associated with sarcopenia and its components in Chinese aged 60 and above. *Front Public Health*. 2023 Mar 13;11:1081843. doi: 10.3389/fpubh.2023.1081843. (Q1/IF=5.2)

Conference presentations in the PhD period

1. 2025 The 61st EASD Annual Meeting
   Location: Vienna, Austria
   Late-breaking Abstract OP #31
   Fixed-dose vs loose-dose combination antidiabetic therapy and cardiorenal risk in type 2 diabetes. **Q. Liu**, P. Welsh, C. Celis-Morales, J. Lees, P. Mark, L. Pazzagli*

2. 2024 The 60th EASD Annual Meeting
   Location: Madrid, Spain
   Short Oral #394
   Association of glycaemic control with intraocular pressure in a large general population: results from the UK Biobank.
   Q. Liu*, C. Celis-Morales, N. Sattar, P. Welsh

   Shor Oral #397
   Genes involved in the manifestation of proliferative diabetic retinopathy in people with diabetic retinopathy.
   Q. Liu*, A. Aman, C. Celis-Morales, P. Welsh

Acknowledgement

This acknowledgement has not been retouched, and the writing style is casual rather than academic. This is because only the raw one can be a faithful reflection of my thoughts, offering the "original taste." In the storm of retouching that no manuscript can escape from, publishing a raw text has become a luxury.

It is clear that, at the moment of drafting this acknowledgement, my PhD study is coming to its end, theoretically. After carefully considering how to arrange the sequence, I think the most rational approach is to express my thankfulness by "relevancy," from "directly relevant" to "indirectly relevant."

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There are many others that I should say thank you. I thought about listing their names, yet such lists are often read only briefly, much like the credits at the end of a film. People come and leave, and now I am leaving. So, let us end this part here, and start the next one.

Author's Declaration

I declare that this thesis is the result of my own work. In those cases where the contributions of others were used, explicit references were included. The manuscripts included are open access; therefore, they can be openly shared in this thesis.

The contents of this thesis have not been submitted for any other degree at the University of Glasgow or any other institution.

Qiaoling Liu
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1. Chapter 1 – Background

1.1. Structure and function of the nephron

The nephron is the fundamental structural and functional unit of the kidneys, with each kidney containing about one million nephrons.[1] Its specialized structure allows it to perform filtration, reabsorption, and secretion, all vital for removing metabolic waste and maintaining homeostasis.

Compromised nephrons can lead to impaired filtration, reduced waste clearance, and electrolyte disturbances. Since the nephron is often the primary site of damage in chronic diseases like diabetes and hypertension, understanding its structure and function is critical for comprehending disease mechanisms and developing effective treatments.

Structure of the nephron

A nephron has two main parts: the renal corpuscle and the tubular system.[1] Located in the renal cortex, the renal corpuscle initiates urine formation by filtering the blood. The tubular system then modifies and regulates this primary filtrate, progressively turning it into final urine.

Structure of the renal corpuscle

The renal corpuscle has two parts: the glomerulus and Bowman's capsule. The glomerulus is a cluster of capillaries. Blood enters the glomerulus via the afferent arteriole and passes through the capillary endothelium, basement membrane, and slit diaphragms between podocyte foot processes to form the filtrate.[1] The endothelial cells of the glomerular capillaries have fenestrations that allow small molecules to pass through. The basement membrane, which carries a negative charge, prevents large molecules from passing. The podocyte forms the final layer of the filtration barrier, which consists of interdigitating foot processes.

Bowman's capsule surrounds the glomerulus and consists of two layers.[2] The inner visceral layer is composed of podocytes that closely adhere to the surface of the glomerular capillaries. The outer parietal layer is formed by flattened epithelial cells. The space between these two layers is called the capsular space, which collects the filtrate. From here, the primary urine flows into the renal tubule.[3] (Figure 1-1)

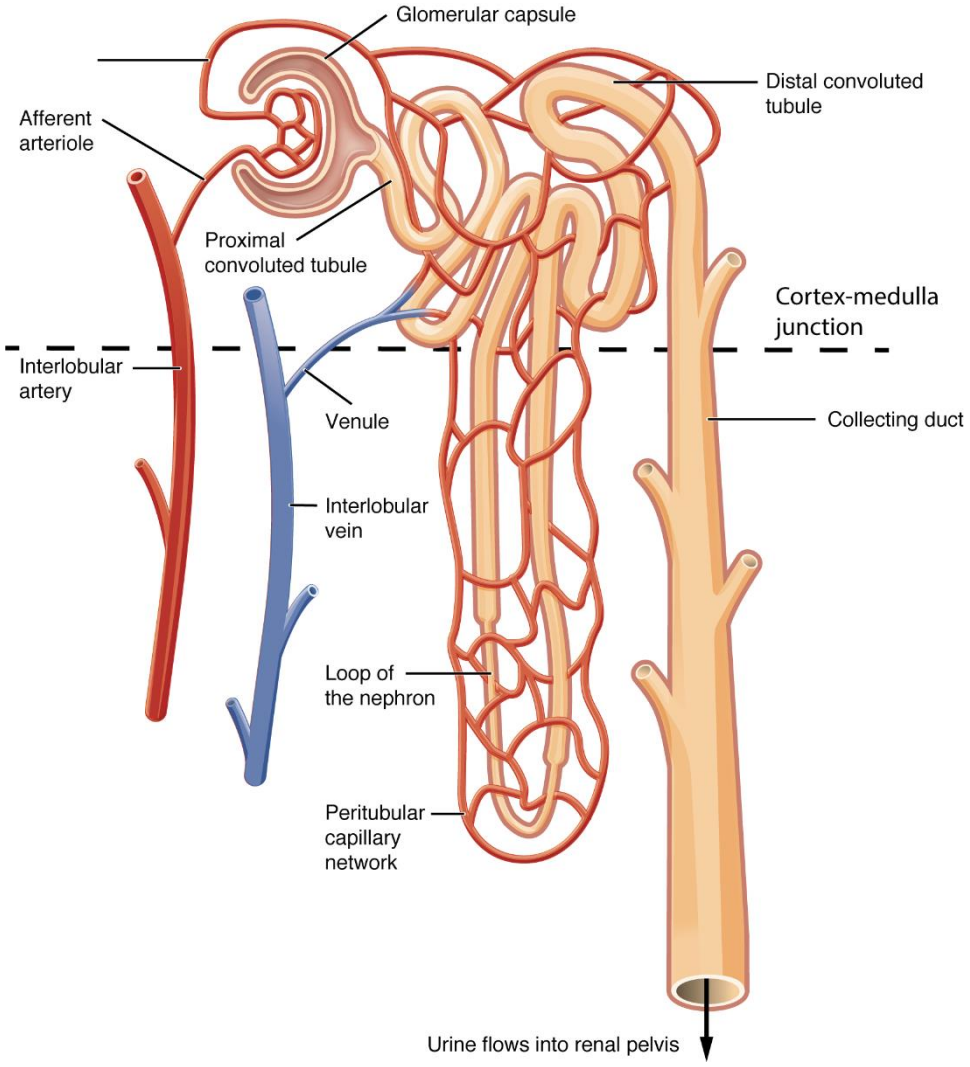

Figure 1-1 Structure of a nephron (Adapted from Lindsay B, 2025) [3]

Segmental structure of the renal tubule system

The renal tubule system begins at Bowman's capsule and is sequentially divided into the proximal tubule, loop of Henle, distal tubule, and collecting duct.[4] Each segment has distinct structural and functional characteristics (Figure 1-2) [5]:

- **Proximal tubule**: It consists of a convoluted part and a straight part. Approximately 65% of water and sodium ions, along with nearly all glucose and amino acids, are reabsorbed here.[6] Its epithelial cells are rich in mitochondria and have a brush border, which facilitates active transport.

- **Loop of Henle**: Also called “loop of the nephron”. It consists of a thin descending limb and a thick ascending limb. The two limbs form a U-shaped loop that extends into the renal medulla. The descending limb is permeable to water,

while the ascending limb is impermeable to water but actively transports sodium, potassium, and chloride ions. This countercurrent mechanism creates a hyperosmotic environment in the medulla, aiding in urine concentration.

- **Distal tubule**: Located in the renal cortex, it regulates the reabsorption and secretion of sodium, potassium, calcium, and hydrogen ions. Its function can be influenced by hormones like aldosterone, playing a key role in fine-tuning of the internal environment.

- **Collecting duct**: It receives fluid from the distal tubules of all the nephrons and ultimately transports urine to the renal pelvis. The collecting duct can modify its water permeability under the influence of antidiuretic hormone (ADH), making it a crucial site for the regulation of urine concentration.

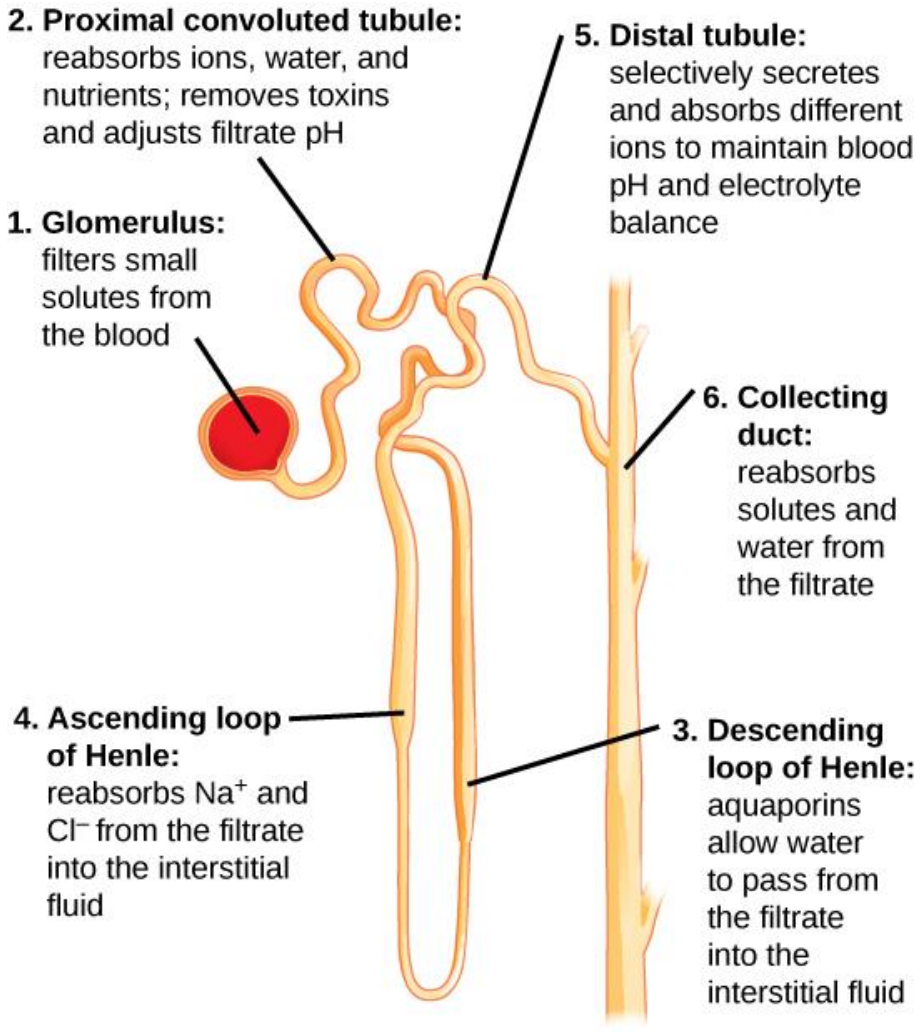

Figure 1-2 Structure of the tubule system (Adapted from Mary Ann C, 2018) [5]

Main functions of the nephron

The main functions of the nephron are filtration, reabsorption, and secretion.

Filtration occurs in the renal corpuscle, where high pressure in the glomerular capillary forces water and small molecules from the plasma into the capsular space, forming primary urine. A healthy adult produces about 180 litres of this primary urine daily.[2]

As primary urine flows through the renal tubule, the majority of its water, electrolytes, and nutrients are reabsorbed into the bloodstream by tubular epithelial cells through active or passive transport. The proximal tubule is the most active site of reabsorption, while the distal tubule and collecting duct are responsible for the fine-tuning of fluid and electrolyte balance. Finally, only about 1–2 litres of final urine are excreted daily.[2]

Meanwhile, tubular epithelial cells secrete certain metabolic waste products, drugs, and hydrogen ions from the blood into the tubular lumen. This process contributes to acid-base balance and the elimination of harmful substances.

Regulation of nephron function

Nephron function is regulated by multiple mechanisms, including blood flow, the sympathetic nervous system, and hormones. During fluctuations in blood pressure, the kidneys can maintain a stable glomerular filtration rate by adjusting the diameter of the afferent and efferent arterioles. Sympathetic nervous stimulation causes renal blood vessel constriction, which reduces blood flow and filtration pressure.

In terms of hormonal regulation, the renin-angiotensin-aldosterone system (RAAS) modulates the reabsorption of sodium and water to maintain blood volume and pressure. ADH influences the collecting duct's water permeability to regulate fluid concentration and water balance. These mechanisms enable the nephron to effectively maintain homeostasis under various conditions.

1.2. Glomerular filtration and its structural and physiological foundations

Glomerular filtration is the primary function of the nephron, a process by which water and solutes are removed from the bloodstream to form the filtrate.[7] This occurs within the glomerulus, which consists of a capillary network, mesangial cells, endothelial cells, and

the surrounding Bowman's capsule.[2] Blood enters the glomerulus through the afferent arteriole and exits via the efferent arteriole. In the process, water and solutes are filtered by three specialized filtration barriers, each of which has a distinct filtering function.

The first filtration barrier is the fenestrated endothelial cells, in which glycocalyx and transcellular pores work together to offer filtration.[8, 9] The luminal side of the cell is coated with a thick layer of glycocalyx. Composed of negatively charged glycoproteins and polysaccharides, the glycocalyx selectively repels proteins through electrostatic interactions. Mice with impaired glycocalyx exhibit albuminuria and proteinuria, highlighting the role of the glycocalyx layer in filtration.[10]

These endothelial cells contain numerous transcellular pores ("fenestration"), typically 60-80 nm in diameter.[8] In other words, these cells resemble a hollow honeycomb. The pores on the luminal side of the endothelial cells, which face the capillary lumen where blood enters the glomerulus, allow free passage of water and small solutes (around 70 nm in diameter) while effectively blocking cells such as erythrocytes (approximately 7,000 nm in diameter).[11] As blood flows into the glomerular capillaries, glomerular capillary hydrostatic pressure (P_{GC}) drives water and small molecules through these pores.[12] After passing through the pores, the filtered water and small molecules exit on the opposite side and immediately meet the second filtration layer.

The second filtration barrier is the glomerular basement membrane (GBM), which is approximately 300 to 350 nm thick.[13] The GBM lies between the fenestrated endothelial cells and the podocytes. Thus, after water and small solutes pass through the pores of the endothelial cells, they reach the GBM for the second stage of filtration.

GBM filters fluids through two main mechanisms. The first is physical filtration. Structural components of the GBM, such as type IV collagen, form the dense network that allows the passage of molecules with a radius less than 4 nm.[13, 14] For a molecule with a diameter between 4 and 8 nm, the molecule's charge (positive/negative) and size determine its passage. For those with a diameter exceeding 8 nm, no passage is allowed.[7]

The second mechanism is electrostatic filtration. GBM contains abundant heparan sulfate proteoglycans (e.g., perlecan). Perlecan possesses numerous sulfate groups that dissociate

hydrogen ions under normal pH, which results in negatively charged sulfate residues. As a result, the GBM functions as a negatively charged electrostatic barrier.[15] Plasma proteins like albumin and immunoglobulin G (IgG) carry a net negative charge and are therefore repelled by the similarly charged GBM. It should be noted that albumin has a diameter of 3.6 nm. Although negatively charged, the albumin molecule is close to the filtration threshold of the GBM. Therefore, albumin cannot be completely restricted from passing through the GBM.

After passing through the GBM, the filtrate encounters the third filtration barrier: the podocyte layer, consisting of interdigitating foot processes of podocytes. The narrow gaps between adjacent foot processes are called filtration slits. The width of these slits can be dynamically regulated by podocyte cytoskeletal contraction. Filtration slits are bridged by a specialized structure known as the slit diaphragm. This slit diaphragm has pores on its surface which act as a size-selective barrier.[16] The third barrier also plays a crucial role in preventing the leakage of albumin that may have passed through the first two layers.

Physical filtration is not the only mechanism of the podocyte layer. The surface of podocytes is covered by a layer of glycocalyx.[8] The glycocalyx is mainly formed by sialic acid and glycosaminoglycans. These components dissociate and carry negative charges that further repel negatively charged plasma proteins. Moreover, because podocytes carry negative charges and the filtration slits are formed between adjacent podocytes, electrostatic repulsion helps prevent podocyte foot processes from adhering to each other. After three filtration barriers, water and solutes enter Bowman's capsule, forming the filtrate. After tubular reabsorption and secretion, this filtrate becomes the final urine.

Aside from physical and electrical barriers, hydrostatic and oncotic pressures within the glomerulus also play an important role in filtration.[3, 17] In brief, the net filtration pressure of the glomerulus can be defined as follows:

Net filtration pressure=$(P_{GC}-P_{BS}) - (\pi_{GC}-\pi_{BS})$

Table 1-1 Starling forces involved in glomerular filtration

	Name	Definition	Direction	Mechanism
P_{GC}	Glomerular capillary hydrostatic pressure	The pressure generated by blood flow through the glomerular capillaries.	Favors filtration (drives fluid into Bowman's capsule)	The main driving force, approximately 55 mmHg
P_{BS}	Bowman's space hydrostatic pressure	The opposing pressure exerted by the filtrate in Bowman's capsule.	Opposes filtration (resists movement into Bowman's capsule)	The main opposing force, approximately 15 mmHg
π_{GC}	Glomerular capillary oncotic pressure	The oncotic pressure generated by plasma proteins that remain in the blood.	Favors reabsorption (draws water back into the capillaries)	The second opposing force, approximately 30 mmHg.
π_{BS}	Bowman's space oncotic pressure	The oncotic pressure generated by the protein concentration in the filtrate.	Favors filtration (pulls water into Bowman's capsule)	Usually negligible, as an intact filtration barrier effectively prevents proteins from entering Bowman's capsule

Beyond structural factors and pressure, glomerular filtration is also regulated by neurohumoral mechanisms. For example, increased sympathetic nervous activity can induce renal vasoconstriction, reducing blood flow into the glomerulus and thereby lowering P_{GC}, which leads to a decrease in glomerular filtration rate (GFR). Activation of the RAAS, on the other hand, can constrict the efferent arteriole, increase P_{GC}, and help maintain GFR.

Under pathological conditions, the structure and function of the glomerular filtration barrier can be affected. For example, diabetic nephropathy involves GBM thickening and podocyte loss, while systemic lupus erythematosus can cause immune complex deposition that "blocks" filtration.

Clinically, kidney function is expressed as estimated GFR (eGFR) using creatinine-based or cystatin C-based equations. Due to differences in molecular weight between these markers, a novel filtration pattern, Selected Glomerular Hypofiltration Syndrome (SGHS), has been proposed. In SGHS, creatinine-based eGFR is significantly higher (or lower, although less common) than cystatin C-based eGFR, differing by more than 15 ml/min/1.73 m^2 or 30 to 40 percent.[18] This suggests reduced filtration of larger molecules. One proposed key mechanism underlying SGHS is shrunken pore syndrome, where GBM thickening leads to narrowing or elongation of pores, limiting the passage of high-molecular-weight solutes.[19] The exact mechanism is still under exploration.

Overall, renal filtration is achieved collectively by the three barriers of the glomerulus. The effectiveness of these barriers is affected by various structural and physiological factors. Aside from filtration, the kidneys are also important in regulating body fluid and in maintaining electrolyte balance.

1.3. Integrative roles of the kidneys in fluid, electrolyte, and metabolic homeostasis

The kidneys are important for maintaining homeostasis, including fluid, electrolyte, and metabolic balance. As sensors and effectors, the kidneys coordinate physiological responses to both acute and chronic disturbances, sustaining systemic homeostasis.

Fluid volume and osmolality homeostasis

The kidneys control extracellular fluid volume and plasma osmolality via excretion and reabsorption of water and sodium to maintain fluid homeostasis.[20]

Water balance is primarily regulated by arginine vasopressin (AVP). During dehydration, increased AVP secretion leads to the excessive insertion of aquaporin-2 proteins into the cell membranes of collecting ducts.[20] Aquaporin-2 is a transmembrane protein that allows water to pass through. The increased insertion of this protein and the hyperosmolar environment of the renal medulla allow a large amount of water to be reabsorbed from the urine back into the bloodstream, thereby restoring water balance during dehydration.[21] Conversely, when the body retains excess water, AVP secretion decreases and more water is excreted. This process of water regulation is closely associated with sodium balance,

which is the primary determinant of extracellular fluid volume and a key factor in maintaining osmotic stability.

Sodium balance is mainly regulated by the RAAS and atrial natriuretic peptide (ANP). When blood volume or pressure decreases, the kidneys secrete renin, triggering the production of angiotensin II (Ang II).[22] Ang II causes vasoconstriction to raise blood pressure and stimulates the production of aldosterone. Elevated aldosterone levels promote the reabsorption of sodium in the renal tubules with water following osmotically, increasing blood volume and pressure.[23] When blood volume is excessive, renin secretion decreases, while the heart releases ANP in response to volume overload. In addition to its natriuretic effect, ANP acts on the kidneys by increasing the hydrostatic pressure in the glomerular capillaries.[24] This drives excess water into the tubules, increasing urine output to help maintain effective circulating volume and osmotic stability.

Electrolyte homeostasis

The kidneys maintain plasma electrolyte concentrations by selectively reabsorbing or excreting key ions. Among the most important are sodium and potassium, which are the primary extracellular and intracellular cations, respectively.

The kidneys maintain potassium balance primarily through secretion, though reabsorption is also involved. While most filtered potassium is reabsorbed in the proximal tubule and loop of Henle, the final amount excreted in the urine is determined by secretion in the distal tubules and collecting ducts.[25] This secretion process is mainly regulated by aldosterone and blood potassium levels.

Beyond sodium and potassium, the kidneys also regulate the homeostasis of calcium and phosphate via filtration, reabsorption, and hormonal regulation.[26] Parathyroid hormone (PTH) inhibits phosphate reabsorption in the proximal tubules while enhancing calcium reabsorption in the distal tubules. Fibroblast growth factor 23 (FGF23) mainly acts on the proximal tubules to suppress phosphate reabsorption.[26] In addition, the kidneys convert vitamin D into calcitriol, which promotes intestinal absorption of calcium and phosphate. These mechanisms work together to support mineral balance.

Acid–Base homeostasis

The kidneys maintain the body's acid-base balance through several key mechanisms.[27] First, the kidneys reabsorb nearly all filtered bicarbonate in the proximal tubules, preventing the loss of it in the urine. Second, to neutralize the body's acid load, tubular cells actively secrete hydrogen ions into the urine. This process occurs mainly in the proximal tubule and the collecting ducts. Third, during chronic acidotic states, the kidneys generate new bicarbonate through ammoniagenesis. Meanwhile, they excrete hydrogen ions and return the newly formed bicarbonate to the circulation. These coordinated actions allow the kidneys to compensate for metabolic acid production and maintain systemic pH.

Endocrine functions and metabolic homeostasis

The kidneys perform crucial metabolic and endocrine functions. As endocrine organs, they produce erythropoietin in response to tissue hypoxia, thereby stimulating erythropoiesis in the bone marrow.[28] Juxtaglomerular cells secrete renin, initiating the RAAS to influence systemic vascular tone and sodium retention.[22]

In the proximal tubule, the enzyme 1α-hydroxylase converts 25-hydroxyvitamin D into calcitriol, which enhances the intestinal absorption of calcium and phosphate.[29] Furthermore, the kidneys contribute to gluconeogenesis and ammonia production[30], and they are central to eliminating metabolic waste products like urea and creatinine.

Central coordination of homeostasis

The kidneys act as a central coordinator of homeostasis, receiving and interpreting signals from the endocrine, nervous, and cardiovascular systems to adjust function according to the body's demands. This integration ensures that balances are maintained across a wide range of physiological conditions.

Impairment of the kidneys leads to widespread disruptions in homeostasis, including volume overload, electrolyte disturbances, metabolic acidosis, anaemia, and mineral bone disorders. A comprehensive understanding of renal physiology is therefore essential for the study of both health and disease.

1.4. Renal regulation of fluid and osmolality homeostasis

The kidneys play a vital role in managing fluid volume and osmolarity through hormonal systems and local feedback mechanisms that maintain the body's internal stability.

Volume regulation and the initial concentration of the primary filtrate

Water reabsorption is the key mechanism for regulating fluid volume. After glomerular filtration, about 65% of the filtered water in the primary filtrate is reabsorbed in the proximal tubule via passive osmosis. The reabsorption is driven by osmotic gradients created by active solute transport.[31]

On the basolateral membrane, Na^+/K^+-ATPases actively pump Na^+ out of tubular epithelial cells.[32] This creates a gradient that facilitates sodium entry from the tubular lumen through sodium-glucose cotransporters and sodium-hydrogen exchangers. The resulting osmotic shift promotes the passive movement of water, and water enters the epithelial cells. From there, it continues into the interstitial space and peritubular capillaries.

Loop of Henle and the medullary concentration gradient

After leaving the proximal tubule, the primary filtrate enters the loop of Henle for concentration. The thin descending limb is highly permeable to water but has minimal solute transport capability.[31] As the filtrate moves into the hyperosmotic medullary interstitium, water passively diffuses out, and the tubular fluid's osmolality increases.

In contrast, the thick ascending limb is largely impermeable to water. It actively reabsorbs Na^+, K^+, and Cl^- via Na^+-K^+-$2Cl^-$ (NKCC2) cotransporters.[31] This action reduces luminal osmolality and amplifies the medullary osmotic gradient. Epithelial cells in this segment lack major hormone receptors, so reabsorption here is largely hormone-independent and driven by local ionic gradients. Once the fluid passes through the Loop of Henle, it enters the distal convoluted tubule (DCT).

Fluid regulation at the distal convoluted tubule

Upon entering the DCT, the dilute filtrate from the thick ascending limb undergoes hormone-mediated fine-tuning. The DCT is largely impermeable to water and primarily reabsorbs sodium and chloride through the sodium-chloride cotransporter.[31] Therefore, the osmolality is further decreased at this segment. The DCT is also involved in calcium and

magnesium reabsorption. Since water is not reabsorbed alongside these solutes, the filtrate is further diluted.

Regulation of water permeability in the collecting duct

After solute reabsorption is completed in the DCT, the filtrate moves into the collecting duct system. Water permeability here is highly variable and is mainly regulated by ADH.[33]

Elevated ADH level triggers the insertion of aquaporin-2 water channel protein (AQP2) into the apical membrane of epithelial cells, significantly enhancing water permeability.[21] In the presence of the hyperosmotic medullary interstitium, water passively flows along the osmotic gradient into the surrounding interstitium and capillaries. This process concentrates the urine and conserves body water. Conversely, reduced ADH secretion or impaired responsiveness prevents AQP2 insertion, decreasing water permeability and resulting in the excretion of large volumes of dilute urine.

Urea recycling and augmentation of the medullary concentration gradient

Aside from water and sodium, urea is important in sustaining the hyperosmotic environment of the medulla. In response to ADH, epithelial cells in the inner medullary collecting duct express urea transporters UT-A1 and UT-A3.[34] These transporters facilitate the passive movement of urea from the tubular lumen into the medullary interstitium. A portion of this urea then re-enters the tubular lumen through the thin segment of the loop of Henle and cycles back to the collecting duct. This recycling concentrates urea in the inner medulla and enhances water reabsorption in the collecting duct.

To conclude, the kidneys maintain fluid volume and osmotic balance through a precisely regulated system. It begins with glomerular filtration, followed by selective reabsorption and secretion in the renal tubules and the loop of Henle. In the collecting duct, water permeability is finely adjusted by hormones. After the collecting duct, urine composition is no longer modified.

1.5. Sodium balance and circulating volume regulation

As the main extracellular cation, sodium determines plasma osmolality and influences water distribution across different fluid compartments. The kidneys' regulation of sodium

balance involves renal blood flow dynamics, the pressure natriuresis response, the RAAS system, and the natriuretic peptide system.

The pressure natriuresis response is fundamental for body fluid balance and blood pressure control. The kidneys adjust sodium excretion based on changes in perfusion pressure. When systemic arterial pressure rises, renal arterial pressure also increases. This increases renal blood flow and raises hydrostatic pressure within the glomerular capillaries.[35]

These changes increase the glomerular filtration rate and affect tubular sodium reabsorption through two mechanisms: elevated peritubular physical forces and filtered load. Increased renal blood flow raises peritubular capillary pressure, reducing fluid reabsorption from the interstitium.[35] The associated rise in filtration rate accelerates tubular flow, leaving less time for sodium reabsorption. Together, these changes enhance natriuresis.

When renal perfusion pressure falls, the RAAS is activated to preserve sodium and volume. Pressure sensors detect decreased pressure, and juxtaglomerular cells in the afferent arteriole are stimulated to release renin.[36] Renin converts plasma angiotensinogen into angiotensin I (Ang I), which is then converted into Ang II by angiotensin-converting enzyme (ACE) on endothelial cells.[37] Ang II constricts the efferent arteriole, thereby preserving glomerular filtration pressure during systemic hypoperfusion.[7] It also enhances sodium reabsorption in the proximal tubule and stimulates aldosterone secretion. Aldosterone targets the principal cells in the distal tubule and collecting duct. It binds to intracellular mineralocorticoid receptors, which stimulate the synthesis and activity of epithelial sodium channel and Na^+/K^+-ATPase.[38] This process increases sodium reabsorption and potassium excretion, expanding extracellular fluid volume and raising blood pressure.

In case of fluid overload, the natriuretic peptide system is activated and counteracts the RAAS. Increased volume stretches atrial myocytes and triggers ANP release.[39] ANP dilates afferent arterioles while constricting efferent arterioles, thereby increasing the glomerular filtration rate. It also reduces sodium reabsorption in the collecting duct and suppresses the secretion of renin, aldosterone, and ADH, further promoting natriuresis and diuresis.[40]

1.6. Potassium homeostasis and acid–base regulation

Potassium is the main intracellular cation and is essential for regulating nerve conduction, cardiac electrophysiology, and acid–base balance. Over 98% of total body potassium resides within cells, leaving only about 2% in the extracellular fluid. Even slight alterations in transmembrane potassium flux can significantly alter serum potassium concentrations.[41] Most filtered potassium is reabsorbed in the proximal tubule and thick ascending limb, while secretion occurs mainly in the distal tubule and collecting duct under the control of aldosterone.

In metabolic acidosis, the blood H^+ concentration rises. To buffer excessive H^+, cells take up H^+ and release K^+ into the extracellular space. The transmembrane shift raises serum potassium and results in redistributive hyperkalaemia. Serum potassium may be high despite depleted total body stores.[41] Acidosis also suppresses Na^+/K^+-ATPase activity in tubular epithelial cells and reduces potassium excretion in the distal nephron.

In contrast, alkalosis causes a shift of K^+ into the intracellular compartment as extracellular H^+ concentration falls. This redistribution can result in hypokalaemia, particularly in chronic alkalosis or in patients receiving diuretics.[42] Alkaline urine stimulates H^+–K^+ exchange in the collecting duct, promoting urinary potassium loss. Additionally, alkalosis augments the effects of aldosterone, increasing sodium reabsorption and potassium secretion, thereby worsening hypokalaemia.

The kidneys' ability to generate and excrete ammonia is also important for acid-base balance.[43] When the body's acid load increases, the kidneys increase ammonia production. Ammonia is primarily produced in the renal tubular epithelial cells as a byproduct of amino acid metabolism. Through deamination, the kidneys release ammonia from amino acids. Ammonia then combines with hydrogen ions to form ammonium ions, which are subsequently excreted from the body.[43] This process helps the kidneys remove excess hydrogen ions and maintain the body's acid-base balance.

1.7. Renal regulation of the calcium-phosphorus-bone axis

The calcium-phosphorus-bone axis is a complex network vital for normal physiological function. The kidneys regulate blood calcium and phosphorus levels through hormones and enzymes.

The kidneys adjust calcium levels by reabsorption in the proximal convoluted tubule and the thick ascending limb of the loop of Henle.[44] When blood calcium levels drop, PTH secretion rises, stimulating osteoclast activity to release more calcium from the bone and enhancing calcium reabsorption in the kidneys while decreasing phosphate reabsorption.[44]

The kidneys are also important for phosphorus metabolism. Healthy kidneys efficiently excrete phosphate to prevent hyperphosphatemia. People with CKD have a high prevalence of hyperphosphatemia. Chronic hyperphosphatemia disrupts calcium absorption in the intestines, thus decreases blood calcium levels.[45] In response to hypocalcaemia, the parathyroid gland compensates by continuously overproducing PTH. This sustained elevation of PTH prompts the release of calcium from the bones, which increases the risk of osteoporosis.[45]

The kidneys also synthesise 1,25-dihydroxyvitamin D3 (active vitamin D) to regulate the calcium-phosphorus-bone axis.[46] This is a hormone essential for promoting calcium and phosphorus absorption in the intestines. Impaired kidneys reduce active vitamin D production, leading to insufficient mineral absorption and bone demineralisation.

In conclusion, the kidneys maintain calcium-phosphorus balance by regulating calcium reabsorption, controlling phosphate excretion, and synthesizing active vitamin D. This intricate regulation is essential for bone health and overall equilibrium.

1.8. Integration of renal homeostatic networks

The kidneys maintain homeostasis through systemic coordination across multiple regulatory pathways. Their functions in water and salt metabolism, electrolyte balance, acid–base regulation, calcium–phosphate–bone metabolism, and hematopoietic control are highly integrated.

First, electrolyte metabolism and acid–base regulation are closely interrelated. Blood potassium levels are directly influenced by acid–base status, while acid–base balance relies on the transmembrane transport and excretion of potassium ions. Similarly, calcium–phosphate metabolism is governed by tubular transport and a hormonal network involving PTH, FGF23, and active vitamin D, all of which are linked to renal function.

Second, volume regulation and endocrine functions are also connected. The RAAS system is a central regulator of blood pressure and sodium balance, while the accompanying hemodynamic changes also indirectly influence the secretion of renin and erythropoietin. This is also seen in the dynamic equilibrium of the heart–kidney axis, where ANP from the heart works in concert with renal-produced renin and prostaglandins.

Loss of kidney function disrupts this integrated homeostatic network, leading to systemic complications such as volume overload, hyperkalaemia, metabolic acidosis, anaemia, and mineral–bone disorders. Understanding this integrative role is crucial for recognising the multisystem impact of kidney disease.

1.9. Assessment of kidney function and key clinical markers

While the kidneys excrete metabolic wastes, some wastes such as creatinine have been used as biomarkers of kidney function in clinical practice. Creatinine is the byproduct of muscle metabolism. Its elevation indicates the impairment of kidney function in removing wastes.[47]

To evaluate kidney function, the glomerular filtration rate (GFR) has been proposed and become a widely accepted parameter. GFR is defined as the volume of plasma that travels from the glomerulus into Bowman's space per minute, reflecting the overall filtration capacity of the kidneys.[48]

Based on the characteristics of filtration markers, GFR is categorized into measured GFR (mGFR) and estimated GFR (eGFR). Measured GFR is the GFR assessed using the clearance of exogenous filtration markers that are freely filtered by the glomerulus and neither secreted nor reabsorbed by the tubules. Several markers have been identified (e.g., inulin) or synthesized (e.g., iohexol).

However, the process of mGFR measurement requires multiple blood and/or urine tests over time, as well as a 3 to 6 hours testing period. The complexity of the procedure and the need for resources and expertise restrict the widespread application of mGFR in routine clinical practice.[48] Therefore, eGFR is mostly used in clinical practice and research. eGFR is calculated using endogenous biomarkers that are routinely measured in clinical laboratories, namely serum creatinine (SCr) and cystatin C (CysC).

Serum creatinine has long served as the cornerstone of eGFR calculations. Historically, several key equations have been developed to get creatinine-based eGFR (eGFRcr), including the Cockcroft-Gault (CG) equation (measuring creatinine clearance which can be converted to eGFRcr) back to 1976 [49], the Modification of Diet in Renal Disease (MDRD) study equation in 1999 and a updated version in 2006 [50, 51], the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation in 2009 [52], and a revised race-independent version in 2021.[53]

These creatinine-based equations have evolved from early exploratory tools to standardized models with higher precision. The CG equation is the earliest widely adopted equation. Its main limitation lies in the study population and laboratory methods. The equation was derived from only 249 hospitalized white men, which restricts its applicability to diverse populations. In addition, serum and urine creatinine concentrations were measured using the Jaffe reaction in developing the CG equation. This colorimetric method lacks specificity because substances like glucose and ketones can react with picric acid and lead to a systematic overestimation of creatinine. As a result, the original CG equation is not compatible with modern standardized creatinine values that are traceable to isotope dilution mass spectrometry (IDMS) and is therefore no longer used.

Later with continued methodological advances, the MDRD equation marked a major improvement. The initial year 1999 version with six variables and the simplified four variable version were derived from 1,628 people with predominately nondiabetic kidney diseases. This equation performed well in populations with reduced eGFR but not in those with higher eGFR. Research has shown that the MDRD equation can underestimate mGFR by 29% in healthy population and by about 6.2% in people with CKD.[54] Variability among clinical laboratories in calibration of serum creatinine assays further contributes to measurement error. To address this issue, the MDRD equation was re-expressed in 2006 using IDMS standardized serum creatinine assays.[51, 55]

Because the MDRD equation was developed in patients with CKD, it inherently generates lower estimates in healthier individuals.[52] The CKD-EPI equation was therefore developed. It was derived from a larger and more heterogeneous cohort of 8,254 participants that included both CKD and non-CKD populations and covered a broader GFR range. The CKD-EPI creatinine-based equation improved accuracy at higher GFR levels while

maintaining compatibility with IDMS standardized creatinine. The most recent 2021 version further removed the race coefficient to enhance equity in clinical practice, although its applicability is still under debate.

However, the level of SCr can be influenced by many factors such as race, diet, and muscle mass.[56-58] Compared to creatinine, CysC has fewer determinants and is not affected by race, diet, or muscle mass. It is a low molecular weight protein produced by all nucleated cells and freely filtered by the glomerulus. In 2012, the first equation for cystatin C-based eGFR (eGFRcys) was developed.[59] Within the same year, the Kidney Disease Improving Global Outcomes (KDIGO) guideline recommended using eGFRcys to reconfirm eGFRcr when a clinical decision is needed.[60] Equations that combine SCr and CysC have also been developed and were considered to have greater accuracy than equations using solely SCr or CysC.[59]

The above biomarkers and equations, while robust for the general population, may not accurately reflect kidney function in certain subgroups. For instance, in elderly patients, the decline in muscle mass can lead to lower serum creatinine levels, potentially overestimating kidney function. A few population-specific equations have been designed to address these challenges. The Berlin Initiative Study (BIS) equation was specifically developed for estimating GFR in the elderly population, while kinetic GFR models are used to reflect rapid changes in kidney function in patients with acute kidney injury.[61, 62] Another population-specific equation recently gained attention across European countries is the European Kidney Function Consortium (EKFC) equation, published in 2020.[63] By incorporating variable "Q", the age- and sex-specific population median of serum creatinine, the EKFC equation demonstrates improved accuracy in estimating kidney function in children and adolescents compared with CKD-EPI equations. A cystatin C-based version of the EKFC equation was later developed, showing similar improvements.[64]

Regardless of equations, the categorisation of kidney function follows the same standard. A key threshold is an eGFR below 60 ml/min/1.73 m^2.[65] According to the KDIGO clinical guidelines, if two eGFR measurements taken three months apart both fall below 60 ml/min/1.73 m^2, clinical intervention is warranted.[66] (Figure 1-3)

				Persistent albuminuria		
				A1	A2	A3
				Normal-mildly ↑	Moderately ↑	Severely ↑
				<3 mg/mmol <30 mg/g	3-30 mg/mmol 30-300 mg/g	>30 mg/mmol >300 mg/g
Glomerular filtration rate (ml/min/1.73m²)	G1	Normal or high	≥90			
	G2	Mildly ↓	60-89			
	G3a	Mildly-moderately ↓	45-59			
	G3b	Moderately-severely ↓	30-44			
	G4	Severely ↓	15-29			
	G5	Kidney failure	<15			

Figure 1-3 Staging of Chronic Kidney Disease Based on GFR and Albuminuria
(Adapted from Mayne, 2024) [65]

Typically, when eGFRcr and eGFRcys are measured simultaneously, eGFRcys tends to be slightly lower than eGFRcr. That said, the current KDIGO guidelines for CKD recommend using eGFRcr as the initial test and switching to the combined eGFRcr-cys if cystatin C is available. One reason is because eGFRcr-cys is generally considered more accurate than either eGFRcr or eGFRcys alone.[67]

However, due to concerns regarding cost-effectiveness and limited evidence for improved accuracy, the widespread use of routine cystatin C testing is not currently recommended in the NICE guidelines. In clinical situations where precise assessment of kidney function is critical, such as in kidney transplantation, isotope-based methods like ^{99m}Tc-DTPA clearance should be employed.[65]

Aside from reduced eGFR, albuminuria is an important marker of kidney damage and a key determinant of cardiovascular risk. Albuminuria is the abnormal presence of albumin in the urine.[68] Under normal conditions, albumin is unable to cross the glomerular filtration barrier. When the charge or structural integrity of the basement membrane is compromised, albumin can leak into the urine, resulting in albuminuria. The mechanisms underlying albuminuria are heterogeneous. The most common is glomerular proteinuria, which arises from injury to the filtration barrier. It can present as selective proteinuria, characterized

predominantly by albumin leakage and typically observed in mild glomerular disease, or as non-selective proteinuria, in which multiple proteins including immunoglobulin G (IgG) are excreted, usually in more severe diseases.[69]

Proteinuria can also arise from extrarenal causes. There is prerenal proteinuria (e.g., due to the overproduction of low-molecular-weight proteins in the plasma) and postrenal proteinuria (e.g., due to inflammation of the urinary tract), which are not attributable to intrinsic kidney disease.

The detection of albuminuria mainly relies on either the measurement of urinary albumin excretion or the urine albumin to creatinine ratio (UACR). The latter is preferred for routine screening due to its simplicity and ability to account for variations in urine concentration. According to KDIGO 2024 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease, albuminuria is classified into three categories: A1 (normal to mildly increased, UACR <30 mg/g), A2 (moderately increased, UACR 30–300 mg/g), and A3 (severely increased, UACR >300 mg/g).[66] This classification is used not only for staging CKD but also for assessing the risk of disease progression.[70] (Figure 1-3)

In summary, the assessment of kidney function relies on the integration of measured and estimated approaches, each with its own advantages and limitations. Understanding the strengths and weaknesses of each method, along with the choice of appropriate biomarkers and equations, is important for accurately measuring kidney function in diverse clinical settings.

1.10. Kidney impairment and its associated cardiovascular diseases

Kidney impairment is associated with an elevated risk of cardiovascular disease. Epidemiological studies consistently demonstrate that impaired kidneys are an independent and robust factor of a series of cardiovascular diseases, including HF, MI, and others.

One comprehensive individual-participant meta-analysis incorporating data from over 27 million individuals of 114 cohorts, demonstrated that a decline in eGFRcr (measured using race-free CKD-EPI equation) was associated with increased risks of multiple CVDs. Specifically, people with eGFRcr between 45 and 59 ml/min/1.73m^2 and with UACR

below 10 mg/g have a hazard ratio (HR) of 1.6 (95% confidence interval [CI]: 1.5,1.7) for HF compared to those with eGFRcr between 90 and 104 ml/min/1.73m^2 in the same UACR category. Among people with the same eGFRcr category but a UACR between 10 and 29 mg/g, the HR increased to 1.8 (95%CI: 1.7, 2.0). Similar associations were observed for MI, stroke, and cardiovascular mortality.[71]

Albuminuria also consistently predicts cardiovascular morbidity and mortality across diverse populations.[72] It is noteworthy that even after adjusting for key cardiovascular biomarkers reflecting cardiac damage and overload, an elevated UACR remains associated with higher risks of MI, HF hospitalization, and CVD mortality.[73] Furthermore, people with a UACR between 10 and 30 mg/g, even though not reaching the clinical threshold for albuminuria (30 mg/g), also exhibit higher risks of cardiovascular disease and mortality compared with those with a UACR <10 mg/g.[73] This highlights albuminuria as not only a marker of kidney injury but also an independent indicator of systemic vascular damage.

Moreover, accumulating evidence suggests that proteinuria may accelerate renal fibrosis and disease progression through induction of chemokine expression, complement activation, and inflammatory cell infiltration, with macrophages playing a pivotal role.[74]

Kidney impairment is also associated with elevated risk of atherosclerosis. Dallas Heart Study demonstrated that people with an eGFRcr<60 ml/min/1.73m^2 were associated with higher coronary artery calcification (CAC) scores, and those with diabetes have even higher CAC scores.[75] In a later population-based cohort study, 3,364 people were followed for 2 years, and the decrease in creatinine clearance was associated with an increase in carotid intima-media thickness. Those in the lowest clearance quartile had a faster increase in the thickness compared to those in the highest quartile (0.024 mm/year vs 0.0077 mm/year).[76]

The observed association between kidney impairment and cardiovascular disease can arise from non-exclusive processes: 1. shared upstream pathology (systemic atherosclerosis, endothelial dysfunction, and low-grade inflammation) that harms the heart and kidneys in parallel; 2. downstream consequences of reduced GFR that amplify cardiovascular risk (RAAS/AVP activation, disordered mineral metabolism, anaemia, and toxin accumulation); and 3. reverse and bi-directional causation whereby cardiac disease (e.g., heart failure)

worsens kidney function via venous congestion, low effective arterial volume, or nephrotoxic exposures.

First, systemic atherosclerosis and arteriosclerosis reduce arterial compliance and impair microvascular perfusion across organs. In the kidneys, these forms of sclerosis manifest as albuminuria and eGFR decline, and in the heart, as coronary ischaemia, remodelling, and heart failure. Albuminuria is also a marker of generalised endothelial dysfunction, which helps explain its robust association with CVD independent of eGFR.[77] On this view, kidney impairment and CVD may often be co-products of the same vascular disease.

Second, impaired kidneys have a pathological overactivation of the RAAS. Impaired kidneys experience local hypoperfusion despite overall fluid overload. This triggers RAAS overactivation and facilitates sodium and water reabsorption.[78] These actions collectively elevate both cardiac preload and afterload, leading to sustained hypertension, which is a well-established major risk factor for various CVDs.[79] Decline in kidney function reduces the amount of phosphate filtered from the blood, resulting in hyperphosphatemia.[80] Hyperphosphatemia, synergistically with an elevated calcium-phosphate product (Ca×P), leads to calcification in the arterial media.[80] Furthermore, kidney injury also disrupts vitamin D metabolism. Calcitriol deficiency caused by a decline in kidney function and hypocalcaemia stimulates the parathyroid glands to secrete extra PTH. Persistent elevation of PTH contributes to skeletal degradation and cardiovascular calcification.[81] The limited clearance of metabolic waste products and uremic toxins in impaired kidneys also contributes to atherosclerosis through endothelial dysfunction and inflammation.[82]

Third, causality is bi-directional: cardiac disease can precipitate or accelerate kidney injury (Type 1/2 cardiorenal syndromes), while AKI and CKD worsen cardiac outcomes (Type 3/4). Together, these pathways yield the strong epidemiological links seen across eGFR and albuminuria strata.

1.11. eGFR discordance: definition, determinants, and clinical outcomes

With the increase in the clinical application of eGFRcys in the past decades, the intraindividual difference between eGFRcr and eGFRcys measured at a single timepoint has gained attention. The absolute difference between the two eGFRs can be 15 ml/min/1.73m^2 or larger. In relative terms, eGFRcr can be 70% or lower than eGFRcys.[67]

That said, the thresholds of 15 ml/min/1.73 m^2 and a 70% relative difference listed above are largely based on experience rather than on studies that provide a clear, evidence-based definition of eGFR discordance. The rationale for adopting a threshold of 15 ml/min/1.73 m^2 was that this value approximately corresponds to one standard deviation of the difference between eGFRcr and eGFRcys and reflects the boundary between CKD stages (i.e. Stage 3a and above).[83] With regard to the relative threshold, there appears to be no published justification for the use of 70% (and occasionally 60%) at the time of writing this thesis.

This discordance is not merely a mathematical calculation but has implications for health outcomes. Across multiple cohort studies, individuals with lower eGFRcys compared to eGFRcr were found to be at higher risk of death, CVD events, and kidney disease progression. These associations were robust across studies using different populations and eGFR equations, including CKD-EPI, Caucasian and Asian paediatric and adult subjects (CAPA) equation, and the Lund-Malmö revised creatinine-based eGFR equation (LMrev).[84]

In one cohort study of 4,956 individuals with CKD, 33.1% had eGFR discordance. Those with lower eGFRcys than eGFRcr had a higher risk of mortality (HR=1.86, 95%CI: 1.40, 2.48) compared to those with consistent eGFRcys and eGFRcr. Conversely, those with higher eGFRcys had lower risks of mortality (HR=0.68, 95%CI: 0.58, 0.81).[85] In another study which contained three different population-based cohorts, Incident, Development, and Prognosis of Diabetic Kidney Disease (INDEED) in China, National Health Nutrition Examination Survey (NHANES) in the United States, and UK Biobank, the prevalence of eGFR discordance was around 40%. A one standard deviation increase in eGFRcys - eGFRcr difference was associated with lower risks of all-cause mortality with a HR between 0.66 and 0.77 among cohorts.[86]

In terms of cardiovascular outcomes, eGFR discordance was associated with heart failure, myocardial infarction, atherosclerosis, and composite endpoints. One study on people with CKD showed that those with lower eGFRcys relative to eGFRcr were associated with a higher risk of HF hospitalization (HR=1.20, 95%CI: 1.07, 1.34). When eGFR discordance is measured by the slope of eGFR discordance, a decrease in the slope was associated with

increased HF incidence.[87] Another study demonstrated an elevated risk of atherosclerotic CVD, HF, and CVD mortality in those with lower eGFRcys.[88]

Evidence also supports an association between eGFR discordance and renal outcomes. Several cohort studies found that individuals with lower eGFRcys than eGFRcr were at increased risk of diabetic kidney disease (DKD), end-stage kidney disease (ESKD), and AKI. In a study focusing on individuals with diabetes, those with eGFRcys-eGFRcr < –15 ml/min/1.73m^2 had a 63% increased risk of incident DKD (HR=1.63; 95%CI: 1.50, 1.76) [89], and a similar association was observed for ESKD and AKI in other studies.[90]

The potential modifying effect of CRP and Body Mass Index (BMI) on the association between eGFR discordance and health outcomes has been observed. People with elevated CRP over 2 mg/L or BMI over 30 kg/m^2 had stronger associations with adverse outcomes than those who were healthier, suggesting interplay with metabolic status.[84]

However, the observed associations between eGFR discordance and adverse outcomes do not necessarily imply that eGFR discordance is a good predictor of future risk. Evidence regarding the predictive utility of eGFR discordance remains scarce. Specifically, it is unclear whether incorporating eGFR discordance into established risk prediction models (such as the Framingham Risk Score) meaningfully improves model performance, in terms of changes in the C-index, net reclassification improvement, or model calibration. Furthermore, it remains to be determined whether eGFR discordance provides incremental predictive value independent of eGFR and albuminuria.

Several mechanisms have been proposed to explain this discordance. One hypothesis is the Shrunken Pore Syndrome, wherein the glomerular basement membrane selectively blocks the filtration of mid-sized cystatin C (13 kDa), while allowing creatinine (113 Da) to pass freely.[91] Another explanation is non-GFR factors. Some well-acknowledged non-GFR factors for serum creatinine are age, sex, diet, and physical activity. In contrast, non-GFR factors are different for serum cystatin C, including thyroid disease, systemic inflammation, adiposity, and medications like steroids.[92]

In a recent study published in August 2025, eGFR difference (defined as eGFRcys-eGFRcr) was shown to correlate with classical non-GFR factors, including markers of muscle mass,

middle-molecule clearance, obesity, inflammation, and protein intake. However, these factors accounted for only 36% of the variance in eGFR difference, suggesting that this metric may capture additional biological processes that remain unknown or are not yet fully understood.[93] Potential variation in eGFR discordance across other domains, such as race or socioeconomic status, also warrants investigation. From a practical perspective, it is suggested that in the presence of eGFR discordance, eGFRcr-cys should be prioritised, as it provides the most accurate estimates.[94]

In sum, eGFR discordance remains conceptually and methodologically underdeveloped. Thresholds used to define discordance are inconsistently applied and often based on experience. Moreover, while associations with adverse outcomes are well-described, the potential of eGFR discordance to improve risk prediction remains largely unexplored. Current literature lacks studies that test its incremental value over established predictors such as eGFR and UACR. This study addresses this important gap.

1.12. Therapeutic strategies in diabetes and their impact on cardiorenal outcomes

Patients with diabetes face a significantly increased risk of developing CKD and CVDs. Thus, this vulnerable population has been the main group receiving attention on cardiorenal protection.

Historically, efforts in diabetes care focused predominantly on achieving glycaemic control. Landmark trials such as the Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) have shown that lowering haemoglobin A1c reduces microvascular complications.[95, 96] However, in recent years, the treatment paradigm has evolved beyond glucose-centric goals to a more comprehensive approach that extends to cardiorenal protection.

The focus of diabetes therapy began shifting significantly toward cardiorenal protection in the late 2000s, gaining considerable traction by the mid-2010s. This evolution was driven by emerging evidence that some antidiabetic medications provide benefits beyond blood sugar control, notably reducing cardiovascular and renal risks. Indeed, the landscape of diabetes management was reshaped by a series of landmark cardiovascular outcome trials that demonstrated these benefits.

In 2015, the Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients–Removing Excess Glucose (EMPA-REG OUTCOME) trial demonstrated that people who had established cardiovascular disease and were taking empagliflozin had a lower rate of composite CVD outcomes (CVD mortality, non-fatal MI, and non-fatal stroke).[97] Two years later in 2017, the Canagliflozin Cardiovascular Assessment Study (CANVAS) Program confirmed that among people with type 2 diabetes and high cardiovascular risk, canagliflozin lowered the risk of composite renal outcomes (including eGFR reduction, initiation of renal-replacement therapy, and death from kidney causes) compared to placebo.[98] Expanding this evidence base, the Dapagliflozin Effect on Cardiovascular Events–Thrombolysis in Myocardial Infarction 58 (DECLARE-TIMI 58) trial in 2019 further showed that dapagliflozin reduced the risk of heart failure hospitalization and renal events in people with diabetes who had or were at risk for atherosclerotic cardiovascular disease.[99]

Almost at the same time, another class of medications, GLP-1 receptor agonists (GLP-1 RAs), also demonstrated significant cardiovascular and renal benefits. In 2016, the Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER) trial showed that taking liraglutide was associated with lower cardiovascular mortality in high-risk patients.[100] The Researching cardiovascular Events with a Weekly INcretin in Diabetes (REWIND) trial in 2019 showed that GLP-1 RAs are associated with reductions in composite cardio and renal outcomes, suggesting their renoprotective potential.[101]

These pivotal trials catalysed a major evolution in clinical practice guidelines. Since 2019, the American Diabetes Association (ADA) has increasingly emphasized cardiorenal protection in its Standards of Care.[102] Recent ADA guidelines refine this approach, recommending the use of SGLT2 inhibitors and GLP-1 receptor agonists for patients with high cardiovascular or renal risk, regardless of baseline HbA1c levels or metformin use.[103] This represents a substantial departure from earlier treatment models centred primarily on glucose indicators.

With extended usage of antidiabetic drugs, current strategies for diabetes management increasingly favour earlier combination therapy rather than sequential drug escalation. This combination approach is driven by the understanding that concurrent use of multiple agents

with complementary mechanisms can provide more robust protection against disease progression. Research has shown the combination of SGLT2i, GLP-1 RA, and non-steroidal mineralocorticoid receptor antagonist (ns-MRA) leads to better prognosis.[104] However, despite growing enthusiasm for combination regimens, there remains a lack of research directly comparing the cardiorenal outcomes of fixed-dose combination formulations versus co-administration of their individual components. This represents a gap in the evidence base.

In all, the landscape of diabetes management has expanded to cardiorenal protection alongside glycaemic optimization. This paradigm shift, informed by robust clinical trial data and reflected in evolving guidelines, underscores the importance of individualized, risk-based treatment approaches. As combination therapy gains more attention, future research is needed to elucidate its long-term impact on cardiorenal outcomes, which will help refine clinical practice and guide strategies for improving patient prognosis.

1.13. General aims and objectives

The aim of this thesis is to advance the understanding of kidney function as a cardiorenal risk factor. This involves defining distinct kidney-function phenotypes, quantifying their relationships with modifiable behaviours, and evaluating whether specific treatment strategies can improve cardiorenal outcomes in population settings.

To achieve this aim, the following objectives are addressed:

1. To examine the role of physical activity in kidney health by investigating its association with kidney-related biomarkers from a dynamic perspective in the general population.
2. To characterize kidney-function phenotypes**,** with a particular focus on the discordance between creatinine-based and cystatin C-based eGFR, using evidence from systematic review and meta-analysis.
3. To assess the prognostic significance of eGFR discordance**,** including its associations with all-cause mortality. To determine whether this discordance provides additional predictive value beyond standard measures such as baseline eGFR and UACR.
4. To evaluate the effectiveness of pharmacological treatment strategies in people with type 2 diabetes, particularly comparing fixed-dose and loose-dose combination

therapies, and to explore whether medication adherence mediates the effectiveness in real-world settings.

By integrating these objectives, this thesis aims to provide population-based insights that bridge the gap between renal measurement and clinical management, contributing to improved risk identification and therapeutic decision-making in kidney and cardiovascular care.

1.14. Thesis structure

This thesis is based on nine chapters. Chapter 1 provides a comprehensive background on the physiology of the kidneys, the kidneys' roles in filtration and homeostasis, and the association with cardiovascular diseases. The first chapter also introduces key biomarkers used for assessing kidney health and the outcomes related to kidney impairment. Chapter 2 focuses on the primary data source, the UK Biobank, detailing participant recruitment, cohort characteristics, data collection methods, and quality control processes. Chapter 3 expands on additional data sources, including several Swedish national registers, and outlines the exposures and outcomes explored in this thesis.

Chapters 4 through 8 present the full content of the five published, peer-reviewed manuscripts incorporated into this thesis. Chapter 4 discusses the effect of exercise on kidney-related biomarkers in the general population through a systematic review and meta-analysis. Chapter 5 evaluates the impact of physical activity changes on kidney function, based on a UK Biobank cohort study. Chapter 6 explores discordance between creatinine-based and cystatin C-based eGFR and their health outcomes. Chapter 7 investigates the association between eGFR discordance and mortality in the general population. Chapter 8 examines the effectiveness of fixed-dose versus loose-dose combination antidiabetic therapies on cardiorenal outcomes in patients with type 2 diabetes.

Finally, Chapter 9 provides a comprehensive discussion of the preceding content, comparing the findings with existing literature, highlighting the strengths and limitations of this thesis, and proposing directions for future studies.

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2. Chapter 2 – Primary data source: the UK Biobank

The UK Biobank is a landmark in cohort studies, providing an unparalleled resource for biomedical research. Enrolling nearly half a million participants aged 40-69 between 2006 and 2010, UK Biobank's primary objective is to deepen our understanding of the causes of common and life-threatening diseases.[1] By linking health, lifestyle, environmental, and genetic data, UK Biobank offers a powerful platform to investigate factors contributing to health and disease. This chapter introduces the UK Biobank, focusing on recruitment, data collection procedures, and the stringent quality control measures applied to its vast collection of biological samples.

2.1. Participant recruitment and cohort characteristics

The recruitment of the UK Biobank cohort was designed to capture a broad section of the UK population. Invitations were mailed to over 9.2 million individuals who were registered with the National Health Service (NHS). Eligible participants lived within 25 miles of one of the 22 dedicated assessment centres. These centres were established across England, Scotland, and Wales.[2]

While the cohort is broadly representative, it is subject to healthy volunteer bias. For example, UK Biobank participants are, on average, more educated and less likely to be obese or smokers, and have fewer self-reported health conditions compared to the general population.[1] Researchers are encouraged to consider this bias when interpreting and extrapolating findings to the wider population. Still, exposure-disease associations derived from the UK Biobank may be generally recognized as comparable to those from the wider population.[1]

2.2. Multi-modal data collection at assessment centres

Data collection was performed in assessment centres. A multi-modal approach with self-reported information and objective measurements was applied. The full process takes approximately two to three hours.

2.2.1. Touchscreen questionnaire

The first stage was a comprehensive touchscreen questionnaire that covered a wide array of domains.[3] Major domains included:

Sociodemographic and lifestyle: Detailed questions on employment, education, and household income, alongside in-depth sections on lifestyle factors. For example, questions on smoking status asked about the current status, as well as the duration, intensity (pack-years), and history of cessation. A modified version of the International Physical Activity Questionnaire was used to quantify weekly activity levels.

Medical and family history: Participants reported on a list of common illnesses, surgeries, and medication use. This was supplemented by questions on family history of diseases like diabetes and cardiovascular diseases.[4]

Mental and cognitive health: A dedicated section on mental health captured data on depression, anxiety, and other conditions. Cognitive function was assessed through tests measuring reaction time, fluid intelligence, and memory.

2.2.2. Nurse-led structured interview

Following the touchscreen component, a trained nurse conducted a one-on-one structured interview. The nurse went through a list of medical conditions, asking for the year of diagnosis and specifics of treatment. The nurses were guided by a central computer system, ensuring that the same questions were asked in a consistent manner across all centres.[5] This process was critical for capturing a nuanced and accurate medical history.

2.2.3. Physical measurements and sample collection

The third major collection was of a standardized set of physical measurements and biological samples. All equipment was calibrated on a regular basis to ensure accuracy.

Data collected included:
Anthropometrics and vitals: This included height, weight, waist and hip circumference, and a series of vital signs. Blood pressure was measured using an automated cuff, and a spirometer was used to assess lung function (e.g., Forced expiratory volume in 1 second).[6]

Biological samples: A total of approximately 45 ml of venous blood and a single 9 ml urine sample were collected. The blood was drawn into several vacutainer tubes (e.g., citrate), each with a specific anticoagulant or preservative for its intended use.[7]

2.3. Biological sample processing and quality control

The integrity and long-term viability of the UK Biobank's biological samples are valuable assets. Upon collection, samples were immediately prepared for transport in temperature-controlled boxes and shipped overnight to a central processing facility.

The samples were sorted and processed using a highly automated robotic system.[8] This minimized human error and contamination. The main steps were: 1. Centrifugation: Blood tubes were spun at high speed to separate plasma, serum, red blood cells, and the buffy coat. 2. Aliquoting: The separated components were carefully pipetted into barcoded cryovials. 3. Barcoding and Tracking: Every single aliquot was assigned a unique barcode. The entire process was managed by a Laboratory Information Management System that tracked each sample from collection to final storage. This system is crucial for ensuring data integrity and preventing mix-ups.[8]

Multiple quality control procedures were implemented to ensure data reliability. Firstly, a specific set of high-throughput analysers (e.g., Beckman Coulter LH750) was used consistently to minimize inter-instrument variability. Secondly, the lab participated in accredited External Quality Assessment Schemes, where an independent body sent blind samples to verify that its results were consistent with those of other high-quality laboratories worldwide.

The precision of the assays was meticulously documented. A key metric for this is the Coefficient of Variation (CV). A CV under 10% was considered to be of high precision and consistency. Among measured biomarkers in the UK Biobank, the CV is generally below 5%.[9] In UK Biobank proteomics studies using Olink technology, the Limit of Detection (LOD) for each assay was established. This process ensures that the LOD accounts for the background noise of the specific plate and varies between plates. The LOD indicates the lowest concentration that can be reliably distinguished from background noise, helping to identify true signal above instrument noise.[10]

2.4. Data linkage to other registers

Another strength of the UK Biobank is its ability to track the health of its participants over decades. This is achieved through data linkage to national health and administrative

registers. All participants provided explicit consent at the time of recruitment for UK Biobank to follow their health status by accessing their medical records.

The UK Biobank has established secure, regular data feeds from a variety of national registries across the UK. Key linkages include [11]:

- Hospital Inpatient Data: This linkage provides detailed records of hospital admissions, including diagnoses, procedures, and surgical interventions. It allows researchers to identify disease incidence and track clinical trajectories post-diagnosis.
- National Cancer Registries: The UK Biobank receives data on all cancer diagnoses for its participants from national cancer registries in England, Scotland, and Wales. This includes information on the type of cancer, date of diagnosis, and tumour histology.
- National Death Registries: For deceased participants, the UK Biobank receives information on the date and cause of death from national death registries.
- Primary Care Data: Primary care data includes information on diagnoses, prescriptions, and test results. As of September 2025, around 230,000 participants have their primary care data linked up to 2016 or 2017 (depending on data supplier). This is a particularly rich source of data, as it captures a wide range of conditions that are managed in a community setting and may not result in hospital admission.

2.5. The role of UK Biobank in the PhD research

UK Biobank is the core element of my PhD research. Its commitment to data integrity and scientific rigor ensures its lasting value as a precious research resource. Since my research aims and objectives are centred on the kidneys, the UK Biobank's extensive data on various kidney biomarkers (e.g., serum creatinine, cystatin C, and albumin), their determinants, and its standardised laboratory protocols and procedures make it an unparalleled resource to support my work.

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3. Chapter 3 – Other data sources and general settings

In addition to the UK Biobank, this thesis utilised individual-level data from four nationwide Swedish registers: the National Prescribed Drug Register (PDR), the National Patient Register (NPR), the National Diabetes Register (NDR), and the Total Population Register (TPR). These registers are maintained by the Swedish government and are linkable through a pseudonymized unique personal identity number, enabling longitudinal studies on health exposures and outcomes at a national scale.

3.1. Swedish national registers

3.1.1. The Swedish prescribed drug register

The Swedish Prescribed Drug Register (PDR) was established in July 2005 and is regulated by the National Board of Health and Welfare.[1] It captures detailed information on all medications prescribed and dispensed by outpatient pharmacies across Sweden. Each record includes a patient identifier, date of dispensing, Anatomical Therapeutic Chemical (ATC) classification codes, dispensed quantity, defined daily dose (DDD), and others. All data are automatically captured and forwarded to the Swedish eHealth Agency. The register is updated on a monthly basis.[2]

Unlike clinical trial datasets or self-reported information, the PDR reflects real-world prescribing and dispensing practices. However, it does not include medications administered during hospitalization or those purchased over the counter. Despite these limitations, the PDR is widely used in pharmacoepidemiological research to investigate drug utilisation patterns, adherence, treatment effectiveness, and adverse drug reactions. It has been estimated that the PDR covers approximately 84% of the total drug sales in Sweden.[1] The high completeness and accuracy of the data, along with its monthly updates, make the PDR a valuable resource for studies examining drug-outcome associations.

3.1.2. The national patient register

The National Patient Register (NPR) is one of the oldest and most comprehensive health registers globally. Data collection for inpatient care began in the 1960s, with complete nationwide coverage achieved by 1987.[3] Since 2001, the register has included data on specialised outpatient care, although primary care data are not included. Each entry contains patient identifiers, admission and discharge dates, primary and secondary diagnoses (using International Classification of Diseases 10th revision [ICD-10] Sweden version), and procedural codes (using Nordic Medico-Statistical Committee [NOMESCO] codes).[4]

For most diagnoses, the NPR demonstrates good accuracy with a median positive predictive value (PPV) for diagnostic codes of 84%. This value is even higher for surgical procedures, with a median PPV of 97%.[4] The NPR enables robust follow-up of clinical outcomes such as hospitalisations, surgical interventions, and disease diagnoses, making it a valuable source for studying disease incidence.

3.1.3. The national diabetes register

The National Diabetes Register (NDR), established in 1996, is a nationwide clinical quality register designed to assess and improve diabetes treatments.[5] It includes patients with type 1 and type 2 diabetes and collects data on demographics, diabetes duration, treatment, and laboratory biomarkers. Key variables include HbA1c levels, lipid profiles, and complications.

Data are entered by healthcare providers in both primary and secondary care settings, and participation is based on informed consent. The coverage of NDR is high. It covers all specialist clinics and approximately 90% of primary care centres in Sweden, representing over 500,000 patients.[6] Annual updates allow researchers to monitor treatment trends and quality of care over time. The NDR provides a rich resource for evaluating long-term outcomes of diabetes care in routine clinical practice. It has been widely used in studies

examining the effectiveness of glucose-lowering therapies, diabetes complications, and healthcare disparities.

3.1.4. The total population register

The Total Population Register (TPR) is maintained by Statistics Sweden and includes demographic information on all Swedish residents.[7] Established in 1968, it records dates of birth and death, migration (both within and outside Sweden), civil status, country of birth, and residential history. The TPR is continuously updated and is considered virtually complete for the entire population.[7]

The TPR is particularly valuable for medical research because of its nearly 100% population coverage, ongoing updates, and linkage to other national health and social registers. Two key variables of TPR are the death status and time, allowing for censoring in longitudinal analyses.

Additionally, the coverage of residential and migration data enables assessments of geographic variations in health, and associations between sociodemographic factors and disease burden.[8] Because of its high quality and minimal loss to follow-up, the TPR is widely used as a foundational data source for studies on healthcare utilization, chronic disease trends, and public health interventions.

3.1.5. Strengths and limitations of the Swedish registers

A major strength of the Swedish register system is the ability to link people across datasets using the personal identity number which is assigned to all residents at birth or upon immigration. This enables comprehensive longitudinal follow-up of disease status, medication use, regional differences, and sociodemographic factors. In this thesis, the integration of PDR, NPR, NDR, and TPR allows for detailed exposure assessment, confounder control, and outcome ascertainment within the Swedish context. Furthermore,

because healthcare in Sweden is tax-funded and universally accessible, these registers are less susceptible to selection bias related to access to care.

Nevertheless, as observational registers, their limitations include potential residual confounding and misclassification of diagnoses or drug exposure. These registers have very limited data on lifestyle factors. In addition, as registers designed for general purposes, their data on kidneys are less ideal than specialized registers (e.g., Chronic Renal Insufficiency Cohort Study).

Despite these limitations, the high validity, completeness, and scale of these national registers make them uniquely suited for population-based research.

3.2. Exposures included in this thesis

This thesis comprises five manuscripts, including three original research articles and two systematic reviews. In general, three exposures were discussed in the five manuscripts: physical activity (one systematic review and one original research), eGFR discordance (one systematic review and one original research), and oral antidiabetic fixed-dose combination drugs (one original research only). This section provides a brief overview of these exposures for quick reference. More detailed descriptions can be found in Chapters 4 to 8.

3.2.1. Physical activity

Physical activity data were obtained from the UK Biobank, which includes three different measures of physical activity. Two of these measures are based on self-reported information, while the third is derived from accelerometer data.

At the assessment centre, UK Biobank participants completed a questionnaire on their physical activity. One section of the questionnaire assessed the frequency of various activities over the past four weeks, such as light or heavy do-it-yourself tasks, stair

climbing, sports participation, and walking for pleasure.[9] A typical question was, *"How many times in the last 4 weeks did you do strenuous sports?"* Responses were categorical (e.g., once a week; 2–3 times a week), and participants were required to select one option only. [10]

The second section of the questionnaire was a modified version of the International Physical Activity Questionnaire (IPAQ). Unlike the first section, which assessed physical activity over the previous four weeks, the modified IPAQ focused on average activity during the past seven days. A typical question was: *"How many minutes did you usually spend doing moderate activities on a typical day?"* In contrast to the categorical format used in the first section, this question required participants to input a specific integer value.[10] Based on the modified IPAQ, Metabolic Equivalent of Task (MET) scores can be calculated.[11] The derived MET score allows for studying the effect of physical activity at a finer granularity.

To objectively assess physical activity, a total of 103,578 participants were enrolled in a 7-day measurement project using a wrist-worn accelerometer.[12] The device was pre-programmed to automatically activate and deactivate at specified times. Participants were instructed to wear the accelerometer on the wrist of their dominant hand and to keep it on throughout the monitoring period. After seven days, the device was to be returned via a prepaid envelope to a coordinating centre, where the physical activity data were extracted. The return rate was exceptionally high, with a loss rate (i.e., devices not returned) as low as 1.2%. From the collected data, several metrics such as acceleration average have been derived and made publicly available for research purposes.[12] A detailed description of the accelerometer and its data collection protocol is available online.[13]

3.2.2. eGFR discordance

The term "eGFR discordance" refers to intra-individual differences between creatinine-based eGFR (eGFRcr) and cystatin C-based eGFR (eGFRcys). Although there is no

universally accepted threshold, eGFR discordance is commonly defined as a difference greater than 15 ml/min/1.73 m^2 between the two estimates.

At the UK Biobank assessment centre, approximately 50 mL of blood was collected from each participant. Samples were aliquoted, centrifuged, stored under appropriate temperature conditions, and then transported via courier service to a central laboratory.[14] In the central laboratory, serum creatinine was measured using enzymatic method on a Beckman Coulter AU5800 analyser, while cystatin C was measured using a latex-enhanced immunoturbidimetric method on a Siemens ADVIA 1800.[15] The laboratory adhered to strict quality control protocols, including routine calibration and regular participation in external quality assessment programs, to ensure the accuracy and comparability of measurements.

In this thesis, both eGFRcr and eGFRcys were mainly calculated using the CKD-EPI equations, which are widely used in clinical practice and applicable to most populations. Alternative equations were used in certain sensitivity analyses. Details are provided in subsequent chapters.

3.2.3. Oral antidiabetic fixed-dose combination drug

Oral antidiabetic fixed-dose combination (FDC) drugs are formulations that combine two or more glucose-lowering agents into a single oral dosage form. These combinations aim to enhance glycaemic control, improve medication adherence by reducing pill burden, and optimize therapeutic convenience.[16] The selected drug components typically act via complementary pathways to address multiple facets of the pathophysiology of type 2 diabetes, such as insulin resistance and glucose reabsorption.

The manufacturing of FDCs requires careful consideration of physicochemical compatibility, pharmacokinetics, and formulation stability. To mitigate potential degradation or interactions between active ingredients, specialized excipients and

polymeric matrix systems are often applied.[17] These matrices can act as protective barriers, minimizing exposure to moisture or oxidative environments during storage and administration. In cases of limited compatibility, the components may be granulated separately and compressed into bilayer tablets, which provide spatial separation and enable differential release kinetics.[18] Additional formulation strategies, such as modified-release layering, enteric coatings, or hydrophobic carriers, can further enhance bioavailability and maintain consistent therapeutic performance across components.[18]

To date, most FDCs are metformin-based. One widely used example is the combination of metformin with a dipeptidyl peptidase-4 (DPP-4) inhibitor, such as Janumet (metformin + sitagliptin) and Jentadueto (metformin + linagliptin). Another major category combines metformin with sodium-glucose co-transporter 2 inhibitors (SGLT2i), such as Xigduo XR (metformin + dapagliflozin) and Synjardy (metformin + empagliflozin). Metformin combinations with classical drugs like sulfonylurea are also available (e.g., Glucovance). Triple combination FDCs are available but rare, such as Trijardy XR, which combines empagliflozin, linagliptin, and metformin in a single extended-release tablet.

In this thesis, FDC was identified by examining ATC codes in the Swedish Prescribed Drug Register. Selected FDCs were identified by ATC codes beginning with A10BD, which denote combinations of blood glucose-lowering drugs excluding insulin.

3.3. Outcomes included in this thesis

This thesis examines a wide range of outcomes, including kidney-related biomarkers, kidney function, cardiovascular disease, chronic kidney disease, and mortality. Given the extensive number of outcome events, this section does not elaborate on specific outcomes (e.g., stroke as a cardiovascular event) to avoid redundancy. Instead, it provides a brief overview of the data sources. Detailed descriptions of the outcomes are presented in Chapters 4 through 8.

Kidney-related biomarkers used in this thesis were obtained either from the UK Biobank or from published literature. Commonly reported biomarkers in both registry data and published studies include serum creatinine, cystatin C, blood urea nitrogen, and urinary albumin. Kidney function was estimated using published serum creatinine- or cystatin C-based equations, or extracted directly from existing literature.

Cardiovascular and kidney diseases were identified using ICD-10 codes in the UK Biobank, based on its linkage to Hospital Episode Statistics. A similar approach was applied to the Swedish national registers. Mortality data, including date of death, were obtained from both the UK Biobank and the Swedish national registers.

3.4. Ethical approval

This thesis has two ethical approvals: 1. The ethical approval of UK Biobank and Swedish registers. 2. The ethical approval of my research.

The UK Biobank has received ethical approval from the North West – Haydock Research Ethics Committee (Ref. 21/NW/0157) as stated in Chapter 2.1. For Swedish registers, the establishment and routine data collection are authorized and mandated by Swedish laws (e.g., the Patient Data Act, SFS 2008:355; Population Register Act, SFS 1991:481).[19, 20]

For the second part, my research using UK Biobank was approved under UK Biobank Application No. 71392. The study based on Swedish registers was approved by the Swedish Ethical Review Authority (dnr 2021–03957, 2023–03824–02).

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4. Chapter 4 – Effect of exercise on kidney-relevant biomarkers in the general population: a systematic review and meta-analysis

The original article of this chapter has been published in BMJ Open on January 8, 2025.

Liu Q, Celis-Morales C, Lees J, Mark P, Welsh P. Effect of exercise on kidney-relevant biomarkers in the general population: a systematic review and meta-analysis. *BMJ Open*. 2025 Jan 8;15(1):e093017. doi: 10.1136/bmjopen-2024-093017.

4.1. Abstract

Objective

Physical activity (PA) has been generally recognized as beneficial for health. The effect of change in PA on kidney biomarkers in healthy individuals without kidney disease remains unclear. This manuscript synthesized the evidence of the association of changes in PA with kidney biomarkers in the general population free from kidney disease.

Design

Systematic review and meta-analysis.

Data sources

Embase, PubMed, MEDLINE, and Web of Science databases searched from inception to March 12, 2023.

Eligibility criteria for selecting studies

Studies of longitudinal or interventional design were initially selected. The following studies were excluded: 1. Case-control studies; 2. Studies where physical activity was measured at a single time point; 3. Populations with known kidney disease; 4. Studies evaluating the impact of a single episode/event of physical activity; and 5. Non-English language studies.

Data extraction and synthesis

Two independent reviewers extracted data to a pre-designed table and assessed the risk of bias using the Cochrane Risk of Bias tool. Data were pooled using random effects model. Hedge's g was used to synthesize effect sizes and obtain an overall estimate. Heterogeneity between studies was measured using I2. Funnel plots and Egger's test were performed to evaluate the risk of biased results.

Results

Sixteen interventional studies with randomized or non-randomized designs involving 500 participants were identified. The median follow-up was 84 days. Ten studies were at high risk of bias. Studies with low quality were published prior to the year 2000. Changes in PA were found only to have a positive association with serum creatinine (SCr) (Hedge's g=0.69, 95%CI: 0.13, 1.24, I2=81.37%) and not with plasma renin activity, urea, or urine albumin-to-creatinine ratio. The positive association was only observed in people with obesity and those who exercised for more than 84 days.

Conclusions

Higher levels of PA are associated with increased SCr levels in healthy people. It remains unclear if this association is related to impaired kidney function or gain in muscle mass, as data on other kidney biomarkers did not support a certain link.

4.2. Background

Cardiovascular disease (CVD) is a major global health issue, causing approximately 17.9 million deaths annually, or 32% of all global fatalities as of 2019 [1]. CVD also imposes a significant economic burden on healthcare systems worldwide [2, 3]. Physical inactivity has been recognized as a risk factor for CVD event [4, 5] while performing physical activity (PA) is beneficial to the prevention of CVD, along with other chronic conditions such as chronic kidney disease (CKD) [6].

Despite the myriad of benefits of PA on cardiovascular health, its effect on kidney function is not well established. Impaired kidney function is a risk factor for cardiovascular disease [7], it is plausible that PA might also positively affect kidney function [8]. Serum creatinine-based estimated glomerular filtration rate is commonly used in clinical practice and creatinine is a product of muscle metabolism [9]. Therefore, any effect of PA on muscle metabolism may indirectly affect the measurement of kidney function. There is also

evidence suggesting that extreme levels of PA may induce kidney damage via rhabdomyolysis or dehydration [10].

Evidence from randomised controlled trials suggests that PA is associated with multiple metrics of kidney function. However, the evidence is controversial. PA is inversely associated with the risk of kidney function decline in people aged over 65, with an average estimated glomerular filtration rate (eGFR) of around 80 mL/min/1.73m^2 [11]. Yet, the same association was not observed in a younger general population (age 26-65 years) with a much higher average eGFR of 108 mL/min/1.73m^2 [12]. Studies assessing PA intensity include data showing that accelerometer-measured low- and moderate-intensity PA are positively associated with eGFR in a general Japanese population (age 35-79 years, average eGFR 92.6 mL/min/1.73m^2) across sexes and ages [13].

PA has also been linked to urinary albumin excretion. As the dysfunction of the kidney endothelial barrier and atherosclerosis contribute to the leakage of albumin into the urine, microalbuminuria has been suggested as an indicator of kidney endothelial dysfunction [14]. The association between high PA levels and lower microalbuminuria has been observed consistently across variant populations [15]. Novel biomarkers of kidney impairment, such as liver-type fatty acid-binding protein, have also been found to be negatively impacted by habitual physical activities [16]. The degree of stress on the proximal tubule may be attenuated through physical activity, regardless of the kidney functional reserve, suggesting PA's health benefits on the kidney structure.

Although the effects of PA on the kidneys have been studied, many articles focus on the acute effect of physical activity, and they are not instructive on the effects of changing habitual PA. The study population is often restricted to patients with chronic kidney disease (CKD) / end-stage kidney disease (ESKD) including those who undergo dialysis. These research findings may not be applicable to the general population without known kidney diseases. A number of intervention studies discussed the effect of PA in

combination with other treatments, like diet and pharmaceutical approaches; thus, it is difficult to measure PA's direct effect. To date, there is a lack of systematic review of the literature which has been conducted on the effect of changes in physical activity on kidney health in populations without pre-existing kidney diseases. In this context, this study aimed to conduct a systematic review and meta-analysis to bridge the knowledge gap.

4.3. Methods

This review has been registered on PROSPERO (CRD42023407820). In this review, we followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Statement (PRISMA) [17] and the Cochrane Handbook for Systematic Reviews of Interventions [18]. We followed the Population, Intervention, Comparison, Outcomes and Study (PICOS) framework to develop our search strategy [19].

To be specific:

Population: Adults without known kidney diseases

Intervention: Physical activity

Comparator: Kidney-relevant biomarkers before physical activity

Outcome: Changes in kidney-relevant biomarkers after physical activity

Study Design: Cohort or interventional study

The research theme is: "In adults without known kidney diseases, to what extent does physical activity affect the levels of kidney-relevant biomarkers compared to baseline levels?"

One author (QL) systematically searched Embase, PubMed, MEDLINE, and Web of Science databases from inception to March 12, 2023. The inclusion criteria were: 1. Cohort studies or interventional studies (randomized / non-randomized); 2. Studies in which the duration and/or intensity of physical activity was measured at least twice; 3. Studies in which the study population is based on the general population. It is expected that people with chronic kidney disease will form around 10% of the general population, so we

additionally extracted baseline eGFR and Urine Albumin-to-Creatinine Ratio (UACR) (where available) as an indicator of the baseline level of kidney function in these studies. We also carefully examined the description of the study population in selected studies. Studies that met the following criteria were excluded: 1. Case-control studies; 2. Studies in which physical activity was measured at a single timepoint; 3. Studies conducted specifically in populations with pre-existing kidney diseases, such as chronic kidney disease, dialysis, and kidney transplantation.; 4. Studies evaluating the impact of a single episode of physical activity, such as a sporting event; and 5. Studies that were not published in English. The detailed search terms can be found in the Supplemental materials (Table S1).

Two authors (QL and PW) independently decided which studies should be included in this study, and any disagreements were resolved through a discussion with two other authors (CC and PBM). To maximize the coverage of sources, one author (QL) checked the references of the selected articles and evaluated their relevance after reading the full text. Additively, one author (QL) performed manual searches on relevant studies.

Study exposure

The study exposure was the change in physical activity. The change in physical activity was denoted as a categorical variable, i.e., from being sedentary to being active.

Study outcome

The primary study outcome was the change in kidney-relevant biomarkers, including but not limited to serum creatinine, cystatin C, and UACR. This change was defined as the difference in a biomarker's level after the completion of a change in physical activity, for example, the difference in serum creatinine levels before and after a 12-week aerobic exercise programme. In addition, long-term kidney outcomes, such as the first diagnosis of chronic kidney disease and the presence of microalbuminuria, were also collected if relevant literature was identified.

Quality assessment

All the selected studies were interventional studies. The risk of bias for each selected study was assessed using the Cochrane Risk of Bias tool [20] by two reviewers independently. Seven domains of bias (sequence generation, allocation concealment, blinding of participants and personnel/outcome assessment, incomplete outcome data, selective reporting, and other biases) were assessed. The overall risk was categorized as low, high, critical, unclear, or no information. A study was biased if the loss to follow-up was 20% or above [21]. Any disparities in judgment raised between the two reviewers were resolved through discussion with the help of a third author as needed.

Data synthesis

Using a predesigned table, information was extracted on the first author's family name, publication year, study type, study location, baseline characteristics of exercise groups, type of exercise, length/frequency/intensity of exercise, and outcomes. In case a study has both exercise and sedentary groups, only the information of the group which performed physical activity was included to align with our research theme.

For studies with multiple measurements, we used the baseline and the final measurement to calculate the change. For example, if a kidney biomarker was measured at exercise week 0 (the baseline week), week 3, and week 6 (the final week), the change in the biomarker between week 0 and week 6 was used. In cases where subgroup findings were reported, those findings were extracted and compiled for meta-analysis subject to data availability. The median (interquartile range [IQR]) of reported data was converted to the mean (standard deviation [SD]) following established methods [22]. In case the standard error of the mean (SEM) was provided only, the SD was calculated from SEM multiplied by the square root of the number of study size.

As between-study heterogeneity was anticipated, we constructed random-effects models [23] to combine the mean (SD) of selected studies and applied the inverse variance weighting

method. The Hedge's g expresses the difference of the means in units of the pooled standard deviation; it measures the effect size for the difference between the means. This study used it to synthesize effect sizes and obtain an overall estimate of the effect of physical activity. It incorporated a correction factor for small sample sizes, which is useful as many PA interventions were of small scales [24]. For interpretation, a value of 0.2, 0.5, and 0.8 was regarded as small, medium, and large effects [25]. Heterogeneity between studies was examined using the I^2 statistic and an I^2 above 50% means substantial heterogeneity [26, 27]. Subgroup analyses and meta-regressions were conducted to investigate heterogeneity across age, obesity, and length of exercise. Due to insufficient data, some subgroup analyses and regressions were not performed for all outcomes. Funnel plots and Egger's test were performed to evaluate the risk of biased results [28]. Statistics analyses were performed using STATA 17 (StataCorp, USA). Data were visualized using Robvis (https://mcguinlu.shinyapps.io/robvis/) [29].

Sensitivity analysis

Leave-one-out analysis was performed to identify influential studies by conducting the meta-analysis multiple times while removing one of the included studies during each iteration. Results were presented as leave-one-out figures. A cumulative meta-analysis was also performed for each outcome according to publication year to identify secular trends.

Patient and Public Involvement

It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

4.4. Results

Identification of studies

After removing duplicated studies, 10294 potentially relevant studies were identified. Initial screening based on title and abstracts resulted in 155 studies retrieved for further evaluation. Following full-text assessment, 150 studies were excluded, leaving five studies.

Additionally, 20 studies were identified through hand search and reading citations, of which nine were excluded, leaving 11 studies. In total, 16 studies were included in the study. (Figure S1).

Characteristics of the included studies

All the 16 included studies were of interventional design. The duration of intervention ranged between one month to nine months with a median duration of 2.8 months (11 weeks). The identified 16 studies have a total study population of 500 people (range 4 to 162 individuals). The average age of participants was 50.1 years. Ten studies recruited people with essential hypertension [30-39], one study recruited people with type 2 diabetes mellitus [40], two studies recruited people with obesity [41, 42], two studies recruited healthy people [43, 44], and one study recruited patients with heart failure [45]. Participants in 12 studies were required to perform aerobic exercise only [30-39, 44, 45]; two studies involved aerobic exercise and its combinations with strength training [40, 42], and two others involved resistance training only [41, 43]. All the studies have a similar exercise frequency of 3-5 sessions per week, while the length of sessions varies according to the exercise intensity, with a median of 12 weeks. Seven studies have an attrition rate of 20% or above [30, 31, 33, 38, 39, 41, 42], with a maximum of 30.9% [33].

Eight studies used maximum oxygen uptake (VO_{2max}) to measure the intensity of aerobic exercise [30, 32, 36-39, 44, 45], with a few studies using heart rate reserve [33], maximum heart rate [34, 35, 42], and lactate threshold [31, 40]. For resistance and strength training, repetition maximum (RM) was used to measure the exercise intensity (Table S2).

Measurement of physical activities

Fourteen studies required participants to perform on-site physical activity under close supervision. The low-workload group in the study by Hagberg et al. [30] was supervised for the first month, and relied on self-reported forms for the remaining eight months. All the participants in the study by Passino et al. [45] had self-conducted exercises with their

compliance to the instructions checked at the beginning and near the end of the study. All the studies have reported the arrangement of physical training.

Measurement of the outcomes

Serum creatinine (SCr), plasma renin activity (PRA), and urea were the most measured biomarkers in selected studies. Two studies reported eGFR [36, 42], one study [42] reported urine albumin-to-creatinine ratio (UACR), and one study [31] was on angiotensin II (Ang II). Twelve of these studies measured fasting biomarkers, while three studies have not specified the fasting status [42, 44, 45]; one study explicitly measured biomarkers after participants have "a light breakfast" [39]. All the biomarkers were measured under resting conditions.

Potential bias and quality assessment

Overall, the selected studies have medium to low quality. Ten out of sixteen studies were evaluated as having a high risk of bias. Most studies had a less representative cohort, especially those published decades ago as early as in the 1980s [30, 31]. Studies with better population representation were published after 2000 [41, 42]. The incomplete outcome data (attrition bias) was another major source of inferior quality, with seven studies having a high attrition rate of over 20% [30, 31, 33, 38, 39, 41, 42]. It was impossible to blind participants in supervised situations due to the nature of the physical activity as an exposure. Considering the nature of the intervention design and the objective evaluation of outcomes through laboratory testing, all studies have a high risk for blinding of participants and personnel (performance bias) and a low risk for blinding of outcome assessment (detection bias). Six studies with a randomized design have provided information on how the random sequence was generated [30, 38, 40, 42, 43, 45]. Some studies also have a higher risk of measurement error for exposure and outcome (Table S3, Figure 4-1, 4-2).

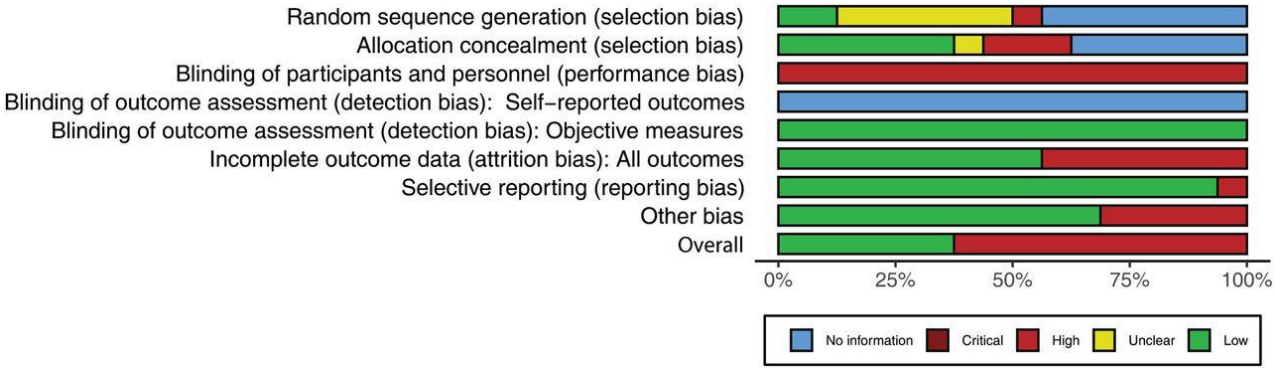

Figure 4-1 Summary of the risk of bias by the Cochrane Risk of Bias tool.

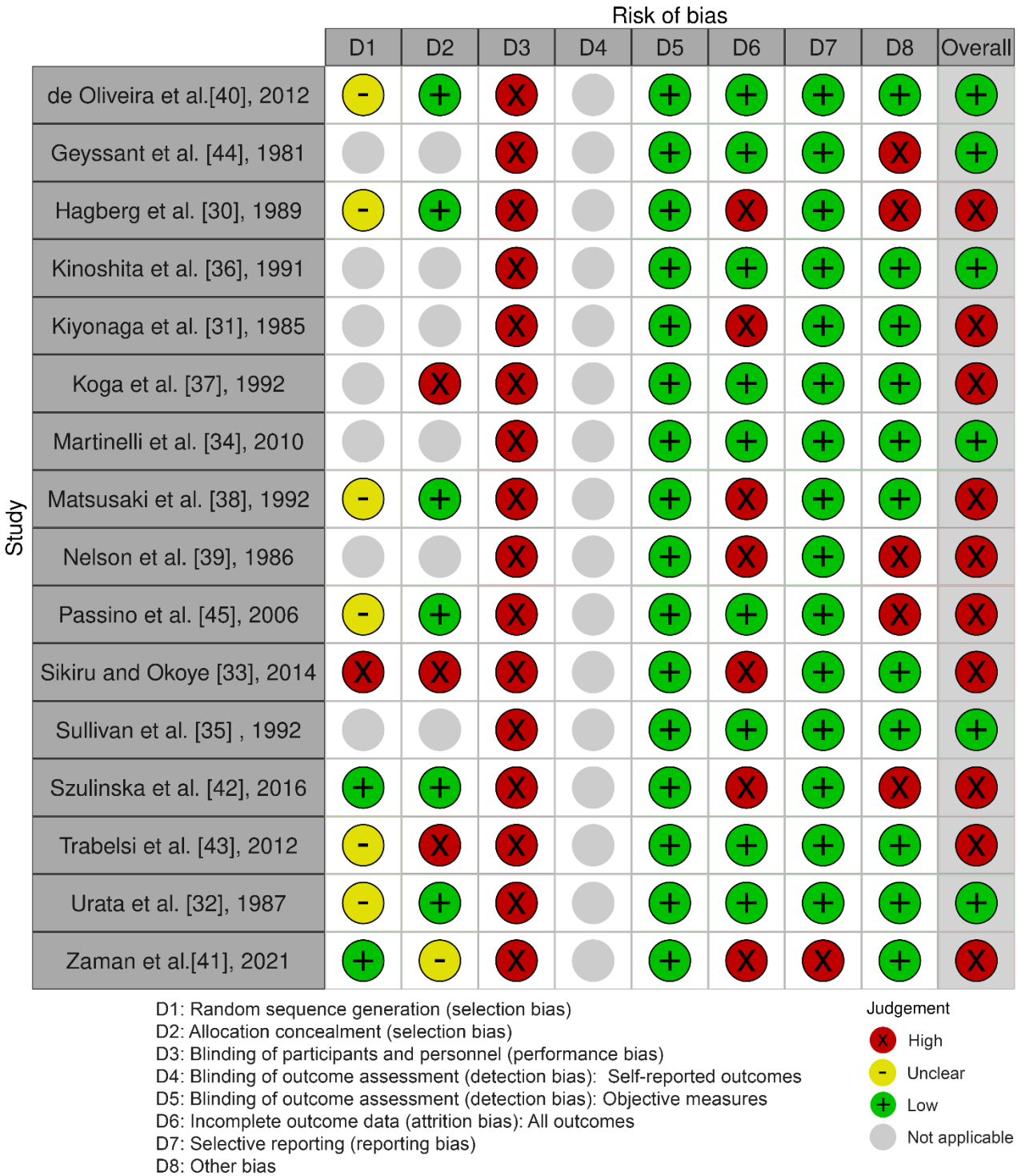

Figure 4-2 Study-specified risk of bias using the Cochrane Risk of Bias tool.

Changes in physical activity and serum creatinine

The meta-analysis included six study populations from four studies [33, 41-43], including 197 participants with an average PA duration of 73 days. The majority of findings have a mean Hedge's g on the right side of the reference line with a wide 95%CI. The pooled result showed a moderate positive effect of PA on SCr (Hedge's g=0.69, 95%CI: 0.13, 1.24). Substantial heterogeneity was detected among cohorts (I^2=81.37%). (Figure 4-3a).

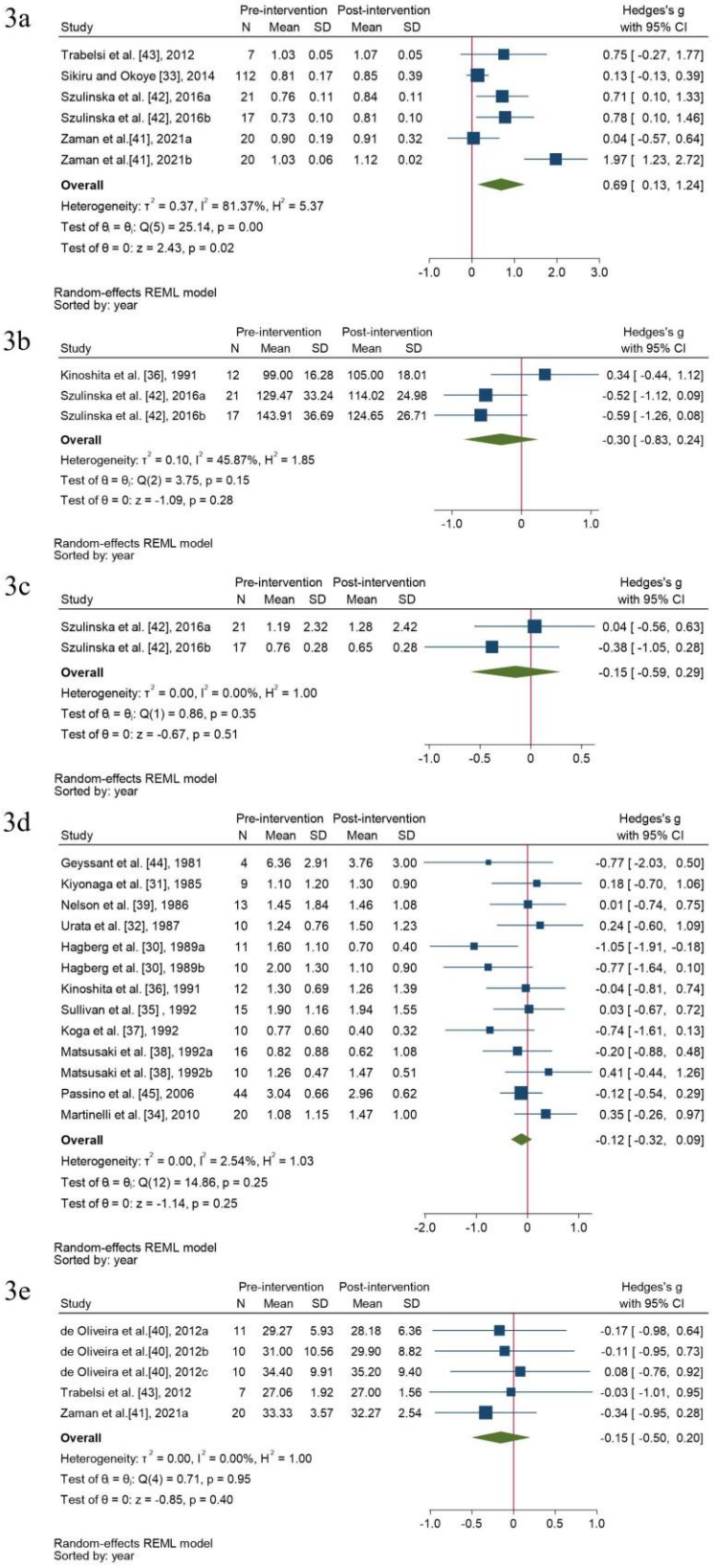

Figure 4-3 Meta-analysis on the associations of changes in physical activity (PA) with kidney-relevant biomarkers: (a) serum creatinine; (b) estimated glomerular filtration rate;

(c) urine albumin-to-creatinine ratio; (d) plasma renin activity; (e) urea. Note: Szulinska et al:[42] patients received endurance training. Szulinska et al:[42] patients received both endurance and strength training. Zaman et al:[41] patients with obesity. Zaman et al:[41] patients without obesity. Hagberg et al:[30] patients performed low-intensity PA. Hagberg et al:[30] patients performed moderate-intensity PA. Matsusaki et al:[38] patients performed low-workload PA. Matsusaki et al:[38] patients performed high-workload PA. de Oliveira et al:[40] patients performed aerobic training. de Oliveira et al:[40] patients performed strength training. de Oliveira et al:[40] patients performed aerobic and strength training. Zaman et al:[41] patients with obesity. REML, Restricted maximum likelihood.

Stratifying by obesity, only two groups of participants with obesity from a single study [42] have a statistically significant pooled effect (Hedge's =0.74, 95%CI: 0.29, 1.20). Stratified by the median of the length of exercises (12 weeks), only two cohorts from one study who have undergone exercise over 12 weeks have a significant pooled effect (Hedge's =0.74, 95%CI: 0.29, 1.20) (Figure S2a-b).

The funnel plot showed mild asymmetry, and Egger's test showed no small-study effects (P value=0.21). Sensitivity analysis showed a consistent result as that of the primary analysis; the removal of one cohort from Zaman [41] largely attenuated the pooled effect (Hedge's =0.38, 95%CI: 0.05, 0.72). Obesity was identified as the only important source of heterogeneity. Cumulative meta-analyses according to the year of publication showed significant evidence of secular trends for SCr (Figure S2c-e).

Changes in physical activity and eGFR

Three cohorts from two studies [36, 42] included 50 people with an average exercise duration of 12 weeks were identified. No significant effect was found in the pooled result of the exercise on eGFR (Hedge's g=-0.30, 95%CI: -0.83, 0.24, I^2=48.57%) (Figure 4-3b).

Changes in physical activity and urine albumin-to-creatinine ratio

Two cohorts from one study [42] included 38 people with an average exercise duration of three months were identified. No statistical significance was found in the pooled result of the exercise on UACR (Hedge's g=-0.15, 95%CI: -0.59, 0.29) (Figure 4-3c).

Changes in physical activity and plasma renin activity

Thirteen cohorts from eleven studies [30-32, 34-39, 44, 45] included 184 people with an average exercise duration of 129 days in the meta-analysis. No association between PA and PRA was observed (Hedge's g=-0.12, 95%CI: -0.32, 0.09). Minor heterogeneity was found among cohorts (I^2=2.54%) (Figure 4-3d).

In stratified analyses, no associations were found by obesity status, exercise length, and age- groups. There was no statistically significant effect of exercise on PRA in people aged 60 and above, the upper 95%CI was close to zero (Hedge's =-0.54, 95%CI: -1.14, 0.06) (Figure S3a-c).

The funnel plot showed good symmetry. Egger's test showed no small-study effects (P value =0.39). Sensitivity analysis showed a consistently insignificant result as that of the primary analysis, with no influential single studies. Meta-regression showed no important source of heterogeneity. Cumulative meta-analysis showed no significant changes in research findings (Figure S3d-f).

Changes in physical activity and serum urea

Five cohorts from three studies [40, 41, 43] included 48 people with an average exercise duration of 73 days. No association was found between PA and urea (Hedge's g=-0.15, 95%CI: -0.50, 0.20). No heterogeneity was found among cohorts (I^2=0.00%) (Figure 4-3e). Data were consistent in subgroup analyses. (Figure S4a).

The funnel plot showed good symmetry, and Egger's test showed no small-study effects (P value =0.39). Sensitivity analysis showed a consistently insignificant result as that of the primary analysis, with no influential single studies. Meta-regression showed no important source of heterogeneity. Cumulative meta-analyses showed no significant changes in research findings (Figure S4b-d).

Changes in physical activity and other kidney-related biomarkers

Five cohorts from three studies [40, 41, 43] included 48 people with an average exercise duration of 73 days. No association was found between PA and urea (Hedge's g=-0.15, 95%CI: -0.50, 0.20). No heterogeneity was found among cohorts (I^2=0.00%) (Figure 4-3e). Data were consistent in subgroup analyses.

4.5. Discussion

In this systematic review and meta-analysis of 16 interventional studies involving 500 people without known kidney diseases, we evaluated the available data exploring the association of change in PA with kidney function. Change in PA was found only to have a positive association with SCr, not with eGFR. There was some limited evidence that participants with obesity and people who exercised for more than 12 weeks may have a larger increase in SCr as compared to their counterparts. Sensitivity analysis was in line with the primary analysis; mild publication bias and a secular trend were found. The general quality of studies was suboptimal to make robust conclusions, and the number and size of studies were generally small (ranging from 4 to 112 participants).

Due to the possibility of physical activity to induce muscle growth, which is the primary source of SCr, the role of body composition in the association between physical activity and SCr deserves discussion. Among three studies reporting on SCr and body composition, Szulinska et al.[42] reported a significant increase in lean body mass and SCr, and a decrease in body fat% in a population receiving endurance and strength training for three months; Trabelsi et al.[43] reported no significant changes in body fat% but a significant increase in

SCr in a population receiving one-month resistance training, while Sikiru et al.[33] reported no significant change in body fat% and a likely increase in SCr in a population receiving eight weeks of aerobic training. It is noteworthy that the study populations of the above studies had markedly different body compositions, with the latter two having low baseline body fat% (11.9% and 13.5%, respectively), while the population in the first study had an average body fat% of over 33%. Additionally, Kinoshita et al.[36] reported no significant change in eGFR in 12 non-obese people after a ten-week aerobic exercise, which implied a possible insignificant change in SCr. Therefore, the impact of PA on SCr levels may be related to body composition.

In the pooled result, there was no statistically significant association of exercise with UACR (Figure 3c). The pooled result came from two cohorts of one study with a small study population [42]. It should be noted that while urinary albumin and urinary creatinine both increased after endurance-strength training, their ratio did not change much, implying a balanced increase of albumin and creatinine. In people who only performed endurance training, no increases in albumin, creatinine, or UACR were found. Therefore, the increase in albumin and creatinine might be caused by increased muscle mass or post-activity proteinuria.

As an important hormone secreted by the kidney for regulating blood pressure, renin has long been a topic of interest. Among the biomarkers under discussion, renin is the most extensively researched, with the earliest studies dating back to the 1980s. However, nine of the eleven studies on renin were published in 1992 or earlier, with only two published after 2000. The studies involved a small number of participants, with most having between 10 and 20 individuals. Nevertheless, the cumulative meta-analysis based on publication year revealed a progressively narrowing 95% confidence interval with an upper limit approaching zero and a consistently negative effect size. In the research conducted by Matsuaki et al. [38]; despite the absence of a significant difference in baseline PRA between the low- (50% VO_{2max}) and the high-workload (75% VO_{2max}) group, both cohorts

manifested a similar pattern characterized by two interlocking M shapes throughout six measurements conducted at baseline and week 1/2/4/7/10. The PRA pattern exhibited by the low-workload group between Week 1 and Week 10 was similar to that of the high-workload group between Week 0 and Week 7. The PRA change in the low-workload group was "delayed" by one week compared to the high-workload group. Specifically, the PRA in the low-workload group experienced a slight decline in the first week, followed by an increase in the second week, whereas the PRA in the high-workload group increased in the first week. The underlying mechanism of this finding remains elusive.

Kiyonaga et al. [31] reported a significant increase in Angiotensin II after 20 weeks of mild aerobic exercise in eight patients, yet no significant increase was observed by week 10. As renin secretion is the first step in the production of Angiotensin II, it can be speculated that an exercise lasting over 20 weeks may significantly impact renin (and thus Angiotensin II). Renin is rarely measured in clinical practice and is affected by many antihypertensive drugs. Although these findings are interesting, any effect of PA on renin is unlikely to translate into information used to inform clinical guidelines.

Urea is clinically measured to evaluate kidney impairment although to a lesser degree than SCr. There was a lack of a significant association between exercise and urea levels. One possible explanation is that, considering the absence of kidney disease in all study participants at baseline, the closely supervised, low to moderate-intensity exercise did not result in kidney damage or alterations that exceeded the kidney compensation, thus precluding significant observable variations in urea levels.

To the best of our knowledge, this is the first systematic review and meta-analysis to investigate the association between changes in physical activity and kidney biomarkers in people without known kidney diseases. The studies included underwent rigorous assessment based on strict criteria. We observed low heterogeneity among most of the biomarkers studied. Sensitivity analyses aligned with our primary findings.

It is unlikely that any rise in serum creatinine with PA represents an adverse effect of PA on kidney function, given the widespread benefits of PA on cardiovascular health. It is theoretically plausible that PA reduces glomerular perfusion and hence creatinine rises. This effect is seen in people taking both medications inhibiting the renin-angiotensin system [46] and sodium-glucose transporter 2 inhibitors [47]. This transient rise in serum creatinine is associated with long-term cardiovascular and kidney benefits with these agents. Studies of PA with long durations are required to determine if any change in creatinine with PA is associated with benefit or harm on cardiorenal health.

Although efforts have been made in this study, there are several limitations. Firstly, most studies had a very small sample size, with only a few exceptions. This may, in part, be attributed to a general insufficiency of resources, such as funding and personnel. Secondly, over 50% of the studies were found to have considerable bias, primarily stemming from high attrition rates, negatively impacting the quality of these studies. Finally, some studies were conducted decades ago, which could introduce potential issues with measurement methods, accuracy, and lab standards. This underscores the pressing requirement for updated and standardized research.

In conclusion, by examining the changes in physical activity among individuals without diagnosed kidney diseases, the findings of this study supported the positive association of physical activity with SCr. However, the association with kidney function specifically could not be confirmed by existing data on other kidney biomarkers. Given the global advocacy for increased physical activity by governments and medical professionals, and the clinical importance of kidney function, further research should be conducted in the general population to investigate the association of change in PA with kidney function.

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4.7. Supplementary tables and figures for Chapter 4

Table S1 Search Terms

Ovid MEDLINE(R) and In-Process, In-Data-Review & Other Non-Indexed Citations, 1946 Year

Steps	Terms	Hits
1	exp physical activity/ or exp exercise test/	291234
2	exp physical education/ or exp physical fitness/ or exp cardiorespiratory fitness/	47746
3	(exercise* or sport* or walking* or cycling or swimming or running or jogging or sedentary or inactiv*).ti,ab.	974933
4	(physical* adj3 (activ* or inactiv* or treat* or exercis* or exertion*)).ti,ab.	189257
5	or/1-4	1150170
6	(controlled clinical trial).ti,ab.	18436
7	exp clinical trial/	960936
8	(randomized controlled trial).ti.	58191
9	(randomized or trial).ti.	353729
10	exp cohort studies/	2439066
11	(retrospective or prospective or longitudinal).ti.	346157
12	or/6-11	3359654
13	5 and 12	172209
14	(exp animal/ or nonhuman/) not exp human/	5086829
15	13 not 14	170034
16	(interven* or change* or traject* or shift* or switch* or alternat* or differen* or variat* or revamp*).ti,kw.	1810596
17	15 and 16	19570

18	(uremi* or uraemi* or albuminuria* or proteinuria* or urin* or albumin* or protein* or glomerular filtration rate* or ?GFR).ti,ab.	4047257
19	exp kidney/ or exp proteinuria/	400331
20	(kidney or renal or disease or insufficien* or failure* or nephro*).ti,ab.	5132016
21	or/18-20	8407973
22	(kidney or renal).mp. and (transplan* or graft*).ti.	94451
23	21 not 22	8319260
24	17 and 23	4571
25	(conference abstract or conference paper or conference review).pt.	0
26	24 not 25	4571
27	editorial/ or letter/ or case reports/ or comment/ or note/	4217780
28	26 not 27	4540

Embase 1947-Present, updated daily

Steps	Terms	Hits
1	exp physical activity/ or exp exercise test/	623139
2	exp physical education/ or exp physical fitness/ or exp cardiorespiratory fitness/	65532
3	(exercise* or sport* or walking* or cycling or swimming or running or jogging or sedentary or inactiv*).ti,ab.	1279181
4	(physical* adj3 (activ* or inactiv* or treat* or exercis* or exertion*)).ti,ab.	260815
5	or/1-4	1707972
6	(controlled clinical trial).ti,ab.	24269
7	exp clinical trial/	1820578
8	(randomized controlled trial).ti.	71118
9	(randomized or trial).ti.	499542
10	exp cohort studies/	962599
11	(retrospective or prospective or longitudinal).ti.	484101
12	or/6-11	3118564
13	5 and 12	193380
14	(exp animal/ or nonhuman/) not exp human/	7826893
15	13 not 14	190775
16	(interven* or change* or traject* or shift* or switch* or alternat* or differen* or variat* or revamp*).ti,kw.	2269582
17	15 and 16	22019
18	(uremi* or uraemi* or albuminuria* or proteinuria* or urin* or albumin* or protein* or glomerular filtration rate* or ?GFR).ti,ab.	5179794
19	exp kidney/ or exp proteinuria/	643196
20	(kidney or renal or disease or insufficien* or failure* or nephro*).ti,ab.	7596748
21	or/18-20	11605916

22	(kidney or renal).mp. and (transplan* or graft*).ti.	156070
23	21 not 22	1145933 1
24	17 and 23	5916
25	(conference abstract or conference paper or conference review).pt.	5452713
26	24 not 25	4217
27	editorial/ or letter/ or case reports/ or comment/ or note/	2784877
28	26 not 27	4193

Pubmed

Steps	Terms	Hits
1	"exercise"[mesh] OR "exercise test"[mesh]	291459
2	"Physical Education and Training"[mesh] OR "physical fitness"[mesh] OR "cardiorespiratory fitness"[mesh]	47747
3	(exercise*[tiab] OR sport*[tiab] OR walking*[tiab] OR "cycling"[tiab] OR "swimming"[tiab] OR "running"[tiab] OR "jogging"[tiab] OR "sedentary"[tiab] OR inactiv*[tiab])	991749
4	(physical*[tiab] AND (activ*[tiab] OR inactiv*[tiab] OR treat*[tiab] OR exercis*[tiab] OR exertion*[tiab]))	459726
5	#1 OR #2 OR #3 OR #4	1384590
6	("controlled clinical trial"[tiab])	18695
7	"clinical trial"[pt]	961591
8	("randomized controlled trial"[ti])	58192
9	("randomized"[ti] OR "trial"[ti])	353735
10	"cohort studies"[mesh]	2441995
11	("retrospective"[ti] OR "prospective"[ti] OR "longitudinal"[ti])	346215
12	#6 OR #7 OR #8 OR #9 OR #10 OR #11	3362395
13	#5 AND #12	210509
14	"animals"[mesh]	26116055
15	#13 NOT #14	8628
16	interven*[ti] OR change*[ti] OR traject*[ti] OR shift*[ti] OR switch*[ti] OR alternat*[ti] OR differen*[ti] OR variat*[ti] OR revamp*[ti]	1765363
17	#15 AND #16	1223
18	(uremi*[tiab] OR uraemi*[tiab] OR albuminuria*[tiab] OR proteinuria*[tiab] OR urin*[tiab] OR albumin*[tiab] OR protein*[tiab] OR glomerular filtration rate*[tiab] OR "eGFR"[tiab] OR "mGFR"[tiab])	4147077

19	"kidney"[mesh] OR "proteinuria"[mesh]	400520
20	("kidney"[tiab] OR "renal"[tiab] OR "disease"[tiab] OR insufficien*[tiab] OR failure*[tiab] OR nephro*[tiab])	5250858
21	#18 OR #19 OR #20	8585949
22	(("kidney"[tiab] OR "kidney"[mh]) OR ("renal"[tiab])) AND (transplan*[ti] OR graft*[ti])	87814
23	#21 NOT #22	8498135
24	#17 AND #23	295
25	"editorial"[pt] OR "letter"[pt] OR "comment"[pt]	2129064
26	#24 NOT #25	294

Web of Science

Steps	Terms	Hits
1	TS=(physical activity OR exercise test OR physical fitness OR exercise OR sport OR sedentary OR inactive OR exertion)	2869900
2	TS=(controlled clinical trial OR clinical trial OR randomized controlled trial OR cohort study OR retrospective study OR prospective study OR longitudinal study)	4391262
3	#1 AND #2	230548
4	TS=(animal OR nonhuman OR non-human)	30545714
5	#3 NOT #4	118699
6	TS=(interven* or change* or traject* or shift* or switch* or alternat* or differen* or variat* or revamp*)	46832576
7	#5 AND #6	82111
8	TS=(uremi* or uraemi* or albumin* or proteinuria* or glomerular filtration rate* or eGFR or mGFR or kidney* or renal failure* or nephro*)	2677866
9	TS=(kidney or renal)	2378506
10	TS=(transplan* or graft*)	2270415
11	#9 AND #10	323241
12	#8 NOT #11	2405699
13	#7 AND #12	1320
14	TS=(editorial or letter or case report* or comment)	3433986
15	#13 NOT #14	1267

Table S2 General characteristics of the included studies

Author, year	Study type	Country	Exercise Group Characteristics	Baseline Size of Exercise Group	Attrition	Study Population Age	Type of Exercise	Exercise Frequency	Exercise Length	Exercise Intensity	Kidney-relevant outcome	Findings
de Oliveira *et al*.[40], 2012	RCT	Brazil	Patients with type 2 diabetes mellitus of a diabetes ambulatory clinic.	Aerobic training: 12 people Strength training: 12 people Combination training: 12 people	Aerobic training: 9.1% Strength training: 16.7% Combination training: 16.7%	Mean (SD), years: Aerobic training: 52.09 (8.71) Strength training: 54.10 (8.94) Combined training: 57.90 (9.82)	Aerobic training, strength training, and combined training	One hour/session, three sessions/week	12 weeks	Not used VO2peak in aerobic and combined training due to unable to get accurate value, used lactate threshold. Strength training: 50% of 1 RM for the Week 1&2, 8-12 RM for Week 3&4.	Urea	Pre/Post Exercise, mean (SD), mg/dL Aerobic training: Urea 29.27 (5.93) / 28.18 (6.36) Strength training: Urea 31.00 (10.56) / 29.90 (8.82) Combined training: Urea 34.40 (9.91) / 35.20 (9.40)
Geyssant *et al*. [44], 1981	CT	France	Healthy male.	4 people	0%	Mean (SD), years: 36 (6.4)	Aerobic	One hour/session, four sessions/week	5 months	87% VO2max	PRA	Pre/Post Exercise, mean (SD), ng/l/mn PRA, 106.08 (48.48)/ 62.5 (49.9)
Hagberg *et al*. [30], 1989	RCT	United States of America	Patients with essential hypertension.	Low-intensity: 14 people Moderate-intensity: 10 people	Low-intensity: 21.4% Moderate-intensity: 0%	Mean (SD), years: all groups 64 (3)	Aerobic	Low-intensive: one hour/session, max three sessions/week Moderate-	9 months	Low-intensity: 50% VO2max Moderate-intensity: 70-85% VO2max	PRA	Pre/Post Exercise, mean (SD), ng/ml/hr Low-intensity: PRA 1.6 (1.1) / 0.7 (0.4) Moderate-intensity:

									intensive: 45 to 60 minutes/session, 3 sessions/week for at least the last 4-5 months of training				PRA 2.0 (1.3) / 1.1 (0.9)
Kinoshita *et al*. [36], 1991	CT	Japan	Patients with essential hypertension.	12 people	0%	Mean (SD), years: 51.7 (2.3)	Aerobic	One hour/session, three sessions/week	10 weeks	50% VO2max	PRA, eGFR	Pre/Post Exercise, mean (SE), ng/ml/h PRA 1.3 (0.2) / 1.26 (0.4) Pre/Post Exercise, mean (SE), ml/min eGFR 99 (4.7) / 105 (5.2)
Kiyonaga *et al*. [31], 1985	CT	Japan	Patients with essential hypertension.	12 people	At 10 weeks: 0% At 20 weeks: 25%	Mean (Range), years: 46 (34 to 56)	Aerobic	One hour/session, three sessions/week	20 weeks	Used lactate threshold, but claimed to have a 50% VO2max although data were not published.	PRA Ang II	Pre/Post Exercise, mean (SE), ng/ml/hr PRA 11 (4) / 13 (3) Pre/Post Exercise, mean (SE), pg/ml Ang II 58 (8) / 91 (12)
Koga *et al*. [37], 1992	CT	Japan	Female atients with essential hypertension	10 people	0%	Mean (SEM), years: 49 (2)	Aerobic	One hour/session, three sessions/week	10 weeks	50% VO2max	PRA	Pre/Post Exercise, mean (SE), ng/ml/h PRA 0.77 (0.19) /

												0.4 (0.1)
Martinelli *et al*. [34], 2010	CT	Brazil	Overweight patients with hypertension.	20 people	0%	Mean (SD), years: 57 (7.1)	Aerobic	40 min/session, three sessions/week	16 weeks	60-80% HRmax	PRA	Pre/Post Exercise, median (IQR), ng/ml/h PRA 0.8 (0.45 - 2.0) / 1.45 (0.8 - 2.15)
Matsusaki *et al*. [38], 1992	CT	Japan	Patients with hypertension.	Low-workload: 16 people High-workload: 14 people	Low-workload: 0% High-workload: 28.6%	Mean (SEM), years: all groups 47.2 (1.5)	Aerobic	Low-workload: one hour/session, three sessions/week High-workload: 30-40 min/session, three sessions per week	10 weeks	Low-workload: 50% VO2max High-workload: 75% VO2max	PRA	Pre/Post Exercise, mean (SE), ng/ml/h Low-workload: PRA 0.82 (0.22) / 0.62 (0.27) High-workload: PRA 1.26 (0.15) / 1.47 (0.16)
Nelson *et al*. [39], 1986	CT	Australia	Patients with essential hypertension of a risk-evaluation clinic.	17 people	23.5%	Mean (Range) , years: 44 (25 to 62)	Aerobic	Three levels of activity for one month each successively. First month: normal sedentary, no training Second month: 45 min/session, three	2 months (exclude the first sedentary month)	60-70% VO2max	PRA	Pre/Post Exercise, mean (SEM), ng/ml/h PRA 1.45 (0.51) / 1.46 (0.30)

								sessions/week Third month: 45 min/session, seven sessions/week					
Passino *et al*. [45], 2006	RCT	Italy	Patients with heart failure.	47 people	6.4%	Mean (SD), years: 60 (2)	Aerobic	Minimum 30 min/day, three days/week	9 months	Heart rate at 65% VO2max	PRA	Pre/Post Exercise, mean (SD), ng/ml/h PRA 3.04 (0.66) / 2.96 (0.62)
Sikiru and Okoye [33], 2014	RCT	Nigeria	Patients with essential hypertension of a hypertensive clinic.	162 people	30.9%	Mean (SD), years: 58.63 (7.22)	Aerobic	45 min/session, three sessions/week for Week 1 and 2 One hour/session, three sessions/week, for Week 3-8	8 weeks	60-79% of HR reserve	SCr	Pre/Post Exercise, mean (SD), mg/dL SCr 0.81 (0.17) / 0.85 (0.39)
Sullivan *et al*. [35] , 1992	CT	United States of America	Male patients with uncomplicated essential hypertension.	15 people	0%	Mean (SD), years: 42.3 (1.0)	Strenuous Aerobic	18 min/session, three sessions/week	6 weeks	90% HRmax	PRA	Pre/Post Exercise, mean (SE), ng/ml/h PRA 1.9 (0.3) / 1.94 (0.4)
Szulinska *et al*. [42], 2016	RCT	Poland	Women with obesity.	Endurance training: 22 people Endurance+strength	Endurance training: 4.5% Endurance+strength	Mean (SD), years: Endurance 51.3 (8.3)	Endurance and Endurance+strength training	One hour/session, three	3 months	Endurance group: 50-80% HRmax Endurance+strength	SCr eGFR UACR	Pre/Post Exercise, mean (SD) Endurance group

				training: 22 people	training: 22.7%	Endurance+strength 48.2 (11.2)		sessions/week	group: 50-80% HRmax for endurance training, unclear intensity for strength exercise.			SCr, mg/dL 0.76 (0.11) / 0.84 (0.11) eGFR-MDRD, 87.81 (18.43) / 77.90 (12.65) eGFR-CG, 129.47 (33.24) / 114.02 (24.98) UACR, mg/mmol cr 1.19 (2.32) / 1.28 (2.42) Endurance+strength group SCr, mg/dL 0.73 (0.10) / 0.81 (0.10) eGFR-MDRD, 93.58 (17.87) / 82.54 (12.01) eGFR-CG, 143.91 (36.69) / 124.65 (26.71) UACR, mg/mmol cr 0.76 (0.28) / 0.65 (0.28)
Trabelsi *et al*. [43], 2012	CT	Turnisa	Male recreational bodybuilders.	non-faster: 7 people	0%	Mean (SD), years: non-faster 26 (3)	Resistance	Four sessions/week	1 month	Four sets with a load of 10 RM for each exercise.	Urea SCr	Pre/Post Exercise, mean (SD), mmol/L Urea 4.51 (0.32) / 4.5 (0.26)

												Pre/Post Exercise, mean (SD), μmol/L SCr 91.14 (4.45) / 94.29 (4.31)
Urata *et al*. [32], 1987	RCT	Japan	Patients with essential hypertension.	10 people	0%	Mean (SE), years: 51.4 (2.8)	Aerobic	One hour/session, three sessions/week	10 weeks	40-60% VO2max	PRA	Pre/Post Exercise, mean (SE), ng/mL/min PRA 1.24 (0.24) / 1.50 (0.39)
Zaman *et al*.[41], 2021	RCT	Saudi Arabia	Obese and non-obese male people.	Obese resistance training: 25 Non-obese resistance training: 25	Obese resistance training: 20% Non-obese resistance training: 20%	Range, years: 35 to 60	Resistance	50 min/session, three days/week	12 weeks	50-70% of 1 RM	Urea	Pre/Post Exercise, mean (SD), mg/dl Obese people Urea 33.33 (3.57) / 32.27 (2.54),

Ang II,angiotensin II; CT, clinical trial; eGFR, estimated glomerular filtration rate; HR, heart rate; PRA, plasma renin activity; RCT, randomized clinical trial; RM, repetition maximum; SCr, serum creatinine; SD, standard deviation;SE, standard error; UARC, urine albumin-to-creatinine ratio; VO2, maximum rate of oxygen.

Note: Conversion factors for units: serum creatinine in mg/dL to μmol/L, ×88.4; urea nitrogen in mg/dL to mmol/L, ×0.357.

Table S3 Cochrane Risk of Bias Assessment Form

Author, Year	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias): Self-reported outcomes	Blinding of outcome assessment (detection bias): Objective measures	Incomplete outcome data (attrition bias): All outcomes	Selective reporting (reporting bias)	Other bias	Overall
de Oliveira *et al.*[40], 2012	Unclear	Low	High	Not applicable	Low	Low	Low	Low	Low
Geyssant *et al.*[44], 1981	Not applicable	Not applicable	High	Not applicable	Low	Low	Low	Low	Low
Hagberg *et al.*[30], 1989	Unclear	Low	High	Not applicable	Low	High	Low	Low	High
Kinoshita *et al.*[36], 1991	Not applicable	Not applicable	High	Not applicable	Low	Low	Low	Low	Low

Kiyonaga *et al*.[31], 1985	Not applicable	Not applicable	High	Not applicable	Low	High	Low	Low	High
Koga *et al*.[37], 1992	Not applicable	High	High	Not applicable	Low	Low	Low	Low	High
Martinelli *et al*.[34], 2010	Not applicable	Not applicable	High	Not applicable	Low	Low	Low	Low	Low
Matsusaki *et al*.[38], 1992	Unclear	Low	High	Not applicable	Low	High	Low	Low	High
Nelson *et al*.[39], 1986	Not applicable	Not applicable	High	Not applicable	Low	High	Low	Low	High
Passino *et al*.[45], 2006	Unclear	Low	High	Not applicable	Low	Low	Low	Low	Low
Sikiru and Okoye[33], 2014	High	High	High	Not applicable	Low	High	Low	Low	High
Sullivan *et*	Not	Not applicable	High	Not applicable	Low	Low	Low	Low	Low

al.[35], 1992	applicable								
Szulinska *et al*.[42], 2016	Low	Low	High	Not applicable	Low	High	Low	Low	High
Trabelsi *et al*.[43], 2012	Unclear	High	High	Not applicable	Low	Low	Low	Low	High
Urata *et al*.[32], 1987	Unclear	Low	High	Not applicable	Low	Low	Low	Low	Low
Zaman *et al*.[41], 2021	Low	Unclear	High	Not applicable	Low	High	High	Low	High

Figure S1 PRISMA workflow

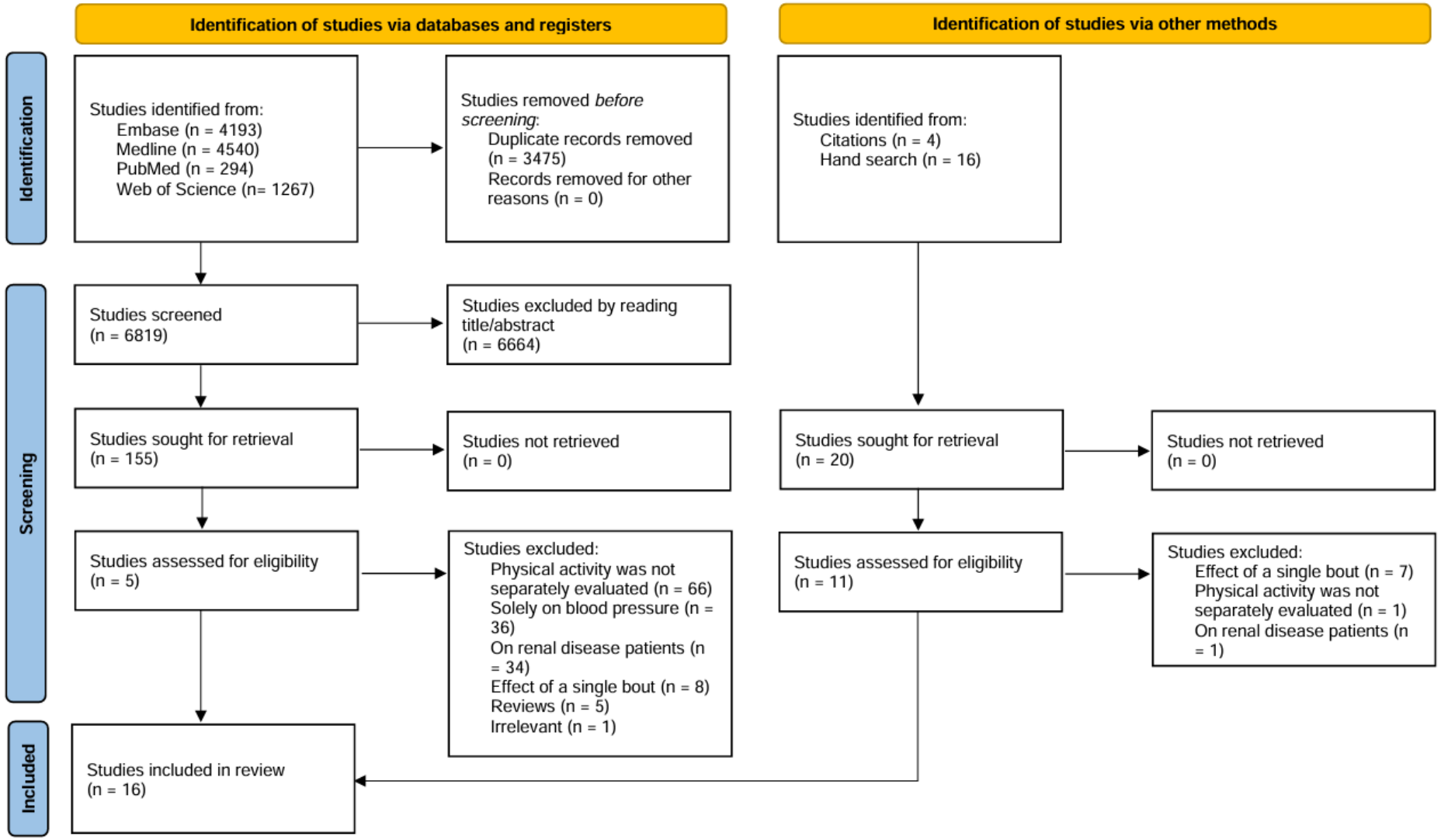
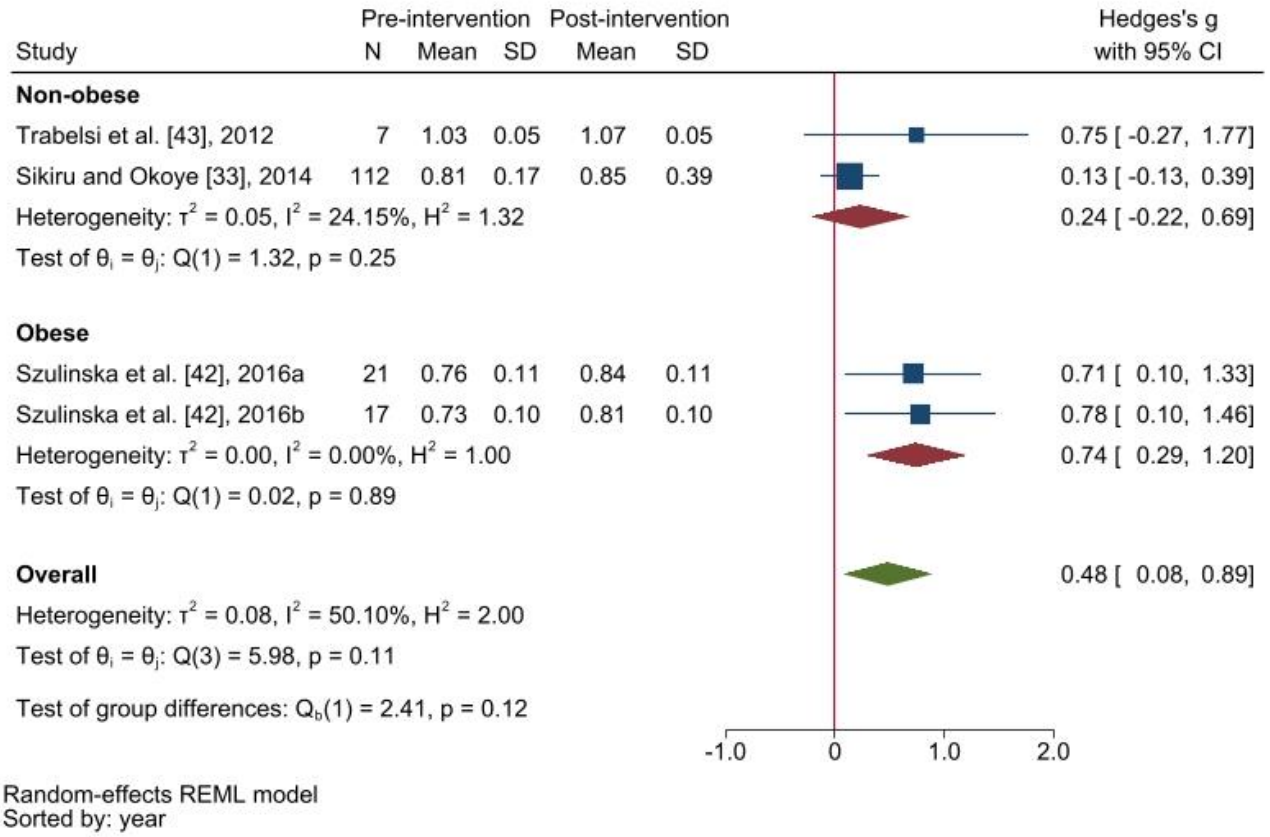


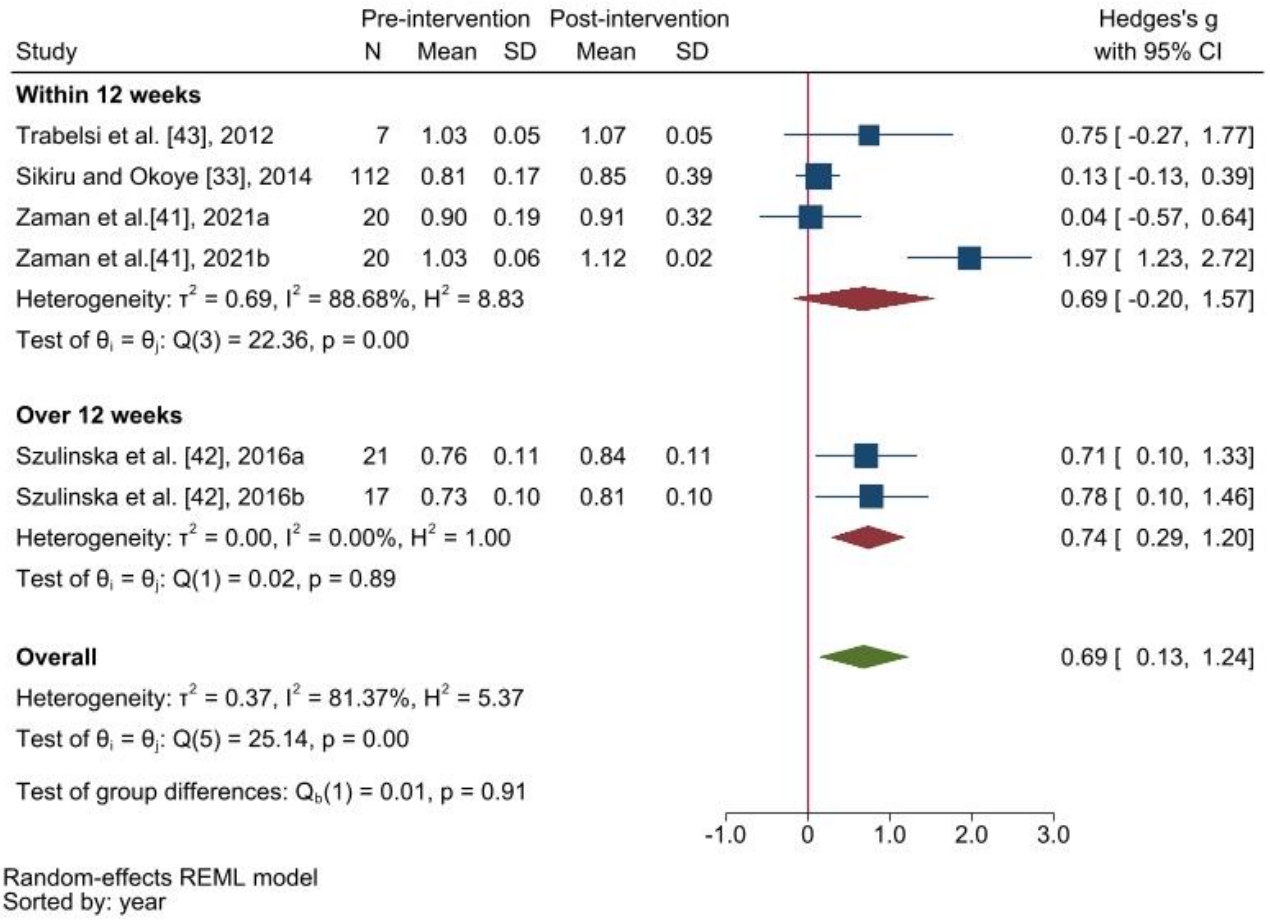
Figure S2a Obesity stratified meta-analysis on the association of changes in physical activity with serum creatinine.



Szulinska *et al.* [42], 2016a: Patients received endurance training.

Szulinska *et al.* [42], 2016b: Patients received both endurance and strength training.

Figure S2b Exercise duration stratified meta-analysis on the association of changes in physical activity with serum creatinine.



Szulinska *et al.* [42], 2016a: Patients received endurance training.

Szulinska *et al.* [42], 2016b: Patients received both endurance and strength training.

Zaman *et al.* [41], 2021a: Patients with obesity

Zaman *et al*.[41], 2021b: Patients without obesity

Figure S2c. Funnel plot of studies on the association of changes in physical activity with serum creatinine

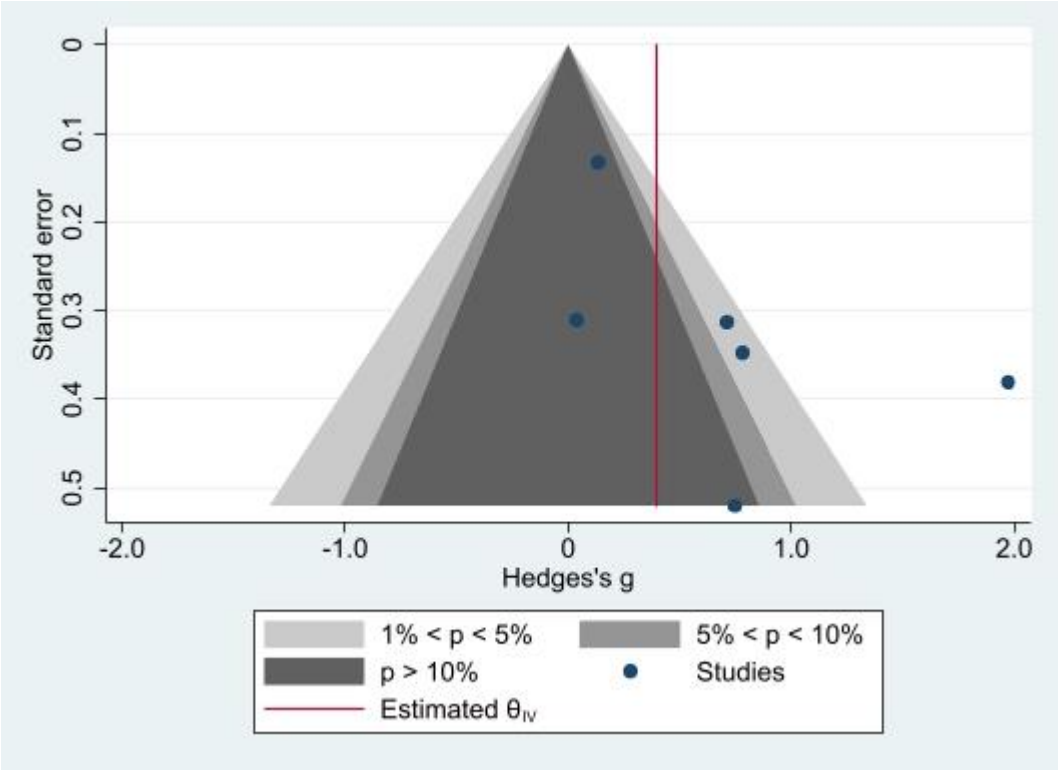
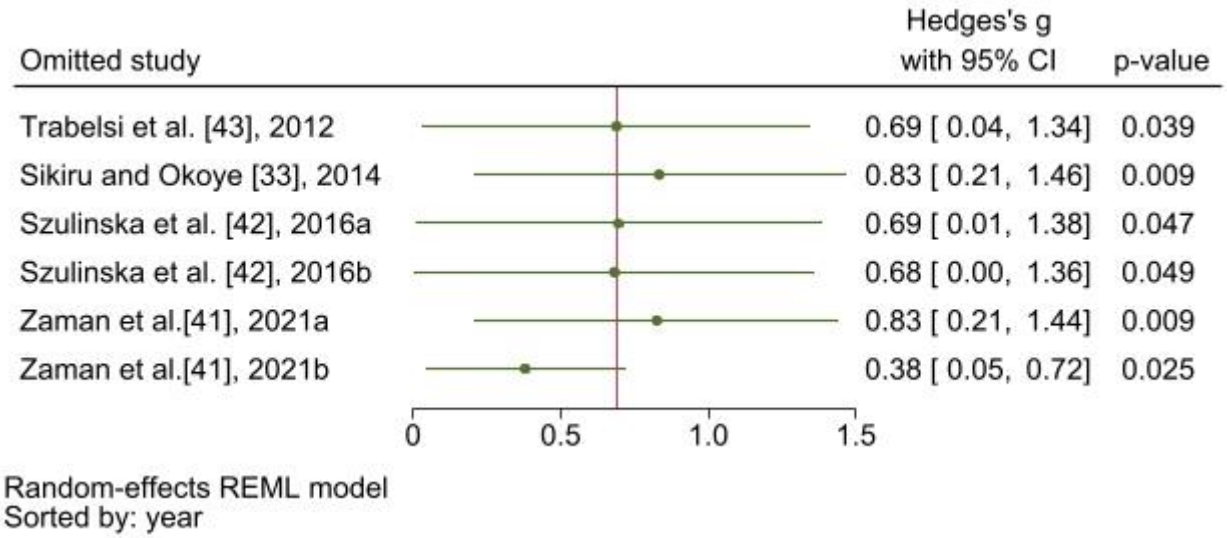


Figure S2d Leave-one-out figure of studies on the association of changes in physical activity with serum creatinine



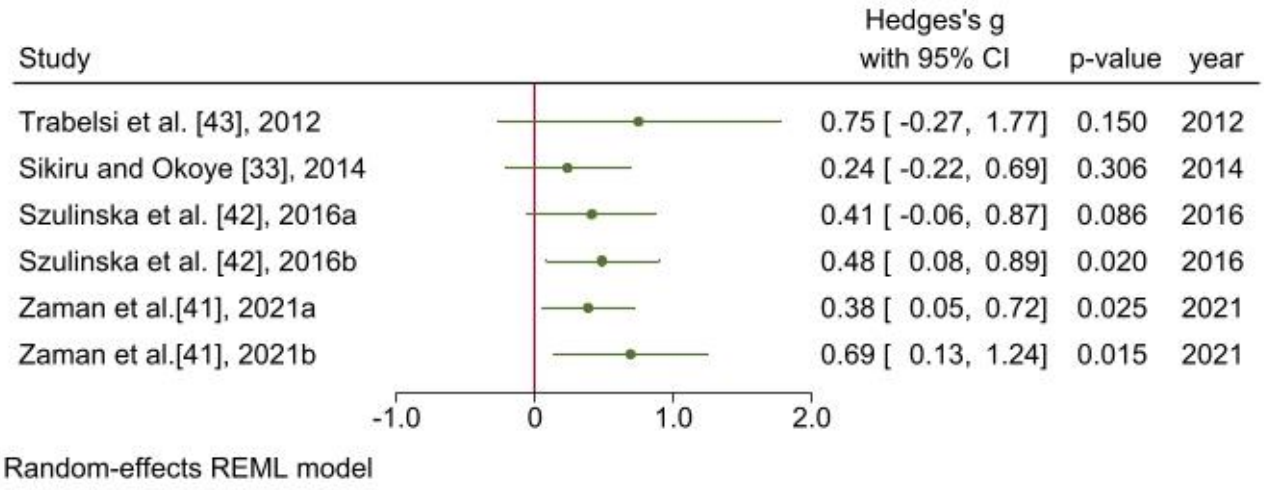
Szulinska *et al.* [42], 2016a: Patients received endurance training.

Szulinska *et al.* [42], 2016b: Patients received both endurance and strength training.

Zaman *et al.* [41], 2021a: Patients with obesity

Zaman *et al*.[41], 2021b: Patients without obesity

Figure S2e Cumulative meta-analysis of the association of changes in physical activity with serum creatinine



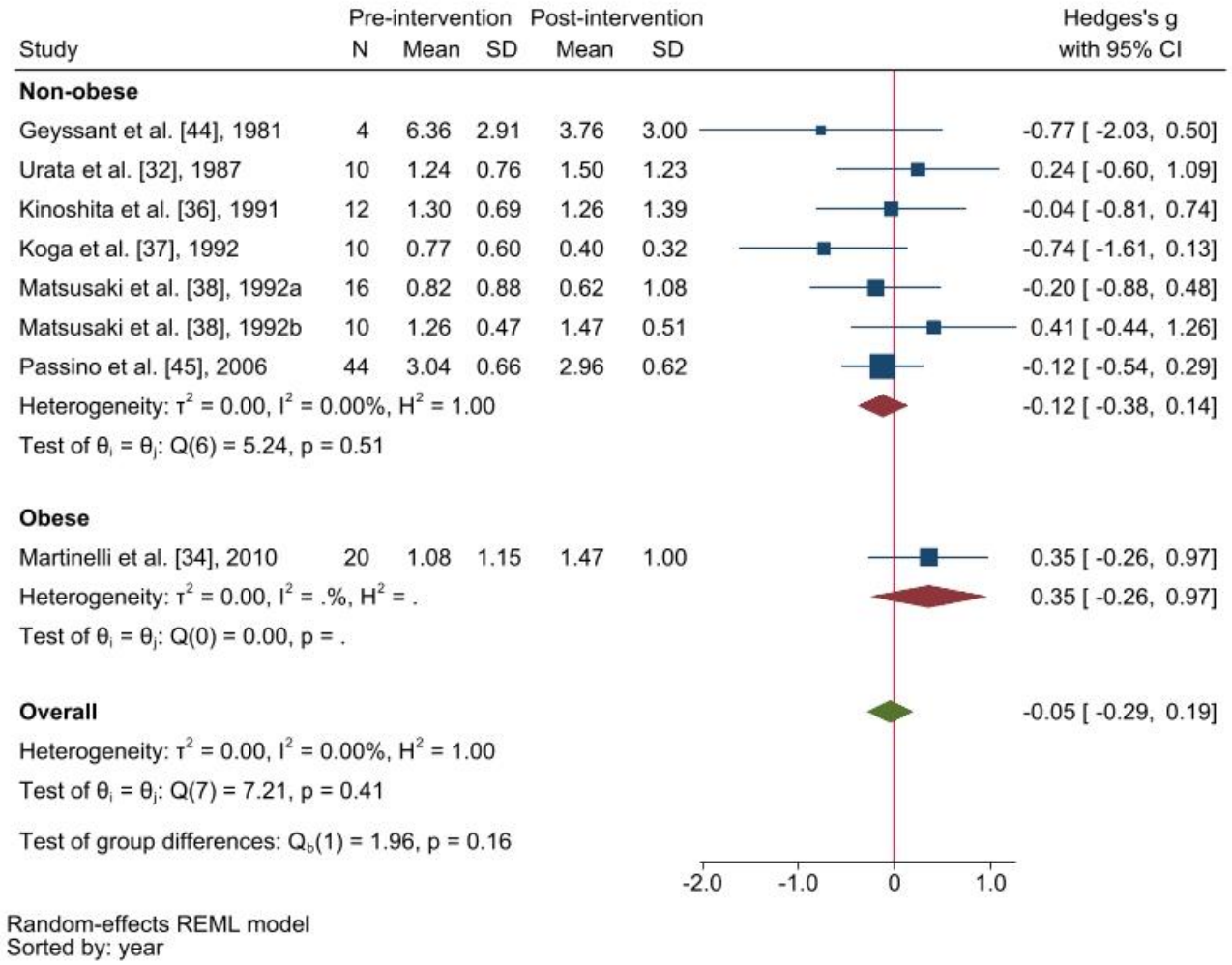
Szulinska *et al.* [42], 2016a: Patients received endurance training.

Szulinska *et al.* [42], 2016b: Patients received both endurance and strength training.

Zaman *et al.* [41], 2021a: Patients with obesity

Zaman *et al*.[41], 2021b: Patients without obesity

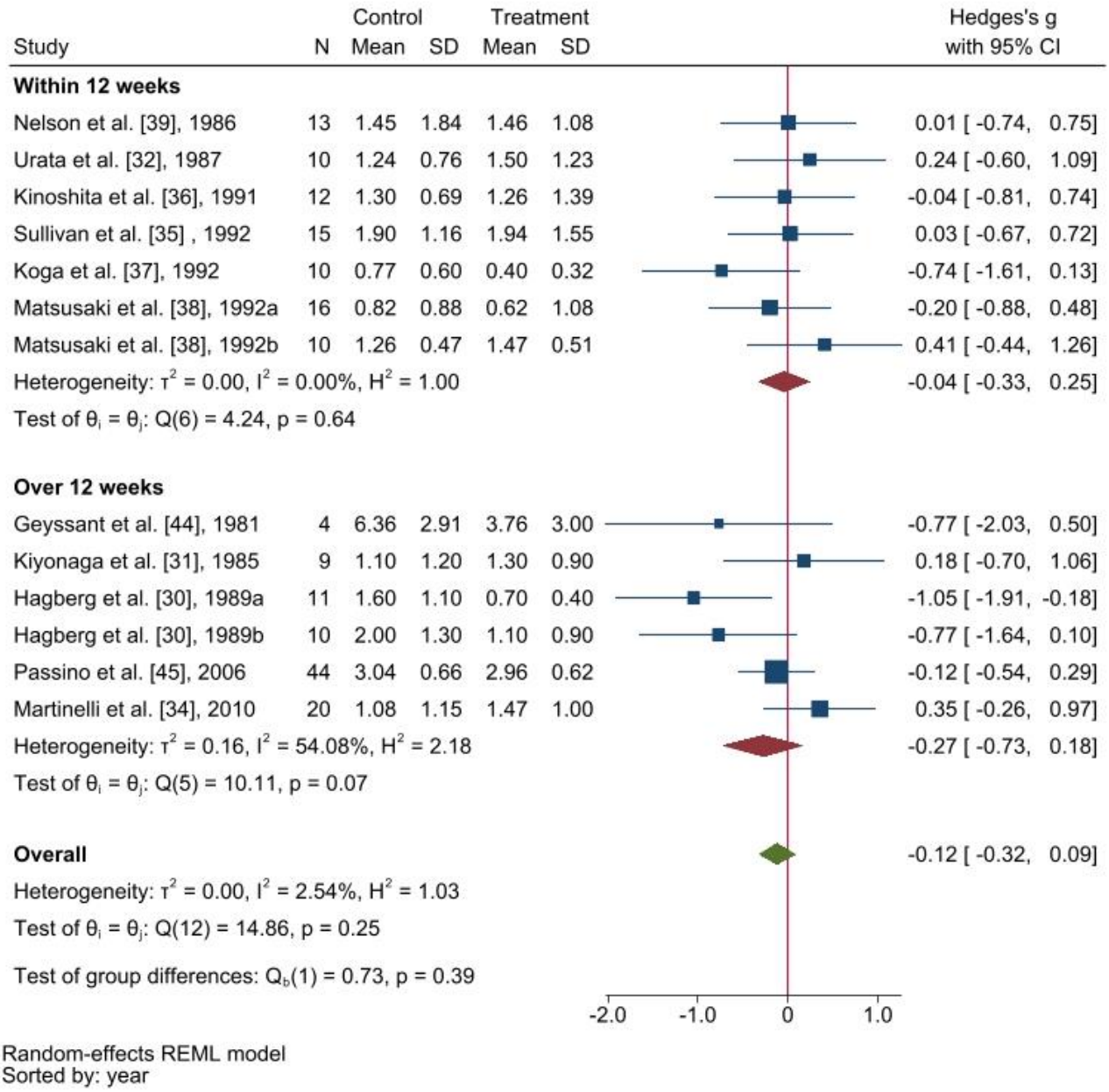
Figure S3a Obesity stratified meta-analysis on the association of changes in physical activity with plasma renin activity



Matsusaki *et al.*[38], 1992a: Patients performed low-workload physical activity.

Matsusaki *et al.*[38], 1992b: Patients performed high-workload physical activity.

Figure S3b Exercise duration stratified meta-analysis on the association of changes in physical activity with plasma renin activity



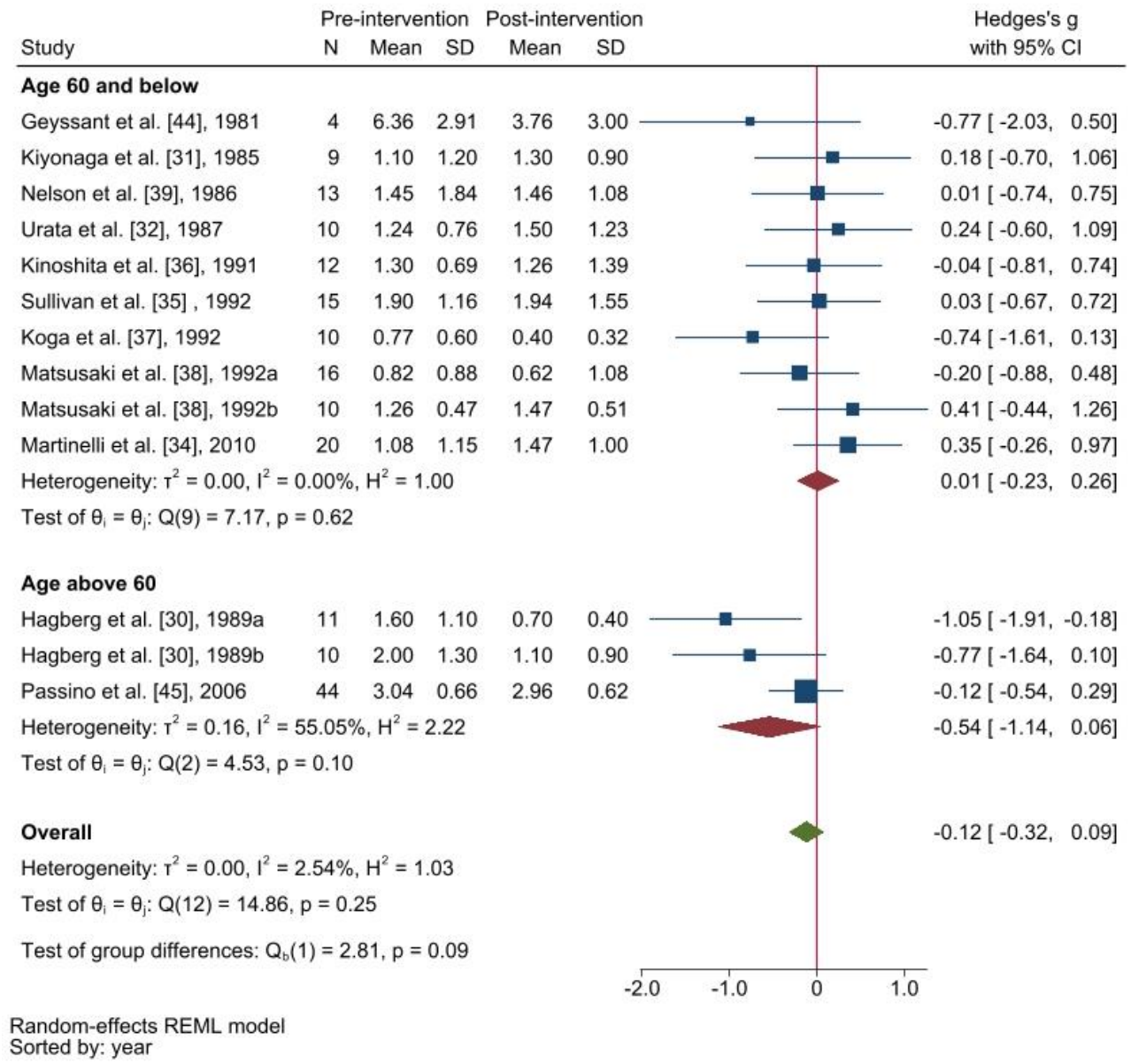
Hagberg *et al.* [30], 1989a: Patients performed low-intensity physical activity.

Hagberg *et al.* [30], 1989b: Patients performed moderate-intensity physical activity.

Matsusaki *et al.*[38], 1992a: Patients performed low-workload physical activity.

Matsusaki *et al.*[38], 1992b: Patients performed high-workload physical activity.

Figure S3c Age stratified meta-analysis on the association of changes in physical activity with plasma renin activity



Hagberg *et al.* [30], 1989a: Patients performed low-intensity physical activity.

Hagberg *et al.* [30], 1989b: Patients performed moderate-intensity physical activity.

Matsusaki *et al.*[38], 1992a: Patients performed low-workload physical activity.

Matsusaki *et al.*[38], 1992b: Patients performed high-workload physical activity.

Figure S3d. Funnel plot of studies on the association of changes in physical activity plasma renin activity

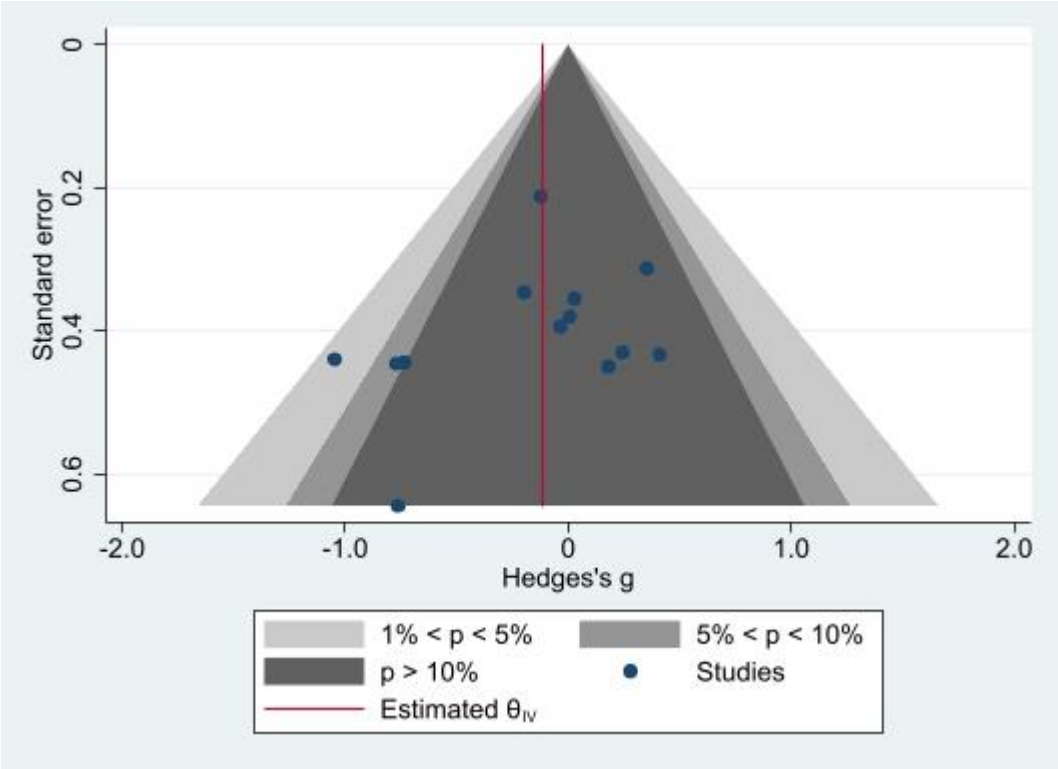
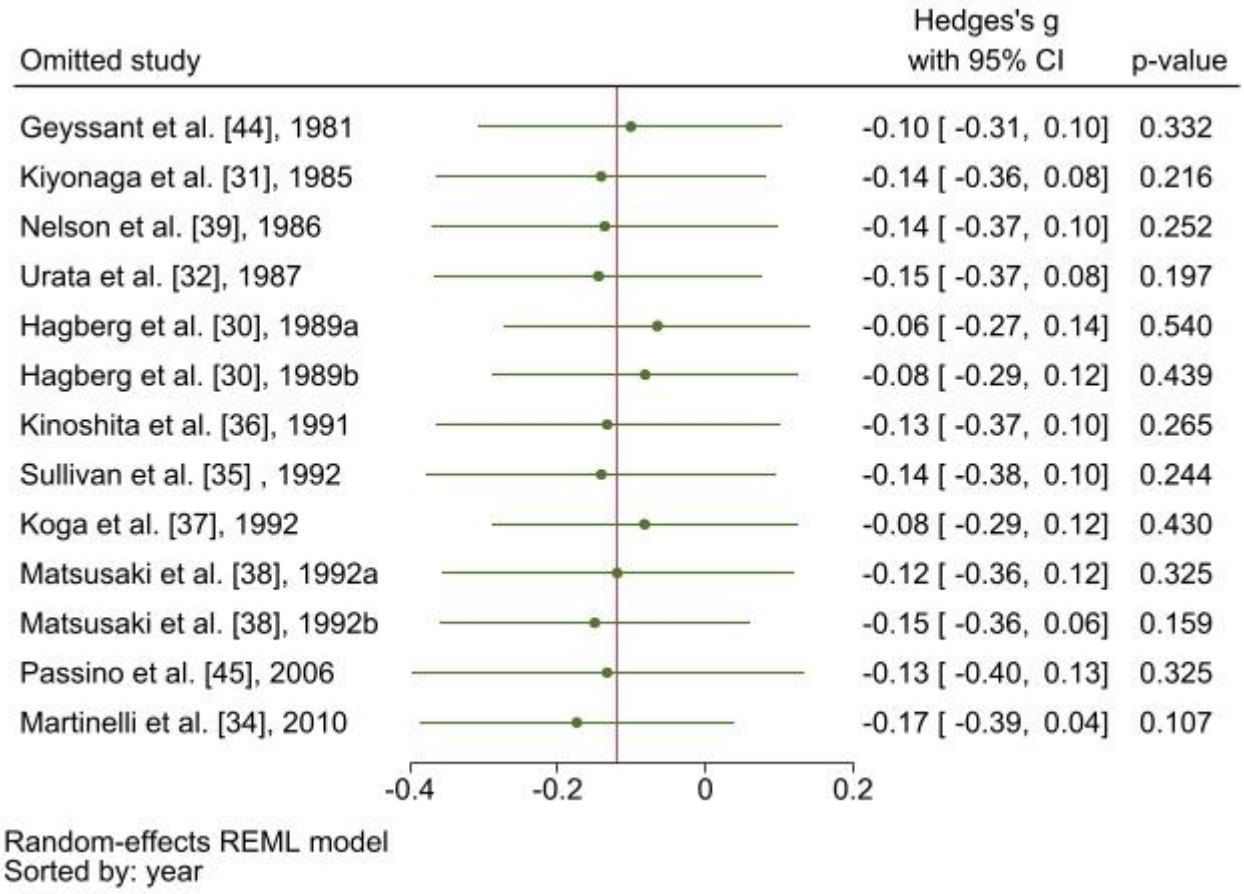


Figure S3e. Leave-one-out figure of studies on the association of changes in physical activity with plasma renin activity



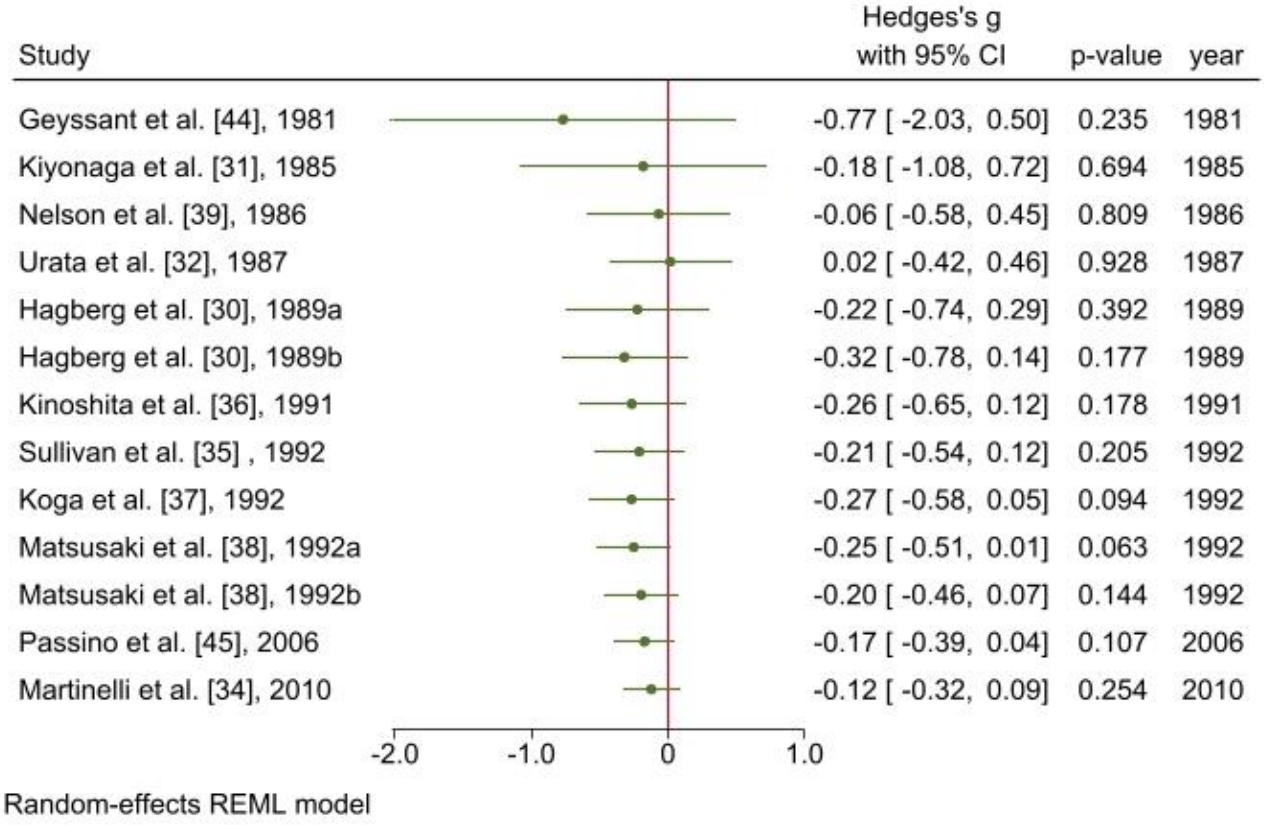
Hagberg *et al.* [30], 1989a: Patients performed low-intensity physical activity.

Hagberg *et al.* [30], 1989b: Patients performed moderate-intensity physical activity.

Matsusaki *et al.*[38], 1992a: Patients performed low-workload physical activity.

Matsusaki *et al.*[38], 1992b: Patients performed high-workload physical activity.

Figure S3f. Cumulative meta-analysis on the association of changes in physical activity with plasma renin activity



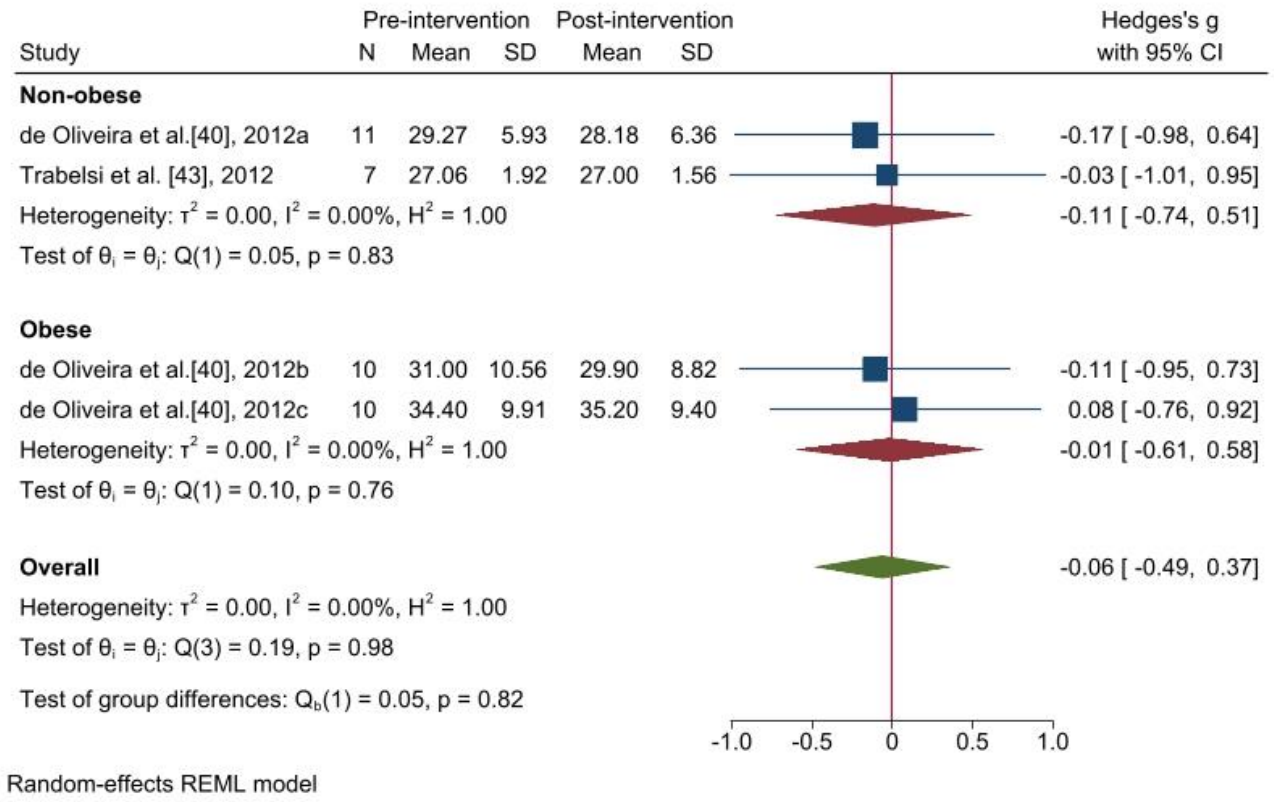
Hagberg *et al.* [30], 1989a: Patients performed low-intensity physical activity.

Hagberg *et al.* [30], 1989b: Patients performed moderate-intensity physical activity.

Matsusaki *et al.*[38], 1992a: Patients performed low-workload physical activity.

Matsusaki *et al*.[38], 1992b: Patients performed high-workload physical activity.

Figure S4a Obesity stratified meta-analysis on the association of changes in physical activity with urea



de Oliveira *et al.* [40], 2012a: Patients performed aerobic training.

de Oliveira *et al.* [40], 2012b: Patients performed strength training.

de Oliveira *et al.* [40], 2012c: Patients performed aerobic and strength training.

Figure S4b Funnel plot of studies on the association of changes in physical activity with urea

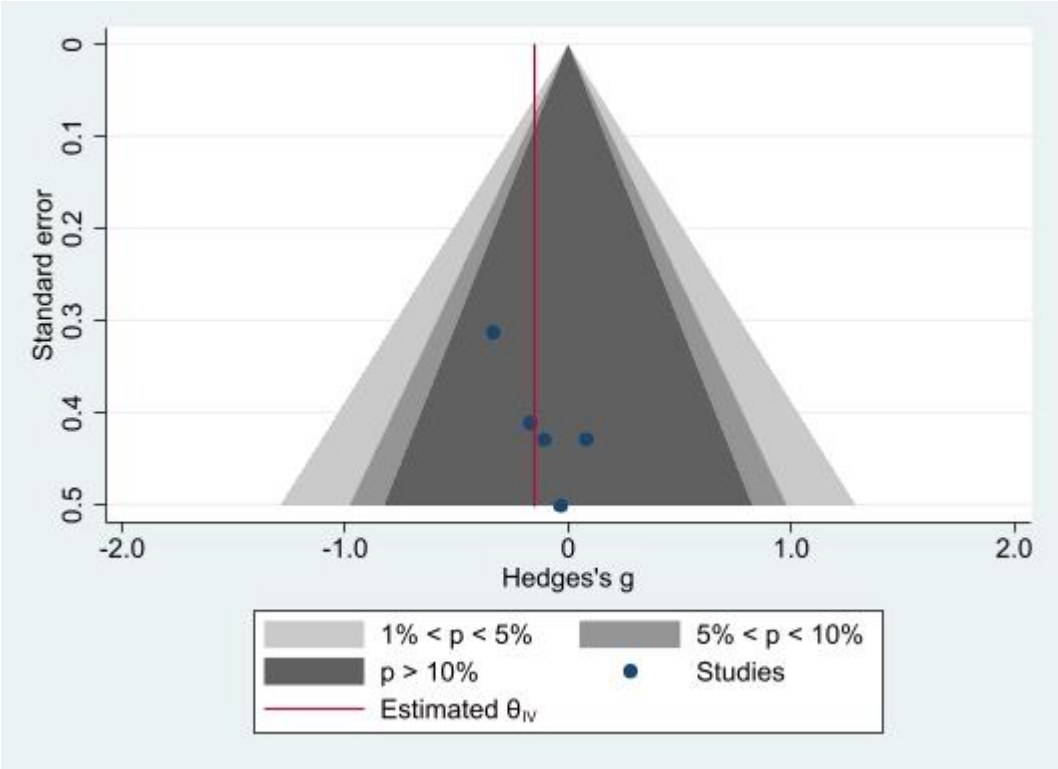
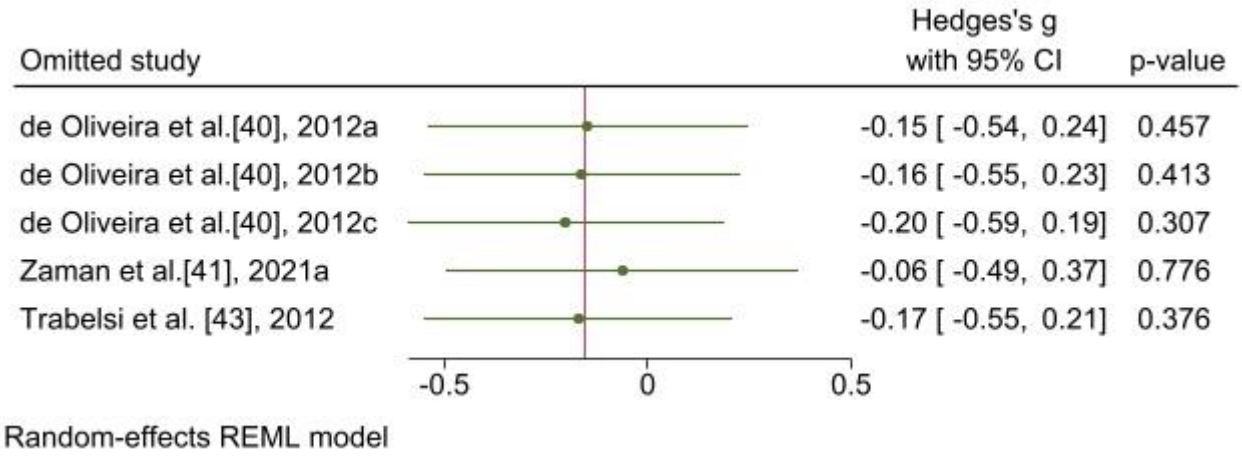


Figure S4c Leave-one-out figure of studies on the association of changes in physical activity with urea



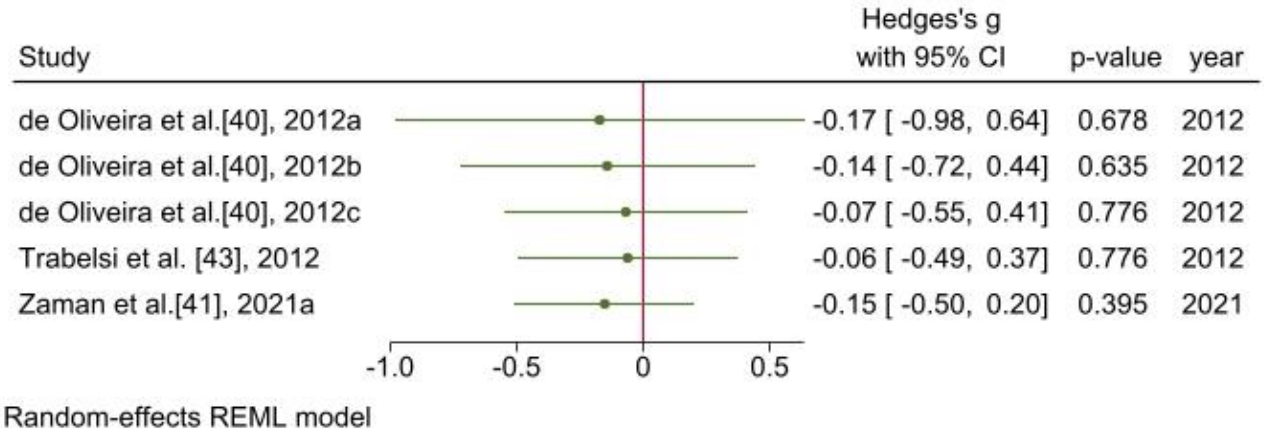
de Oliveira *et al.* [40], 2012a: Patients performed aerobic training.

de Oliveira *et al.* [40], 2012b: Patients performed strength training.

de Oliveira *et al.* [40], 2012c: Patients performed aerobic and strength training.

Zaman *et al.* [41], 2021a: Patients with obesity

Figure S4d Cumulative meta-analysis on the association of changes in physical activity with urea



de Oliveira *et al.* [40], 2012a: Patients performed aerobic training.

de Oliveira *et al.* [40], 2012b: Patients performed strength training.

de Oliveira *et al.* [40], 2012c: Patients performed aerobic and strength training.

Zaman *et al.* [41], 2021a: Patients with obesity

5. Chapter 5 – Change in physical activity and its association with decline in kidney function: A UK Biobank-based cohort study

The original article of this chapter has been published in Journal of Cachexia, Sarcopenia and Muscle on August 18, 2024.

Liu Q, Celis-Morales C, Lees JS, Sattar N, Ho FK, Pell JP, Mark PB, Welsh P. Change in physical activity and its association with decline in kidney function: A UK Biobank-based cohort study. J Cachexia Sarcopenia Muscle. 2024 Oct;15(5):2046-2055. doi: 10.1002/jcsm.13551.

5.1. Abstract

Background

Previous research on the association between physical activity (PA) and kidney function is inconsistent. The association between muscle mass and serum creatinine (SCr) may have implications for interpreting the effect of PA on estimated glomerular filtration rate (eGFR). Few studies have reported changes in physical activity and changes in kidney function.

Methods

A cohort study was constructed using the UK Biobank. Changes in physical activity were self-reported as metabolic equivalent task (MET) minutes/week. eGFR was calculated using SCr and cystatin C (CysC). Cox and nonlinear regressions with restricted cubic splines were applied to explore the association between changes in physical activity and rapid decline of kidney function (RDKF, eGFR annual decrease ≥3/ml/min/1.73m^2), and the annual change of eGFR. An exploratory analysis of cardiorespiratory fitness as the exposure was conducted.

Results

Among 11,757 participants, the median follow-up time was 4.4 years. Participants whose PA decreased by 1,000 MET minutes/week at the follow-up assessment had a 2% reduction in risk of developing $RDKF_{SCr}$ (HR=0.98, 95%CI: 0.96, 1.00). In contrast, a 1,000 MET minutes/week increase in PA was associated with a 4% reduction in risk of developing $RDKF_{CysC}$ (HR=0.96, 95%CI: 0.93, 0.99). A PA increase of 1,000 MET minutes/week was associated with $eGFR_{CysC}$ annual increase of 0.04 ml/min/1.73m^2 (95%CI: 0.03, 0.06); but no significant changes in $eGFR_{SCr}$.

Conclusions

In this general population study, there are differing associations between changes in PA and changes in kidney function depending on the kidney biomarker used. Increasing PA is modestly associated with improving annual $eGFR_{CysC}$ and reduced risk of RDKF.

5.2. Introduction

While current international physical activity (PA) guidelines recommend engaging in 150 to 300 minutes of moderate-intensity physical activity or its equivalent weekly, a

significant proportion of adults fail to achieve these recommendations [1]. The international PA recommendation is backed by substantial evidence that suggests regular physical activity offers multiple health benefits [2].

Physical activity has a myriad of health benefits and is generally recommend for those with chronic kidney disease (CKD): moderate-intensity regular exercise recommended for a cumulative duration of at least 150 minutes per week for patients with high blood pressure (BP) and CKD [3]. However, these recommendations are mainly based on the cardiovascular benefits of PA, with the impact of exercise on kidney function across a wide range of kidney functions uncertain. Kidney function is currently measured using estimated glomerular filtration rate (eGFR) based on the levels of serum creatinine (SCr), whilst the use of serum cystatin C (CysC) based eGFR is not widespread in clinical practice. Serum creatinine is a byproduct of muscle metabolism. At normal GFR, muscle mass and function are the most important determinants of eGFR based on serum creatinine ($eGFR_{SCr}$) [4]. Increased physical activity, leading to an increase in muscle mass, could affect kidney function estimates. On the other hand, CysC is freely filtered at the glomerulus and is unaffected by muscle mass, suggesting it may be a more reliable marker of declining kidney function when muscle mass is changeable [5, 6].

Existing research on the association of physical activity with kidney function is inconsistent and often focuses on patients with chronic kidney disease. A meta-analysis of randomized controlled trials targeting patients with CKD showed that aerobic exercise can reduce serum creatinine levels, improving $eGFR_{SCr}$ [7]. Patients with CKD may present different associations than the general population. One clinical trial demonstrated that physical activity was inversely associated with the risk of deteriorating kidney function in individuals aged over 65 years, who had an average $eGFR_{CysC}$ of approximately 80 $mL/min/1.73m^2$ [8]. However, a similar association was not observed in a younger general population (aged 26-65) with a significantly higher average $eGFR_{CysC}$ of 108 $mL/min/1.73m^2$ [9].

While patients with CKD represent a specific subset of the population, the general population consists of a broader demographic. Preventing CKD is critical due to its irreversible nature. Focusing on the broader population, exploring how physical activity affects kidney health in individuals without CKD is vital. This approach could lead to

lifestyle guidelines that help maintain kidney function longer. Thus, while managing CKD is crucial, prioritizing research on physical activity's role in kidney health is equally imperative for those not affected by CKD.

Most current cohort studies measure the association between a single baseline value of exposure at entry time and the outcome that occurs by the exit time. A potentially better approach is to use the change in exposure over time. Such results might better elucidate causality [10]. Here, we studied the impact of changes in physical activity on eGFR; a dynamic approach may better reflect real-world scenarios [11].

Furthermore, given that various equations exist for assessing kidney function—each relying on different biomarkers—and that the associations between physical activity and these biomarkers may differ, it is essential to compare outcomes across different eGFR estimating equations. We utilized the extensive data from the UK Biobank to provide simultaneous creatinine and cystatin measurements and its first follow-up assessment to carry out a cohort study. Our objective is to explore the association between longitudinal changes in physical activity and kidney function in the general population.

5.3. Methods

Study population

In this research, the study population was drawn from the UK Biobank, a large-scale population cohort designed to enhance the prevention, diagnosis, and treatment of numerous diseases. Initiated in 2006, UK Biobank recruited around half a million participants, aged 40 to 69 years, from across the UK. A follow-up assessment was conducted between August 2012 and June 2013, involving approximately 20,000 baseline UK Biobank participants. To exclude participants with possible prevalent CKD, those with baseline eGFR lower than 60 ml/min/1.73m^2 were excluded.

Derivation of the primary exposure

The primary exposure in this study was the change in physical activity among participants between the baseline and the follow-up assessments. In this study, data on physical activity was collected through a self-reported questionnaire adapted from the International Physical Activity Questionnaire (IPAQ). Questions like "How many minutes did you usually spend doing moderate activities on a typical DAY?" and "In a typical WEEK, on how many days

did you walk for at least 10 minutes at a time? (Include walking that you do at work, travelling to and from work, and for sport or leisure)" were asked. The detailed questionnaire can be assessed online [12]. The questionnaire categorized physical activity into light, moderate, and vigorous intensity physical activity. Following the IPAQ guideline [13], we calculated the metabolic equivalent of task (MET) minutes for each participant in the three intensity categories and summed up the three values to obtain total physical activity expressed as MET minutes/week. Changes in physical activity were estimated by subtracting the total PA at baseline from the total physical activity at follow-up, 116 and 115 extreme values were replaced by the values at 1st and 99th percentiles respectively.

Derivation of the secondary exposure

The study's secondary exposure was the change in cardiorespiratory fitness between the baseline and the follow-up. Cardiorespiratory fitness was assessed in a subset of participants (n=2,040) using a 6-min incremental ramp cycle ergometer test with workload calculated according to age, height, weight, resting heart rate, and sex [14]. Following validated formulae, cardiorespiratory fitness was presented as maximal oxygen uptake (VO_2 max, ml/kg/min) [15].

Study outcomes

The main outcome of this study was the rapid decline of kidney function (RDKF), defined as an annual decrease in eGFR of ≥ 3/ml/min/1.73m^2 [16]. The annual change of eGFR was obtained through calculating the differences of eGFRs between the baseline and the follow-up visit then divided by the follow-up length in years. We applied three equations published by the CKD Epidemiology Collaboration (CKD-EPI), including the race-independent 2021 $eGFR_{SCr}$ equation and $eGFR_{SCr-CysC}$ equation [17], and the 2012 $eGFR_{CysC}$ equation [18] to calculate eGFR. In the UK Biobank, SCr and CysC were collected and measured by enzymatic analysis on a Beckman Coulter AU5800. The detailed procedure can be found in the published UK Biobank report on laboratory procedures [19]. The secondary outcome was the average annual change in eGFR, based on two recordings taken at baseline and follow-up (median 4.41, interquartile interval: 3.68 – 5.05 years after baseline). For each individual, the change in eGFR between the baseline and the follow-up assessment was divided by the follow-up length in years to get the average annual change in eGFR.

Covariates

Several socio-demographic, lifestyle and health-related covariates of this study were selected *a priori* based on published literatures [8, 9], including sex, race (white, black, South Asian, and other), baseline age (years), Townsend socioeconomic deprivation index, baseline systolic and diastolic blood pressures, baseline body mass index (BMI), smoking status (never/previous/current smoker), self-reported use of a statin, and self-reported non-cancer illness: hypertension, diabetes, chronic heart disease, chronic obstructive pulmonary disease (COPD), stroke, atrial fibrillation, heart failure, and myocardial infarction. More details of how each covariate was assessed can be found in Supplementary Table 1-3.

Statistical methods

In our study, we first employed Cox regression models to evaluate the impact of changes in self-reported total physical activity on RDKF, estimating hazard ratios (HRs) and their corresponding 95% confidence intervals (CIs). The entry time was the date of the baseline assessment. The exit time was the date of attending the assessment centre for the first repeat assessment or the date of withdrawal, whichever was earlier. The proportional hazards assumptions were verified using Schoenfeld residuals. To capture potential nonlinear associations, we utilized restricted cubic splines (RCS) with three knots in our Cox regression models. The choice of using only three knots was made as four knots or above lead to overfitting, which could lead to spurious associations and reduce the model's generalizability. The locations of the knots were determined based on Harrell's recommended percentiles. The reference level of RCS models was zero, meaning no exposure changes between the baseline and the follow-up period. To evaluate the association between changes in physical activity and eGFR, we adopted nonlinear RCS regressions using the same settings as above. As kidney function formulae were different between the sexes, sex-specific analyses were conducted to explore the possible differences in associations. Since it is possible that a higher baseline PA leads to beneficial renal results (e.g., delay in decline in kidney function) during the follow-up period, participants were stratified into two groups according to their baseline PA using with a threshold of 1,000 MET minutes/week (the upper end of the recommend PA level of current guidelines [20]).

All analyses were adjusted for confounding factors measured at baseline, which included sex, age, Deprivation, systolic and diastolic blood pressure, BMI, smoking status, self-

reported statin use, and self-reported non-cancer illnesses (hypertension, diabetes, chronic heart disease, COPD, stroke, atrial fibrillation, heart failure, and myocardial infarction). In the Cox-regression analyses, age and BMI were incorporated as time-varying covariates [21]. All statistical analyses were performed using STATA MP 17.0 (Texas, USA).

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5.4. Results

A total of 20,343 individuals participated in the first repeated assessment, of whom 16,546 had baseline SCr and CysC data available. After retaining those with eGFR results greater than 60 ml/min/1.73m^2, 15,966 individuals remained. After excluding those lacking data on physical activity and covariates, 11,757 individuals were finally included in the study. Among them, 2,040 participants also had cardiorespiratory fitness data (Figure 5-1).

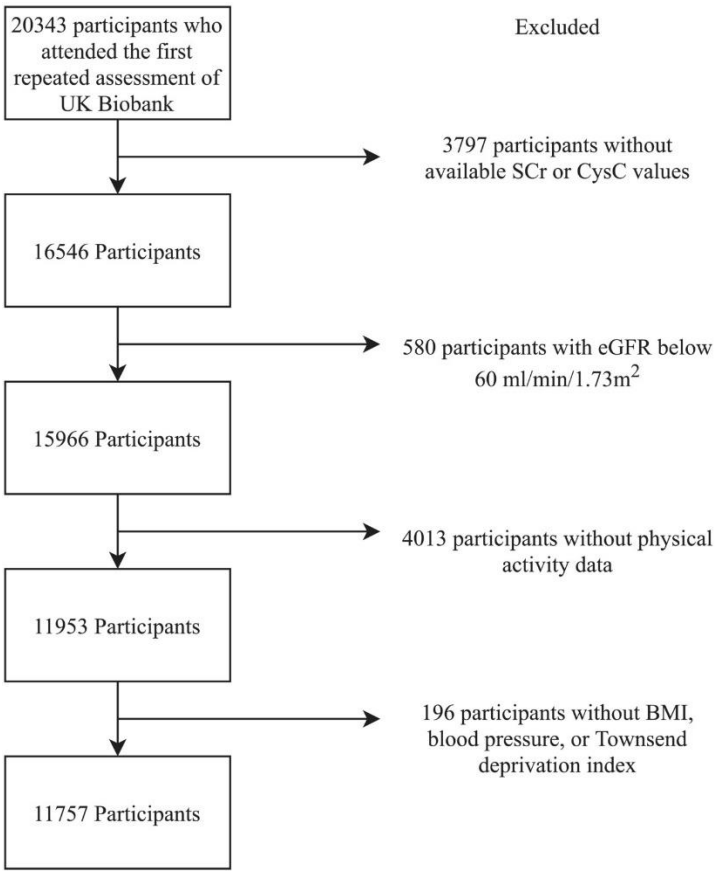

Figure 5-1 Study flowchart

In the 11,757 participants, the median follow-up time was 4.4 years (interquartile range, [IQR] 1.25 years). At follow-up, 77.6% (n=9,118) had a decline in $eGFR_{SCr}$, and 71.3% (n=8,378) had a decline in $eGFR_{CysC}$. The mean (standard deviation) $eGFR_{SCr}$ and $eGFR_{CysC}$ were 95.4 (10.9) and 91.3 (13.2) ml/min/1.73m^{2,} respectively, at baseline; and

fell to 90.6 (11.9) and 87.7 (14.9) ml/min/1.73m^2 at the follow-up assessment. There were more males (52.16% vs 47.46%) and lower C-reactive protein (1.03 vs 1.24 mg/L) among participants with baseline PA≥1,000 MET minutes/week versus <1000 MET minutes/week, but few other substantial differences were found comparing these groups (Supplementary Table 4).

At follow-up, roughly half of the study population (49.2%, 5,789 participants) had decreased their total PA, with a median change of -1,095 MET minutes/week (IQR: 2,004 MET minutes/week). A total of 5,736 participants (48.8%) had increased their total PA over follow-up, with a median change of 1,065 MET minutes/week (IQR: 1,823 MET minutes/week). The remaining 232 individuals reported no change in their total PA levels. At the follow-up visit, more participants had developed comorbidities or were using cholesterol lowering medication than at baseline. The median (IQR) annual change of $eGFR_{SCr}$ and $eGFR_{CysC}$ was -0.92 (1.90) and -0.88 (1.75) ml/min/1.73m^2 respectively (Supplementary Table 5).

More individuals were identified as having RDKF at follow-up using $eGFR_{SCr}$ than $eGFR_{CysC}$ (1,731 vs 1,285 participants). Participants with $RDKF_{SCr}$ were less likely to be male (50.2% vs 57.9%), have hypertension (52.9% vs 58.8%), use a statin (14.2% vs 19.3%), and smaller median of C-reactive protein (1.14 mg/L vs 1.26 mg/L) compared those with RDKF based on $eGFR_{CysC}$ (Table 5-1).

Table 5-1 Baseline characteristics of the study population, stratified by the status of RDKF at the end of follow-up period

	All	RDKF by $eGFR_{SCr}$		RDKF by $eGFR_{CysC}$	
		Yes	No	Yes	No
Sample size, n (%)[a]	11757 (100)	1731 (14.72)	10026 (85.28)	1285 (10.93)	10472 (89.07)
Age, years	57.25 (7.35)	57.26 (7.34)	57.25 (7.35)	58.79 (6.93)	57.06 (7.38)
Male, n (%)	5978 (50.85)	869 (50.20)	5109 (50.96)	744 (57.90)	5234 (49.98)
Ethnicity, n (%)					
White	11530 (98.07)	1701 (98.27)	9829 (98.04)	1259 (97.98)	10271 (98.08)
Black	47 (0.40)	6 (0.35)	41 (0.41)	3 (0.23)	44 (0.42)
South Asian	61 (0.52)	6 (0.35)	55 (0.55)	9 (0.70)	52 (0.50)
Others	119 (1.01)	18 (1.04)	101 (1.01)	14 (1.09)	105 (1.00)
Body mass index, kg/m^2	26.46 (4.13)	26.74 (4.18)	26.41 (4.12)	27.13 (4.37)	26.38 (4.09)
Smoking, n (%)					
Never	7030 (59.79)	1037 (59.91)	5993 (59.77)	735 (57.20)	6295 (60.11)
Previous	4026 (34.24)	582 (33.62)	3444 (34.35)	460 (35.80)	3566 (34.05)
Current	701 (5.96)	112 (6.47)	589 (5.87)	90 (7.00)	611 (5.83)
Townsend deprivation index, median (IQR)	-2.76 (3.13)	-2.83 (3.18)	-2.76 (3.12)	-2.69 (3.12)	-2.77 (3.12)
Systolic blood pressure, mmHg	137.72 (18.15)	139.60 (18.94)	137.40 (17.99)	141.43 (18.85)	137.26 (18.01)
Diastolic blood pressure, mmHg	81.52 (9.79)	82.33 (10.07)	81.38 (9.74)	82.28 (10.12)	81.43 (9.75)

Serum creatinine, mg/dL	0.81 (0.15)	0.81 (0.13)	0.81 (0.15)	0.83 (0.15)	0.81 (0.15)
Serum cystatin C, mg/L	0.87 (0.11)	0.89 (0.11)	0.87 (0.11)	0.88 (0.09)	0.87 (0.11)
C-reactive protein, mg/L, median (IQR)	1.08 (1.64)	1.14 (1.74)	1.08 (1.60)	1.26 (1.72)	1.06 (1.61)
Estimated glomerular filtration rate, mean (SD)					
$eGFR_{SCr}$, ml/min/1.73m^2	95.44 (10.90)	96.50 (8.91)	95.25 (11.19)	93.67 (10.95)	95.65 (10.87)
$eGFR_{CysC}$, ml/min/1.73m^2	91.28 (13.24)	89.65 (13.16)	91.56 (13.23)	90.50 (11.14)	91.37 (13.47)
Comorbidities, n (%)					
Atrial fibrillation	79 (0.67)	17 (0.98)	62 (0.62)	18 (1.40)	61 (0.58)
Chronic obstructive pulmonary disease	99 (0.84)	20 (1.16)	79 (0.79)	13 (1.01)	86 (0.82)
Coronary heart disease	365 (3.10)	69 (3.99)	296 (2.95)	66 (5.14)	299 (2.86)
Diabetes	405 (3.44)	79 (4.56)	326 (3.25)	81 (6.30)	324 (3.09)
Heart failure	6 (0.05)	2 (0.12)	4 (0.04)	0 (0.00)	6 (0.06)
Hypertension	5867 (49.90)	916 (52.92)	4951 (49.38)	756 (58.83)	5111 (48.81)
Myocardial infarction	199 (1.69)	36 (2.08)	163 (1.63)	31 (2.41)	168 (1.60)
Stroke	156 (1.33)	21 (1.21)	135 (1.35)	10 (0.78)	146 (1.39)
Use of statin, n (%)	1632 (13.88)	246 (14.21)	1386 (13.82)	248 (19.30)	1384 (13.22)
Total MET minutes/week of physical activity, median (IQR)	1896 (2723)	1848 (2602)	1904 (2755)	1893 (3048)	1898 (2688)
VO_2max, ml/kg/min, median (IQR)[b]	38.19 (73.87)	37.40 (76.18)	38.59 (73.30)	37.32 (63.06)	38.62 (76.11)

CysC Cystatin C, eGFR estimated glomerular filtration rate, IQR Interquartile range, MET Metabolic equivalent task, RDKF Rapid decline of kidney function,

SCr Serum creatinine, SD standard deviation

% is the column percentage unless otherwise specified

[a]row percentage is presented

[b]sample size=2,040 participants

Association between changes in total physical activity and RDKF incidence

The association between changes in total physical activity and RDKF was observed to yield almost opposite results depending on the kidney function biomarkers used. Using $eGFR_{SCr}$ to ascertain RDKF, the risk decreased as physical activity levels declined. Compared to individuals with consistent physical activity levels from baseline to follow-up, those who reduced their weekly physical activity by 1,000 MET-min/week over follow-up had a 2% lower risk of developing RDKF (HR=0.98, 95%CI: 0.96, 1.00). A reduction of 2,000 MET-min/week decreased the risk further by 5% (HR=0.95, 95%CI: 0.91, 0.99) (Figure 5-2). There was no interaction by sex (P-value=0.75) (Supplementary Figure 1a, 1b).

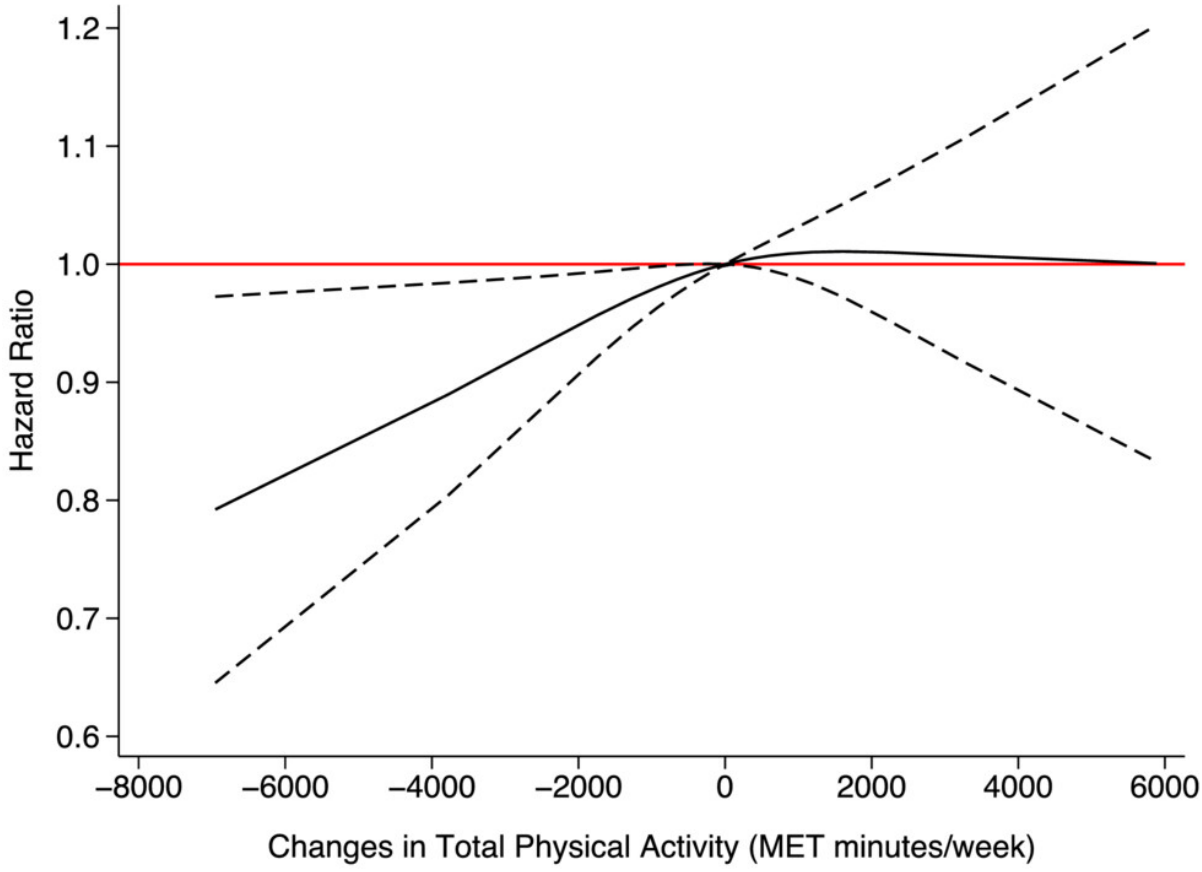

Figure 5-2 Association between changes in physical activity and RDKF incidence using $eGFR_{SCr}$

The solid black line represents the regression line. Dashed lines on either side of the solid black line show the 95% confidence interval (CI). The red line is for easy reference, and a 95%CI below or above the line is regarded as a meaningful association. Adjusted for sex, race, baseline age, smoking, body mass index, Townsend deprivation index, baseline

systolic blood pressure, baseline diastolic pressure, use of statin, hypertension, diabetes, coronary heart disease, chronic obstructive pulmonary disease, stroke, atrial fibrillation, heart failure, myocardial infarction.

Using $eGFR_{CysC}$, there was a decrease in the incidence of RDKF as physical activity levels rose. Specifically, individuals who increased their weekly physical activity by 1,000 MET minutes/week (equivalent to walking 264 minutes/week) at the follow-up, compared to those with stable activity levels from baseline, experienced a 4% reduction in the risk of developing RDKF (HR=0.96, 95%CI: 0.93, 0.99). An increase of 2,000 MET minutes/week (equivalent to walking 528 minutes/week) was associated with to a 9% reduction in risk (HR=0.91, 95%CI: 0.85, 0.97) (Figure 5-3). There was no evidence of a sex-interaction (P-value=0.17) (Supplementary Figure 2a, 2b). There were no associations of changes in total physical activity with RDKF incidence measured by $eGFR_{SCr\text{-}CysC}$ as the outcome (Supplementary Figure 3a-c).

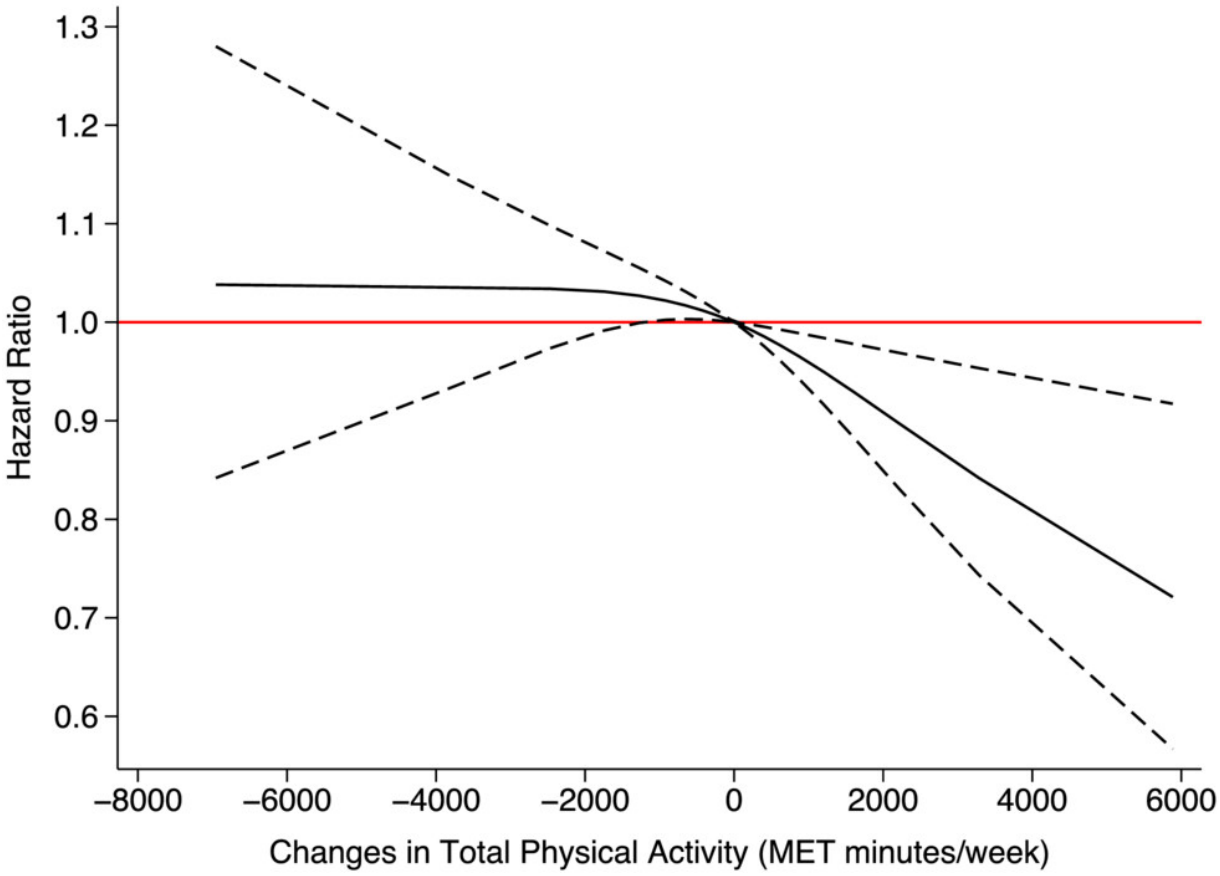

Figure 5-3 Association between changes in physical activity and RDKF incidence using $eGFR_{CysC}$

The solid black line represents the regression line. Dashed lines on either side of the solid black line show the 95% confidence interval (CI). The red line is for easy reference, and a 95%CI below or above the line is regarded as a meaningful association. Adjusted for sex, race, baseline age, smoking, body mass index, Townsend deprivation index, baseline systolic blood pressure, baseline diastolic pressure, use of statin, hypertension, diabetes, coronary heart disease, chronic obstructive pulmonary disease, stroke, atrial fibrillation, heart failure, myocardial infarction.

A total of 3,285 participants (27.94%) had baseline PA below the 1,000 MET minutes/week threshold. The association of changes in total physical activity with RDKF ascertained by $eGFR_{SCr}$, $eGFR_{CysC}$, and $eGFR_{SCr-CysC}$ were explored in both strata (above and below 1,000 MET minutes/week). No interactions by baseline PA categories for RDKF ascertained by $eGFR_{SCr}$ (P-value=0.63) and $eGFR_{SCr-CysC}$ (P-value=0.37), and a marginal interaction was observed when RDKF ascertained by $eGFR_{CysC}$ (P-value=0.06) (Supplementary Figure 1c-d, 2c-d, 3d-e).

Association between changes in total physical activity and annual change in eGFR

No association was observed between the change in physical activity and the annual change of $eGFR_{SCr}$ (Figure 5-4, Supplementary Figures 4a, 4b). However, there was a highly consistent association with the annual change of $eGFR_{CysC}$ (Figure 5-5, Supplementary Figures 5a, 5b).

In both the overall and sex-specific analyses, individuals who exhibited an increase in total PA, compared to those who maintained consistent levels from baseline to follow-up, had a small but statistically significant increase in $eGFR_{CysC}$. Within the general population, individuals whose PA rose by 1,000 MET-min/week from baseline had an $eGFR_{CysC}$ increase of 0.04 ml/min/1.73m^2 (coefficient=0.04, 95%CI: 0.03, 0.06). After additional adjustment for baseline and change in body fat and C-reactive protein, the association was slightly attenuated (coefficient=0.03, 95%CI: 0.01, 0.05). For those with a total PA increment of 2,000 MET-min/week, the increase was 0.10 ml/min/1.73m^2 (coefficient=0.10, 95%CI: 0.06, 0.14).

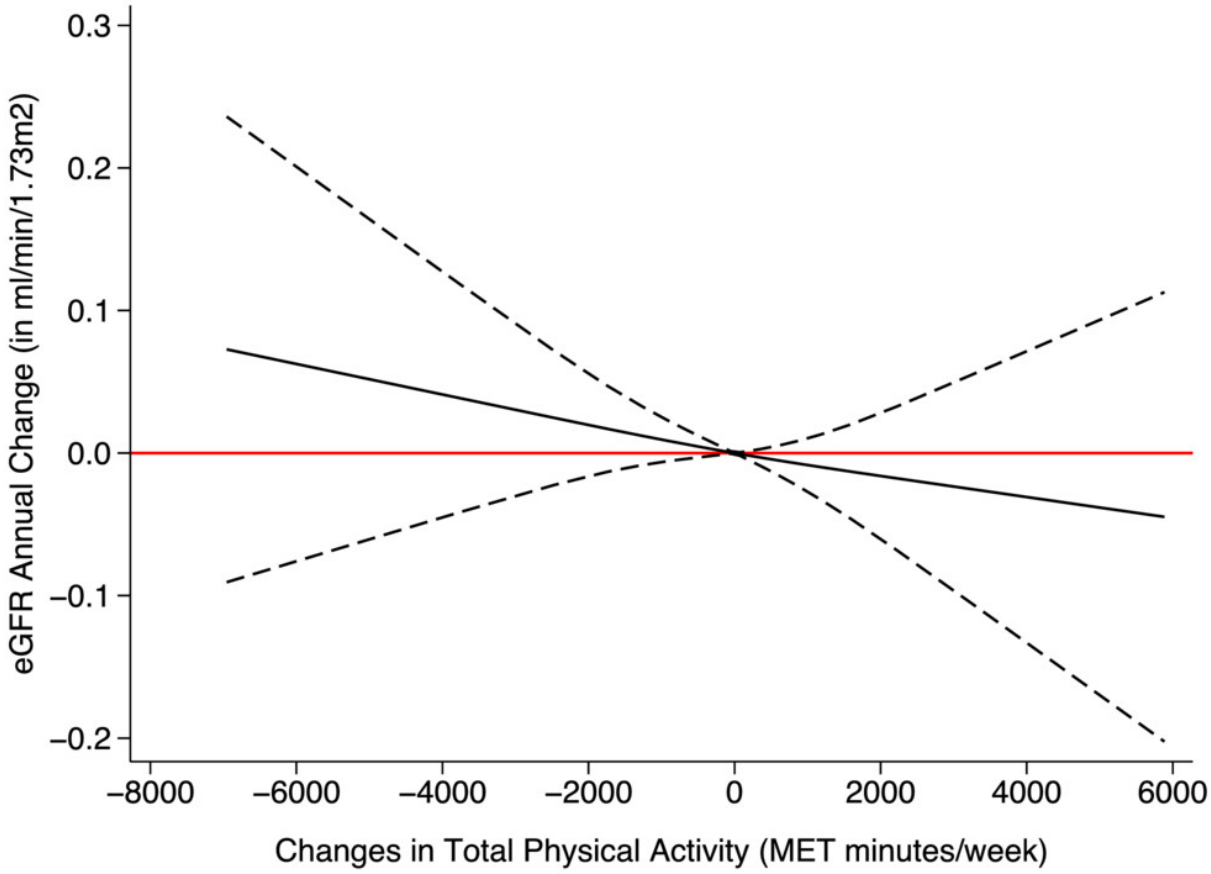

Figure 5-4 Association between changes in physical activity and the annual change of eGFR$_{SCr}$

The solid black line represents the regression line. Dashed lines on either side of the solid black line show the 95% confidence interval (CI). The red line is for easy reference, and a 95%CI below or above the line is regarded as a meaningful association. Adjusted for sex, race, baseline age, smoking, body mass index, Townsend deprivation index, baseline systolic blood pressure, baseline diastolic pressure, use of statin, hypertension, diabetes, coronary heart disease, chronic obstructive pulmonary disease, stroke, atrial fibrillation, heart failure, myocardial infarction.

Test for interaction showed significant sex difference (P-value=0.02). Notably, among men, a decrease in total physical activity levels from baseline was correlated with a negative annual change in eGFR$_{CysC}$. Specifically, men who reduced their physical activity by 1,000 MET minutes/week had an annual eGFR$_{CysC}$ decline of 0.04 ml/min/1.73m^2 (coefficient= -0.04, 95%CI: -0.06, -0.02). For those with a decrease of 2,000 MET minutes/week, the annual change in eGFR$_{CysC}$ was lowered by 0.07 ml/min/1.73m^2 (coefficient= -0.07, 95%CI: -0.12, -0.03). Associations between changes in total physical activity and eGFR$_{SCr}$-

$_{CysC}$ were also identified, with strong similarity to the regression results using $eGFR_{CysC}$ (Supplementary Figure 6a-c). In participants with baseline PA ≥1,000 vs <1000 MET minutes/week, no significant interaction effect was found (Supplementary Figure 4c-d, 5c-d, 6d-e).

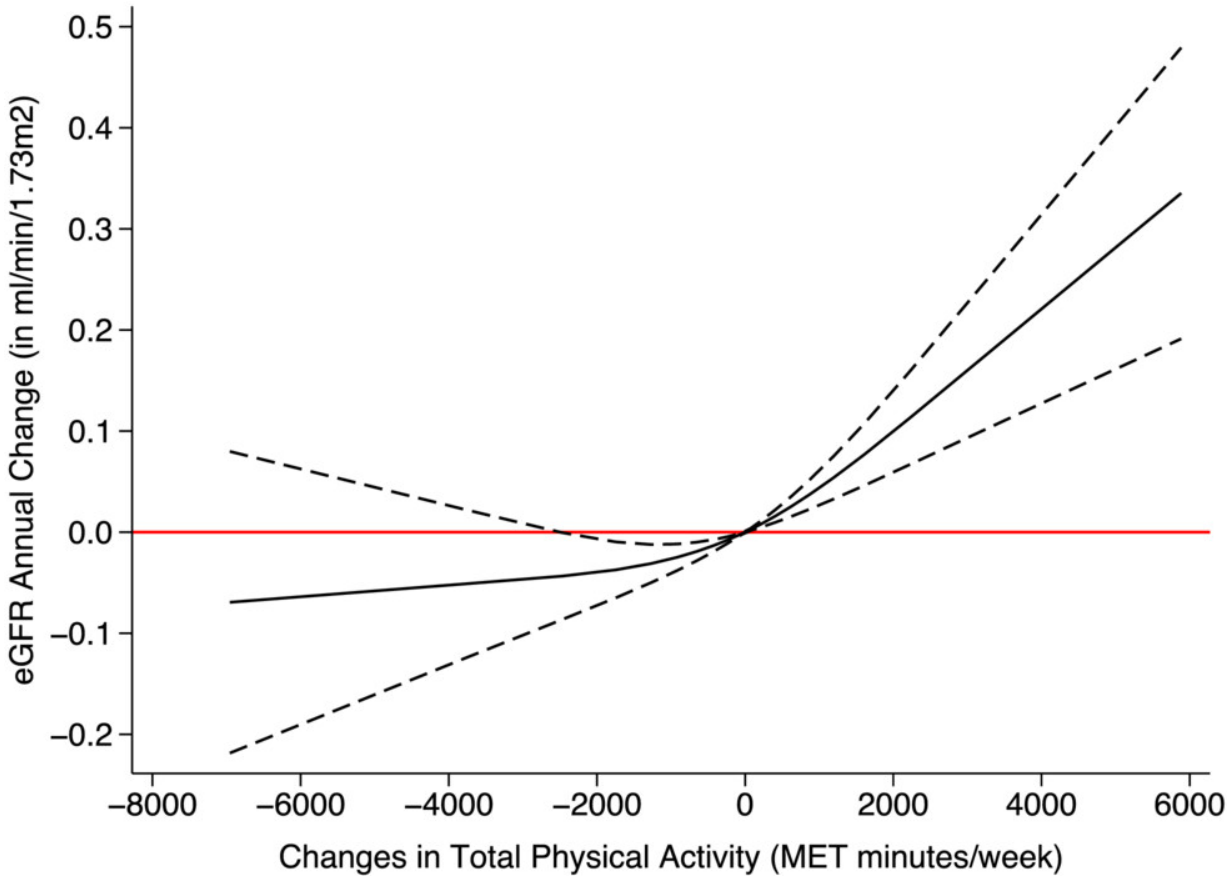

Figure 5-5 Association between changes in physical activity and the annual change of $eGFR_{CysC}$

The solid black line represents the regression line. Dashed lines on either side of the solid black line show the 95% confidence interval (CI). The red line is for easy reference, and a 95%CI below or above the line is regarded as a meaningful association. Adjusted for sex, race, baseline age, smoking, body mass index, Townsend deprivation index, baseline systolic blood pressure, baseline diastolic pressure, use of statin, hypertension, diabetes, coronary heart disease, chronic obstructive pulmonary disease, stroke, atrial fibrillation, heart failure, myocardial infarction.

Association between changes in cardiorespiratory fitness and markers of kidney function

There was no association between change in fitness levels measured by VO_2max and the onset of RDKF, irrespective of the kidney function biomarkers considered (Supplementary Figure 7a-c, 8a-c). While there was no significant link between fitness and the annual change rate of $eGFR_{SCr}$, a modest decrease in the annual change of $eGFR_{CysC}$ was noted among men when their fitness levels decreased from baseline.

In men, for a decrease of 20 ml/kg/min in fitness, the annual $eGFR_{CysC}$ showed an average decline of 0.02 (coefficient=-0.02, 95%CI: -0.04, -0.004). When the fitness reduction reached 30 ml/kg/min, the yearly $eGFR_{CysC}$ decreased by an average of 0.04 (coefficient=-0.04, 95%CI: -0.06, -0.01) (Supplementary Figure 9a-c, 10a-c).

5.5. Discussion

The study findings suggest that changes in physical activity are associated with changes in kidney function. Notably, the research results differed depending on whether SCr or CysC was used to calculate kidney function. Using SCr to assess kidney function, a decrease in physical activity correlated with lower RDKF incidence, and there were no overall associations of change in PA with change in $eGFR_{SCr}$. In contrast, when using CysC to measure kidney function, increased physical activity was associated with lower RDKF incidence and increase in PA was associated with modest increases in $eGFR_{CysC}$ (with a sex-interaction). There was no evidence that baseline PA had any effect on the association between changes in PA and the kidneys.

A study of 199,421 Taiwanese individuals with normal kidney function reported that a higher level of habitual physical activity was linked to a lesser decline in $eGFR_{SCr}$ levels and a reduced risk of developing CKD [22]. Our analyses add to this showing that changes in physical activity may be associated with changes in kidney function, although change in $eGFR_{CysC}$ may be a more optimal way to measure eGFR. Therefore, if these associations are causal, there may be an opportunity for interventions that improve kidney health.

Physical activity, especially leisure-time physical activity (LTPA), has been examined for its dose-response relationship with various health conditions. A meta-analysis of LTPA highlighted a negative nonlinear association with incident metabolic syndrome (MetS),

whereby 150 mins of moderate PA per week (10 MET hours/week) confers a 10% decrease in the risk of MetS, a 20% decrease with doubled PA per week (20 MET hours/week), and a 53% decrease with 70 MET hours/week, compared with inactive participants [23]. Since MetS and related metabolic perturbances such as type 2 diabetes are established risk factors for CKD, this dose-response association indicates that higher levels of physical activity might be mechanistically related to kidney function [24]. One study using 7988 Korean participants also revealed that high physical activity can alleviate the risk for chronic kidney disease regardless of sedentary time [25].

The inverse association between PA and kidney biomarkers may also be explained by the effect of PA on lowering blood pressure. One meta-analysis has revealed a linear 6% reduction in the risk of hypertension for each 600 MET minutes/week increase in LTPA [26]. Blood pressure lowering is known to be associated with improved eGFR and reduced development of end-stage renal disease [27].

The underlying mechanisms by which physical activity could potentially benefit the kidney may also lie in the effect of PA on reducing adiposity, thus easing inflammation, decreasing oxidative stress, and improving endothelial function. These physiological processes are influenced by adipocytokines, which are known to contribute to obesity-related complications, including those impacting kidney health [28].

Several studies have investigated $eGFR_{SCr}$ and $eGFR_{CysC}$ within identical populations, consistently finding that $eGFR_{SCr}$ is less efficient than $eGFRC_{ysC}$ in evaluating health outcomes [5, 29]. Our findings align with this observation. Moreover, when comparing $eGFR_{SCr}$ with directly measured GFR (via Tc99m DTPA plasma clearance), indications suggest that $eGFR_{SCr}$ might bear an intrinsic bias related to muscle mass [4]. Creatinine's primary internal origin stems from muscle metabolism. Hence, individuals with increased muscle mass gained from physical activity inherently produce more creatinine. Engaging in regular physical activity can enhance muscle mass [30, 31], potentially counteracting age-induced muscle attrition [32]. On the other hand, a decline in physical activity accentuates the impact of age and other factors on muscle mass reduction. Notably, men tend to undergo a faster reduction in both absolute and relative muscle mass [33]. Loss of muscle mass subsequently could result in reduced creatinine output, leading to an $eGFR_{SCr}$ increment. This could account for the observed reduced RDKF risk, as characterized by $eGFR_{SCr}$, in

individuals who reduced their physical activity during the follow-up. Such patterns were especially observed in male participants.

Serum CysC levels can vary due to factors such as inflammatory markers, thyroid dysfunction, and adiposity [34, 35]; muscle mass is not a factor that influences CysC. Therefore, using CysC to measure eGFR can avoid the confounding effects caused by muscle mass. CysC is generally considered to be sex-independent [36]. However, Cystatin C is not without confounders, such as inflammation measured by C-reactive protein (CRP) [34, 35, 37]. It is possible that increased physical activity leads to weight loss, reducing resting levels of inflammatory markers and subsequently lowering CRP, which reduces CysC and increases $eGFR_{CysC}$. In the association of changes in PA with $eGFR_{CysC,}$ with baseline and changes in body fat percentage and CRP adjusted for, the effect size of per 1,000 MET minutes/week increase in PA was slightly attenuated from 0.04 (95%CI: 0.03, 0.06) to 0.03 (95%CI: 0.01, 0.05), further supporting the suggested mechanisms.

A characteristic of our study population was that average age was below 60 years, and the average kidney function was in the range of 90-95 ml/min/1.73m^2, suggesting that they were a cohort with relatively normal kidney function. Additionally, the median physical activity for our study group was around 1,900 MET minutes/week, which is considerably above current physical activity guidelines. We believe this is significant because it indicates that even among populations generally considered to be healthy, increasing physical activity is still associated with better kidney health.

Our study demonstrated some sex-specific differences. When $eGFR_{SCr}$-defined RDKF was used as the outcome, a decrease in physical activity was protective of RDKF incidence, but only in men. This may be due to the faster decline in both absolute and relative muscle mass in men compared to women, as aforementioned [33].

As a small exploratory effort, we briefly examined the association between changes in cardiorespiratory fitness and kidney function. Perhaps due to the small sample size, we found almost no meaningful associations. However, we did notice some similarities in the shape of the graphs between this and the primary analysis. It is possible that with a larger sample size, some meaningful findings may be identified.

To the best of our knowledge, no studies have utilized a large sample, along with both $eGFR_{SCr}$ and $eGFR_{CysC}$, to conduct a cohort study of changes in physical activity. One of the major highlights of our study is that we used a large general population study sample to investigate the associations between changes in physical activity and eGFR calculated using different biomarkers. Our findings showed that when measuring the impact of physical activity on kidney function, using CysC yielded more consistent results. With increased physical activity, the incidence of RDKF decreased, and there was an upward trend in the annual change in eGFR. This suggests a potential protective effect of physical activity on kidney function.

However, several points should be noted when interpreting our results. Firstly, physical activity in this study was measured using self-reported questionnaires rather than objective instruments such as accelerometers. This could introduce a certain degree of self-reporting bias in the results. Secondly, although the study suggested a consistent association between changes in physical activity and annual changes in $eGFR_{CysC}$, the effect size was small. Compared to the baseline, an increase of 1,000 MET minutes/week in physical activity was associated with an increase of 0.04 ml/min/1.73m^2 in $eGFR_{CysC}$. Although statistically significant and consistent across different groups, this may not be large enough to be of clinical significance. In our study population, 2,979 participants, or 25.3% of the total, increased their physical activity by 1,000 MET minutes/week or more compared to the baseline. The proportion was relatively high. Moreover, an increase of 1,000 MET minutes/week was associated with a 4% lower risk of RDKF than those who did not modify their physical activity levels over follow-up. Therefore, despite small changes at the individual level, there may be significant population-level benefits. Third, we did not adjust for albuminuria because over 75% of the study participants had urine albumin below the detection threshold. However, all the study participants had available urine creatinine data, meaning the identification of albuminuria could be biased. In addition, albuminuria was regarded as a syndrome of kidney impairment, which was a successor of the study outcome and, therefore, should not be adjusted for. Fourth, GFR was not measured but rather estimated in the UK Biobank. It is plausible that the associations between PA and RDKF were due to non-GFR effects on biomarkers. Fifth, although we observed a consistent association of total changes in physical activity and RDKF identified by $eGFR_{SCr}$, the upper estimate of the 95%CI was close to null. It meant that while the observed trend suggested a clear association, the statistical significance of this association

might not be robust. This indicated the need for cautious interpretation of the data, as the results could be influenced by the narrow confidence intervals. Furthermore, our analysis did not account for some confounders that might increase SCr, such as high-protein dietary habits commonly found in the UK residents. Without adjusting for these confounders, the SCr was overestimated and the $eGFR_{SCr}$ resulting from the decrease of physical activity was underestimated, thus leading to increased risk of RDKF, showing a marginal association. Nevertheless, our study did not discern an association between changes in physical activity and annual changes in $eGFR_{SCr}$. In addition, a larger sample size might be more powerful in identify tiny differences. Even so, our sample size can detect a 6% decrease of HR with 90% power at a significance level of 0.05, we regarded this sample size as large enough. Last, due to data availability, the changes in eGFR and physical activity were only measured twice. More repeated assessments would offer a better overview of trajectories. This could be addressed in future research should data be available.

In conclusion, the study investigated the complex associations between physical activity levels and kidney function, measured through various biomarkers. Importantly, the study underscored the potentially protective effect of increased physical activity on kidney function, particularly when estimated using $eGFR_{CysC}$. While the study relied on self-reported physical activity data and showed only modest effect sizes, its large sample size and comprehensive analysis made a compelling case for further research, highlighting the importance of physical activity for chronic disease.

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5.7. Supplementary tables and figures for Chapter 5

Supplementary Table 1 Definition of covariates

Covariates	Definition
Sex of the participant	Directly obtained from Data-field ID 31
Race (White, Black, South Asian, and others),	Directly obtained from Data-field ID 21000
Baseline age	Directly obtained from Data-field ID 21003
Baseline systolic and diastolic blood pressures	Blood pressures were calculated as the average of manual reading and automatic reading. For SBP, the Data-field IDs are 4080 (automatic reading) and 93 (manual reading). For DBP, the Data-field IDs are 4079 and 94 respectively.
Baseline body mass index	Directly obtained from Data-field ID 23104
Baseline smoking status	Directly obtained from Data-field ID 20116
Self-reported use of statin/anti-cholesterol drugs	Examined Data-field ID 20003, use of statin/anti-cholesterol drugs was identified if the data field contains any of 1141146234,1141146138,1140888594,1140864592,1140888648,1141192414,1140861958,1140881748,1141157416, 1140861942, 1140865576, 1140861936, 1141172214,1140861858, 1141188546, 1141181868, 1140861884,1140861892, 1140861894, 1140861868,1140861848, 1141192740, 1140861954,1141162544,1141192736,1140862026, 1140888590,1141200040
Self-reported non-cancer illness:	
Hypertension	Examined Data-field ID 20002, this disease was identified if the data field contains 1065 or 1072, or if baseline SBP over 140 mmHg or DBP over 90 mmHg.
Diabetes	Examined Data-field ID 20002, this disease was identified if the data field contains 1220, 1222, 1223, 1276, 1468, or 1607.

	Examined Data-field ID 2443, 2976, 5901, and 6177.
Chronic heart disease	Examined Data-field ID 20002, chronic heart disease was identified if the data field contains 1074 or 1075.
Chronic obstructive pulmonary disease	Examined Data-field ID 20002, the disease was identified if the data field contains 1112, 1113, or 1472.
Stroke	Examined Data-field ID 20002, the disease was identified if the data field contains 1081, 1082, 1086, 1491, or 1583
Atrial fibrillation	Examined Data-field ID 20002, the disease was identified if the data field contains 1471
Heart failure	Examined Data-field ID 20002, the disease was identified if the data field contains 1076, 1079, or 1588
Myocardial infarction	Examined Data-field ID 20002, the disease was identified if the data field contains 1075
Townsend deprivation index	Directly obtained from Data-field ID 22189. A higher Townsend index score means a higher degree of deprivation.

Supplementary Table 2 Description of UK Biobank data-field ID and code value

Data-field ID or Code	Description
31	Sex
93	Systolic blood pressure, manual reading
94	Diastolic blood pressure, manual reading
1065	Hypertension
1072	Essential hypertension
1074	Angina
1075	Heart attack/myocardial infarction
1076	Heart failure/pulmonary odema
1079	Cardiomyopathy
1081	Stroke
1082	Transient ischaemic attack
1086	Subarachnoid haemorrhage
1112	Chronic obstructive airways disease
1113	Emphysema/chronic bronchitis
1220	Diabetes
1222	Type 1 diabetes
1223	Type 2 diabetes
1276	Diabetic eye disease
1468	Diabetic neuropathy/ulcers
1471	Atrial fibrillation
1472	Emphysema
1491	Brain haemorrhage
1583	Ischaemic stroke
1588	Hypertrophic cardiomyopathy
1607	Diabetic nephropathy
2443	Diabetes diagnosed by doctor
2976	Age diabetes diagnosed
4079	Diastolic blood pressure, automated reading
4080	Systolic blood pressure, automated reading
5901	Age when diabetes-related eye disease diagnosed

6177	Medication for cholesterol, blood pressure, or diabetes
20116	Smoking status
21000	Ethnic background
21003	Age when attended assessment center
22189	Townsend deprivation index at recruitment
23104	Body mass index

Supplementary Table 3 Medicine ID and Medicine for Identification of Statin and anti-cholesterol drugs

Medicine ID	Medicine
1140861848	Colestid 5g/sachet granules
1140861858	Lopid 300 capsule
1140861868	Nicotinic acid product
1140861884	Maxepa 1g capsule
1140861892	Acipimox
1140861894	Olbetam 250mg capsule
1140861936	Questran 4g/sachet powder
1140861942	Cholestyramine+aspartame 4g/sachet powder
1140861954	Fenofibrate
1140861958	Simvastatin
1140862026	Ciprofibrate
1140864592	Lescol 20mg capsule
1140865576	Cholestyramine
1140881748	Zocor 10mg tablet
1140888590	Colestipol
1140888594	Fluvastatin
1140888648	Pravastatin
1141146138	Lipitor 10mg tablet
1141146234	Atorvastatin
1141157416	Cholestyramine product
1141162544	Lipantil micro 67mg capsule
1141172214	Supralip 160mg m/r tablet
1141181868	Omacor 1g capsule
1141188546	Niaspan 500mg m/r tablet
1141192414	Crestor 10mg tablet
1141192736	Ezetimibe
1141192740	Ezetrol 10mg tablet
1141200040	Zocor heart-pro 10mg tablet

Supplementary Table 4 Baseline characteristics of the study population, stratified by the categories of physical activity

	All	Baseline PA< 1000 MET minutes/week	Baseline PA≥1000 MET minutes/week
Sample size, n (%)[a]	11757 (100)	3285 (27.94)	8472 (72.06)
Age, years	57.25 (7.35)	56.79 (7.34)	57.43 (7.35)
Male, n (%)	5978 (50.85)	1559 (47.46)	4419 (52.16)
Ethnicity, n (%)			
White	11530 (98.07)	3217 (97.93)	8313 (98.12)
Black	47 (0.40)	15 (0.46)	32 (0.38)
South Asian	61 (0.52)	22 (0.67)	39 (0.46)
Others	119 (1.01)	31 (0.94)	88 (1.04)
Body mass index, kg/m^2	26.46 (4.13)	26.93 (4.45)	26.28 (3.98)
Smoking, n (%)			
Never	7030 (59.79)	1979 (60.24)	5051 (59.62)
Previous	4026 (34.24)	1111 (33.82)	2915 (34.41)
Current	701 (5.96)	195 (5.94)	506 (5.97)
Townsend deprivation index, median (IQR)	-2.76 (3.13)	-2.78 (3.13)	-2.76 (3.13)
Systolic blood pressure, mmHg	137.72 (18.15)	136.77 (18.03)	138.09 (18.18)

Diastolic blood pressure, mmHg	81.52 (9.79)	81.58 (9.82)	81.50 (9.79)
Serum creatinine, mg/dL	0.81 (0.15)	0.81 (0.15)	0.81 (0.15)
Serum cystatin C, mg/L	0.87 (0.11)	0.88 (0.11)	0.87 (0.11)
C-reactive protein, mg/L, median (IQR)	1.08 (1.64)	1.24 (1.82)	1.03 (1.56)
Estimated glomerular filtration rate, mean (SD)			
$eGFR_{SCr}$, ml/min/1.73m^2	95.44 (10.90)	95.62 (10.94)	95.36 (10.88)
$eGFR_{CysC}$, ml/min/1.73m^2	91.28 (13.24)	90.82 (13.31)	91.45 (13.21)
Comorbidities, n (%)			
Atrial fibrillation	79 (0.67)	21 (0.64)	58 (0.68)
Chronic obstructive pulmonary disease	99 (0.84)	20 (0.61)	79 (0.93)
Coronary heart disease	365 (3.10)	94 (2.86)	271 (3.20)
Diabetes	405 (3.44)	125 (3.81)	280 (3.31)
Heart failure	6 (0.05)	1 (0.03)	5 (0.06)
Hypertension	5867 (49.90)	1650 (50.23)	4217 (49.78)
Myocardial infarction	199 (1.69)	53 (1.61)	146 (1.72)
Stroke	156 (1.33)	51 (1.55)	105 (1.24)
Use of statin, n (%)	1632 (13.88)	472 (14.37)	1160 (13.69)
Total MET minutes/week of physical activity, median (IQR)	1896 (2723)	594 (348)	2706 (2944)

VO_2max, ml/kg/min, median (IQR)[b]	38.19 (73.87)	36.64 (78.33)	38.65 (72.88)

CysC Cystatin C, eGFR estimated glomerular filtration rate, IQR Interquartile range, MET Metabolic equivalent task, PA physical activity, RDKF Rapid decline of kidney function, SCr Serum creatinine, SD standard deviation

% is the column percentage unless otherwise specified

[a]row percentage is presented

[b]sample size=2,040 participants

Supplementary Table 5 Characteristics of the study population at the follow-up visit, stratified by the status of RDKF at the end of follow-up period

	All	RDKF by $eGFR_{SCr}$		RDKF by $eGFR_{CysC}$	
		Yes	No	Yes	No
Sample size, n (%)[a]	11757 (100)	1731 (14.72)	10026 (85.28)	1285 (10.93)	10472 (89.07)
Age, years	61.53 (7.34)	61.38 (7.35)	61.56 (7.33)	62.82 (7.01)	61.38 (7.36)
Male, n (%)	5978 (50.85)	869 (50.20)	5109 (50.96)	744 (57.90)	5234 (49.98)
Ethnicity, n (%)					
White	11530 (98.07)	1701 (98.27)	9829 (98.04)	1259 (97.98)	10271 (98.08)
Black	47 (0.40)	6 (0.35)	41 (0.41)	3 (0.23)	44 (0.42)
South Asian	61 (0.52)	6 (0.35)	55 (0.55)	9 (0.70)	52 (0.50)
Others	119 (1.01)	18 (1.04)	101 (1.01)	14 (1.09)	105 (1.00)
Body mass index, kg/m^2	26.54 (4.19)	26.99 (4.25)	26.46 (4.18)	27.54 (4.72)	26.42 (4.11)
Smoking, n (%)					
Never	7133 (60.67)	1048 (60.54)	6085 (60.69)	740 (57.59)	6393 (61.05)
Previous	4142 (35.23)	616 (35.59)	3526 (35.17)	471 (36.65)	3671 (35.06)
Current	482 (4.10)	67 (3.87)	415 (4.14)	74 (5.76)	408 (3.90)
Townsend deprivation index, median (IQR)	-2.76 (3.13)	-2.83 (3.18)	-2.76 (3.12)	-2.69 (3.12)	-2.77 (3.12)
Systolic blood pressure, mmHg	139.15 (18.37)	138.46 (18.28)	139.27 (18.38)	140.15 (18.80)	139.02 (18.31)
Diastolic blood pressure, mmHg	80.25 (9.65)	79.96 (9.71)	80.30 (9.63)	80.78 (10.44)	80.19 (9.54)

Serum creatinine, mg/dL	0.84 (0.16)	0.99 (0.17)	0.81 (0.15)	0.91 (0.18)	0.83 (0.16)
Serum cystatin C, mg/L	0.90 (0.13)	0.94 (0.16)	0.89 (0.13)	1.03 (0.14)	0.88 (0.12)
C-reactive protein, mg/L, median (IQR)	1.12 (1.67)	1.22 (1.73)	1.11 (1.65)	1.64 (2.27)	1.06 (1.61)
Estimated glomerular filtration rate, mean (SD)					
$eGFR_{SCr}$, ml/min/1.73m^2	90.67 (11.90)	77.35 (11.12)	92.97 (10.43)	85.16 (13.47)	91.35 (11.52)
$eGFR_{CysC}$, ml/min/1.73m^2	81.71 (14.86)	83.54 (16.12)	88.43 (14.51)	73.78 (12.69)	89.42 (14.19)
eGFR annual change, median (IQR)					
$eGFR_{SCr}$, ml/min/1.73m^2	-0.92 (1.90)	-4.19 (1.76)	-0.72 (1.46)	-1.68 (2.75)	-0.86 (1.78)
$eGFR_{CysC}$, ml/min/1.73m^2	-0.88 (1.75)	-1.42 (2.40)	-0.80 (2.05)	-3.81 (1.29)	-0.68 (1.19)
Comorbidities, n (%)					
Atrial fibrillation	167 (1.42)	33 (1.91)	134 (1.34)	34 (2.65)	133 (1.27)
Chronic obstructive pulmonary disease	132 (1.12)	21 (1.21)	111 (1.11)	15 (1.17)	117 (1.12)
Coronary heart disease	484 (4.12)	84 (4.85)	400 (3.99)	77 (5.99)	407 (3.89)
Diabetes	547 (4.65)	99 (5.72)	448 (4.47)	99 (7.70)	448 (4.28)
Heart failure	15 (0.13)	5 (0.29)	10 (0.10)	4 (0.31)	11 (0.11)
Hypertension	6642 (56.49)	996 (57.54)	5646 (56.31)	813 (63.27)	5829 (55.66)
Myocardial infarction	257 (2.19)	48 (2.77)	209 (2.08)	39 (3.04)	218 (2.08)
Stroke	236 (2.01)	30 (1.73)	206 (2.05)	22 (1.71)	214 (2.04)
Use of statin, n (%)	2432 (20.69)	392 (22.65)	2040 (20.35)	343 (26.69)	2089 (19.95)
Total MET minutes/week of physical activity, median (IQR)	1912 (2604)	1890 (2622)	1920 (2613)	1800 (2507)	1920 (2621)

VO_2max, ml/kg/min, median (IQR)[b]	37.71 (83.32)	37.94 (90.40)	37.59 (81.02)	36.06 (80.40)	37.86 (84.62)

CysC Cystatin C, eGFR estimated glomerular filtration rate, IQR Interquartile range, MET Metabolic equivalent task, RDKF Rapid decline of kidney function,

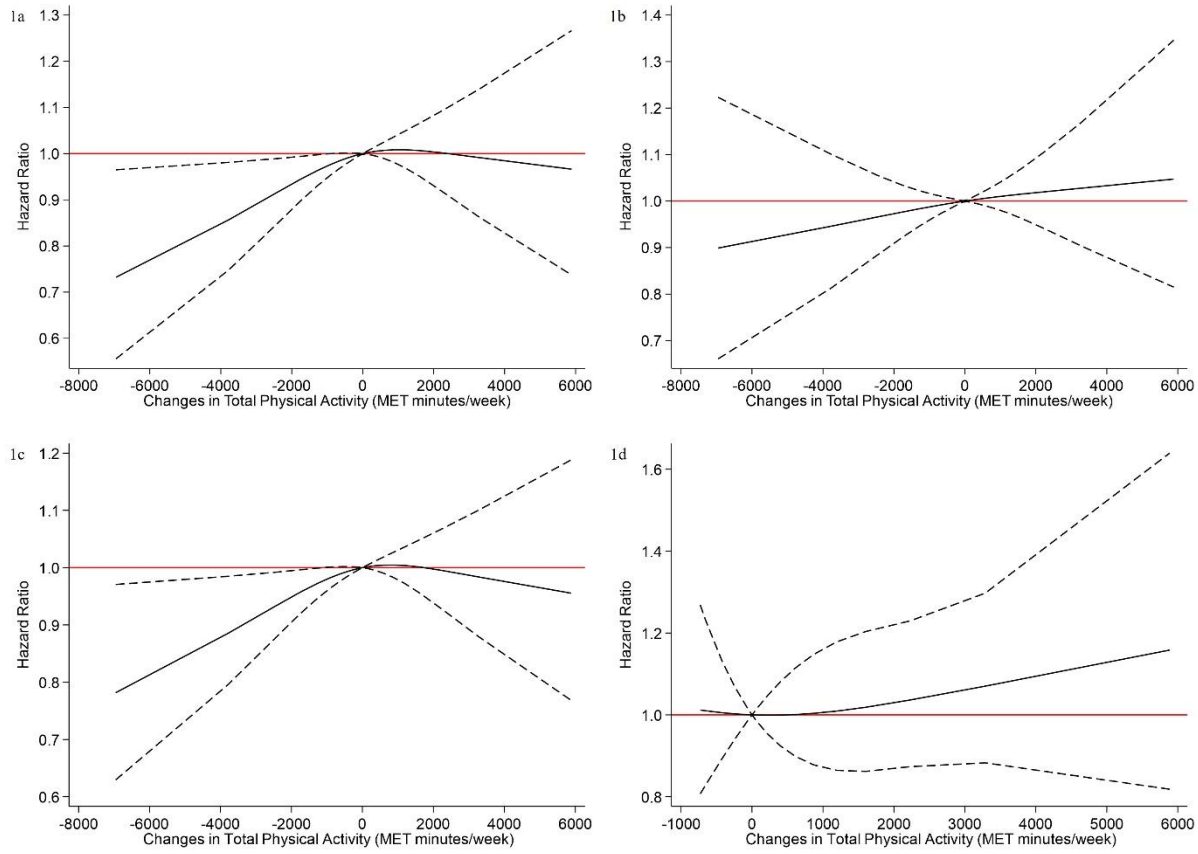
SCr Serum creatinine, SD standard deviation

% is the column percentage unless otherwise specified

[a]row percentage is presented

[b]sample size=2,040 participants

Supplementary Figure 1 Association between changes in physical activity and the RDKF incidence identified using $eGFR_{SCr}$

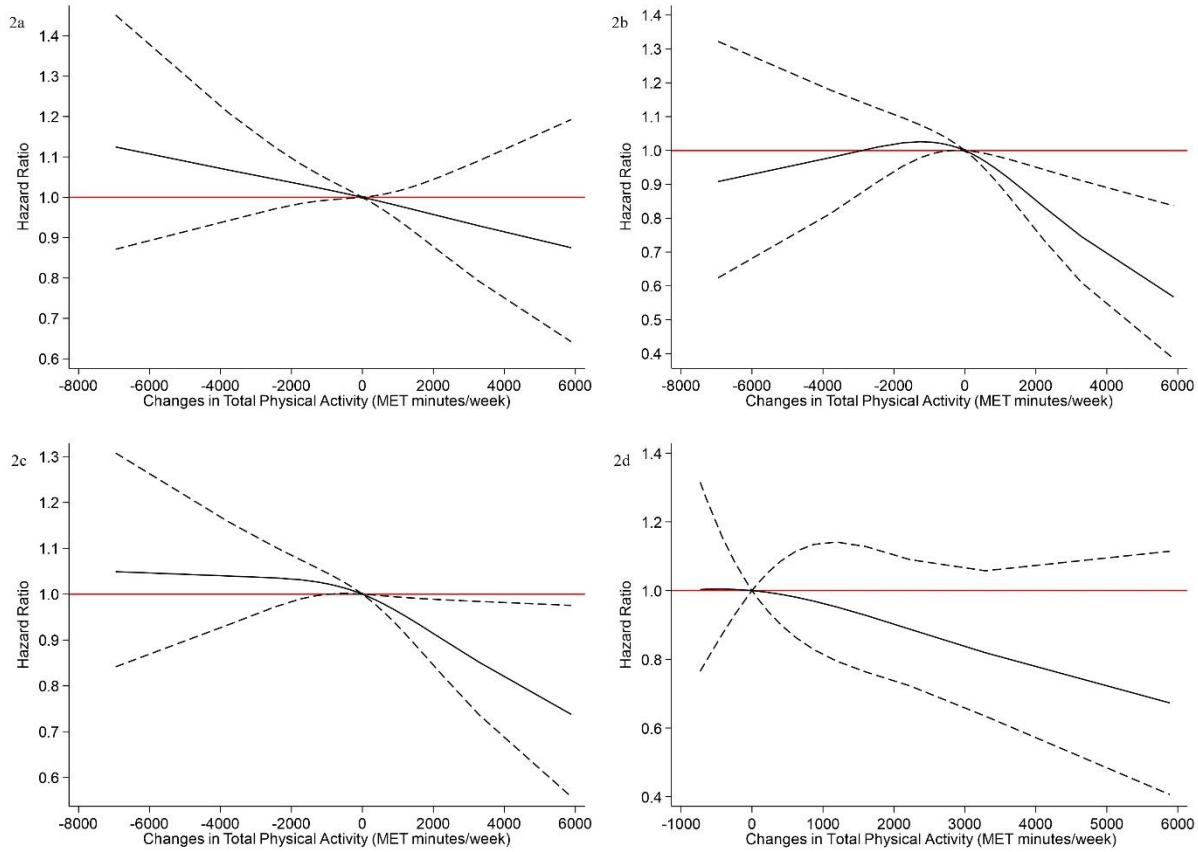


1a: males; 1b: females; 1c: people with baseline physical activity above 1000 MET minutes/week; 1d: people with baseline physical activity below 1000 MET minutes/week

The solid black line represents the regression line. Dashed lines on either side of the solid black line show the 95% confidence interval (CI). The red line is for easy reference, and a 95%CI below or above the line is regarded as a meaningful association.

Adjusted for race, baseline age, smoking, body mass index, Townsend deprivation index, baseline systolic blood pressure, baseline diastolic pressure, use of statin, hypertension, diabetes, coronary heart disease, chronic obstructive pulmonary disease, stroke, atrial fibrillation, heart failure, myocardial infarction.

Supplementary Figure 2 Association between changes in physical activity and the RDKF incidence identified using $eGFR_{CysC}$

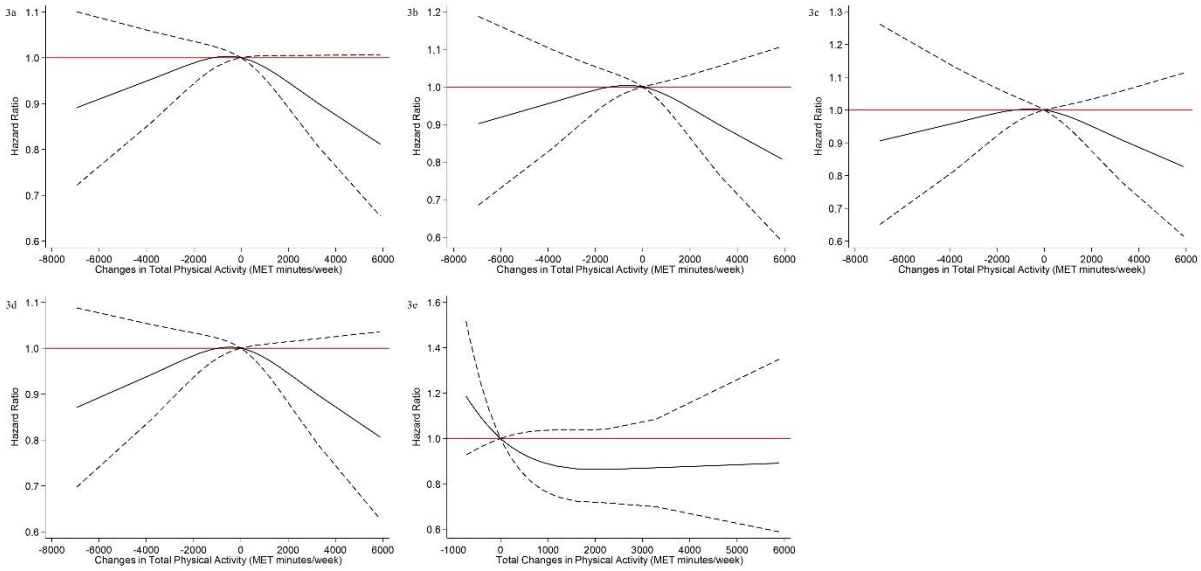


2a: males; 2b: females; 2c: people with baseline physical activity above 1000 MET minutes/week; 2d: people with baseline physical activity below 1000 MET minutes/week

The solid black line represents the regression line. Dashed lines on either side of the solid black line show the 95% confidence interval (CI). The red line is for easy reference, and a 95%CI below or above the line is regarded as a meaningful association.

Adjusted for sex, race, baseline age, smoking, body mass index, Townsend deprivation index, baseline systolic blood pressure, baseline diastolic pressure, use of statin, hypertension, diabetes, coronary heart disease, chronic obstructive pulmonary disease, stroke, atrial fibrillation, heart failure, myocardial infarction.

Supplementary Figure 3 Association between changes in physical activity and the RDKF incidence identified using $eGFR_{SCr\text{-}CysC}$

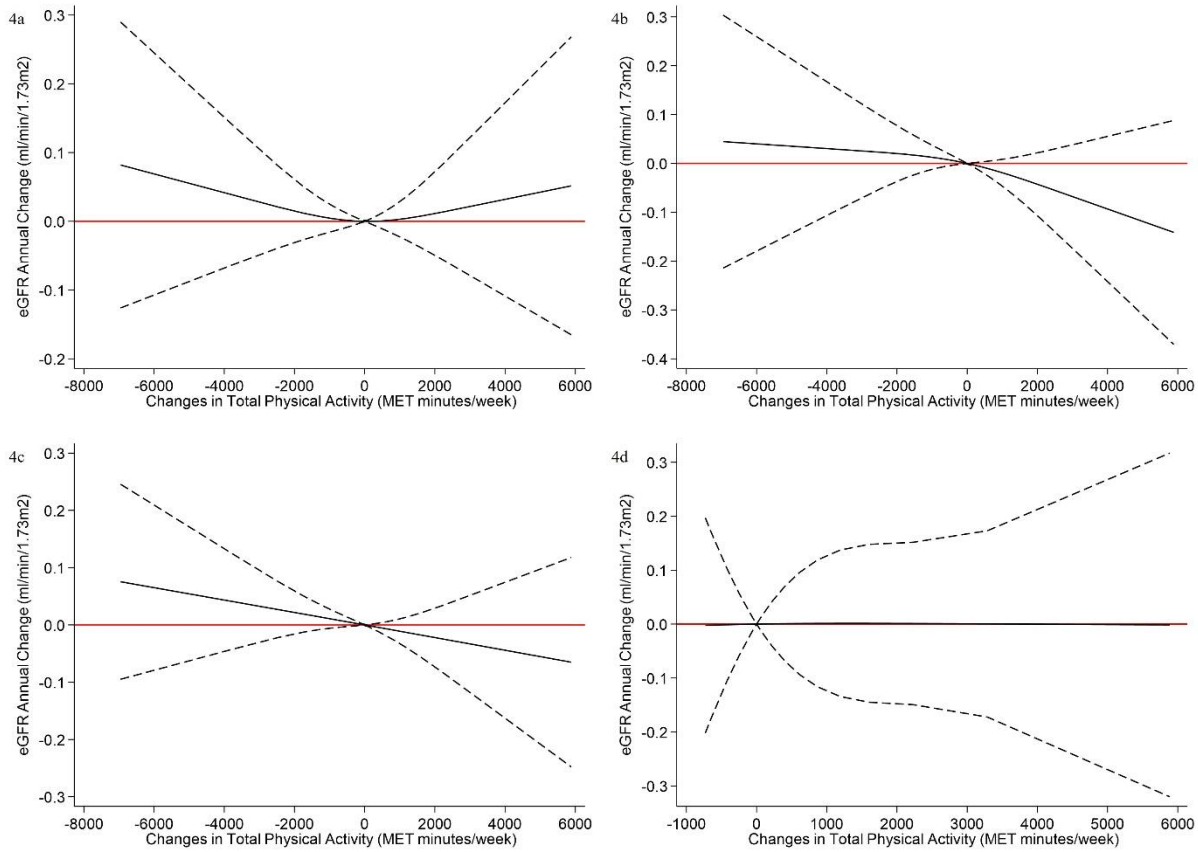


3a: unstratified population; 3b: males; 3c: females; 3d: people with baseline physical activity above 1000 MET minutes/week; 3e: people with baseline physical activity below 1000 MET minutes/week

The solid black line represents the regression line. Dashed lines on either side of the solid black line show the 95% confidence interval (CI). The red line is for easy reference, and a 95%CI below or above the line is regarded as a meaningful association.

Adjusted for sex, race, baseline age, smoking, body mass index, Townsend deprivation index, baseline systolic blood pressure, baseline diastolic pressure, use of statin, hypertension, diabetes, coronary heart disease, chronic obstructive pulmonary disease, stroke, atrial fibrillation, heart failure, myocardial infarction.

Supplementary Figure 4 Association between changes in physical activity and the annual change of $eGFR_{SCr}$

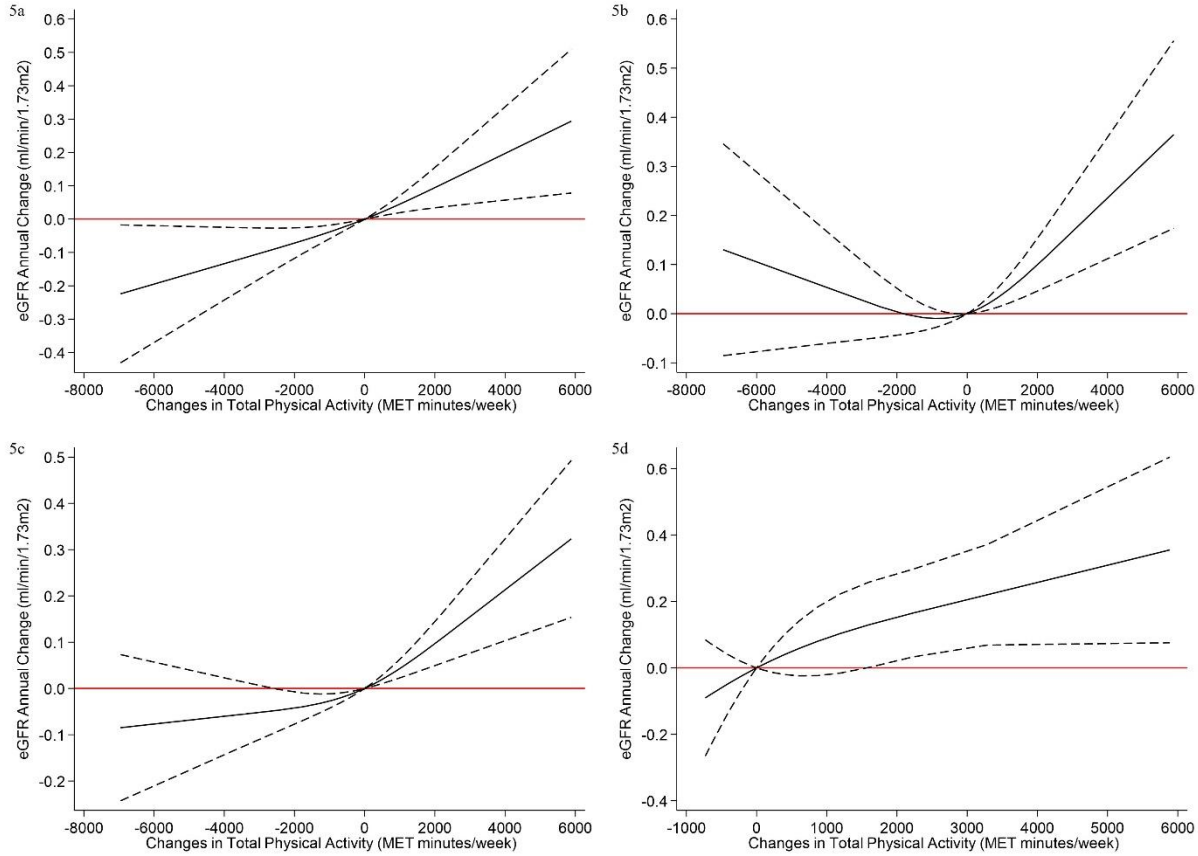


4a: males; 4b: females; 4c: people with baseline physical activity above 1000 MET minutes/week; 4d: people with baseline physical activity below 1000 MET minutes/week

The solid black line represents the regression line. Dashed lines on either side of the solid black line show the 95% confidence interval (CI). The red line is for easy reference, and a 95%CI below or above the line is regarded as a meaningful association.

Adjusted for sex, race, baseline age, smoking, body mass index, Townsend deprivation index, baseline systolic blood pressure, baseline diastolic pressure, use of statin, hypertension, diabetes, coronary heart disease, chronic obstructive pulmonary disease, stroke, atrial fibrillation, heart failure, myocardial infarction.

Supplementary Figure 5 Association between changes in physical activity and the annual change of $eGFR_{CysC}$

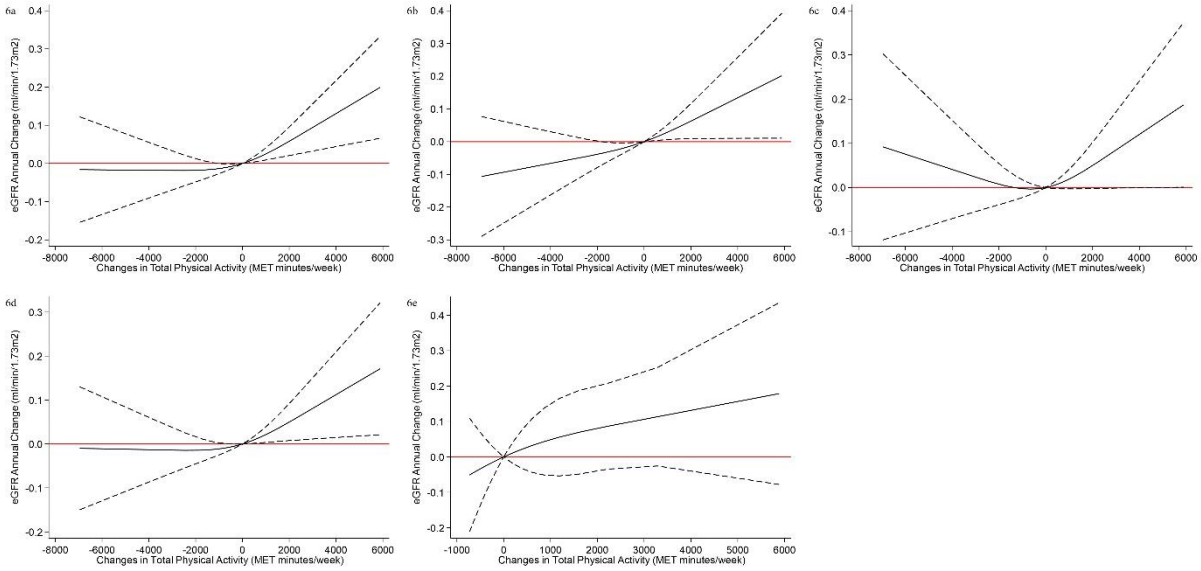


5a: males; 5b: females; 5c: people with baseline physical activity above 1000 MET minutes/week; 5d: people with baseline physical activity below 1000 MET minutes/week

The solid black line represents the regression line. Dashed lines on either side of the solid black line show the 95% confidence interval (CI). The red line is for easy reference, and a 95%CI below or above the line is regarded as a meaningful association.

Adjusted for race, baseline age, smoking, body mass index, Townsend deprivation index, baseline systolic blood pressure, baseline diastolic pressure, use of statin, hypertension, diabetes, coronary heart disease, chronic obstructive pulmonary disease, stroke, atrial fibrillation, heart failure, myocardial infarction.

Supplementary Figure 6 Association between changes in physical activity and the annual change of $eGFR_{SCr-CysC}$

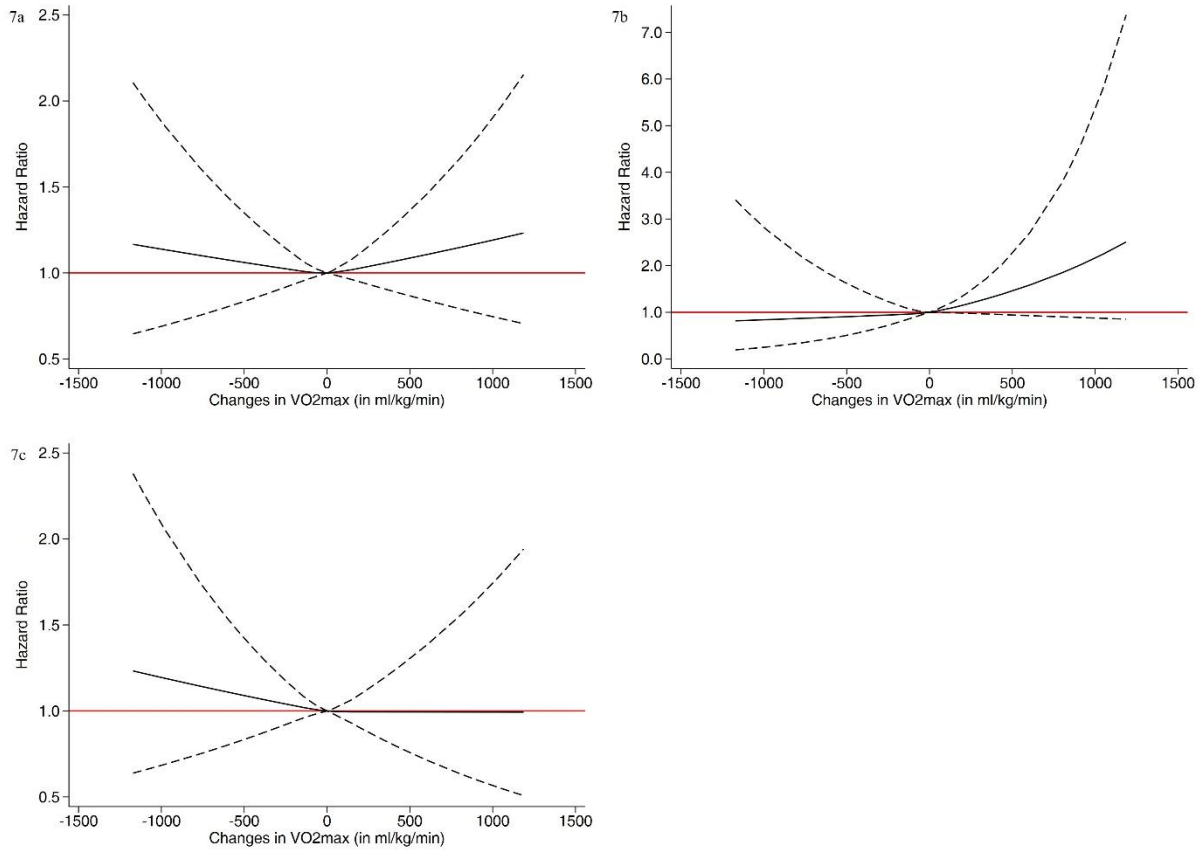


6a: unstratified population; 6b: males; 6c: females; 6d: people with baseline physical activity above 1000 MET minutes/week; 6e: people with baseline physical activity below 1000 MET minutes/week

The solid black line represents the regression line. Dashed lines on either side of the solid black line show the 95% confidence interval (CI). The red line is for easy reference, and a 95%CI below or above the line is regarded as a meaningful association.

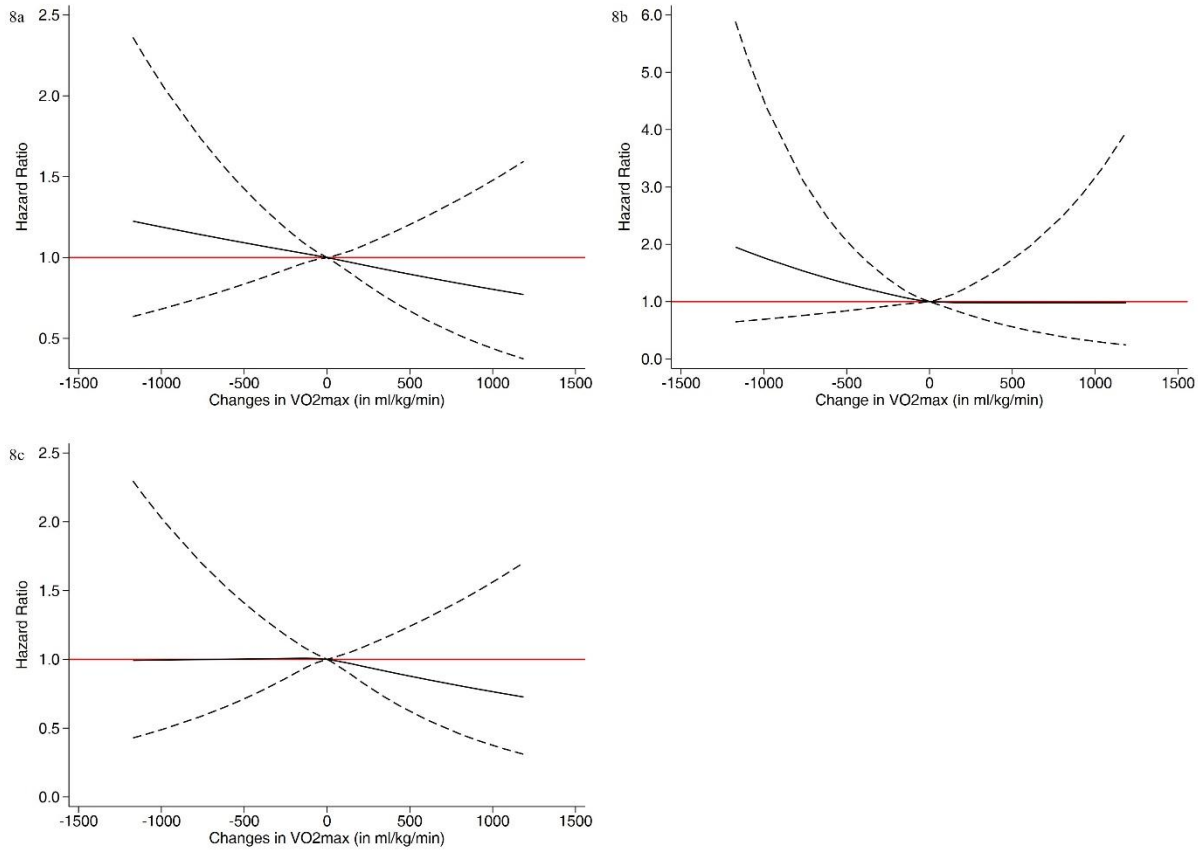
Adjusted for sex, race, baseline age, smoking, body mass index, Townsend deprivation index, baseline systolic blood pressure, baseline diastolic pressure, use of statin, hypertension, diabetes, coronary heart disease, chronic obstructive pulmonary disease, stroke, atrial fibrillation, heart failure, myocardial infarction.

Supplementary Figure 7 Association between changes in VO_2max and the RDKF incidence identified using $eGFR_{SCr}$



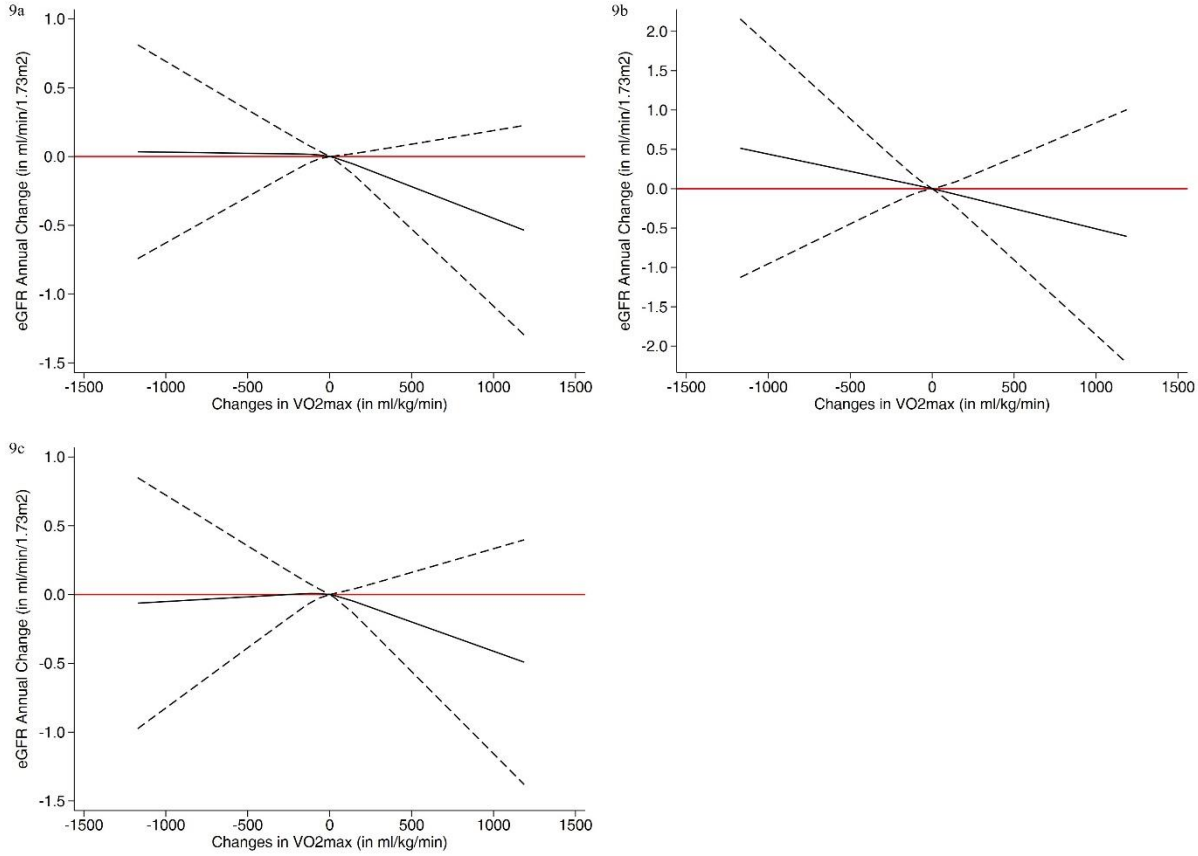
7a: unstratified population; 7b: males; 7c: females; The solid black line represents the regression line. Dashed lines on either side of the solid black line show the 95% confidence interval (CI). The red line is for easy reference, and a 95%CI below or above the line is regarded as a meaningful association. Adjusted for sex, race, baseline age, smoking, body mass index, Townsend deprivation index, baseline systolic blood pressure, baseline diastolic pressure, use of statin, hypertension, diabetes, coronary heart disease, chronic obstructive pulmonary disease, stroke, atrial fibrillation, heart failure, myocardial infarction.

Supplementary Figure 8 Association between changes in VO_2max and the RDKF incidence identified using $eGFR_{CysC}$



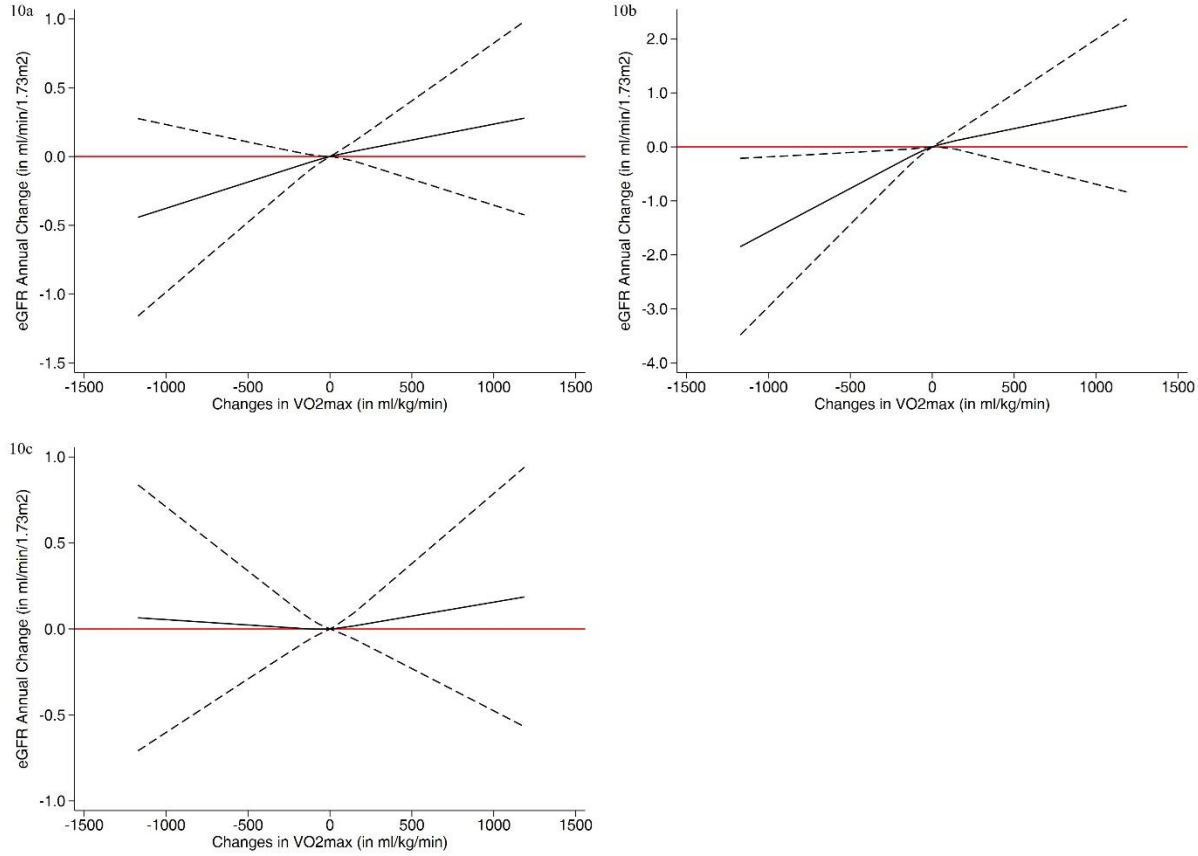
8a: unstratified population; 8b: males; 8c: females; The solid black line represents the regression line. Dashed lines on either side of the solid black line show the 95% confidence interval (CI). The red line is for easy reference, and a 95%CI below or above the line is regarded as a meaningful association. Adjusted for sex, race, baseline age, smoking, body mass index, Townsend deprivation index, baseline systolic blood pressure, baseline diastolic pressure, use of statin, hypertension, diabetes, coronary heart disease, chronic obstructive pulmonary disease, stroke, atrial fibrillation, heart failure, myocardial infarction.

Supplementary Figure 9 Association between changes in VO_2max and the annual change of $eGFR_{SCr}$



9a: unstratified population; 9b: males; 9c: females; The solid black line represents the regression line. Dashed lines on either side of the solid black line show the 95% confidence interval (CI). The red line is for easy reference, and a 95%CI below or above the line is regarded as a meaningful association. Adjusted for sex, race, baseline age, smoking, body mass index, Townsend deprivation index, baseline systolic blood pressure, baseline diastolic pressure, use of statin, hypertension, diabetes, coronary heart disease, chronic obstructive pulmonary disease, stroke, atrial fibrillation, heart failure, myocardial infarction.

Supplementary Figure 10 Association between changes in VO_2max and the annual change of $eGFR_{CysC}$



10a: unstratified population; 10b: males; 10c: females; The solid black line represents the regression line. Dashed lines on either side of the solid black line show the 95% confidence interval (CI). The red line is for easy reference, and a 95%CI below or above the line is regarded as a meaningful association. Adjusted for sex, race, baseline age, smoking, body mass index, Townsend deprivation index, baseline systolic blood pressure, baseline diastolic pressure, use of statin, hypertension, diabetes, coronary heart disease, chronic obstructive pulmonary disease, stroke, atrial fibrillation, heart failure, myocardial infarction.

6. Chapter 6 – Discordance between cystatin C-based and creatinine-based estimated glomerular filtration rate and health outcomes in adults: a systematic review and meta-analysis.

The original article of this chapter has been published in Clinical Kidney Journal on January 08, 2025.

Liu Q, Welsh P, Celis-Morales C, Ho FK, Lees JS, Mark PB. Discordance between cystatin C-based and creatinine-based estimated glomerular filtration rate and health outcomes in adults: a systematic review and meta-analysis. Clin Kidney J. 2025 Jan 8;18(3):sfaf003. doi: 10.1093/ckj/sfaf003

6.1. Abstract

Background

The intra-individual difference in Cystatin C-based and Creatinine-based estimated glomerular filtration rate (eGFRcys, eGFRcr, respectively), i.e., eGFR discordance, has recently been demonstrated to have prognostic implications. It was associated with mortality, cardiovascular, and renal outcomes. We presented a systematic review and meta-analysis to summarize existing literature.

Methods

We searched PubMed, Embase, and MEDLINE up to April 28, 2024 for cohort and cross-sectional studies in English reporting the association of eGFR discordance with mortality, cardiovascular, and renal outcomes. The quality of studies was evaluated by Risk Of Bias In Non-randomized Studies - of Exposure form. Data from studies were extracted to a pre-defined table and pooled using a random-effects model. Stratified and sensitivity analyses were performed.

Results

A total of 1489 studies were initially identified, of which 18 studies with longitudinal or cross-sectional designs were included, with a sample size between 373 and 363494 people. The risk of bias was generally low to some concerns. eGFR was mainly calculated using Chronic Kidney Disease Epidemiology Collaboration equations, while a few studies applied other equations. An eGFR discordance featuring lower eGFRcys, e.g., eGFRcys≤60% of eGFRcr, or eGFRcys - eGFRcr≤ -15 ml/min/1.73m^2 was consistently associated with higher mortality and elevated risk of cardiovascular and renal outcomes. People with lower eGFRcys have a 58% greater risk of mortality (HR=1.58, 95%CI: 1.42, 1.76), and 32% greater risk of cardiovascular events (HR=1.32, 95%CI: 1.25, 1.39. People with higher eGFRcys have a 39% lower risk of mortality (HR=0.61, 95%CI: 0.52, 0.70), and 29% lower risk of cardiovascular events (HR=0.71, 95%CI: 0.62, 0.81). No meta-analysis for renal outcomes was conducted due to data availability.

Conclusions

The eGFR discordance serves as a meaningful indicator of adverse health outcomes. The lack of a consensus on the cut-off value of eGFR discordance and the mixture use of eGFR equations warrants attention.

6.2. Background

An accurate estimated glomerular filtration rate (eGFR) is key in the clinical diagnosis and management of chronic kidney disease (CKD). Common equations for estimating GFR are based on serum creatinine and cystatin C levels. Creatinine is widely used but its levels can be influenced by muscle mass, diet, and medications.[1,2] In contrast, cystatin C is less likely affected by common confounders like muscle mass and diet [3], but may be influenced by alternative non-GFR factors including obesity and glucocorticoids.[4] Although the accuracy among eGFR derived from a single biomarker has been debated [5,6], several studies have highlighted the advantages of using cystatin C-based eGFR (eGFRcys) against creatinine-based eGFR (eGFRcr), as it offers better accuracy in predicting cardiovascular disease (CVD), end-stage kidney disease (ESKD) progression, and mortality.[7-10]

Since both creatinine and cystatin C are influenced by non-GFR determinants, it is common to identify situations where large discordances exist between eGFRcr and eGFRcys.[11] For example, eGFRcys can be as low as 70% of eGFRcr or more than 15 ml/min/1.73m² lower than eGFRcr.[12,13] Such discordances may lead to differences in diagnosis or staging of CKD and/or eligibility for clinical treatments, and cannot be attributed to kidney function alone.

Various hypotheses have been proposed to explain situations where large discordances exist. Grubb et al. (2015) highlighted significantly higher ratios of cystatin C/creatinine,β_2-microglobulin/creatinine, and beta-trace protein/creatinine in people with eGFRcys≤60% of eGFRcr, compared to those with eGFRcys within 90-110% of eGFRcr.[14] Given the similar molecular sizes of cystatin C, β_2-microglobulin, and beta-trace protein, and the fact that their production is not co-regulated, Grubb et al. proposed that a reduction in the pore diameter of the glomerular membrane impaired the filtration of these proteins, resulting in the observed ratio elevations (the “Shrunken pore syndrome” [SPS]).[13,14] Considering that the molecular weight of cystatin C is 13 kDa, while creatinine is merely 113 Da, this presence of eGFR discordance may indicate enhanced selective filtration of medium-sized molecules (5-30 kDa) in the kidneys.[12,15] Thus, another term, Selective Glomerular Hypofiltration Syndrome (SGHS) was proposed in early 2023.[15]

Alternatively, eGFR discordance may relate to the differential production of creatinine and cystatin C.[4] The degree of eGFR discordance is purported to be a useful indicator of

muscle mass.[16] Individuals with the lowest eGFRcys/eGFRcr ratio have the largest absolute underestimation of mGFR by eGFRcys and overestimation by eGFRcr (particularly in the setting of muscle wasting). [17]

Immediately following Grubb et al.'s study in 2015, several researchers reported the association of SPS with an increase in mortality in patients undergoing elective coronary artery bypass grafting [18] and in healthy seniors (aged 60 years and above).[19,20] Since 2020, there is an increase in studies showed that eGFR discordance was associated with various adverse outcomes, including kidney disease, cardiovascular disease, and mortality.[11,21,22] These associations suggested that eGFR discordance could serve as a potential marker for identifying vulnerable populations, which highlights the importance of understanding this phenomenon in depth.

Despite the growing body of literature on eGFR discordance, there is a lack of systematic reviews and meta-analyses to comprehensively evaluate its association with health outcomes. Therefore, this study aims to conduct a systematic review and meta-analysis to assess the pooled effect of eGFR discordance on mortality, kidney, and cardiovascular disease.

6.3. Methods

This review has been registered on PROSPERO (CRD42024540635). We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Statement (PRISMA) [23] and Meta-analysis of Observational Studies in Epidemiology.[24] One author (QL) performed systematic literature searches on Embase, PubMed, and MEDLINE from database inception to April 28, 2024. If study data were not published in the pre-defined discordance categories, authors were contacted via email twice with an interval of a week to obtain the full text if it was not available online. Observational studies of longitudinal and cross-sectional designs were included. Due to resource constraints, we only included English language manuscripts and did not search for unpublished results.

Studies meeting the following criteria were excluded: 1. Studies conducted in populations whose kidney function cannot be reliably evaluated by creatinine-based eGFR, such as patients receiving dialysis.[25]; 2. Studies involving people with cancer, AIDS, or those under intensive care. 3. Studies that did not have mortality, cardiovascular, or kidney

diseases as outcomes. Detailed search terms can be found in the Supplemental Materials (Table S1).

Two authors (QL, PW) independently reviewed and selected studies for inclusion, with any conflicts resolved through consultation with two additional authors (CC and PM). To ensure comprehensive coverage, one author (QL) thoroughly examined the references of the chosen articles and assessed their relevance after reading the full texts. Furthermore, this author (QL) conducted manual searches for additional relevant studies.

Quality assessment

Because all the selected studies were observational studies, their qualities were independently assessed by two reviewers (QL, PW) using the Risk Of Bias In Non-randomized Studies - of Exposure (ROBINS-E), which was designed for evaluating observational studies.[26] The methodological flaws of a study were categorized as low, some concerns, high, or very high based on (1) confounding, (2) measurement of the exposure, (3) selection of participants, (4) post-exposure interventions, (5) missing data, (6) measurement of the outcome, and (7) selection of the reported results. A cohort study was considered biased if the loss to follow-up was 20% or above.[27] Any disparities in judgment raised between the two reviewers were resolved through discussion with a third reviewer (JSL) as needed.

Data synthesis

Using a predesigned table, information on each study was extracted from the first author's family name, publication year, study type, study location, characteristics of the study population, eGFR calculation method, kidney characteristics, study exposure, the prevalence of eGFR discordance defined by the study, study outcome, and main study findings (i.e., subgroup findings were not presented). If a study calculated eGFR using multiple eGFR equations, then the result of each equation would be extracted separately. The outcomes of interest were predefined as mortality, cardiovascular events, and renal events.

In this review, the direction of discordance was arbitrarily defined as the derivation from eGFRcr, which means eGFRcr was the subtrahend or denominator. If a study reported results in the opposite direction, such as eGFRcr being the minuend or numerator, the

results would be reversed to ensure all findings were presented in the same direction for ease of comparison. Data were synthesized and compared among studies if they have the same reference group definition and eGFR discordance calculation methods.

Data reported in the median (interquartile range [IQR]) were converted to the mean (standard deviation [SD]) following established methods.[28] One author (FH) offered advice on methodology and statistical approaches. We used random-effects model with the restricted maximum likelihood method to pool the hazard ratio (HR) or odds ratio (OR) from individual studies.[29] Heterogeneity between studies relative to total variance was examined using the I^2 statistic.[30] Subgroup meta-analyses would be performed subject to data availability. Funnel plots were used to evaluate the risk of biased results.[31] Statistics analyses were performed using STATA 17 (StataCorp, USA) and Robvis (https://mcguinlu.shinyapps.io/robvis/).[32]

Sensitivity analysis

Leave-one-out analysis was performed to identify influential studies by conducting the meta-analysis multiple times while removing one of the included studies during each iteration. Results were presented as leave-one-out figures.

6.4. Results

Identification of studies

After removing duplicated studies, 1489 potentially relevant studies were identified. Initial screening based on title and abstracts resulted in 51 studies retrieved for further evaluation. Following full-text assessment, 33 studies were excluded, leaving 18 studies.[11,17,21,22,33-46] Another nine studies were identified through reading citations, and all were excluded (Figure 6-1). All the included studies were either of cohort design or cross-sectional design or covered both.

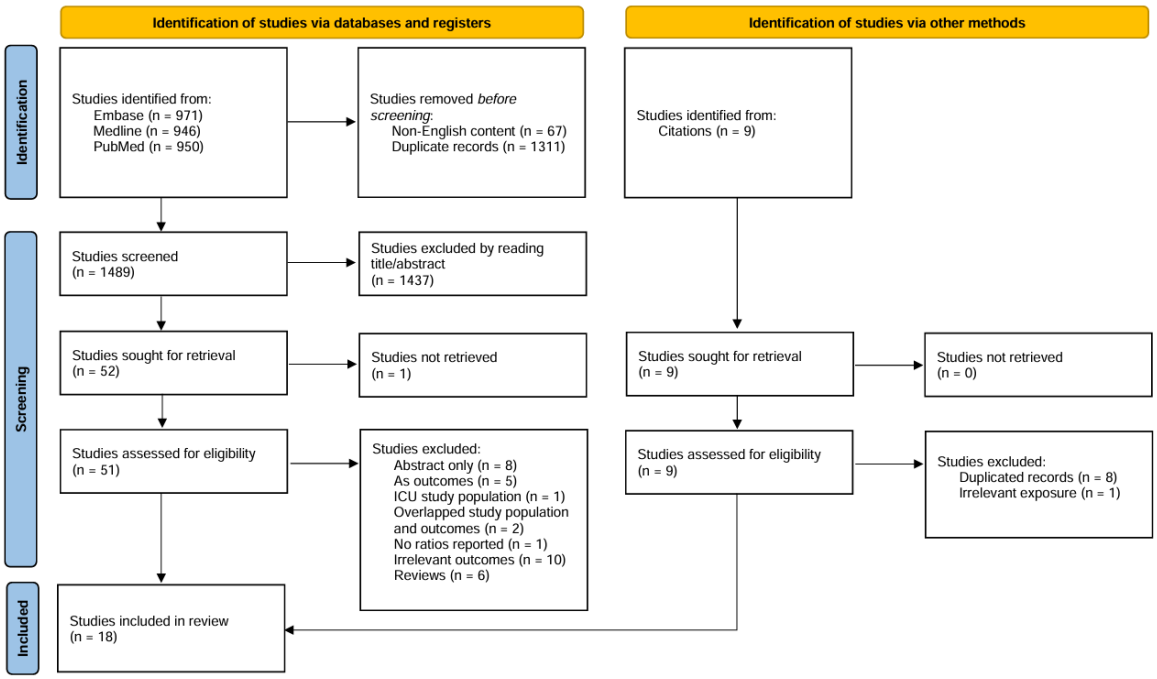


Figure 6-1 The PRISMA flow diagram

Characteristics of the included studies

All selected studies were published between 2020 and 2024. The 18 studies included 20 study populations. Sample sizes ranged from 373 to 363494 individuals, with a median of 4252 individuals. Nineteen out of 20 populations had a median or mean age of 50 years or older, with the oldest age being 84 years. One study had a much younger population with a median (IQR) age of 37 (27 - 48) years.[44] Except for one study where all participants were female, all other studies included both males and females (Table 6-1 and 6-2).[41]

Table 6-1 The study characteristics of the included studies.

ID	Author year	Study Type	Follow-up Duration	Study Population				eGFR calculation method	Exposure	Prevalence of exposure, as categorical variable n(%)	Outcome
				Size	Age	Sex	Country				
1	Akesson, et al., 2020	Cohort	Median, year: 5.6	2781 people from LCS cohort	Median (2.5 - 97.5 percentiles), year: 63 (20 - 85)	Male, n(%): 1449 (51.4) Female, n(%): 1332 (48.6)	Sweden	LMRcr: LMrev equation CAPAcys: CAPA equation CKD-EPIcr, CKD-EPIcys: 2012 CKD-EPI equation mGFR: Plasma clearance of iohexol FAScr, FAScys: FAS equation	CAPAcys/LMRcr ratio CKD-EPIcys/CKD-EPIcr ratio FAScys/FAScr ratio	Size (%) CAPAcys/LMRcr ratio <0.70: 645 (23.2) CAPAcys/LMRcr ratio 0.70-0.84: 699 (25.1) CAPAcys/LMRcr ratio 0.85-0.99: 728 (26.2) CAPAcys/LMRcr ratio ≥1.00: 709 (25.5)	1. All-cause mortality 2. Cause-specific mortality by Cancer, CVD, Diabetes, and CKD
2	Carrero, et al., 2023	Cohort	Median (IQR), year: 4.5 (2.3 - 6.8)	158601 people from SCREAM project	Mean (SD), year: 62 (18)	Male, %: 52 Female, %:48	Sweden	eGFRcr: 2021 CKD-EPI race-free equation eGFRcys: 2012 CKD-EPI equation	Percent eGFRdiff, defined as (eGFRcys-eGFRcr)/eGFRcr Absolute eGFRdiff, defined as eGFRcys-eGFRcr	Not applicable.	1. Kidney failure with replacement therapy 2. Acute kidney injury 3. Heart failure 4. Atherosclerotic cardiovascular disease 5. All-cause death 6. Cardiovascular death
3	Chen, et al., 2022a	Cohort	Incident of end-stage kidney disease Median (IQR), year: 4.7 (2.6 - 7.5)	4956 people from CRIC study	Mean (SD), year: 59.5 (10.5)	Male, n(%): 2800 (56.5) Female, n(%): 2156 (43.5)	USA	eGFRcr: 2021 CKD-EPI race-free equation eGFRcys: 2012 CKD-EPI equation	eGFRdiffcys-cr, defined as eGFRcys-eGFRcr	Size (%) eGFRdiffcys-cr <-15 ml/min/1.73m^2: 390 (7.9) eGFRdiffcys-cr -15 to 15 ml/min/1.73m^2: 3318 (66.9) eGFRdiffcys-cr ≥15 ml/min/1.73m^2: 1248 (25.2)	1. Incident end-stage kidney disease 2. All-cause mortality

			All-cause mortality Median (IQR), year: 7.2 (4.4 - 9.7)								
4	Chen, et al., 2022b	Cohort	Median (IQR), year: 3.5 (1.5 - 7.1)	4512 people from CRIC study, without prevalent heart failure.	Mean (SD), year: 59.4 (10.7)	Male, n(%): 2531 (56.1) Female, n(%): 1981 (43.9)	USA	eGFRcr: 2021 CKD-EPI race-free equation eGFRcys: 2012 CKD-EPI equation	eGFRdiff, defined as eGFRcys-eGFRcr	Size (%) eGFRdiff <-15 ml/min/1.73m^2: 340 (7.5) eGFRdiff -15 to 15 ml/min/1.73m^2: 2977 (66.0) eGFRdiff ≥15 ml/min/1.73m^2: 1195 (26.5)	Incident heart failure hospitalization
5	Farrington et al., 2023	Cohort	Minimum 25 years	13197 people from the ARIC study without prevalent kidney failure at visit 2.	Mean (SD), year: 57 (6)	Male, n(%): 5778 (44) Female, n(%): 7419 (56)	USA	eGFRcr: 2021 CKD-EPI race-free equation eGFRcys: 2012 CKD-EPI equation	eGFRcys/eGFRcr ratio	Size (%) eGFRcys >30% lower than eGFRcr: 937 (7) eGFRcys >30% higher than eGFRcr: 343 (3)	1. Incident kidney failure, heart failure, acute kidney injury 2. Death

6	He, et al., 2024a	Cohort	INDEED Median (IQR), year: 3.83 (3.47 - 4.18) NHANES Median (IQR), year: 15.17 (7.7 - 17.58) UK Biobank Median (IQR), year: 13.51 (12.68 - 14.39)	All with diabetes and without history of cardiovascular diseases. 8129 people from INDEED 1634 people from NHANES 29358 people from the UK Biobank	Mean (SD), year: INDEED: 60.7 (10.0) NHANES: 62.5 (14.4) UK Biobank: 59.4 (7.3)	INDEED: Male, n(%): 6440 (79.2) Female, n(%): 1689 (20.8) NHANES: Male, n(%): 877 (53.7) Female, n(%): 757 (46.3) UK Biobank: Male, n(%): 17787 (60.6) Female, n(%): 11571 (39.4)	China, USA, and UK	eGFRcr: 2021 CKD-EPI race-free equation eGFRcys: 2012 CKD-EPI equation	eGFRabdiff, defined as eGFRcys-eGFRcr eGFRrediff, defined as eGFRcys/eGFRcr	INDEED Size (%) eGFRabdiff <-15 ml/min/1.73m^2: 2750 (33.8) eGFRabdiff -15 to 15 ml/min/1.73m^2: 4584 (56.4) eGFRabdiff ≥15 ml/min/1.73m^2: 795 (9.8) eGFRrediff ≥0.6: 7802 (96.0) eGFRrediff <0.6: 327 (4.0) NHANES Size (%) eGFRabdiff <-15 ml/min/1.73m^2: 211 (12.9) eGFRabdiff -15 to 15 ml/min/1.73m^2: 1104 (67.6) eGFRabdiff ≥15 ml/min/1.73m^2: 319 (19.5) eGFRrediff ≥0.6: 1602 (98) eGFRrediff <0.6: 32 (2.0) UK Biobank Size (%) eGFRabdiff <-15 ml/min/1.73m^2: 11563 (39.4) eGFRabdiff -15 to 15 ml/min/1.73m^2: 16994 (57.9)	1. All-cause mortality 2. Incident cardiovascular events, defined as myocardial infarction, heart failure, atrial fibrillation, and stroke.

									eGFRabdiff ≥15 ml/min/1.73m^2: 801 (2.7) eGFRrediff ≥0.6: 28411 (96.8) eGFRrediff <0.6: 947 (3.2)	
7 2024b	He, et al.,	Cohort	Median (IQR), year: 13.59 (12.77 - 14.43)	25825 people with diabetes free of diabetic microvascular complications from the UK Biobank.	Mean (SD), year: 59.1 (7.3)	Male, n(%): 15589 (60.4) Female, n(%): 10236 (39.6)	UK	eGFRcr: 2021 CKD-EPI race-free equation eGFRcys: 2012 CKD-EPI equation	eGFRabdiff, defined as eGFRcys-eGFRcr eGFRrediff, defined as eGFRcys/eGFRcr	Size (%) eGFRabdiff <-15: 10045 (38.9) eGFRabdiff -15 to 15: 15028 (58.2) eGFRabdiff ≥15: 752 (2.9) eGFRrediff ≥0.6: 25247 (97.8) eGFRrediff <0.6: 578 (2.2)	Incidence of diabetic kidney disease

8	Heo, et al., 2024	Cohort	Median (IQR), year: 11.7	363494 people from the UK Biobank without a prior diagnosis of atrial fibrillation or a history of related procedures, including catheter ablation.	Mean (SD), year: 56.2 (8.1)	Male, n(%): 172004 (47.3) Female, n(%): 191490 (52.7)	UK	eGFRcr: 2009 CKD-EPI race-dependent equation, 2021 CKD-EPI race-free equation (sensitivity analysis only) eGFRcys: 2012 CKD-EPI equation	eGFRdiff, defined as eGFRcys-eGFRcr which was calculated using 2009 CKD-EPI race-dependent equation Race-free eGFRdiff, defined as eGFRcys-eGFRcr which was calculated using 2021 CKD-EPI race-free equation	Size (%) eGFRdiff <-15 ml/min/1.73m^2: 48899 (13.5) eGFRdiff -15 to 15 ml/min/1.73m^2: 276960 (76.2) eGFRdiff ≥15 ml/min/1.73m^2: 37635 (10.4)	Incident atrial fibrillation
9	Herou, et al., 2022	Cohort	Median (IQR), year: 6.5 (5.1 - 8.2)	3993 people underwent CABG, SAVR, or CABG+SAVR	Mean (SD), year: 68 (10)	Male, n(%): 3063 (77) Female, n(%): 930 (23)	Sweden	eGFRcreatinine: 2012 CKD-EPI race-dependent equation eGFRcystatin C: 2012 CKD-EPI equation eGFR LMrev: LMrev creatinine equation eGFR CAPA: CAPA cystatin C equation	Shrunken pore syndrome, defined as eGFRcystatin C ≤ 60% of eGFRcreatinine, and by eGFR CAPA ≤ 60% of eGFR LMrev separately.	Size (%), by CKD-EPI equation eGFRcystatin C ≤ 60% of eGFRcreatinine: 296 (7.4) Size (%), by CAPA and LMrev equation eGFR CAPA ≤ 60% of eGFR LMrev: 92 (2.3)	10-year mortality
10	Jonsson, et al., 2021	Cohort	Not available	996 people with hip fracture.	Median (IQR), year: 84 (77 - 90)	Male, %: 29 Female, %:71	Sweden	eGFRcrea: LMrev creatinine equation eGFRcys: CAPA cystatin C equation	Shrunken pore syndrome, defined as eGFRcys/eGFRcrea ≤ 0.7	Size (%) eGFRcys/eGFRcrea ≤ 0.7: 87 (8.7)	1. All-cause one year mortality 2. All-cause 90-day mortality
11	Kim, et al., 2021	Cohort	Median, year: 4.1	2076 people with CKD from KNOW-CKD study	Mean (SD), year: 53.5 (12.2)	Male, n(%): 1265 (60.9) Female, n(%): 811 (39.1)	Korea	2012 CKD-EPI equation	eGFRdiff, defined as eGFRcreat-eGFRcys	Size (%) eGFRdiff <-2.3 ml/min/1.73m^2: 691 (33.3) -2.3 ≤ eGFRdiff <4.0	Newly occurred MACE, defined as death from cardiovascular causes and any non-fatal

										ml/min/1.73m^2: 690 (33.3) eGFRdiff ≥4.0 ml/min/1.73m^2: 695 (33.4)	cardiovascular events that required hospitalization.
12	Ljungberg, et al., 2019	Case-control	Not applicable	Case: 336 survey participants who received surgery for aortic stenosis. Control: 671 people matched for sex, age, type and date of survey, and geographical area.	Mean (95%CI), year At survey Case: 56.7 (55.8, 57.6) Control: 56.7 (56.0, 57.3)	Female (95%CI), % Case: 48 (43, 53) Control: 48 (44, 52)	Sweden	eGFRcreatinine: LMrev creatinine equation eGFRcystatin C: CAPA cystatin C equation	eGFRcystatin C / eGFRcreatinine Z (ln) ratio, defined as Z-score of ln(eGFRcystatin C / eGFRcreatinine)	Not applicable.	Aortic valve replacement due to aortic stenosis
13	Malmgren, et al., 2022	Cohort	Minimum 10 years	849 females from OPRA cohort, without sarcopenia or taking glucocorticoids.	Mean (SD), year: 75.2 (0.14)	Female, n(%): 849 (100)	Sweden	eGFRcrea: 2012 CKD-EPI race-dependent equation, LMrev creatinine equation (sensitivity analysis only) eGFRcysC: 2012 CKD-EPI equation, CAPA cystatin C equation (sensitivity analysis only)	eGFRcysC/eGFRcrea <0.6	Size (%), by CKD-EPI equation eGFRcysC/eGFRcrea ratio <0.6: 80 (9.4) eGFRcysC/eGFRcrea ratio 0.6-0.69: 85 (10.0) eGFRcysC/eGFRcrea ratio 0.7-0.79: 140 (16.5) eGFRcysC/eGFRcrea ratio 0.8-0.89: 178 (21.0) eGFRcysC/eGFRcrea ratio ≥0.9: 366 (43.1) Size (%), by CAPA and LMrev equation	10-year mortality

											eGFRcysC/eGFRcrea ratio <0.6: 22 (2.6) eGFRcysC/eGFRcrea ratio 0.6-0.69: 39 (4.6) eGFRcysC/eGFRcrea ratio 0.7-0.79: 56 (6.6) eGFRcysC/eGFRcrea ratio 0.8-0.89: 99 (11.7) eGFRcysC/eGFRcrea ratio ≥0.9: 633 (74.5)	
14	Potok, et al., 2020	Cohort	Not available	9092 people from SPRINT study.	Mean (SD), year: 68 (9)	Male, n(%): 5842 (64) Female, n(%): 3250 (36)	USA	2012 CKD-EPI equation	eGFRDiff, defined as eGFRcys-eGFRcr	Size (%) eGFRDiff <-15: 1230 (14) eGFRDiff -15 to 15: 6471 (71) eGFRDiff ≥15: 1391 (15)	1. Cardiovascular events, including myocardial infarction, stroke, acute coronary syndrome, and heart failure. 2. Total mortality

15	Söderström, et al., 2021	Nested case-control	Not applicable	Case: 545 survey participants who experienced first-ever myocardial infarction. Control: 1054 people matched for sex, age, time and type of survey, and geographical area.	Mean, year Male Case: 53.6 Control: 53.4 Female Case: 58.3 Control: 58.1	Male, n(%) Case: 387 (71.0) Control: 752 (71.3)	Sweden	eGFRcreatinine: LMrev creatinine equation eGFRcystatin C: CAPA cystatin C equation	eGFRcystatin C / eGFRcreatinine Z (ln) ratio, defined as Z-score of ln(eGFRcystatin C / eGFRcreatinine)	Not applicable.	First-ever myocardial infarction.
13	Wu, et al., 2022	Cohort	Mean, month: 27.7	536 people with IgA nephropathy or membranous nephropathy	Median (IQR), year: 37 (27 - 48)	Male, n(%): 262 (49) Female, n(%): 274 (51)	China	2012 CKD-EPI equation	Shrunken pore syndrome, defined as eGFRcys < 70% eGFRcr	Size (%) eGFRcys < 70% eGFRcr: 44 (8.2)	1. ESRD, defined as eGFRcr ≤ 15 ml/min/1.73m2 or the initiation of renal placement therapy. 2. Severe eGFR decline, defined as stable serum creatinine doubling or ≥ 50% eGFRcr decline compared with its baseline value.

14	Xhakollari, et al., 2021	Cohort	Median, year: 1.8	373 people hospitalized for newly diagnosed or exacerbated acute heart failure from HARVEST-Malmo Study	Mean (SD), year: 74.8 (12.1)	Male, n(%): 255 (68.4) Female, n(%): 118 (31.6)	Sweden	2012 CKD-EPI equation	Shrunken pore syndrome, defined as eGFRcystatin C ≤ 60% of eGFRcreatinine	Size (%) eGFRcystatin C/eGFRcreatinine ratio ≤0.6: 94 (25.2) eGFRcystatin C/eGFRcreatinine ratio >0.6: 279 (74.8)	1. All-cause mortality. 2. 30-day rehospitalization due to cardiac causes.
15	Zhang, et al., 2023	Cross-sectional	Median, year: 2.8	5050 people underwent elective PCI	Median (IQR), year: Non-Shrunken pore syndrome group: 66 (58 - 73) Shrunken pore syndrome group: 69 (61 - 76)	Non-Shrunken pore syndrome group Male, n(%): 3472 (78.9) Female, n(%): 929 (21.1) Shrunken pore syndrome group Male, n(%): 487 (75.0) Female, n(%): 162 (25.0)	China	eGFRcr: 2021 CKD-EPI race-free equation eGFRcys: 2012 CKD-EPI equation	Shrunken pore syndrome, defined as eGFRcys < 60% eGFRcr, and eGFRcys < 70% eGFRcr, respectively	Size (%) eGFRcys < 60% eGFRcr: 649 (12.9)	1. CA-AKI, defined as a relative increase in SCr ≥50% or an absolute SCr increase ≥0.3 mg/dL within 48 hours after contrast medium exposure. 2. Mortality

ARIC, Atherosclerosis Risk in Communities Study; CA-AKI, Contrast-Associated Acute Kidney Injury; CABG, Coronary Artery Bypass Grafting; CAPA, Caucasian, Asian, Paediatric, and Adult cohorts cystatin C-based equation; CI, Confidence Interval; CKD, Chronic Kidney Disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CRIC, Chronic Renal Insufficiency Cohort; CVD, Cardiovascular Disease; eGFR, estimated Glomerular Filtration Rate; ESRD, End-Stage Renal Disease; FAS, Full Age Spectrum; INDEED, Incidence, Development, and Prognosis of Diabetic Kidney Disease Study; IQR, Interquartile range; KNOW-CKD, Korean Cohort Study for Outcome in Patients With Chronic Kidney Disease; LCS, Lund Cystatin C Standardization Cohort; LMrev, Lund-Malmö revised creatinine-based eGFR equation; MACE, Major Adverse Cardiovascular Events; mGFR, measured Glomerular Filtration Rate; NHANES, National Health and Nutrition Examination Survey; OPRA, Malmo Osteoporosis Prospective Risk Assessment cohort; PCI, Percutaneous Coronary Intervention; SAVR, Surgical Aortic Valve Replacement; SCr, Serum Creatinine; SD, standard deviation; sHR, Subdistribution Hazard Ratio; SPRINT, Systolic Blood Pressure Intervention Trial Study.

Table 6-2 Renal characteristics and the main findings of the included studies.

ID	Author year	Study Type	Renal characteristics	Main findings
1	Akesson, et al., 2020	Cohort	Median (2.5- 97.5 percentiles): CAPAcys, ml/min/1.73m^2: 47 (10 - 107) LMRcr, ml/min/1.73m^2: 61 (11 - 111) CKD-EPIcr, ml/min/1.73m^2: 66 (11 - 127) CKD-EPIcys, ml/min/1.73m^2: 46 (10 - 112) FAScr, ml/min/1.73m^2: 62 (13 - 140) FAScys, ml/min/1.73m^2: 48 (15 - 109) mGFR, ml/min/1.73m^2: 57 (10 - 118) CAPAcys/LMRcr ratio: 0.86 (0.46 - 1.29) CKD-EPIcys/CKD-EPIcr ratio: 0.78 (0.41 - 1.26) FAScys/FAScr ratio: 0.84 (0.47 - 1.34)	1. All-cause mortality, HR (95%CI) CAPAcys/LMRcr ratio <0.60: 3.3 (2.5, 4.5) CAPAcys/LMRcr ratio <0.70: 3.0 (2.4, 3.7) CAPAcys/LMRcr ratio 0.70-0.84: 2.2 (1.8, 2.7) CAPAcys/LMRcr ratio 0.85-0.99: 1.3 (1.1, 1.6) CAPAcys/LMRcr ratio ≥1.00: Reference CKD-EPIcys/CKD-EPIcr ratio <0.70: 2.4 (1.9, 3.1) CKD-EPIcys/CKD-EPIcr ratio 0.70-0.84: 1.5 (1.2, 1.9) CKD-EPIcys/CKD-EPIcr ratio 0.85-0.99: 1.2 (0.9, 1.6) CKD-EPIcys/CKD-EPIcr ratio ≥1.00: Reference FAScys/FAScr ratio <0.70: 2.7 (2.2, 3.5) FAScys/FAScr ratio 0.70-0.84: 1.9 (1.5, 2.3) FAScys/FAScr ratio 0.85-0.99: 1.2 (1.0, 1.5) FAScys/FAScr ratio ≥1.00: Reference 2. Cancer mortality, HR (95%CI) CAPAcys/LMRcr ratio <0.70: 3.3 (2.3, 4.9) CAPAcys/LMRcr ratio 0.70-0.84: 2.4 (1.7, 3.3) CAPAcys/LMRcr ratio 0.85-0.99: 1.3 (1.0, 1.8) CAPAcys/LMRcr ratio ≥1.00: Reference 3. CVD mortality, HR (95%CI) CAPAcys/LMRcr ratio <0.70: 2.4 (1.6, 3.7) CAPAcys/LMRcr ratio 0.70-0.84: 1.7 (1.1, 2.5) CAPAcys/LMRcr ratio 0.85-0.99: 1.2 (0.7, 1.8) CAPAcys/LMRcr ratio ≥1.00: Reference 4. Diabetes mortality, HR (95%CI) CAPAcys/LMRcr ratio <0.70: 2.0 (0.8, 4.7) CAPAcys/LMRcr ratio 0.70-0.84: 1.9 (0.8, 4.7) CAPAcys/LMRcr ratio 0.85-0.99: 1.2 (0.5, 3.3) CAPAcys/LMRcr ratio ≥1.00: Reference 5. CKD mortality, HR (95%CI) CAPAcys/LMRcr ratio <0.70: 5.1 (0.6, 46.0) CAPAcys/LMRcr ratio 0.70-0.84: 3.3 (0.3, 33.7) CAPAcys/LMRcr ratio 0.85-0.99: 2.2 (0.2, 25.6) CAPAcys/LMRcr ratio ≥1.00: Reference

2	Carrero, et al., 2023	Cohort	Mean (SD) eGFRcr, ml/min/1.73m^2: 80 (26) eGFRcys, ml/min/1.73m^2: 73 (31) Median (IQR) %eGFRdiff, -10 [-27 to 6]	1. Kidney failure with replacement therapy Quartiles of Percent eGFRdiff, HR(95%CI) Quartile 1 (eGFRcys<<eGFRcr): 1.36 (1.17, 1.58) Quartile 2 (eGFRcys<eGFRcr): 1.08 (0.94, 1.25) Quartile 3 (eGFRcys≈eGFRcr): Reference Quartile 4 (eGFRcys>eGFRcr): 0.79 (0.69, 0.92) Quartiles of Absolute eGFRdiff, HR(95%CI) Quartile 1 (eGFRcys<<eGFRcr): 2.46 (1.98, 3.05) Quartile 2 (eGFRcys<eGFRcr): 1.20 (1.05, 1.36) Quartile 3 (eGFRcys≈eGFRcr): Reference Quartile 4 (eGFRcys>eGFRcr): 0.57 (0.48, 0.69) 2. Acute kidney injury Quartiles of Percent eGFRdiff, HR(95%CI) Quartile 1 (eGFRcys<<eGFRcr): 2.62 (2.42, 2.85) Quartile 2 (eGFRcys<eGFRcr): 1.53 (1.40, 1.67) Quartile 3 (eGFRcys≈eGFRcr): Reference Quartile 4 (eGFRcys>eGFRcr): 0.67 (0.59, 0.75) Quartiles of Absolute eGFRdiff, HR(95%CI) Quartile 1 (eGFRcys<<eGFRcr): 3.10 (2.85, 3.36) Quartile 2 (eGFRcys<eGFRcr): 1.60 (1.48, 1.72) Quartile 3 (eGFRcys≈eGFRcr): Reference Quartile 4 (eGFRcys>eGFRcr): 0.64 (0.57, 0.72) 3. Atherosclerotic cardiovascular disease Quartiles of Percent eGFRdiff, HR(95%CI) Quartile 1 (eGFRcys<<eGFRcr): 1.42 (1.33, 1.51) Quartile 2 (eGFRcys<eGFRcr): 1.19 (1.11, 1.27) Quartile 3 (eGFRcys≈eGFRcr): Reference Quartile 4 (eGFRcys>eGFRcr): 0.79 (0.73, 0.86) Quartiles of Absolute eGFRdiff, HR(95%CI) Quartile 1 (eGFRcys<<eGFRcr): 1.46 (1.37, 1.56) Quartile 2 (eGFRcys<eGFRcr): 1.19 (1.12, 1.26) Quartile 3 (eGFRcys≈eGFRcr): Reference Quartile 4 (eGFRcys>eGFRcr): 0.78 (0.72, 0.85) 4. Heart failure Quartiles of Percent eGFRdiff, HR(95%CI) Quartile 1 (eGFRcys<<eGFRcr): 2.04 (1.92, 2.17) Quartile 2 (eGFRcys<eGFRcr): 1.33 (1.25, 1.41) Quartile 3 (eGFRcys≈eGFRcr): Reference Quartile 4 (eGFRcys>eGFRcr): 0.76 (0.70, 0.83) Quartiles of Absolute eGFRdiff, HR(95%CI) Quartile 1 (eGFRcys<<eGFRcr): 2.20 (2.07, 2.34) Quartile 2 (eGFRcys<eGFRcr): 1.41 (1.34, 1.49)

				Quartile 3 (eGFRcys≈eGFRcr): Reference Quartile 4 (eGFRcys>eGFRcr): 0.73 (0.67, 0.80) 5. Cardiovascular death Quartiles of Percent eGFRdiff, HR(95%CI) Quartile 1 (eGFRcys<<eGFRcr): 2.48 (2.32, 2.66) Quartile 2 (eGFRcys<eGFRcr): 1.40 (1.30, 1.50) Quartile 3 (eGFRcys≈eGFRcr): Reference Quartile 4 (eGFRcys>eGFRcr): 0.85 (0.77, 0.94) Quartiles of Absolute eGFRdiff, HR(95%CI) Quartile 1 (eGFRcys<<eGFRcr): 2.87 (2.69, 3.06) Quartile 2 (eGFRcys<eGFRcr): 1.50 (1.41, 1.58) Quartile 3 (eGFRcys≈eGFRcr): Reference Quartile 4 (eGFRcys>eGFRcr): 0.78 (0.70, 0.86) 6. All-cause death Quartiles of Percent eGFRdiff, HR(95%CI) Quartile 1 (eGFRcys<<eGFRcr): 2.62 (2.54, 2.72) Quartile 2 (eGFRcys<eGFRcr): 1.46 (1.41, 1.52) Quartile 3 (eGFRcys≈eGFRcr): Reference Quartile 4 (eGFRcys>eGFRcr): 0.80 (0.77, 0.84) Quartiles of Absolute eGFRdiff, HR(95%CI) Quartile 1 (eGFRcys<<eGFRcr): 2.88 (2.79, 2.98) Quartile 2 (eGFRcys<eGFRcr): 1.49 (1.45, 1.54) Quartile 3 (eGFRcys≈eGFRcr): Reference Quartile 4 (eGFRcys>eGFRcr): 0.74 (0.70, 0.77)
3	Chen, et al., 2022a	Cohort	Mean (SD) eGFRcr, ml/min/1.73m^2: 49 (16) eGFRcys, ml/min/1.73m^2: 54 (23) eGFRdiffcys-cr, ml/min/1.73m^2: 6 (16)	1. Incident end-stage kidney disease Baseline measures ,sHR (95%CI) eGFRdiffcys-cr <-15 ml/min/1.73m^2: 1.00 (0.65, 1.52) eGFRdiffcys-cr -15 to 15 ml/min/1.73m^2: Reference eGFRdiffcys-cr ≥15 ml/min/1.73m^2: 0.73 (0.59, 0.89) Time-updated measures, sHR (95%CI) eGFRdiffcys-cr <-15 ml/min/1.73m^2: 1.83 (1.10, 3.04) eGFRdiffcys-cr -15 to 15 ml/min/1.73m^2: Reference eGFRdiffcys-cr ≥15 ml/min/1.73m^2: 0.50 (0.35, 0.71) 2. All-cause mortality Baseline measures, HR (95%CI) eGFRdiffcys-cr <-15 ml/min/1.73m^2: 1.86 (1.40, 2.48)

				eGFRdiffcys-cr -15 to 15 ml/min/1.73m^2: Reference eGFRdiffcys-cr ≥15 ml/min/1.73m^2: 0.68 (0.58, 0.81) Time-updated measures, HR (95%CI) eGFRdiffcys-cr <-15 ml/min/1.73m^2: 3.03 (2.19, 4.19) eGFRdiffcys-cr -15 to 15 ml/min/1.73m^2: Reference eGFRdiffcys-cr ≥15 ml/min/1.73m^2: 0.58 (0.45, 0.75)
4	Chen, et al., 2022b	Cohort	Mean (SD) eGFRcr, ml/min/1.73m^2: 49 (16) eGFRcys, ml/min/1.73m^2: 55 (23) eGFRdiff overall, ml/min/1.73m^2: 6 (16) eGFRdiff <-15 ml/min/1.73m^2: -24 (8) eGFRdiff -15 to 15 ml/min/1.73m^2: 2 (7) eGFRdiff ≥15 ml/min/1.73m^2: 26 (10)	Incident heart failure hospitalization Baseline measures ,sHR (95%CI) eGFRdiff, per -15 ml/min/1.73m^2: 1.20 (1.07, 1.34) eGFRdiff <-15 ml/min/1.73m^2: 1.14 (0.77, 1.70) eGFRdiff -15 to 15 ml/min/1.73m^2: Reference eGFRdiff ≥15 ml/min/1.73m^2: 0.78 (0.61, 1.01) Time-updated measures, sHR (95%CI) eGFRdiff, per -15 ml/min/1.73m^2: 1.36 (1.18, 1.55) eGFRdiff <-15 ml/min/1.73m^2: 1.99 (1.39, 2.86) eGFRdiff -15 to 15 ml/min/1.73m^2: Reference eGFRdiff ≥15 ml/min/1.73m^2: 0.67 (0.49, 0.91)
5	Farrington et al., 2023	Cohort	Mean (SD) eGFRcr, ml/min/1.73m^2: 97 (14) eGFRcys, ml/min/1.73m^2: 91 (19) Percent difference between eGFRcr and eGFRcys: 5 (17) Median (IQR) Percent difference between eGFRcr and eGFRcys: 4 (-5, 16)	1. Kidney failure, HR (95%CI) eGFRcys >30% lower than eGFRcr: 1.53 (1.07, 2.18) 0.7≤ eGFRcys/eGFRcr≤1.3: Reference eGFRcys >30% higher than eGFRcr: 0.62 (0.36, 1.08) 2. Heart failure, HR (95%CI) eGFRcys >30% lower than eGFRcr: 1.58 (1.39, 1.80) 0.7≤ eGFRcys/eGFRcr≤1.3: Reference eGFRcys >30% higher than eGFRcr: 0.90 (0.69, 1.17) 3. Acute kidney injury, HR (95%CI) eGFRcys >30% lower than eGFRcr: 1.76 (1.52, 2.03) 0.7≤ eGFRcys/eGFRcr≤1.3: Reference eGFRcys >30% higher than eGFRcr: 0.65 (0.48,

					0.87) 4. Death, HR (95%CI) eGFRcys >30% lower than eGFRcr: 1.61 (1.47, 1.76) 0.7≤ eGFRcys/eGFRcr≤1.3: Reference eGFRcys >30% higher than eGFRcr: 0.76 (0.63, 0.92)
6	He, et al., 2024a	Cohort	Mean (SD) INDEED eGFRcr, ml/min/1.73m^2: 95.54 (14.70) eGFRcys, ml/min/1.73m^2: 88.73 (22.53) eGFRabdiff, ml/min/1.73m^2: -6.81 (18.17) eGFRrediff, ratio: 0.93 (0.20) NHANES eGFRcr, ml/min/1.73m^2: 84.78 (25.85) eGFRcys, ml/min/1.73m^2: 87.15 (28.78) eGFRabdiff, ml/min/1.73m^2: 2.38 (16.33) eGFRrediff, ratio: 1.04 (0.22) UK Biobank eGFRcr, ml/min/1.73m^2: 92.79 (16.35) eGFRcys, ml/min/1.73m^2: 81.39 (19.46) eGFRabdiff, ml/min/1.73m^2: -11.40 (14.03) eGFRrediff, ratio: 0.80 (0.16)	1. All-cause mortality INDEED, HR (95%CI) eGFRabdiff, per +1 SD: 0.77 (0.69, 0.86) eGFRabdiff <-15 ml/min/1.73m^2: 1.38 (1.12, 1.70) eGFRabdiff -15 to 15 ml/min/1.73m^2: Reference eGFRabdiff ≥15 ml/min/1.73m^2: 0.76 (0.49, 1.19) eGFRrediff, per +10%: 0.88 (0.84, 0.93) eGFRrediff ≥0.6: Reference eGFRrediff <0.6: 1.89 (1.41, 2.53) NHANES, HR (95%CI) eGFRabdiff, per +1 SD: 0.70 (0.65, 0.76) eGFRabdiff <-15 ml/min/1.73m^2: 1.43 (1.18, 1.73) eGFRabdiff -15 to 15 ml/min/1.73m^2: Reference eGFRabdiff ≥15 ml/min/1.73m^2: 0.55 (0.45, 0.67) eGFRrediff, per +10%: 0.87 (0.84, 0.90) eGFRrediff ≥0.6: Reference eGFRrediff <0.6: 2.45 (1.66, 3.62) UK Biobank, HR (95%CI) eGFRabdiff, per +1 SD: 0.66 (0.65, 0.68) eGFRabdiff <-15 ml/min/1.73m^2: 1.66 (1.57, 1.75) eGFRabdiff -15 to 15 ml/min/1.73m^2: Reference eGFRabdiff ≥15 ml/min/1.73m^2: 0.53 (0.42, 0.66) eGFRrediff, per +10%: 0.79 (0.78, 0.80) eGFRrediff ≥0.6: Reference eGFRrediff <0.6: 2.58 (2.35, 2.84) 2. Incident cardiovascular events INDEED, HR (95%CI) eGFRabdiff, per +1 SD: 0.82 (0.74, 0.90) eGFRabdiff <-15 ml/min/1.73m^2: 1.31 (1.08, 1.59)

				eGFRabdiff -15 to 15 ml/min/1.73m^2: Reference eGFRabdiff ≥15 ml/min/1.73m^2: 0.67 (0.44, 1.02) eGFRrediff, per +10%: 0.90 (0.86, 0.95) eGFRrediff ≥0.6: Reference eGFRrediff <0.6: 1.55 (1.10, 2.17) NHANES, HR (95%CI) eGFRabdiff, per +1 SD: 0.68 (0.57, 0.82) eGFRabdiff <-15 ml/min/1.73m^2: 0.96 (0.60, 1.55) eGFRabdiff -15 to 15 ml/min/1.73m^2: Reference eGFRabdiff ≥15 ml/min/1.73m^2: 0.37 (0.22, 0.61) eGFRrediff, per +10%: 0.84 (0.78, 0.91) eGFRrediff ≥0.6: Reference eGFRrediff <0.6: 0.50 (0.12, 2.14) UK Biobank, HR (95%CI) eGFRabdiff, per +1 SD: 0.78 (0.76, 0.81) eGFRabdiff <-15 ml/min/1.73m^2: 1.37 (1.29, 1.45) eGFRabdiff -15 to 15 ml/min/1.73m^2: Reference eGFRabdiff ≥15 ml/min/1.73m^2: 0.71 (0.58, 0.87) eGFRrediff, per +10%: 0.87 (0.85, 0.89) eGFRrediff ≥0.6: Reference eGFRrediff <0.6: 1.42 (1.23, 1.63)
7	He, et al., 2024b	Cohort	Mean (SD) eGFRcr, ml/min/1.73m^2: 95.6 (12.6) eGFRcys, ml/min/1.73m^2: 84.4 (16.5) eGFRabdiff, ml/min/1.73m^2: -11.1 (14.1) eGFRrediff, ratio: 0.9 (0.2)	Diabetic kidney disease HR (95%CI) eGFRabdiff, per -1 SD: 1.56 (1.50, 1.63) eGFRabdiff <-15 ml/min/1.73m^2: 1.63 (1.50, 1.76) eGFRabdiff -15 to 15 ml/min/1.73m^2: Reference eGFRabdiff ≥15 ml/min/1.73m^2: 0.39 (0.31, 0.50) eGFRrediff, per -10%: 1.29 (1.26, 1.33) eGFRrediff ≥0.6: Reference eGFRrediff <0.6: 2.32 (1.94, 2.79) sHR (95%CI) eGFRabdiff, per -1 SD: 1.47 (1.41, 1.54) eGFRabdiff <-15 ml/min/1.73m^2: 1.52 (1.41, 1.65) eGFRabdiff -15 to 15 ml/min/1.73m^2: Reference eGFRabdiff ≥15 ml/min/1.73m^2: 0.39 (0.30, 0.50) eGFRrediff, per -10%: 1.26 (1.22, 1.29) eGFRrediff ≥0.6: Reference eGFRrediff <0.6: 1.84 (1.52, 2.23)

8	Heo, et al., 2024	Cohort	Mean (SD) eGFRcr, ml/min/1.73m^2: 90.7 (13.2) eGFRcys, ml/min/1.73m^2: 91 (16.0) eGFRdiff overall, ml/min/1.73m^2: -0.6 (13.2) eGFRdiff <-15 ml/min/1.73m^2: -22.1 (6.4) eGFRdiff -15 to 15 ml/min/1.73m^2: 0.1 (7.8) eGFRdiff ≥15 ml/min/1.73m^2: 22.2 (6.9)	Incident atrial fibrillation sHR (95%CI) eGFRdiff, per +10 ml/min/1.73m^2: 0.90 (0.88, 0.91) eGFRdiff <-15 ml/min/1.73m^2: 1.25 (1.20, 1.30) eGFRdiff -15 to 15 ml/min/1.73m^2: Reference eGFRdiff ≥15 ml/min/1.73m^2: 0.81 (0.77, 0.87) Race-free eGFRdiff, per +10 ml/min/1.73m^2: 0.90 (0.89, 0.91) Race-free eGFRdiff <-15 ml/min/1.73m^2: 1.23 (1.19, 1.27) Race-free eGFRdiff -15 to 15 ml/min/1.73m^2: Reference Race-free eGFRdiff ≥15 ml/min/1.73m^2: 0.88 (0.79, 0.93)
9	Herou, et al., 2022	Cohort	Mean (SD) eGFRcreatinine, ml/min/1.73m^2: 75 (20) eGFRCystatin C, ml/min/1.73m^2: 64 (22)	10-year mortality, HR (95%CI) By CKD-EPI equation eGFRcystatin C ≤ 60% of eGFRcreatinine: 1.96 (1.63, 2.36) eGFRcystatin C > 60% of eGFRcreatinine: Reference By CAPA and LMrev equation eGFR CAPA ≤ 60% of eGFR LMrev: 1.66 (1.25, 2.21) eGFR CAPA > 60% of eGFR LMrev: Reference
10	Jonsson, et al., 2021	Cohort	Percentage eGFRcrea ≥90 ml/min/1.73m^2: 1% eGFRcrea 60-89 ml/min/1.73m^2: 16% eGFRcrea 30-59 ml/min/1.73m^2: 45% eGFRcrea 15-29 ml/min/1.73m^2: 35% eGFRcrea <15 ml/min/1.73m^2: 3%	1. All-cause one year mortality, HR (95%CI) eGFRcys/eGFRcrea ≤ 0.7: 1.661 (1.155, 2.391) eGFRcys/eGFRcrea > 0.7: Reference 2. All-cause 90-day mortality, HR (95%CI) eGFRcys/eGFRcrea ≤ 0.7: 1.832 (1.095, 3.063) eGFRcys/eGFRcrea > 0.7: Reference
11	Kim, et al., 2021	Cohort	Mean (SD) eGFRcreat, ml/min/1.73m^2: 53.4 (30.9) eGFRcys, ml/min/1.73m^2: 52.8 (32.5) eGFRdiff, ml/min/1.73m^2: 0.6 (11.0)	1. MACE, cause-specific HR (95%CI) eGFRdiff <-2.3 ml/min/1.73m^2: Reference -2.3≤ eGFRdiff <4.0 ml/min/1.73m^2: 1.74 (1.03, 2.93) eGFRdiff ≥4.0 ml/min/1.73m^2: 2.12 (1.26, 3.50) eGFRdiff, per +1 ml/min/1.73m^2: 1.03 (1.01, 1.05) 2. Fatal and non-fatal MI and unstable angina, cause-specific HR (95%CI) eGFRdiff, per +1 ml/min/1.73m^2: 1.01 (0.98, 1.05) 3. Stroke, cause-specific HR (95%CI) eGFRdiff, per +1 ml/min/1.73m^2: 1.03 (0.99,

				1.07) 4. Congestive heart failure, cause-specific HR (95%CI) eGFRdiff, per +1 ml/min/1.73m^2: 1.07 (0.99, 1.14) 5. Symptomatic arrhythmia, cause-specific HR (95%CI) eGFRdiff, per +1 ml/min/1.73m^2: 1.04 (1.00, 1.08)
12	Ljungberg, et al., 2019	Case-control	Mean (95%CI) Case eGFRcreatine, ml/min/1.73m^2: 80.0 (78.6, 81.5) eGFRcystatin C, ml/min/1.73m^2: 88.9 (86.6, 91.4) eGFRcystatin C/eGFRcreatinine: 1.11 (1.09, 1.13) Control eGFRcreatine, ml/min/1.73m^2: 79.7 (78.8, 80.6) eGFRcystatin C, ml/min/1.73m^2: 91.0 (89.6, 92.4) eGFRcystatin C/eGFRcreatinine: 1.14 (1.13, 1.16)	Aortic valve replacement, OR (95%CI) Quartiles of eGFRcystatin C /eGFRcreatinine ratio Quartile 1 (the lowest): 1.00 Quartile 2: 0.91 (0.61, 1.34) Quartile 3: 0.75 (0.51, 1.10) Quartile 4 (the highest): 0.62 (0.40, 0.95) Z (ln) ratio, per +1 SD, OR (95%CI): 0.80 (0.68, 0.95)
13	Malmgren, et al., 2022	Cohort	Mean (SD), by CKD-EPI equation eGFRcrea, ml/min/1.73m^2: 75.3 (12.5) eGFRcysC, ml/min/1.73m^2: 64.8 (17.1) eGFRcysC/eGFRcrea ratio: 0.86 (0.19)	10-year mortality, HR (95%CI) By CKD-EPI equation eGFRcysC/eGFRcrea ratio <0.6: 1.6 (1.1, 2.5) eGFRcysC/eGFRcrea ratio 0.6-0.69: 1.1 (0.7, 1.8) eGFRcysC/eGFRcrea ratio 0.7-0.79: 0.9 (0.6, 1.4) eGFRcysC/eGFRcrea ratio 0.8-0.89: 1.0 (0.7, 1.5) eGFRcysC/eGFRcrea ratio ≥0.9: Reference By CAPA and LMrev equation eGFRcysC/eGFRcrea ratio <0.6: 2.5 (1.4, 4.5) eGFRcysC/eGFRcrea ratio 0.6-0.69: 1.3 (0.7, 2.3) eGFRcysC/eGFRcrea ratio 0.7-0.79: 1.4 (0.9, 2.3) eGFRcysC/eGFRcrea ratio 0.8-0.89: 1.0 (0.7, 1.6) eGFRcysC/eGFRcrea ratio ≥0.9: Reference
14	Potok, et al., 2020	Cohort	Mean (SD) eGFRcr, ml/min/1.73m^2: 72 (20) eGFRcys, ml/min/1.73m^2: 73 (23) eGFRcr-cys, ml/min/1.73m^2: 73 (21) eGFRDiff, ml/min/1.73m^2: 0.5 (15)	1. Cardiovascular events, HR (95%CI) eGFRdiff, per +1 SD: 0.89 (0.81, 0.97) 2. Total mortality, HR (95%CI) eGFRdiff, per +1 SD: 0.71 (0.63, 0.82)

15	Söderström, et al., 2021	Nested case-control	Mean (SD) Male Case eGFRcreatinine, ml/min/1.73m^2: 92.1 (12.9) eGFRcystatin C, ml/min/1.73m^2: 83.6 (19.8) eGFRcystatin C/eGFRcreatinine ratio: 0.91 (0.20) Male Control eGFRcreatinine, ml/min/1.73m^2: 91.2 (10.8) Female Case eGFRcreatinine, ml/min/1.73m^2: 86.3 (14.2) eGFRcystatin C, ml/min/1.73m^2: 74.8 (18.2) eGFRcystatin C/eGFRcreatinine ratio: 0.87 (0.17) Female Control eGFRcreatinine, ml/min/1.73m^2: 97.4 (11.0) eGFRcystatin C, ml/min/1.73m^2: 80.7 (16.35) eGFRcystatin C/eGFRcreatinine ratio: 0.93 (0.17) eGFRcystatin C, ml/min/1.73m^2: 84.8 (20.5) eGFRcystatin C/eGFRcreatinine ratio: 0.93 (0.19)	First-ever myocardial infarction, OR (95%CI) Quartiles of eGFRcystatin C/eGFRcreatinine Quartile 1 (the lowest): 1.00 Quartile 2: 0.94 (0.70, 1.26) Quartile 3: 0.59 (0.43, 0.80) Quartile 4 (the highest): 0.62 (0.45, 0.85) Z (ln) ratio, per +1 SD, OR (95%CI): 0.92 (0.77, 1.09)
13	Wu, et al., 2022	Cohort	Median (IQR): eGFRcr, ml/min/1.73m^2: 95 (69 - 112) eGFRcys, ml/min/1.73m^2: 76 (58 - 100) eGFRcys/eGFRcr ratio: 0.85 (0.74 - 0.96)	Severe eGFR decline, HR (95%CI) eGFRcys < 70% eGFRcr: 1.87 (0.86, 4.06) eGFRcys) ≥70% eGFRcr: Reference eGFRcys/eGFRcr) ratio, per +1 unit: 0.42 (0.06, 2.96)
14	Xhakollari, et al., 2021	Cohort	Mean (SD) eGFRcreatinine, ml/min/1.73m^2: 51.6 (22.6) eGFRcystatin C, ml/min/1.73m^2: 37.8 (17.1)	1. All-cause mortality, HR (95%CI) eGFRcystatin C/eGFRcreatinine ratio ≤0.6: 1.99 (1.23, 3.21) 2. 30-day rehospitalization, HR (95%CI) eGFRcystatin C/eGFRcreatinine ratio ≤0.6: 1.82 (1.04, 3.18)
15	Zhang, et al., 2023	Cohort, Cross-sectional	Not available	1. CA-AKI, OR (95%CI) eGFRcys < 60% eGFRcr: 4.17 (3.17, 5.46) eGFRcys ≥ 60% eGFRcr: Reference eGFRcys < 70% eGFRcr: 3.58 (2.78, 4.62) eGFRcys ≥ 70% eGFRcr: Reference 2. Mortality, HR (95%CI) eGFRcys < 60% eGFRcr: 1.37 (1.08, 1.74) eGFRcys ≥ 60% eGFRcr: Reference eGFRcys < 70% eGFRcr: 1.27 (1.03, 1.57) eGFRcys ≥ 70% eGFRcr: Reference

CA-AKI, Contrast-Associated Acute Kidney Injury; CAPA, Caucasian, Asian, Paediatric, and Adult cohorts cystatin C-based equation; CI, Confidence Interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; FAS, Full Age Spectrum; HR, Hazard Ratio; INDEED, Incidence, Development, and Prognosis of Diabetic Kidney Disease Study; LMrev, Lund-Malmö revised creatinine-based eGFR equation;

MACE, Major Adverse Cardiovascular Events; MI, Myocardial infarction; NHANES, National Health and Nutrition Examination Survey; OR, Odds Ratio; SD, standard deviation; sHR, Subdistribution Hazard Ratio

The study populations mainly came from the United States and Sweden, followed by the United Kingdom and China, with one study involving a Korean population.[36] Most studies focused on the effect of eGFR discordance in patients with kidney diseases or diabetes from the Chronic Renal Insufficiency Cohort (CRIC) Study [11,21], the Lund Cystatin C Standardization (LCS) Cohort [17], the Incidence, Development, and Prognosis of Diabetic Kidney Disease (INDEED) Study [34], the National Health and Nutrition Examination Survey (NHANES) [34], the UK Biobank [34,35], the Korean Cohort Study for Outcome in Patients With Chronic Kidney Disease (KNOW-CKD) [39], the Systolic Blood Pressure Intervention Trial (SPRINT) Study [42], the Northern Sweden Health and Disease Study (NSHDS) [43], and the HARVEST-Malmo Study.[45] Four studies focused on the general population, including people from the Atherosclerosis Risk in Communities (ARIC) Study [22], the Stockholm Creatinine Measurements (SCREAM) project [33], the UK Biobank [36], and the Malmo Osteoporosis Prospective Risk Assessment (OPRA) Cohort.[41] Study populations self-collected by researchers were all patients, including those with fractures [38], individuals who received cardiac surgery [37,40,46], and patients with kidney disease.[44]

In selected cohort studies, the median follow-up time differed among the studies based on the outcome (e.g., the follow-up time for CVD incidence is generally shorter than that for mortality). Two studies did not report the median follow-up time [38,42], while two others provided only the minimum follow-up time.[22,41] For the remaining studies, the median follow-up time ranged from 1.8 to 15.17 years, with an overall median of 5.2 years. Except for one study with an attrition rate of 20%, all the other studies have a low attrition rate.[39]

Measurement of eGFR and eGFR discordances

Except for three studies [38,40,43] that solely applied the eGFR equation based on Caucasian, Asian, Paediatric, and Adult cohorts (CAPA) [47] and the Lund-Malmö revised creatinine-based eGFR equation (LMrev) [48] to calculate eGFR discordance, the remaining 15 studies applied the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations, one study applied both race-independent and race-free CKD-EPI equations.[36]

Of the 15 studies that applied CKD-EPI equations, eight used the latest 2021 race-free eGFRcr equation.[49] Among the remaining seven articles, four studies were published before or around the time the race-free equation was released, which explained why they

did not use the latest equation.[17,39,42,45] However, for the other three studies, there was no clear explanation.[37,41,44] Additionally, four studies used both the CAPA-LMrev and CKD-EPI equations [17,37,41,44], and one of these studies [17] also applied the full age spectrum (FAS) equation.[50] Studies that used the CAPA-LMrev equation were all conducted on Nordic people [17,37,38,40,41,43] except one which was on a Chinese population.[44]

The definition of eGFR discordance in the selected studies was based on absolute differences and/or relative differences. Studies using absolute differences assessed the effects of eGFR discordance in both directions, i.e., when eGFRcys was either lower or higher than eGFRcr. In contrast, studies using relative values to measure eGFR discordance predominantly assessed the effect when eGFRcys was less than eGFRcr, with two studies examined the effect in both directions.[22,33] All the selected studies, except for one that used eGFRcr as the minuend [39], used eGFRcr as the subtrahend or denominator.

The cutoff values for eGFR discordance were largely consistent across studies. For the absolute difference, most studies considered eGFRcys - eGFRcr less than -15 ml/min/1.73m² and greater than 15 ml/min/1.73m² as indicative of eGFR discordance, and eGFRcys - eGFRcr between -15 and 15 ml/min/1.73m^2 serving as the reference group. The rationale for using 15 ml/min/1.73m² as the cutoff value is that it approximates one standard deviation in most populations and signifies a clinically meaningful difference that indicates CKD stages.[21] One study evaluated eGFR discordance based on tertiles of eGFRcys - eGFRcr [39], while another study used the quartile of the same difference.[33] For the relative difference, most studies defined eGFR discordance as eGFRcys being less than or equal to 60% or 70% of eGFRcr. One study explored health effects when eGFRcys was 30% higher than eGFRcr.[22] Another study used the quartiles of the percentage difference between eGFRcys and eGFRcr, defined as (eGFRcys-eGFRcr)/eGFRcr.[33]

Measurement of outcomes

Among the included studies, 11 studies have mortality as the research outcome.[11,17,22,33,34,37,38,41,42,45,46] Mortality included both long-term and short-term mortality (e.g., 90-day mortality). Most studies reported all-cause mortality, while one study specifically examined cause-specific mortality.[17]

Five studies discussed the association between eGFR discordance and long-term kidney outcomes, including kidney failure, ESKD, severe eGFR decline (SCr doubling or≥50% eGFRcr decline compared with the baseline), and diabetic kidney disease (DKD) [11,22,33,35,44]. Additionally, three studies investigated acute kidney injury (AKI) [22,33,46]. Research outcomes were confirmed through patient records or database linkage.

Of the ten studies that used CVD incidence as an outcome [21,22,33,34,36,39,40,42,43,45], one focused solely on heart failure (HF) [21], another reported results for HF and atherosclerotic CVD separately [33], one studied aortic stenosis[40], and one focused on myocardial infarction (MI) [43] while the remaining studies combined multiple CVDs, including MI, atrial fibrillation (AF), stroke, and HF.

Potential bias and quality assessment

The overall quality of the selected studies was good. The ROBINS-E overall scale was "Low" or "Some concerns" in 13 out of 18 studies. Four studies were categorized as high risk of bias because their insufficient adjustments for confounding effect.[17,37,40,43] One study was graded as of very high risk of bias because its authors used CAPA and LMrev equations in defining study exposure but then used CKD-EPI equations in follow-up assessments in primary analysis (Table 6-3).[44]

Table 6-3. Risk Of Bias In Non-randomized Studies - of Exposure (ROBINS-E) Assessment Form

Author, Year	Risk of bias due to confounding	Risk of bias arising from measurement of the exposure	Risk of bias in selection of participants into the study (or into the analysis)	Risk of bias due to post-exposure interventions	Risk of bias due to missing data	Risk of bias arising from measurement of the outcomes	Risk of bias in selection of the reported results	Overall
Akesson, et al. ,2020	High	Low	Low	Low	Low	Low	Low	High
Carrero et al., 2023	Some concerns	Low	Low	Low	Low	Low	Low	Low
Chen, et al. ,2022a	Low	Low	Low	Low	Low	Low	Low	Low
Chen, et al. ,2022b	Low	Low	Low	Low	Low	Low	Low	Low
Farrington et al. ,2023	Low	Some concerns	Low	Low	Some concerns	Low	Low	Some concerns
He, et al. ,2024a	Low	Low	Low	Low	Some concerns	Low	Low	Some concerns
He, et al. ,2024b	Low	Low	Low	Low	Low	Low	Low	Low
Heo, et al. ,2023	Low	Low	Low	Low	Low	Some concerns	Low	Some concerns
Herou, et al. ,2022	High	Low	Low	Low	Low	Low	Low	High
Jonsson, et al. ,2021	Some concerns	Low	Low	Low	Low	Low	Low	Some concerns

Kim, et al. ,2021	Low	Low	Low	Low	Some concerns	Low	Low	Some concerns
Ljungberg, et al., 2019	High	Low	Low	Low	Low	Low	Low	High
Malmgren, et al. ,2022	Low	Low	Low	Low	Low	Low	Low	Low
Potok, et al. ,2020	Low	Low	Low	Low	Low	Low	Low	Low
Söderström, et al., 2021	High	Low	Low	Low	Low	Low	Low	High
Wu, et al. ,2022	Low	Very high	Low	Low	Low	Very high	Low	Very high
Xhakollari, et al. ,2021	Some concerns	Low	Low	Low	Low	Low	Low	Some concerns
Zhang, et al. ,2023	Some concerns	Low	Low	Low	Low	Low	Low	Some concerns

Aligned with Table 3, the main risk of bias was due to confounding, followed by missing data. Measurement of exposure and outcome were generally accurate (Supplementary Figure S1, Supplementary Figure S2).

Systematic review on the association of eGFR discordances with mortality

Across 11 studies, mortality was elevated when eGFRcys was less than eGFRcr [11,17,22,33,34,37,38,41,42,45,46]. Compared to people with eGFRcys-eGFRcr within ±15 ml/min/1.73m², those with lower eGFRcys-eGFRcr showed an elevated risk of mortality. For each 1 SD increase in eGFRcys-eGFRcr, there was a significant decrease in the risk of mortality. People with an eGFRcys/eGFRcr≤0.6 or 0.7 have elevated mortality, compared to those with a higher ratio. Studies using CKD-EPI, CAPA-LMrev, and FAS equations have similar results [17,37,38,41].

Eight studies conducted stratified analyses [11,17,22,33,34,37,42,45], and only a few studies tested for interaction effects. Two studies found that the association between eGFR discordance and mortality was significant for both sexes, although no interaction with sex was observed [11,34]. Another study also showed no interactions of sex, but subgroup results were not presented.[42] A fourth study observed the interaction effect of sex on the association of eGFR discordance with all-cause mortality, but not with CVD mortality.[33]

Three studies tested the interaction effect of age, using 60 or 65 years as the threshold, with inconsistent results. In Chen et al.'s study on the CRIC cohort [11], and He et al.'s study on the INDEED cohort [34], no interaction was found. However, an interaction of age was observed in the NHANES and UK Biobank populations in the same study by He et al.[34] The study by Carrero et al. showed consistent interaction effect of age that people aged 65 years or above have attenuated effect size.[33]

Five studies performed eGFR-stratified analyses based on eGFR calculated using CKD-EPI equations.[11,22,33,34,37] Various eGFR cutoff values were applied, including 45 and 90 ml/min/1.73m^2 [11,34], and by CKD stages.[22,33,37] The interaction effect of baseline eGFR was inconsistent. In a subset of the UK Biobank population, a consistent significant interaction was found; people with baseline eGFR < 90 ml/min/1.73m^2 have a smaller effect size.[34] Similar findings were also presented in another study where patients with a higher CKD

stage. One study showed Black people have lesser risk (P for interaction=0.042) [11], but another study did not.[42]

Meta-analysis on the association of eGFR discordances with all-cause mortality

Three studies were not included in the meta-analysis because their reference groups were different from that of other studies thus incomparable.[17,33,41] Among the remaining studies, only one study focused on community dwellers. [22] Thus, a meta-analysis for the general population could not be conducted. Subject to data consistency, only studies using the CKD-EPI eGFR equation were used in the meta-analysis. Studies using different versions of CKD-EPI equations were jointly analysed for simplicity.

One study used an eGFRcys/eGFRcr <0.6 as the criterion for eGFR discordance [34], while the other three studies used a ratio of ≤ 0.6.[37,45,46] To simplify the analysis, we combined these studies. We regarded the slight difference in the definition of eGFR discordance had a minimal impact on the study results and was therefore acceptable.

With a total population of 19414 people from two studies [11,34], the pooled result showed a significant positive association of eGFR discordance with mortality when eGFRcys-eGFRcr below -15 ml/min/1.73m^2, compared to people with eGFRcys-eGFRcr between ±15 ml/min/1.73m^2 (HR=1.58, 95%CI: 1.42, 1.76). Heterogeneity among studies was moderate (I^2=42.92%, 95%CI: 22.93%, 62.91%). Leave-one-out analyses showed positive associations, funnel plot showed possible bias that studies with smaller sizes tend to report larger effect sizes (Figure 6-2a, Supplementary Figure S3a, S4a).

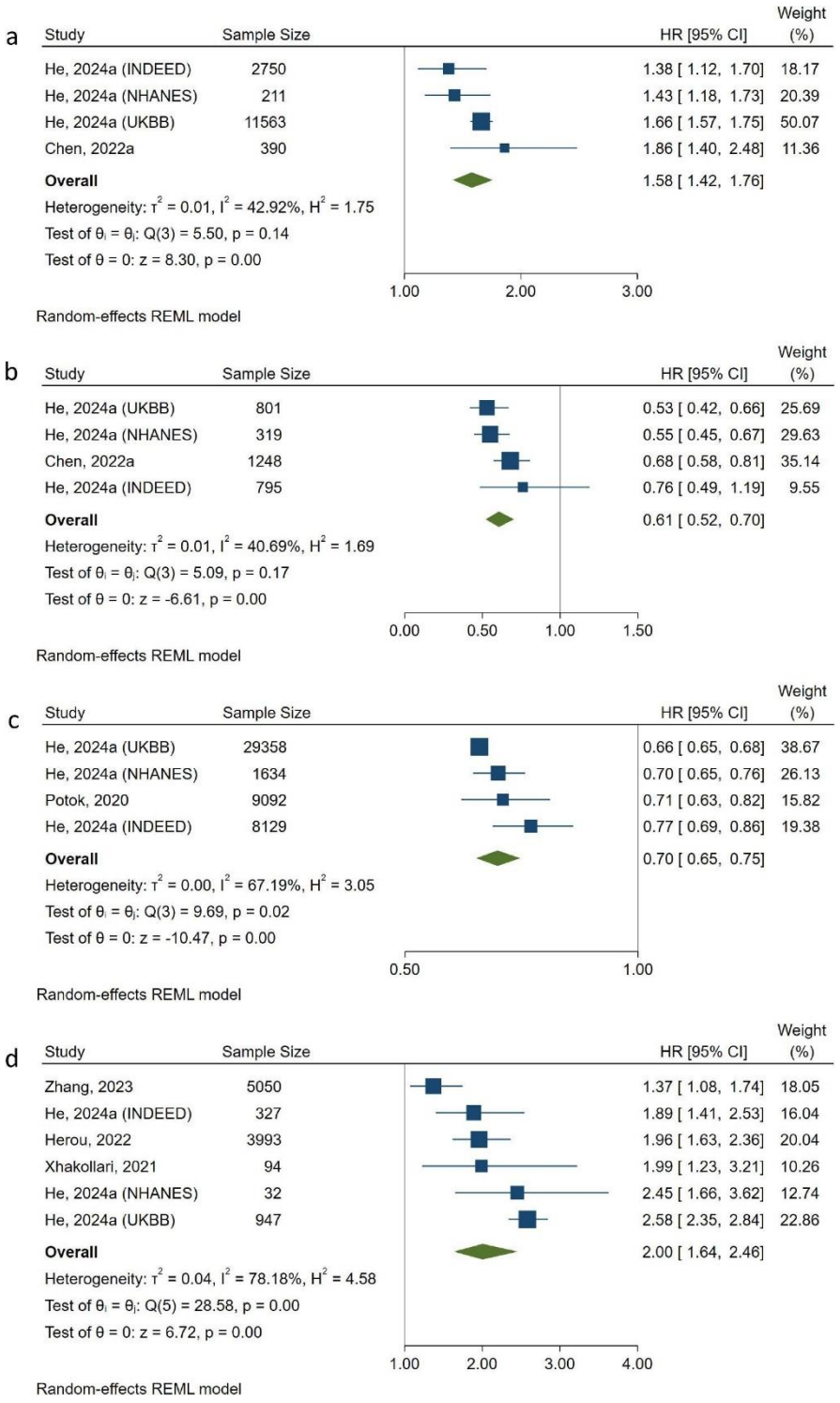


Figure 6-2 Forest plot of meta-analysis on the association between eGFR discordance and mortality.

(a) eGFR discordance was defined as eGFRcys-eGFRcr <–15 mL/min/1.73 m^2. (b) eGFR discordance was defined as eGFRcys-eGFRcr ≥15 mL/min/1.73 m^2. (c) Per 1 SD increase of eGFRcys-eGFRcr. (d) eGFR discordance was defined as eGFRcys/eGFRcr ≤0.6.

The pooled result of the association of eGFR discordance with mortality when eGFRcys-eGFRcr≥15 ml/min/1.73m^2 in a total population of 3163 people showed significance.[11,34] Those people have a 39% lower risk of mortality (HR=0.61, 95%CI: 0.52, 0.70) as compared to those with eGFRcys-eGFRcr between ±15 ml/min/1.73m^2, with moderate heterogeneity (I^2=40.69%, 95%CI: 19.36%, 62.02%). Leave-one-out analyses showed consistent effects and potential bias was found (Figure 6-2b, Supplementary Figure S3b, S4b).

Treating eGFRcys-eGFRcr as a continuous variable, the pooled result in a total population of 48213 people showed per one standard deviation elevation of eGFRcys-eGFRcr was associated with a 30% lower mortality risk (HR=0.70, 95%CI: 0.65, 0.75).[34,42] Heterogeneity was substantial (I^2=67.19%, 95%CI: 56.24%, 78.14%). Leave-one-out analyses showed a consistent effect. Serious bias was found that small sample-size studies were more likely to underestimate the effect (Figure 6-2c, Supplementary Figure S3c, S4c).

Using eGFRcys/eGFRcr to denote eGFR discordance, in a total population of 10443 people with eGFRcys/eGFRcr≤0.6, the pooled result showed a doubled risk of mortality (HR=2.00, 95%CI: 1.64, 2.46),[34,37,45,46] albeit with substantial heterogeneity (I^2=78.18%, 95%CI: 73.90%, 82.46%). Leave-one-out analyses showed a consistent effect, small sample-size studies were likely to overestimate the effect (Figure 6-2d, Supplementary Figure S3d, S4d).

Subject to data availability, only stratified meta-analysis on median C-reactive protein (CRP) levels and obesity (BMI≥ 30 kg/m^2) were performed. In people with eGFRcys-eGFRcr <-15 ml/min/1.73m^2, those with CRP≥2mg/L have a stronger association of discordance with mortality compared to those with lower CRP levels (HR=1.67, 1.41, respectively), the between-group difference was significant (P=0.03) (Supplementary Figure S5a-b). People with obesity have a significantly higher pooled effect of eGFRcys/eGFRcr≤ 0.6 on mortality compared to non-obese counterparts (P<0.001, HR=2.57, 1.66, respectively) (Supplementary Figure S5c).

Studies on the association of eGFR discordances with kidney outcomes

The association of eGFR discordances with kidney outcomes was measured by several models, including Cox regression [22,33,35,44], logistic regression [46], and Fine-Gray proportional subhazards model with mortality as a competing risk.[11,35] One study also presented findings using time-updated covariates.[11]

In general, eGFR discordance was significantly associated with the incidence of kidney outcomes. Compared to people with similar eGFRcys and eGFRcr, people with eGFRcys-eGFRcr<-15 ml/min/1.73m^2 have 63% higher risk of DKD incidence (HR=1.63, 95%CI: 1.50, 1.76), and those with eGFRcys-eGFRcr≥15 ml/min/1.73m^2 have 61% lower risk of DKD incidence (HR=0.39, 95%CI: 0.31, 0.50), with similar findings in Fine-Gray models.[35]

The association between eGFR discordance and ESKD incidence was only significant when eGFRcys-eGFRcr was modelled as a time-updated covariate, people with eGFRcys-eGFRcr<-15 ml/min/1.73m^2 had 83% elevated risk of ESKD (subhazard ratio [SHR]=1.83, 95%CI: 1.10, 3.04), while those with eGFRcys-eGFRcr≥15 ml/min/1.73m^2 have the risk dropped by 50% (SHR=0.50, 95%CI: 0.35, 0.71).[11] One study showed that people in the lowest quartile of eGFRcys-eGFRcr have 210% elevated risk of AKI (HR=3.10, 95%CI: 2.85, 3.36) and 36% elevated risk of kidney failure with replacement therapy (HR=1.36, 95%CI: 1.17, 1.58), compared to those with similar eGFRcys and eGFRcr.[33]

When eGFR discordance was defined as eGFRcys/eGFRcr≤0.6 or <0.7, its association with DKD, ESKD, and AKI was significantly positive.[22,35,46] Similar findings were observed when the quartile of the percentage difference between eGFRcys and eGFRcr was used.[33] No association with severe eGFR decline was found (HR=1.87, 95%CI: 0.86, 4.06) in females with IgA nephropathy or membranous nephropathy during a median follow-up of 2.3 years.[44]

Four studies conducted stratified analyses and all have interaction effects tested [11,33,35,46]. In three studies, no interaction of age, sex, black ethnicity, diabetes, and hypertension was found.[11,35,46] Yet, in one large-scale study (N=158,601), the interaction of age, baseline eGFR category, and hypertension on the association of eGFR discordance with kidney

failure and AKI was consistently found.[33] Compared to younger individuals, those over 65 years of age have a lower risk of renal outcomes. Similarly, individuals with lower baseline eGFR (as calculated using the CKD-EPI equation), or those with hypertension, have a reduced renal risk compared to those with higher baseline eGFR or without hypertension. [33] There was a significant interaction (P for interaction<0.05) of CVD history on the association between eGFR discordance and DKD incidence, people with historical CVD have a lower risk, but no subgroup values were reported.[35]

Limited by outcome data, no meta-analysis on the association of eGFR discordance with kidney outcomes was performed.

Studies on the association of eGFR discordance with cardiovascular outcomes

The association of eGFR discordance with cardiovascular outcomes has been explored in classical Cox regression [22,33,34,42,45], cause-specific Cox regression [39], logistic regression[40,43], and Fine-Gray proportional subhazards model with mortality as a competing risk.[21,34,36]

Compared to individuals with similar eGFRcys and eGFRcr, those with lower eGFRcys-eGFRcr have an elevated risk of CVD incident in general [21,33,34,36], despite a few discrepancies among cohorts.[34] Those with higher eGFRcys-eGFRcr have a lower risk of CVD incidents, although cohort discrepancies existed.[21,33,34,36] Per one SD greater in eGFRcys-eGFRcr has a consistent negative association with CVD incidence.[34,42] A study showed that people in the highest tertile of eGFRcr-eGFRcys had an elevated incidence of major adverse cardiovascular events (MACE), indirectly confirming that a higher eGFRcys-eGFRcr was associated with a lower incidence [39].

People with eGFRcys/eGFRcr≤0.6 or <0.7 have an elevated risk of heart failure and 30-day rehospitalization due to cardiac causes, compared to those with a higher eGFRcys/eGFRcr ratio. [22,45] Two studies showed people in the highest quartile of eGFRcys/eGFRcr (i.e., higher eGFRcys/eGFRcr) have a reduced risk of surgery for aortic stenosis and a lower risk of myocardial infarction in women, compared to those in the lowest quartile of eGFRcys/eGFRcr.[40,43]

Nine studies conducted stratified analyses [21,33,34,36,39,40,42,43,45], among which six studies have interaction test results available.[21,33,34,36,39,42] The interaction of age, baseline eGFR (by CKD-EPI equation), and sex was not found in most analyses, except in a few studies.[33,34,39,42] Specifically, an interaction of age was observed when the cutoff value was set at 65 or 75 years, but not at 60 years. In individuals aged 65 or 75 years and above, the effect of eGFR discordance was attenuated.[33,34,42] Females (P for interaction = 0.016) [39] and those with baseline eGFR above 90 ml/min/1.73 m^2 (P for interaction = 0.035) [34] have a higher risk of MACE. No consistent interactions of albuminuria, BMI, hypertension, obesity, and race were found.[21,33,34,36,39,42]

Meta-analysis on the association of eGFR discordance with cardiovascular outcomes

Although three studies were conducted on the general population, a meta-analysis could not be performed because their eGFR discordance was measured differently.[22,33,36] Another study was excluded from the meta-analysis because it reported a cause-specific hazard ratio that could not be compared with other studies.[39] Two cross-sectional studies were also excluded because their outcomes were not comparable with each other.[40,43] Studies that reported both Fine-Gray subhazard ratios and Cox hazard ratios had these ratios synthesized separately, with the pooled results of the Fine-Gray subhazard ratios presented in the main text.

With a total population of 11702 people from two studies [21,34], the pooled result showed a significantly elevated risk of CVD incidences in people with eGFRcys-eGFRcr< -15 ml/min/1.73m^2, compared to those with similar eGFRcys and eGFRcr (HR=1.32, 95%CI: 1.25, 1.39). Heterogeneity among studies was small (I^2=0.00%, 95%CI: 0.00%, 23.79%). Leave-one-out analyses showed the association would be nullified if either of the two largest study populations was omitted. Studies with small sample sizes tended to underestimate the association (Figure 6-3a, Supplementary Figure S6a, S7a).

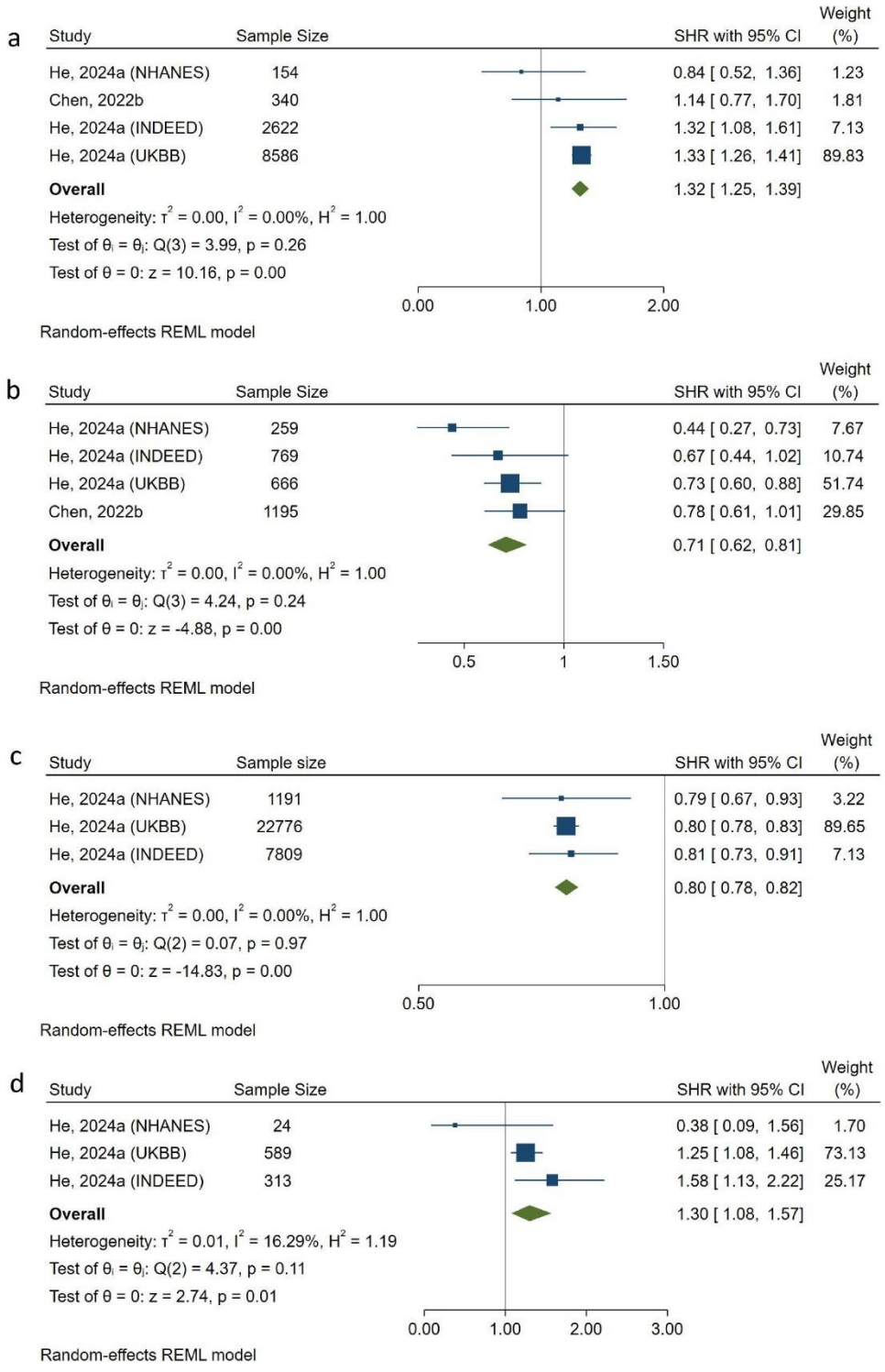


Figure 6-3 Forest plot of meta-analysis on the association between eGFR discordance and cardiovascular incidence, Fine–Gray competing risk model.

(a) eGFR discordance was defined as eGFRcys-eGFRcr <–15 mL/min/1.73 m^2. (b) eGFR discordance was defined as eGFRcys-eGFRcr ≥15 mL/min/1.73 m^2. (c) Per 1 SD increase of eGFRcys-eGFRcr. (d) eGFR discordance was defined as eGFRcys/eGFRcr ≤0.6.

The pooled 2889 people with eGFRcys-eGFRcr ≥ 15 ml/min/1.73m^2 have a pooled result of 29% lower risk of CVD incidence (HR=0.71, 95%CI: 0.62, 0.81) compared to those with similar eGFRcys and eGFRcr.[21,34] Heterogeneity among studies was small (I^2=0.00%, 95%CI: 0.00%, 23.66%). Leave-one-out analyses showed consistent associations. Studies with small sample sizes tended to underestimate the association (Figure 6-3b, Supplementary Figure S6b, S7b). In addition, the pooled results of three populations from a single study showed that per 1 SD increase in eGFRcys-eGFRcr was associated with a 20% lower risk of CVD incidence (HR=0.80, 95%CI: 0.78, 0.82).[34] Heterogeneity among studies was small (I^2=0.00%, 95%CI: 0.00%, 39.91%).Results were less likely to be biased and were consistent in leave-one-out analyses (Figure 6-3c, Supplementary Figure S6c, S7c).

In the pooled population of 926 people with eGFRcys/eGFRcr <0.6, they have a 30% more risk (HR=1.30, 95%CI: 1.08, 1.57) of CVD incidence compared to those with higher eGFRcys/eGFRcr ratio.[34] Heterogeneity was low (I^2=16.29%, 95%CI: 0.00%, 32.82%). Yet, in leave-one-out analyses, the results were insignificant if either of the two largest study populations was excluded. Small-size study populations were likely to underestimate the association (Figure 6-3d, Supplementary Figure S6d, S7d). Studies reported Cox hazard ratio has similar pooled results (Supplementary Figure S8a-d, S9a-d, and S10a-d). No stratified meta-analysis was conducted due to data availability.

6.5. Discussion

Overall, this meta-analysis affirms that eGFR discordance characterized by eGFRcys being lower than eGFRcr is common and is positively and consistently associated with adverse health outcomes, including mortality, ESKD, and HF incidence. Conversely, eGFR discordance characterized by eGFRcys being higher than eGFRcr is significantly associated with lower mortality and cardiovascular diseases. The interaction effects of sex, age, race, and comorbidities remained inconclusive, and most studies have reported insignificant interactions. The association between eGFR discordance and mortality was enhanced by higher CRP levels (cutoff: 2 mg/L, P for interaction<0.001), which is a factor not previously addressed in selected studies.

Our observations suggest that people with a large discordance between eGFRcys and eGFRcr have a different pattern of risks on mortality, CVD events, and renal events, compared to people with similar eGFRcys and eGFRcr. Current research suggests that eGFR discordance may arise from a reduced pore diameter in the glomerular basement membrane, making it more difficult for cystatin C to be filtered while smaller molecules like creatinine pass through easily. [12,15] This results in the accumulation of circulating levels of cystatin C.[13] In patients with SPS, proteins with molecular weights similar to cystatin C, despite having almost completely different functions, have their filtration impeded, resulting in elevated levels.[51] This indicates that eGFR discordance may at least partly be caused by physical, rather than chemical, factors.

Non-GFR factors are assumed to play a key role in explaining the association between eGFR discordance and health outcomes. Declining health may lead to reduced physical activity, leading to muscle loss and decreased creatinine production, which may prevent changes in eGFRcr from falling alongside declining kidney function. In contrast, the impact of declining health on cystatin C is independent of muscle mass, though may be related to accrual of metabolic disease, resulting in a more linear decrease in eGFRcys. As these factors are more likely to occur with increasing age, people with the lowest eGFRcys/eGFRcr ratio tend to be older [33,34,42] and have a higher prevalence of sarcopenia.[34] Sarcopenia is associated with multiple adverse health outcomes, including frailty and disability, and often with other age-related conditions like osteoporosis, cardiovascular disease, and metabolic disorders—all of which contribute to increased morbidity and mortality in older adults. [52] It is characterized by the reduction in muscle mass which leads to lower creatinine production, potentially resulting in eGFRcr overestimating actual kidney function. Sharma et al. (2014) showed that in obese people (defined as total body fat% above the sex-specific 60th percentile, measured using dual-energy x-ray absorptiometry) with sarcopenia, 97.7% were misclassified as not obese by BMI.[53] Therefore, non-GFR factors may be a key contributor in the association between eGFR discordance and health outcomes, especially in older adults.

Additionally, studies have found that people with an eGFRcys/eGFRcr ratio<0.6 have higher levels of proteins related to atherosclerosis and cell proliferation, such as IL6,

CXCL10, and FGF23.[51] These proteins are known risk factors for mortality, kidney disease, and CVD.[54-56]

Notably, in a study using iohexol-measured mGFR, the mGFR category (<30, 30-59, and 60-89 ml/min/1.73m² respectively, reference group = 90 ml/min/1.73m²) was not the risk factor for all-cause mortality.[17] Instead, a low eGFRcys/eGFRcr ratio defined by the CAPA-LMrev equations was a risk factor (reference group = ratio ≥ 1.0) in the same study.[17] This further suggests that eGFR discordance may indicate the influence of non-GFR factors.

A notable limitation in several selected studies is the inadequate adjustment for confounding factors. Several known factors of creatinine levels and renal health, such as muscle mass, sarcopenia, and albuminuria, were not included as explanatory variables.[17,37,40,43] While insufficient adjustment for confounding factors is a recognized disadvantage of using existing study populations, inadequate adjustment may have inflated eGFR discordances, particularly in studies with small sample sizes.[40,43]

Another issue that is worth attention comes from the large difference in the follow-up duration of cohort studies. In the meta-analysis of all-cause mortality, the median of follow-up duration ranged from 3.83 to 15.17 years.[11,34] In the meta-analysis of CVD events, the median of follow-up duration ranged from 3.5 to 15.92 years.[21,34] This variability makes it difficult to distinguish the short and long-term effects of eGFR discordance, as shorter durations may miss late-onset events and chronic exposure effects.

The standard for eGFR discordance has not been uniformly defined, and the current standard is based on consensus rather than evidence-based approaches. The prevalence of eGFR discordance can vary significantly depending on the equations used (e.g., 11.5% with the CKD-EPI equation and 2.3% with the CAPA-LMrev equation) [17] , although the association between eGFR discordance and health outcomes remains consistent.[37] It is worth investigating whether to define different eGFR discordance criteria for different equations or to develop an equation-independent criterion.

Several studies have used both eGFRcys-eGFRcr and eGFRcys/eGFRcr to assess eGFR discordance. A recent study demonstrated that these two approaches have nearly identical predictive accuracy for mortality, as reflected by similar Harrell's C-index values. [57] However, they classified different subsets of individuals as having discordant eGFR values, which may reflect variations in the underlying non-GFR factors influencing the results. For example, 6.2% (172 out of 2781) of the study population had an eGFRcys-eGFRcr between 0 and -15 ml/min/1.73m² and an eGFRcys/eGFRcr < 0.7, highlighting differences between the two approaches. Whether these differences impact outcomes beyond mortality or reflect specific pathophysiological processes remains uncertain, as insufficient data exist to determine whether eGFRcys or eGFRcr better reflects mGFR in this subgroup.

Therefore, future research on eGFR discordance may seek to: 1. Establish a consensus on the definition of eGFR discordance; 2. Discuss the possible role of eGFR discordance as a mediator on the pathway between other known risk factors; 3. Explore the extent of eGFR discordance that can explain the residual risks after adjusting for known risk factors and 4. Explore the association between eGFR discordance and health outcomes in the general population.

The limitations of the study include: 1. Some studies did not adequately adjust for confounding factors, which may lead to biased results. 2. Due to data availability, our meta-analysis focused only on eGFR discordance measured by the CKD-EPI equation. This limits the generalizability of findings. 3. A meta-analysis on the association between eGFR discordance and kidney outcomes was not performed, affecting the comprehensive evaluation of kidney outcomes.4. Due to data availability, we did not conduct subgroup meta-analyses on important variables such as sex and age, restricting our understanding of the effect of eGFR discordance in different populations. 5. Follow-up lengths of selected studies vary significantly, which may affect the results. This variation could lead to heterogeneity, impacting the comparability and interpretation of findings. 6. The study included primarily populations with specific diseases, primarily from Europe and the United States, which may not be applicable to the general population or other populations. 7. Grey literature was excluded from this review. Although this exclusion might only bring very limited impact to the conclusions, if any; readers should be aware of potential alternation to our findings.

In conclusion, our study thoroughly explored the association between eGFR discordance and adverse health outcomes, providing the first systematic insights to our best knowledge. Despite certain limitations, our research, based on high-quality studies and comparative analyses of various eGFR discordance measurement methods, may lay the groundwork for further understanding the clinical significance and practical value of eGFR discordance.

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6.7. Supplementary tables and figures for Chapter 6

Supplementary Table S1 Search terms for PubMed, Embase, and MEDLINE.

PubMed

Search number	Query	Results
1	Cystatin*[Title/Abstract] AND(creatin*[Title/Abstract] OR SCr[Title/Abstract] OR serum creatinine*[Title/Abstract])	4,762
2	difference*[Title/Abstract] OR discordance*[Title/Abstract] OR discrepanc*[Title/Abstract] OR divergenc*[Title/Abstract] OR disparit*[Title/Abstract] OR compar*[Title/Abstract] OR agreemen*[Title/Abstract]	8,746,800
3	Intra-individual*[Title/Abstract] OR intraindividual*[Title/Abstract]	13,546
4	#1 AND (#2 OR #3)	2,778
5	selective glomerular hypofilt*[Title/Abstract] OR SGHS[Title/Abstract] OR shrunken pore[Title/Abstract]	80
6	#4 OR #5	2,833
7	"cohort studies"[MeSH]	2,597,749
8	"cross-sectional studies"[MeSH Terms]	499,871
9	#7 OR #8	3,004,862
10	#6 AND #9	971
11	"Animals"[Mesh] NOT "Humans"[Mesh]	5,215,064
12	#10 NOT #11	963
13	editorial[pt] OR letter[pt] OR comment[pt] OR meta-analysis[pt] OR review[pt]	5,640,329
14	#12 NOT #13	950

Embase 1947-Present, updated daily

Search number	Query	Results
1	(Cystatin* and (creatin* or SCr or serum creatinine*)).ti,ab.	7984
2	difference*.ti,ab. or discordance*.ti,ab. or discrepanc*.ti,ab. or	11893923

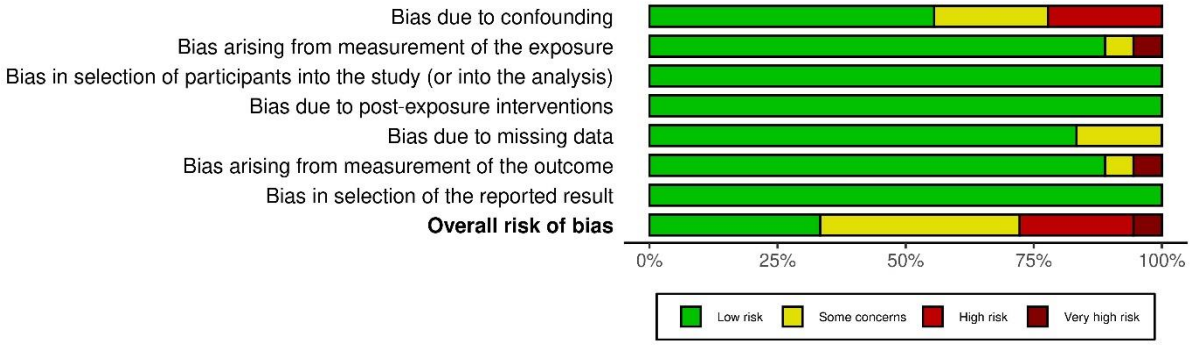
	divergenc*.ti,ab. or disparit*.ti,ab. or compar*.ti,ab. or agreement*.ti,ab.	
3	Intra-individual*.ti,ab. or intraindividual*.ti,ab.	17202
4	1 and (2 or 3)	4920
5	selective glomerular hypofilt*.ti,ab. or SGHS.ti,ab. or shrunken pore*.ti,ab.	103
6	4 or 5	4989
7	exp cohort studies/	1152510
8	exp cross-sectional studies/	630718
9	7 or 8	1735479
10	6 and 9	983
11	exp Animals/ not exp Humans/	6039093
12	10 not 11	981
13	exp editorial/ or exp letter/ or exp meta-analysis/ or exp review/	5422522
14	12 not 13	971

Ovid MEDLINE(R) ALL 1946 to April 28, 2024

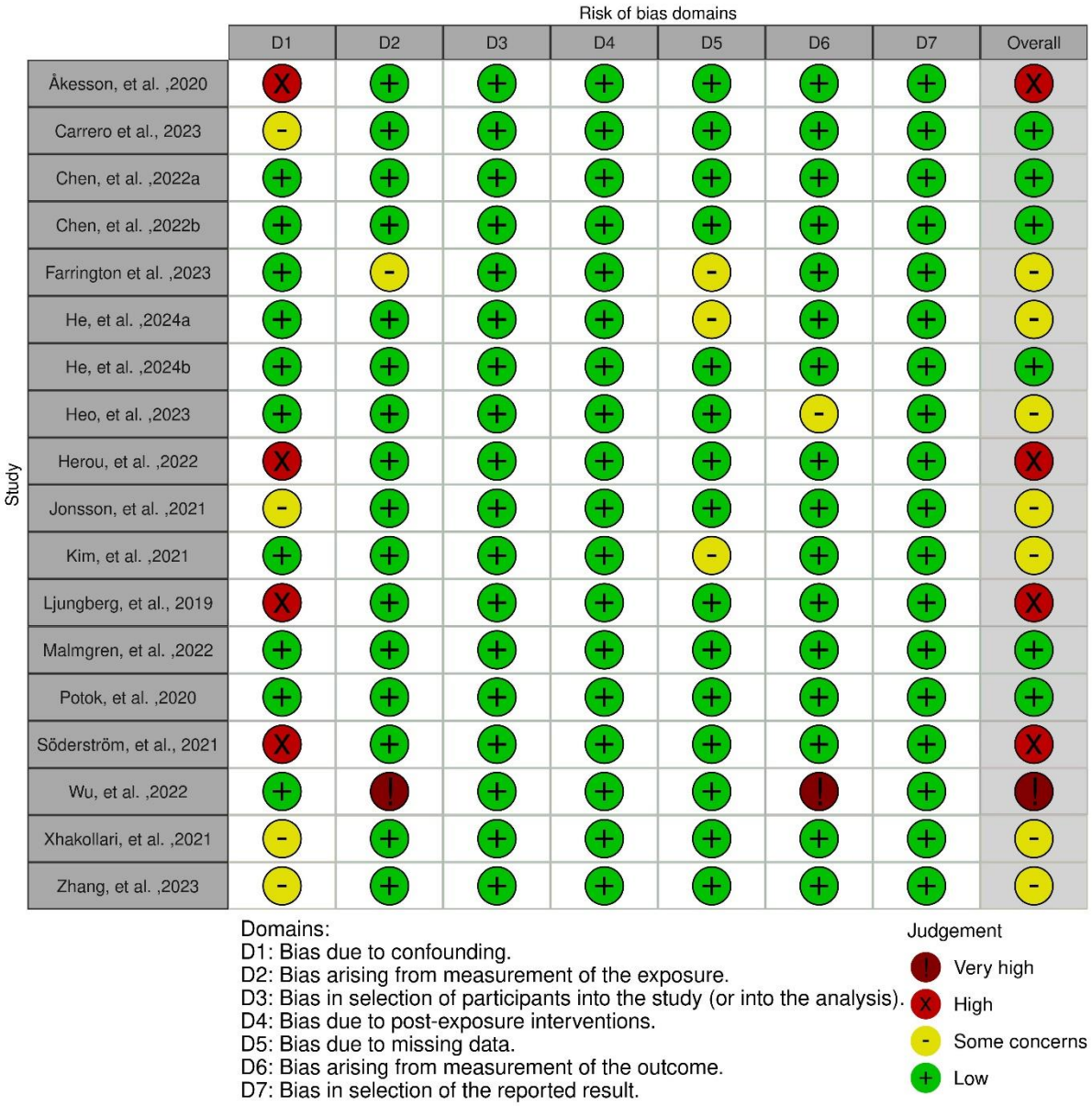
Search number	**Query**	**Results**
1	(Cystatin* and (creatin* or SCr or serum creatinine*)).ti,ab.	4690
2	difference*.ti,ab. or discordance*.ti,ab. or discrepanc*.ti,ab. or divergenc*.ti,ab. or disparit*.ti,ab. or compar*.ti,ab. or agreement*.ti,ab.	8732743
3	Intra-individual*.ti,ab. or intraindividual*.ti,ab.	13371
4	1 and (2 or 3)	2750
5	selective glomerular hypofilt*.ti,ab. or SGHS.ti,ab. or shrunken pore*.ti,ab.	81
6	4 or 5	2808
7	exp cohort studies/	2598859
8	exp cross-sectional studies/	500474
9	7 or 8	3006471
10	6 and 9	966

11	exp Animals/ not exp Humans/	5215958
12	10 not 11	958
13	exp editorial/ or exp letter/ or exp meta-analysis/ or exp review/	5350763
14	12 not 13	946

Supplementary Figure S1 Summarized risk of bias of included studies based on ROBINS-E scale.

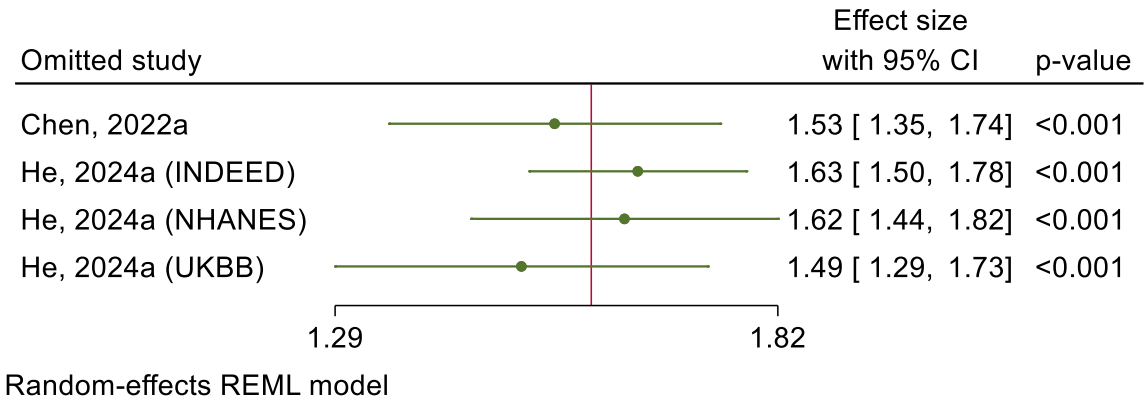


Supplementary Figure S2 Detailed risk of bias of included studies based on ROBINS-E scale.

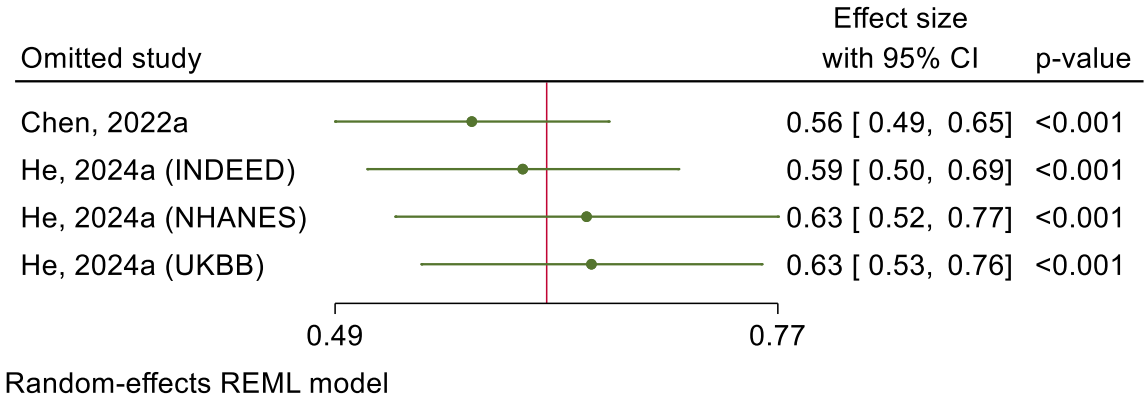


Supplementary Figure S3 Leave-one-out analyses of the association between eGFR discordance and mortality

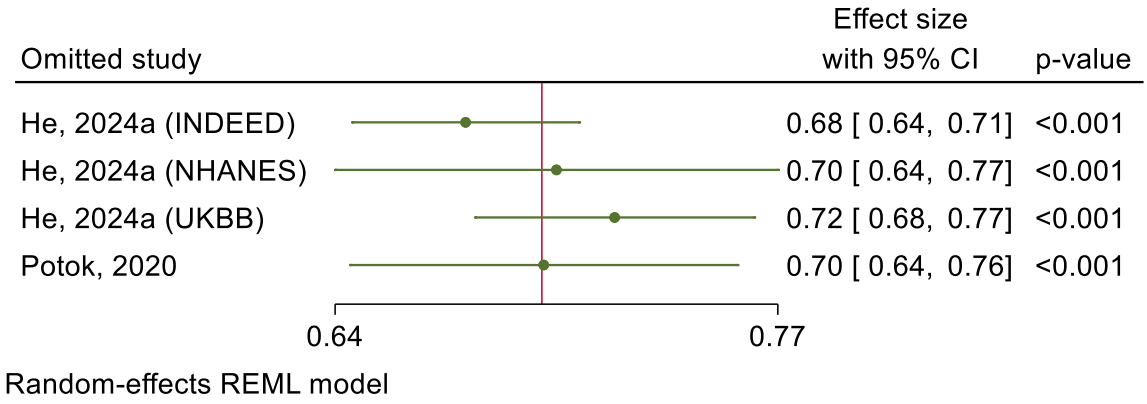
a. eGFRcys-eGFRcr < -15 ml/min/1.73m^2, hazard ratio



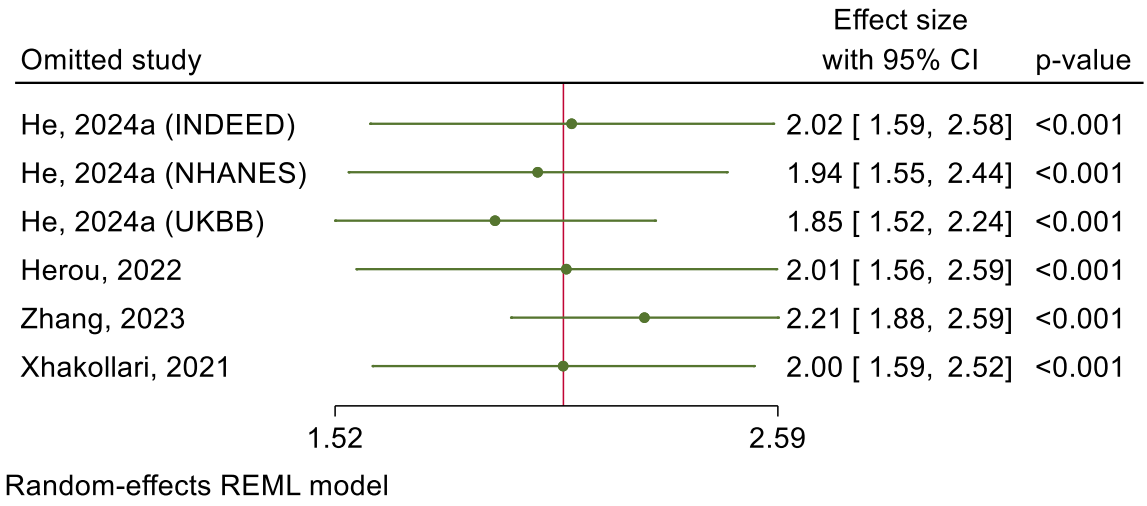
b. eGFRcys-eGFRcr ≥ 15 ml/min/1.73m^2, hazard ratio



c. eGFRcys-eGFRcr per +1 SD, hazard ratio

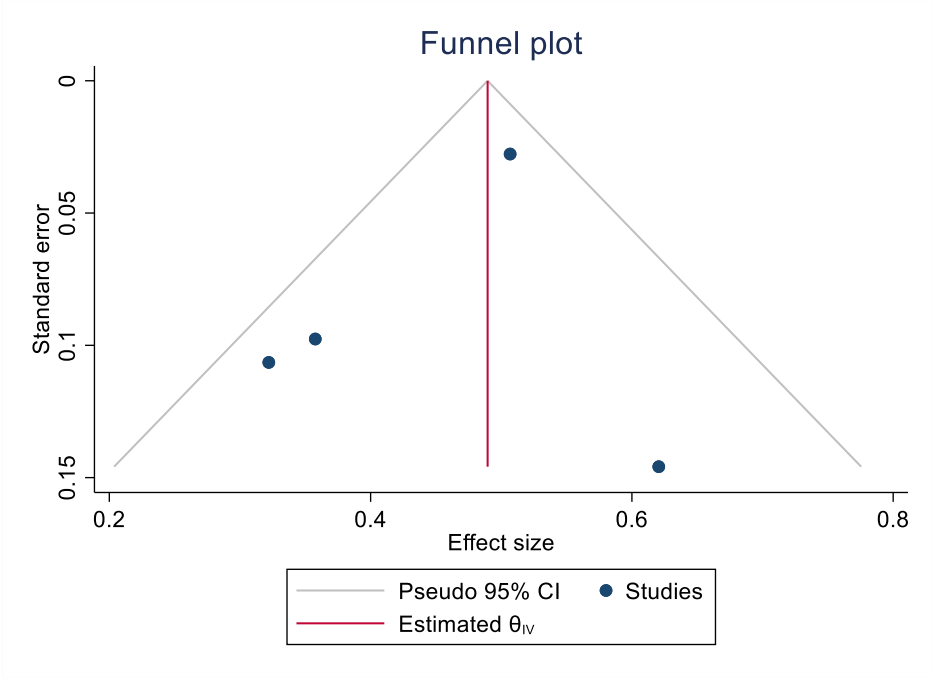


d. eGFRcys/eGFRcr≤0.6, hazard ratio

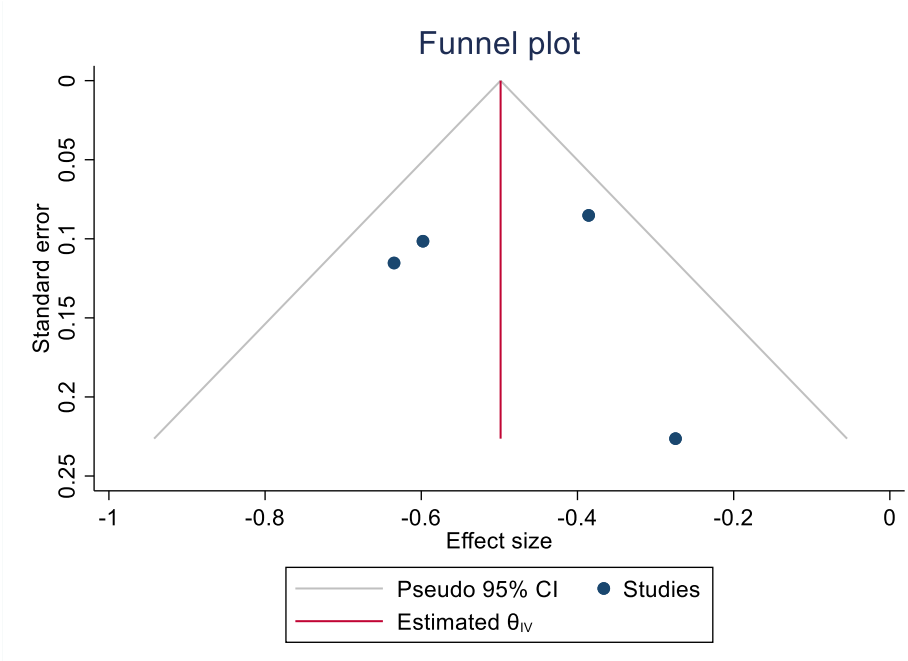


Supplementary Figure S4 Funnel plot of the association between eGFR discordance and mortality

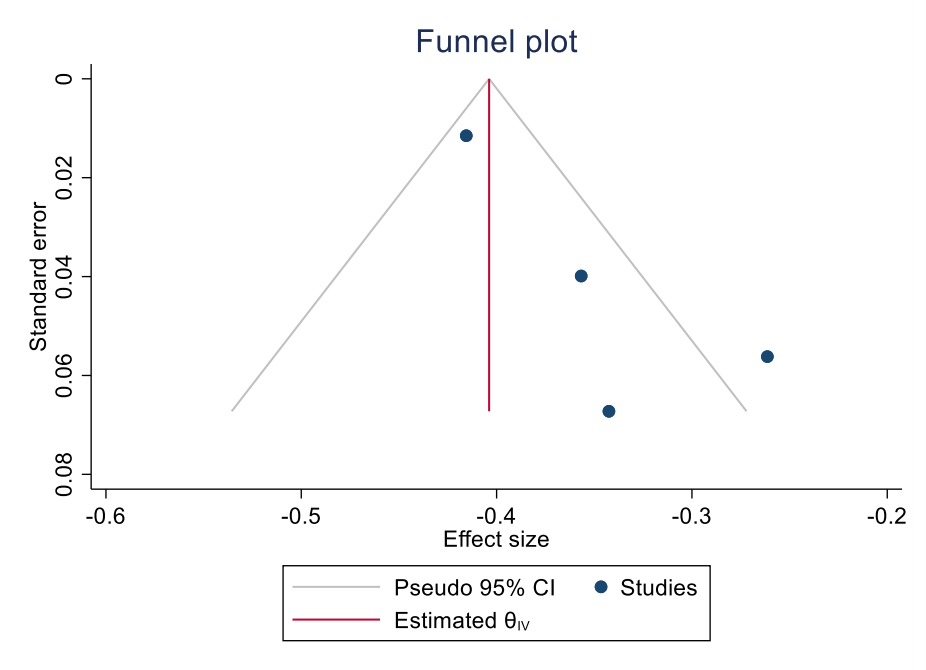
a. eGFRcys-eGFRcr < -15 ml/min/1.73m^2



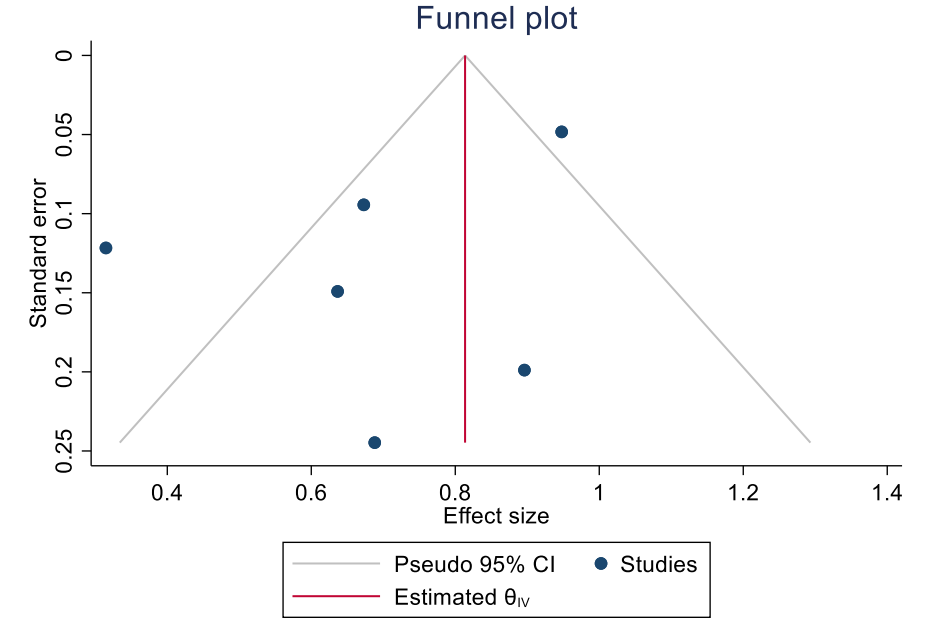
b. eGFRcys-eGFRcr ≥ 15 ml/min/1.73m^2



c. eGFRcys-eGFRcr per +1 SD

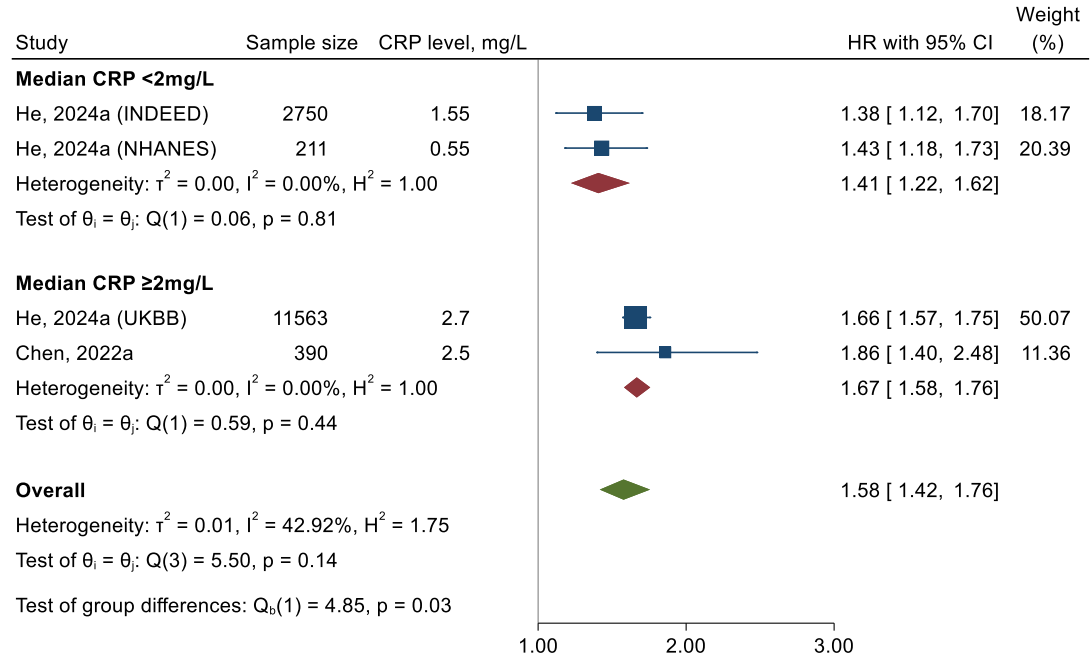


d. eGFRcys/eGFRcr≤0.6

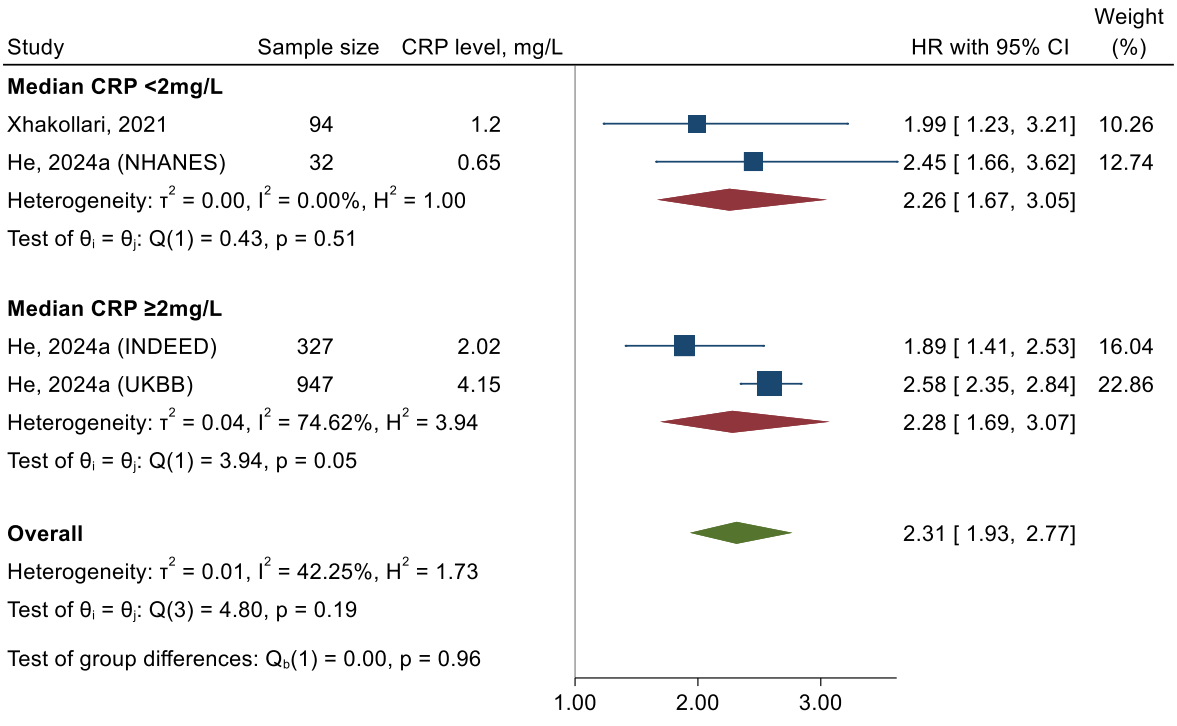


Supplementary Figure S5 Stratified meta-analyses on the association of eGFR discordance with mortality

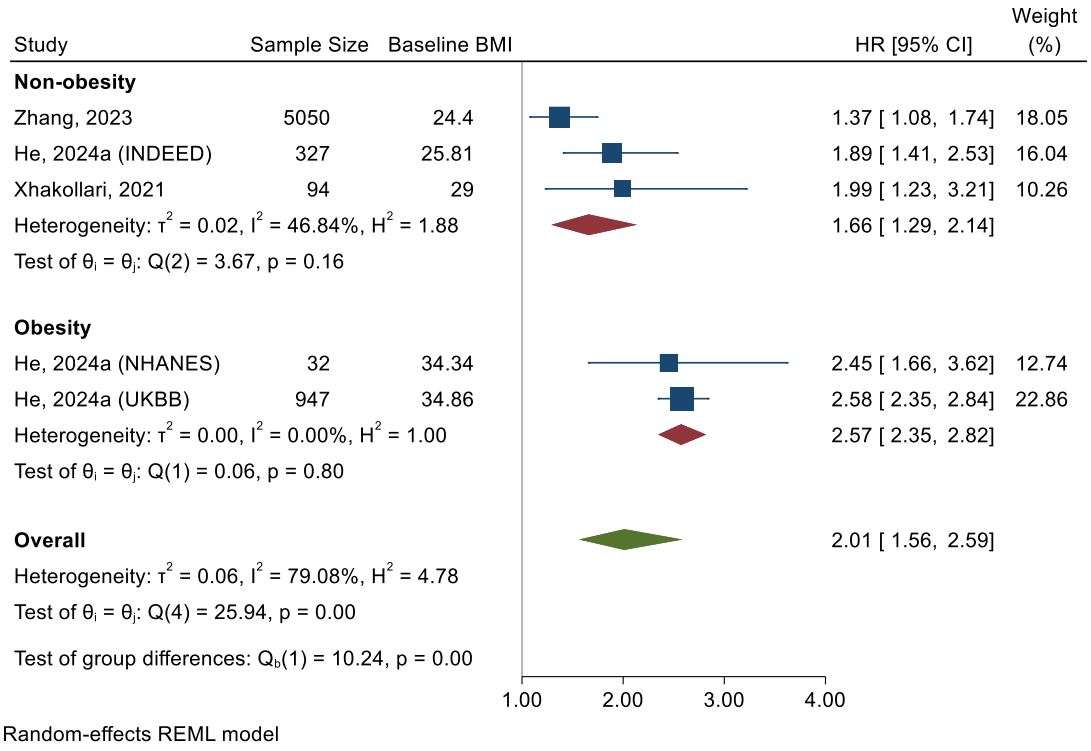
a. eGFRcys-eGFRcr < -15 ml/min/1.73m^2, stratified by C-reactive protein level



b. eGFRcys/eGFRcr⩽0.6, stratified by C-reactive protein level

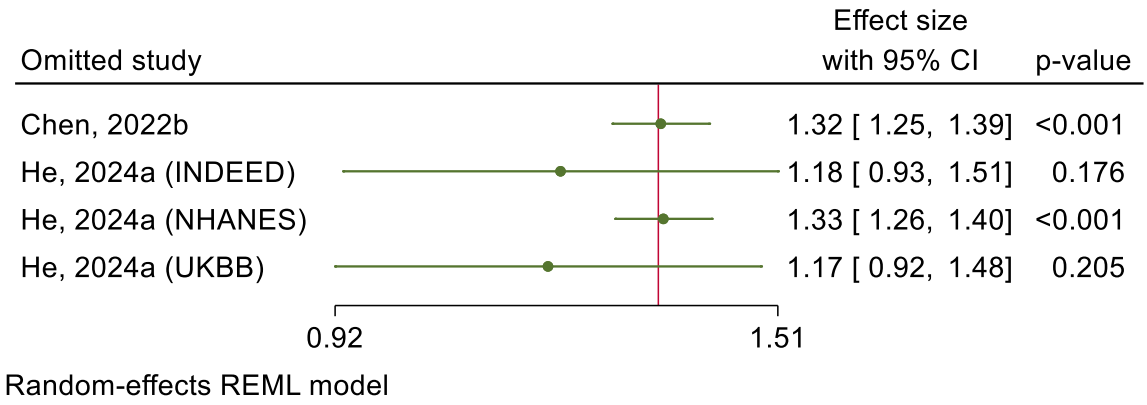


c. eGFRcys/eGFRcr≤0.6, stratified by obesity status

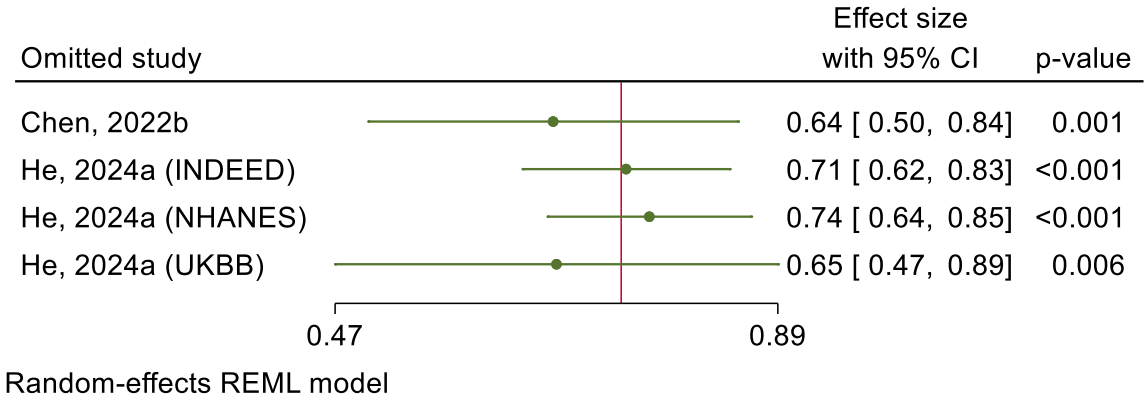


Supplementary Figure S6 Leave-one-out analyses of the association between eGFR discordance and cardiovascular incidence, Fine-Gray competing risk model

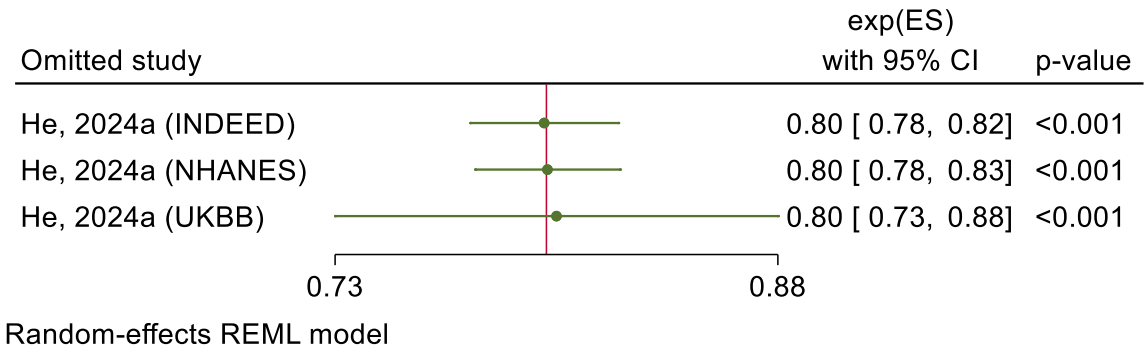
a. eGFRcys-eGFRcr < -15 ml/min/1.73m^2, subhazard ratio



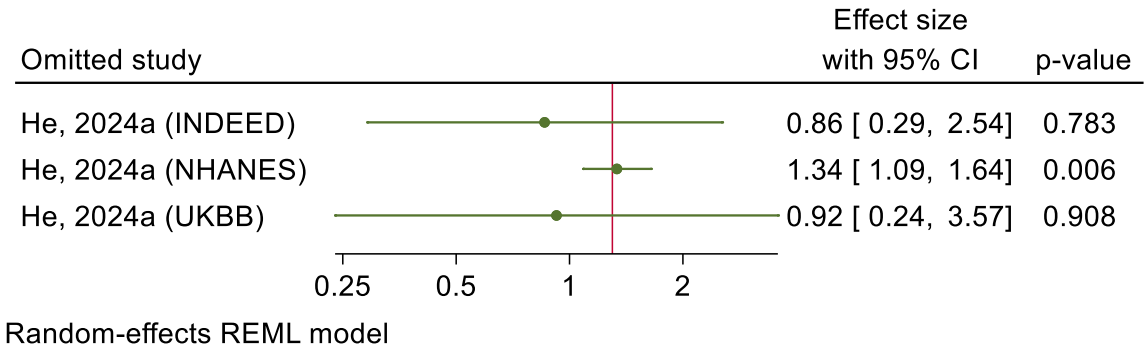
b. eGFRcys-eGFRcr ≥ 15 ml/min/1.73m^2, subhazard ratio



c. eGFRcys-eGFRcr per +1 SD, subhazard ratio

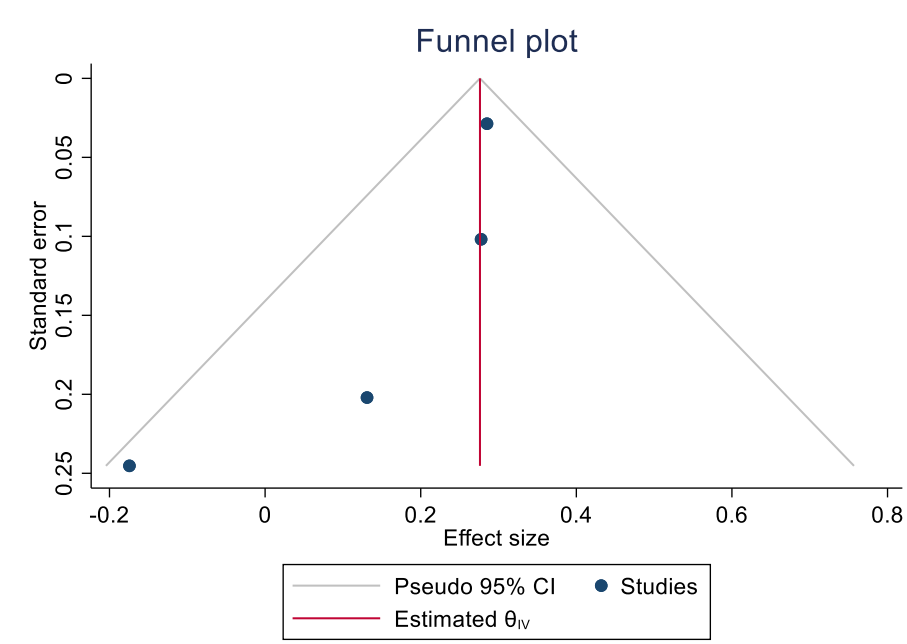


d. eGFRcys/eGFRcr<0.6, subhazard ratio

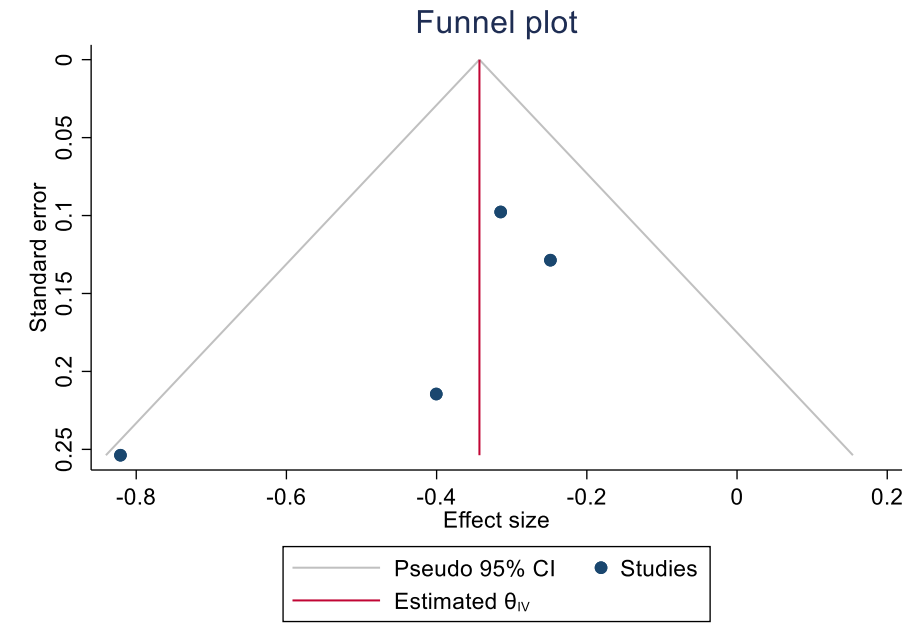


Supplementary Figure S7 Funnel plot of the association between eGFR discordance and cardiovascular incidence, Fine-Gray competing risk model

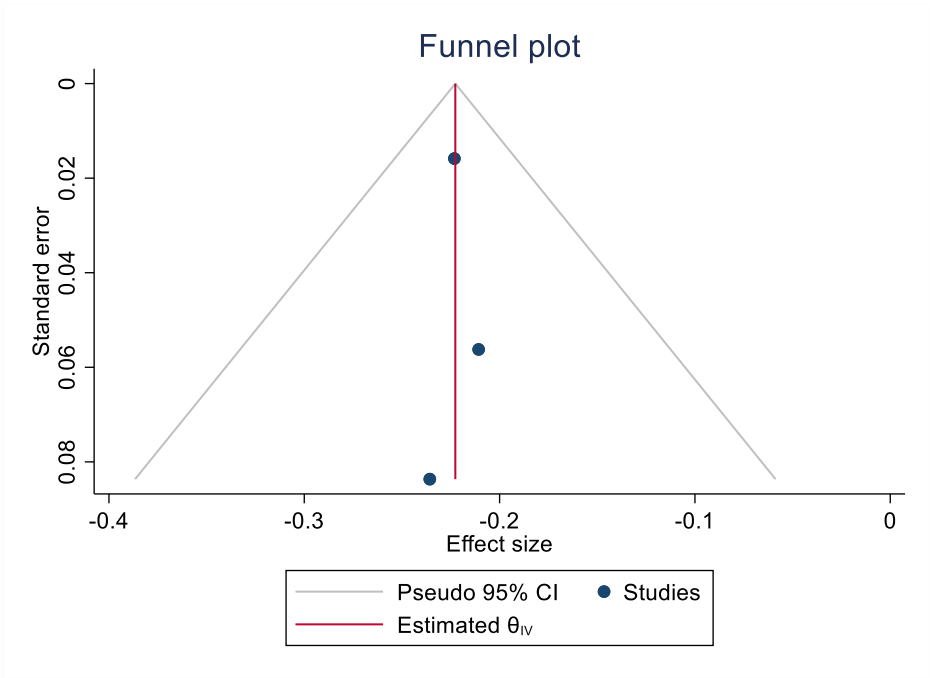
a. eGFRcys-eGFRcr < -15 ml/min/1.73m^2



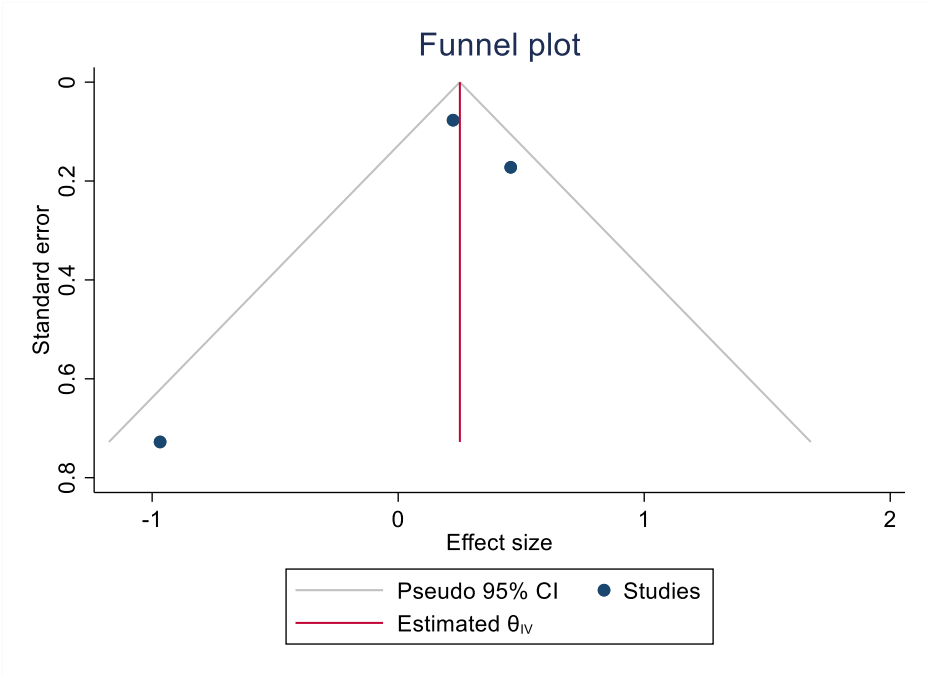
b. eGFRcys-eGFRcr ≥ 15 ml/min/1.73m^2



c. eGFRcys-eGFRcr per +1 SD

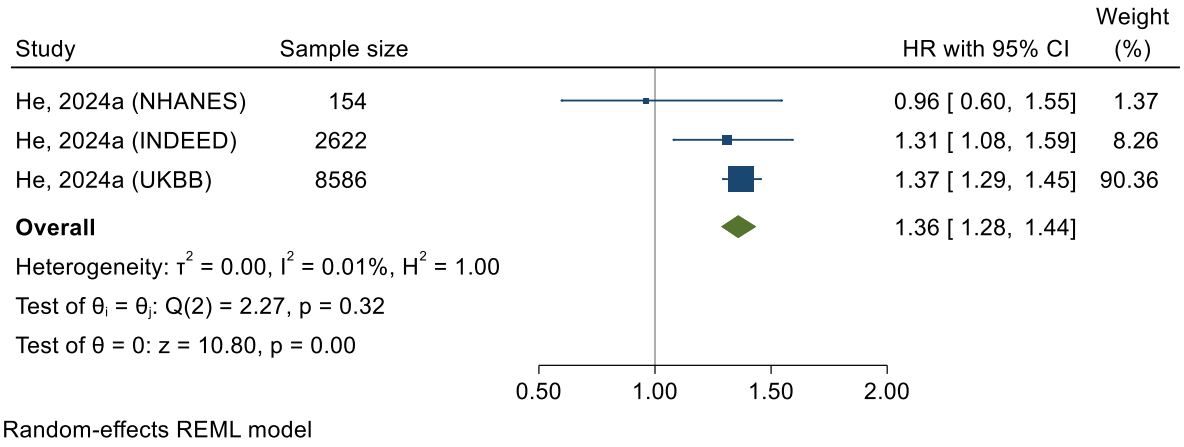


d. eGFRcys/eGFRcr<0.6

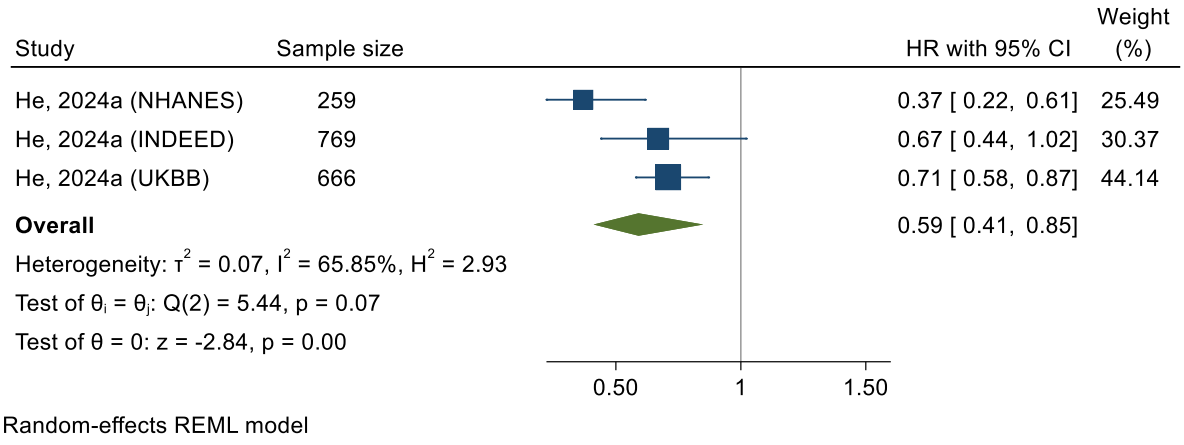


Supplementary Figure S8 Meta-analyses of the association between eGFR discordance and cardiovascular incidence, Cox regression model

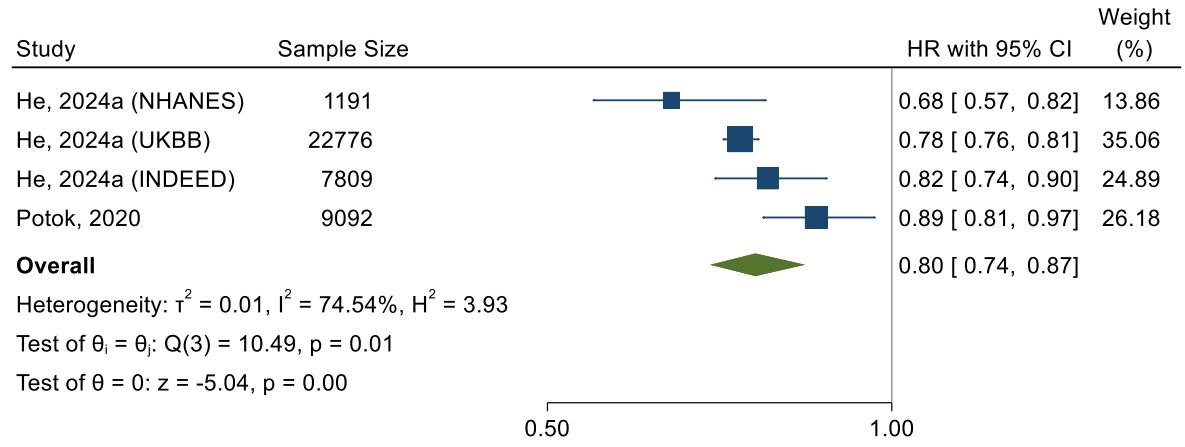
a. eGFRcys-eGFRcr < -15 ml/min/1.73m^2



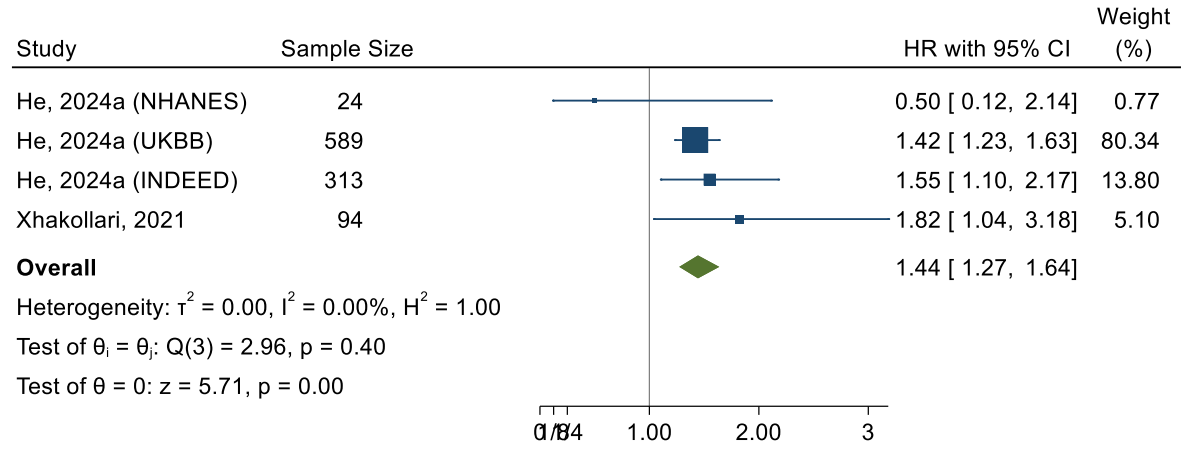
b. eGFRcys-eGFRcr ≥ 15 ml/min/1.73m^2



c. eGFRcys-eGFRcr per +1 SD

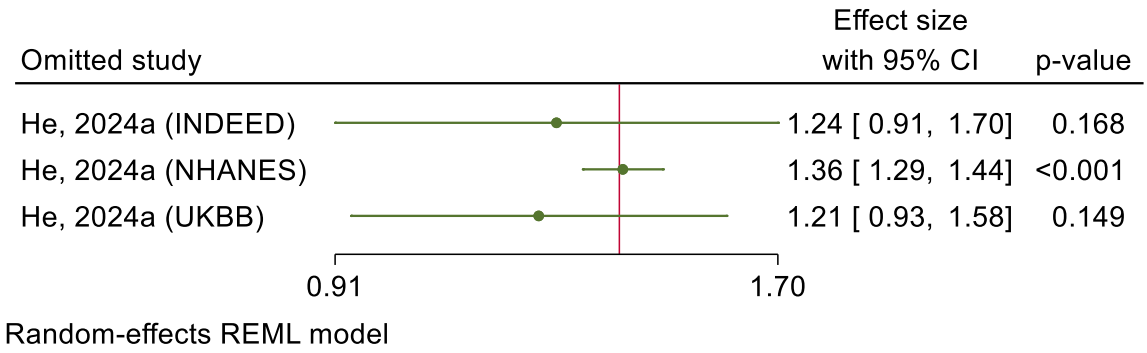


d. eGFRcys/eGFRcr<0.6

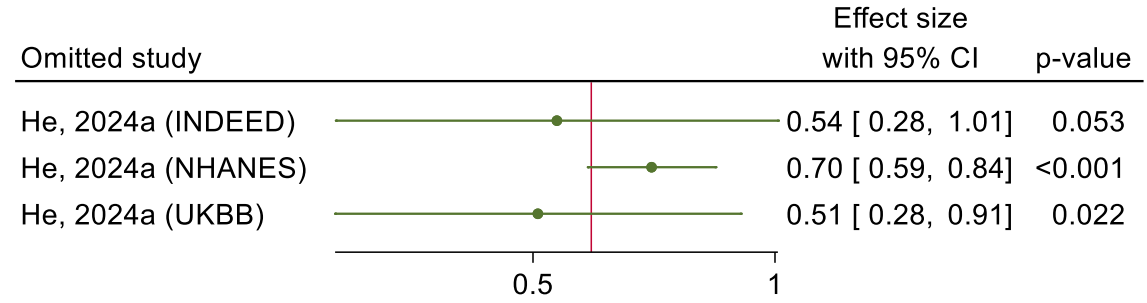


Supplementary Figure S9 Leave-one-out analyses of the association between eGFR discordance and cardiovascular incidence, Cox regression model

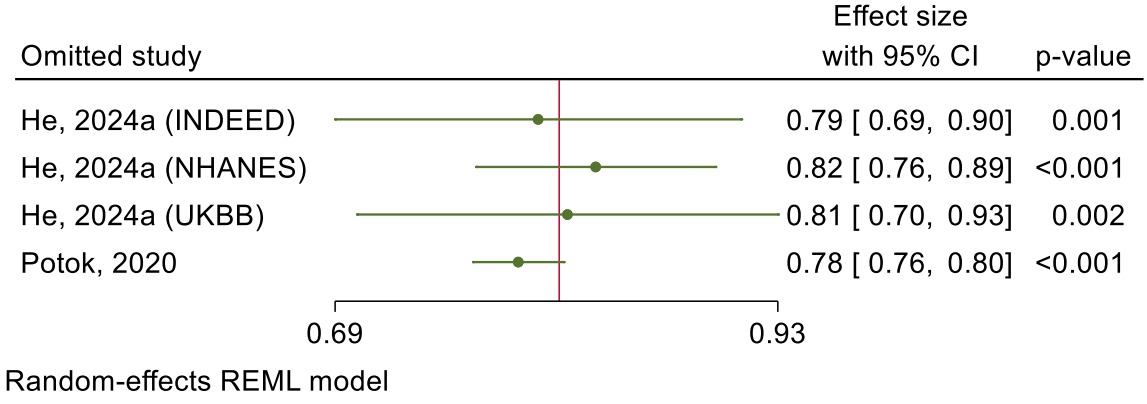
a. eGFRcys-eGFRcr < -15 ml/min/1.73m^2



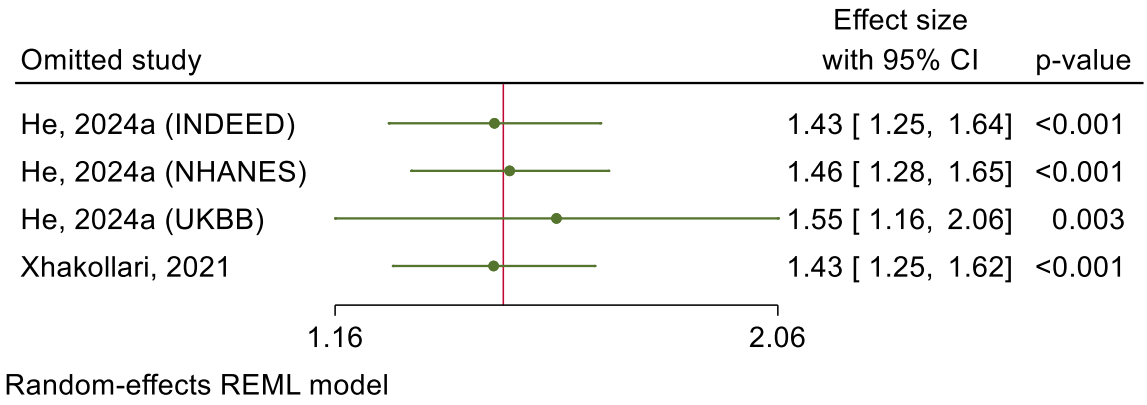
b. eGFRcys-eGFRcr ⩾ 15 ml/min/1.73m^2



c. eGFRcys-eGFRcr per +1 SD

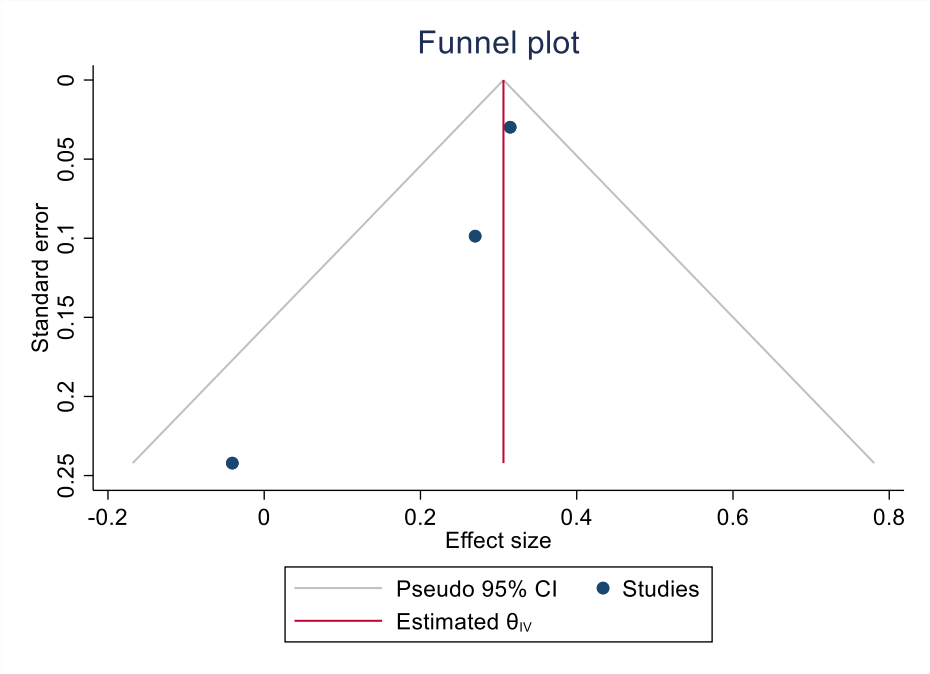


d. eGFRcys/eGFRcr<0.6

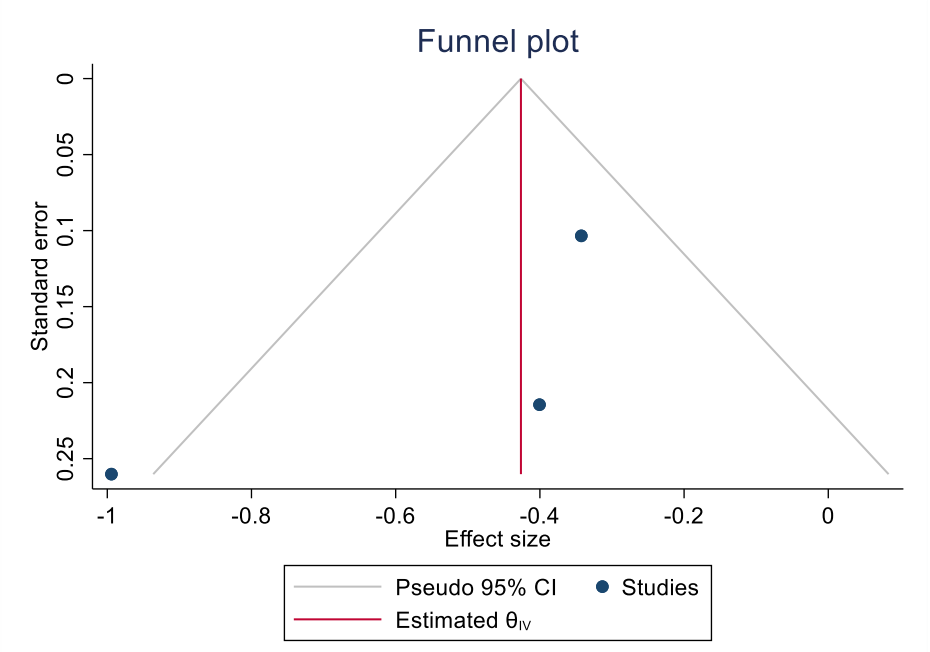


Supplementary Figure S10 Funnel plot of the association between eGFR discordance and cardiovascular incidence, Cox regression model

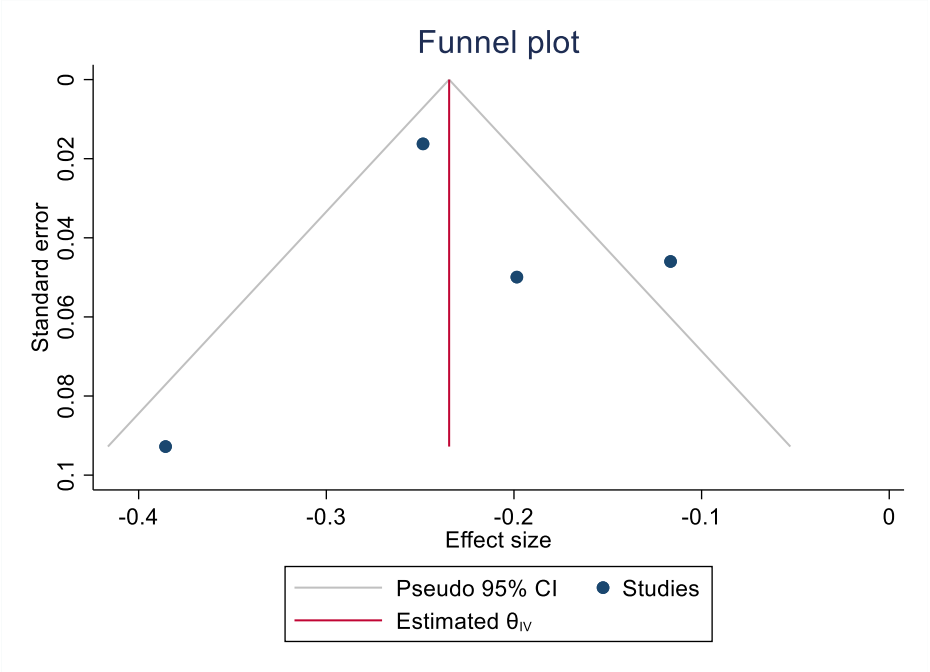
a. eGFRcys-eGFRcr < -15 ml/min/1.73m^2



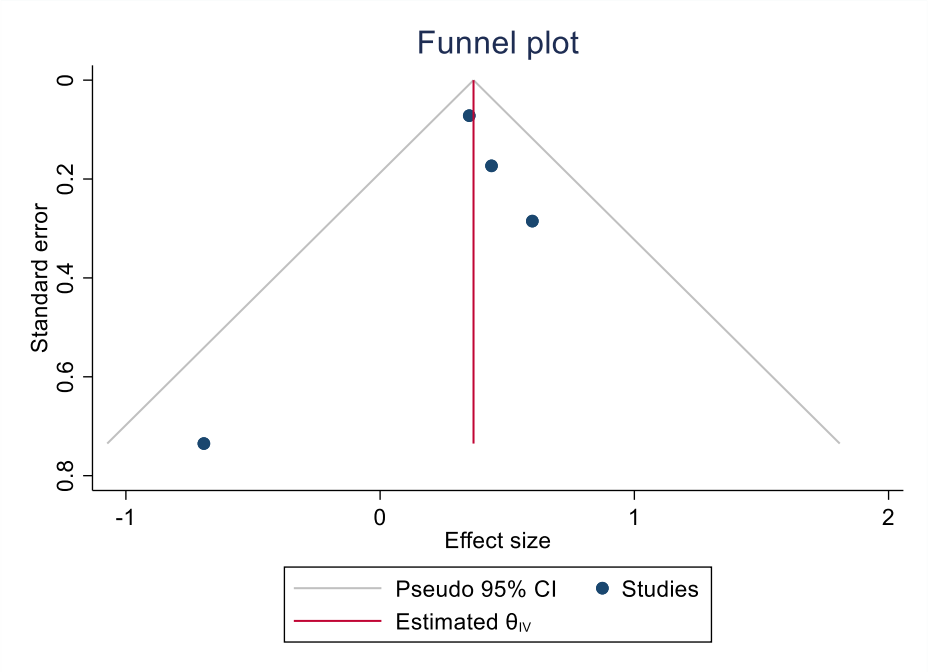
b. eGFRcys-eGFRcr ≥ 15 ml/min/1.73m^2



c. eGFRcys-eGFRcr per +1 SD



d. eGFRcys/eGFRcr<0.6



7. Chapter 7 – Discordance between Cystatin C-based and Creatinine-based estimated glomerular filtration rate and mortality in general population.

The original article of this chapter has been published in Clinical Chemistry on June 04, 2025.

Liu Q, Celis-Morales C, Lees JS, Sattar N, Ho FK, Pell JP, Mark PB, Welsh P. Discordance between Cystatin C-Based and Creatinine-Based Estimated Glomerular Filtration Rate and Mortality in the General Population. Clin Chem. 2025 Aug 1;71(8):858-869. doi: 10.1093/clinchem/hvaf063.

7.1. Abstract

Background: The consequences for health outcomes of the discordance in cystatin C-based ($eGFR_{cys}$) and creatinine-based ($eGFR_{cr}$) estimated glomerular filtration rates are gaining attention. However, the association of discordance with all-cause mortality in the general population has not been explored.

Methods: A total of 325,356 UK Biobank participants aged 40 to 69 years were followed for a median of 13.7 years. eGFR was calculated using both the CKD Epidemiology Collaboration (CKD-EPI) 2009/2012 equations and the European Kidney Function Consortium (EKFC) equations. Differences were expressed as the absolute difference ($eGFR_{cys}$-$eGFR_{cr}$: where discordance was defined as ±15 ml/min/1.73 m^2 difference) and relative difference ($eGFR_{cys}$/$eGFR_{cr}$: where discordance was defined as $eGFR_{cys}$ <60% $eGFR_{cr}$). Hazard ratios (HRs) for mortality were estimated using multivariable Cox proportional hazards models.

Results: Among the participants, 15.5% had a discordant lower absolute $eGFR_{cys}$, and 8.5% had a discordant higher absolute $eGFR_{cys}$. Participants with discordant lower absolute $eGFR_{cys}$ (CKD-EPI and EKFC equations) were older, more frequently male, had higher BMI and blood pressure, more comorbidities, and did less physical activity. A total of 26,465 deaths occurred. Participants with discordant lower $eGFR_{cys}$ had a 53% higher risk of mortality (HR=1.53, 95%CI: 1.48, 1.57), while those with discordant higher $eGFR_{cys}$ had a 30% lower risk (HR=0.70, 95%CI: 0.66, 0.75) compared to those with concordant eGFR. Those with discordance of lower relative $eGFR_{cys}$ had doubled risk of mortality (HR=2.25, 95%CI: 2.04, 2.47).

Conclusions: eGFR discordance was prevalent and associated with mortality in general populations. These results support broader use of cystatin C for risk stratification of mortality.

7.2. Introduction

Estimated glomerular filtration rate (eGFR) has been widely used to assess kidney function in clinical settings. The two common biomarkers for calculating eGFR are serum

creatinine (SCr) and cystatin C (CysC). SCr has been used as the primary biomarker as it achieves a balance of availability, accuracy and cost.[1]

Circulating concentrations of SCr and CysC are influenced by various non-GFR determinants. For SCr, common influencing factors include diet and muscle mass, while for CysC, factors such as adiposity, steroid administration, and inflammation are more significant. [2] Notably, SCr-based eGFR ($eGFR_{cr}$) and CysC-based eGFR ($eGFR_{cys}$) differ in their ability to stratify health risks. CysC-based eGFR has a better performance in identifying cardiovascular and kidney events.[3]

Due to differences in calculation methods and biomarkers, it is reasonable to expect that the $eGFR_{cr}$ and the $eGFR_{cys}$ measured in the same individual at the same time may not give the same result. However, recent studies have shown that the discrepancy between $eGFR_{cr}$ and $eGFR_{cys}$ for the same individual measured at the same time can be unexpectedly large, with an absolute difference of 15 ml/min/1.73m², or a relative difference of 30%, or more.[4]

Because such a difference could be clinically important, terms such as Shrunken Pore Syndrome (SPS) and Selective Glomerular Hypofiltration Syndrome (SGHS) have been suggested.[4] Current research has shown a strong association between eGFR discordance and the incidence of cardiovascular diseases, diabetes complications, and frailty.[5-7] Additionally, studies have highlighted a close link of eGFR differences with all-cause mortality and cause-specific mortality.[8] In general, negative discordance ($eGFR_{cys}<eGFR_{cr}$) is associated with increased risk of adverse outcomes, while positive discordance ($eGFR_{cys}>eGFR_{cr}$) associates with reduced risk.

Much of the work on eGFR discordance was in cohorts with chronic kidney disease, i.e. eGFR<60ml/min/1.73m^2.[3] A recent systematic review on 18 studies showed that existing research on the health effects of eGFR discordance has been primarily focused on individuals with specific diseases (such as diabetes and kidney disease), with a lack of studies conducted on the general population.[9] However, eGFR discordance has also been found in those with normal measured GFR (mGFR).[4, 10] A study of 1300 patients with mGFR≥60 ml/min/1.73m^2 showed those whose $eGFR_{cys}$ was less than 70% $eGFR_{cr}$ faced nearly a fourfold higher risk of all-cause mortality. [11] Another large-scale study on the

general population from the Stockholm Creatinine Measurements (SCREAM) project showed the percentage difference between $eGFR_{cys}$ and $eGFR_{cr}$ (i.e. $(eGFR_{cys} - eGFR_{cr})/eGFR_{cr}$) is associated with mortality. [12] The above observations shed light on the need to study the potential health effects of eGFR discordance in the general population.

Therefore, we used the UK Biobank, a large-scale multicenter survey in the United Kingdom, to explore the association between eGFR discordance and mortality in a large general population, with progressive adjustment for covariates.

7.3. Materials and Methods

Study population

This study utilized data from the UK Biobank: a large-scale, population-based cohort initiated in 2006. The UK Biobank enrolled more than 500,000 participants aged 40 to 69 years from across Great Britain. [13] Ethical approval for the UK Biobank was obtained from the North West Multi-Centre Research Ethics Committee (Reference 21/NW/0157), and all participants provided electronic informed consent.

For a total of 502,369 participants, we excluded 33,483 participants without either SCr or CysC measurement and further excluded 143,530 with missing values for covariates, leaving 325,356 participants in the final cohort.

This study was conducted in accordance with the ethical standards of the Declaration of Helsinki and was performed under UK Biobank Application Number. 71392. Participants with missing baseline values of SCr, CysC or other covariates were excluded.

Glomerular filtration rate estimation

In the UK Biobank, single values of SCr and CysC were obtained at the baseline study visit and measured through enzymatic analysis on a Beckman Coulter AU5800 at a centralized laboratory, following standard procedures. The detailed laboratory procedures can be found in published documents.[14]

We calculated eGFR using four different methods. In the primary analysis, we first employed the eGFR equations published by the CKD Epidemiology Collaboration (CKD-

EPI), namely the 2009 race-dependent SCr-based equation [15] and the 2012 CysC-based equation [16] to calculate eGFR. The 2009 SCr-based equation was selected as: i) it is the equation recommended in current guidance in the United Kingdom for estimation of kidney function, and ii) the newer race-independent CKD-EPI $eGFR_{cr}$ 2021 has been shown to overestimate GFR in European populations.[17]

Secondly, considering that UK Biobank participants were all middle-aged to older adults at the time of assessment and spanned a wide age range, and given that factors such as muscle mass, diet, and metabolism in older adults might influence eGFR, we applied the European Kidney Function Consortium (EKFC) SCr-based and CysC-based equations to ensure the continuity of eGFR measurement across ages and a higher accuracy in European populations.[18, 19]

Measurement of eGFR discordance

This study measured eGFR discordance using two approaches. One approach was the absolute difference, defined $eGFR_{cys}$ minus $eGFR_{cr}$. The second approach was the relative difference, derived from the ratio of $eGFR_{cys}$ to $eGFR_{cr}$.

As different eGFR equations were used in this study, we used the following pairs of equations to calculate eGFR discordance:

eGFR absolute difference:

1. CKD-EPI 2012 $eGFR_{cys}$ - CKD-EPI 2009 race-dependent $eGFR_{cr}$
2. EKFC $eGFR_{cys}$ - EKFC $eGFR_{cr}$

eGFR relative difference:

1. CKD-EPI 2012 $eGFR_{cys}$/CKD-EPI 2009 race-dependent $eGFR_{cr}$
2. EKFC $eGFR_{cys}$/EKFC $eGFR_{cr}$

The eGFR absolute difference was divided into three categories: negative discordance (indicates lower $eGFR_{cys}$, eGFR absolute difference <-15 ml/min/1.73m^2), concordant eGFR (eGFR absolute difference ≥ -15 and < 15 ml/min/1.73m^2), and positive discordance (indicates higher $eGFR_{cys}$, eGFR absolute difference ≥15 ml/min/1.73m^2). These thresholds were selected because 15 ml/min/1.73m² roughly equals one standard deviation

from the eGFR absolute difference in this study population and indicates a clinically significant variation of eGFR. For eGFR relative difference, we classified it into two groups: lower $eGFR_{cys}$ ratio ($eGFR_{cys}$ <60% $eGFR_{cr}$) and normal $eGFR_{cys}$ ratio ($eGFR_{cys}$ ≥60% $eGFR_{cr}$). These categories have been used in previous studies.[4-6]

Measurement of all-cause mortality

All-cause mortality was selected as the primary outcome as it was a definite outcome related to overall health status at baseline. Mortality data were sourced from the National Health Service (NHS) England for participants from England and Wales, and from the NHS Central Register and National Records of Scotland for participants from Scotland.

Covariates

Drawing from existing studies and clinical knowledge, information about a range of covariates was collected. Specifically, we collected demographic data, health status, laboratory results, and the use of drugs which can affect eGFR biomarkers.[4-6, 20-27]

In detail, demographic data were age, sex, self-reported race (White, Black, South Asian, and other), and Townsend deprivation index. The Townsend deprivation index is a composite measure used to assess material deprivation in a geographical area. The smaller the numerical Townsend deprivation index, the greater the relative affluence, with a score of zero indicating an area with the overall mean deprivation level. Therefore, a negative index indicates an area more affluent than the mean, and a positive index indicates an area less affluent than the mean [28].

Health status included: smoking status, fat-free mass status proxied by appendicular lean soft tissue (ALST) mass indexed to $height^2$ ($ALST/height^2$) [27], self-reported weekly physical activity, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), self-reported and diagnosed comorbidities (hypertension, angina, heart failure, myocardial infarction, stroke, diabetes, thyroid diseases, asthma, chronic bronchitis, chronic obstructive pulmonary disease, and cancer). Self-reported and diagnosed comorbidities were collected through interviews and questionnaires on the day of the first UK Biobank assessment. [29]

Laboratory results were high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), C-reactive protein (CRP), neutrophil-to-lymphocyte ratio (NLR), urine albumin-creatinine ratio (UACR) and uric acid. All the tests were performed following standard procedures.[30, 31] Use of following drugs was self-reported: cimetidine [21], co-trimoxazole [22], fibrates [23], steroids [24], trimethoprim [22], and statins. A detailed list of drugs can be found in Supplementary Table S1.

Statistical analysis

Continuous variables were summarized as mean ± standard deviation (SD) if normally distributed, and median (25^{th}, 75^{th} percentile) if not. Categorical variables were summarized as numbers (percentage).

Cox regression models estimated hazard ratios (HRs) with 95% confidence intervals (CIs) to examine associations between eGFR discordance and mortality. Proportional hazards assumptions were examined using Schoenfeld residuals.

Confounders were adjusted in a comparative approach to illustrate their effect. Model 1 was adjusted for common confounders (namely, age, sex, race, Townsend deprivation index, smoking status, BMI, SBP, DBP, comorbidities, HDL-C, LDL-C, TG, NLR, baseline eGFRcr, UACR, and weekly physical activity). Model 2 was adjusted for confounders in Model 1 plus factors that affect SCr, namely ALST/Height^2, the use of cimetidine, co-trimoxazole, fibrate, and trimethoprim. Model 3 was adjusted for Model 1 plus factors that affect CysC, namely thyroid disease, C-reactive protein, use of steroids, and uric acid. Model 4 was the final model which was adjusted Model 1 plus and factors that affect either SCr (as in Model 2) or CysC (as in Model 3).

Apart from primary analysis, we performed stratified analysis and tests for interactions over a selected set of baseline covariates. Stratifications included sex (male or female), age<65 or ≥65 years, race (Black or non-Black), BMI<30 or ≥30 kg/m^2, CRP< 2 or ≥2mg/L, baseline CKD stages (stage 1, stage 2, stage 3a, and stage 3b/4/5) measured by $eGFR_{cr}$ [1], and ALST/Height^2 (within mean ±1 SD, more than 1 SD below the mean, more than 1 SD above the mean). Tests for interactions between eGFR discordance and the selected baseline characteristics were conducted using likelihood ratio tests.

To evaluate the robustness of study findings, we conducted a sensitivity analysis. A two-year landmark analysis on the subset of the study populations with the exposure time set to two years after the assessment date to minimize the risk of reverse causation. All statistical analyses were conducted using STATA 17.0 (StataCorp, College Station, Texas, USA). All tests were two-tailed with statistical significance set at $P<0.05$.

7.4. Results

Baseline characteristics

Among 325,356 UK Biobank participants, 152,569 (46.9%) were male, mean (SD) age was 56.6 (8.1) years (Table 1). A total of 309,478 (95.1%) participants were White, 4,618 (1.4%) were Black, and 4,565 (1.4%) were South Asian. The median follow-up time was 13.7 years.

The mean baseline $eGFR_{cr}$ was higher than $eGFR_{cys}$ for both CKD-EPI and EKFC equations (Table 7-1). The prevalence of participants with CKD-EPI $eGFR_{cr}<60$ ml/min/1.73m^2 was 2.1% (6725 participants), and 4.0% (12925 participants) for CKD-EPI $eGFR_{cys}<60$ ml/min/1.73m^2. The prevalence of participants with EKFC $eGFR_{cr}<60$ ml/min/1.73m^2 was 2.9 % (9505 participants), and 2.8 % (9231 participants) for EKFC $eGFR_{cys}<60$ ml/min/1.73m^2.

Table 7-1 Baseline characteristics of the study population, stratified by CKD-EPI eGFR discordance categories.

Characteristics		Overall	eGFR absolute difference[a]		$eGFR_{cys}/eGFR_{cr}$	
		Lower $eGFR_{cysc}$	Concordant eGFR	Higher $eGFR_{cysc}$	<0.6	≥ 0.6
Size, n(row%)	325356 (100)	50414 (15.5)	247210 (76.0)	27732 (8.5)	1354 (0.4)	324002 (99.6)
Age, year	56.6±8.1	58.2±7.7	56.5±8.1	54.0±8.1	60.2±7.0	56.6±8.1
Male, n(%)	152569 (46.9)	24723 (49.0)	115584 (46.7)	12462 (44.9)	661 (48.8)	151908 (46.9)
Race, n(%)						
White	309478 (95.1)	46873 (93.0)	236366 (95.6)	26239 (94.6)	1194 (88.2)	308284 (95.2)
Black	4618 (1.4)	1051 (2.1)	3003 (1.2)	564 (2.0)	48 (3.6)	4570 (1.4)
South Asian	4565 (1.4)	1551 (3.1)	2832 (1.2)	182 (0.7)	84 (6.2)	4481 (1.4)
Other	6695 (2.1)	939 (1.9)	5009 (2.0)	747 (2.7)	28 (2.1)	6667 (2.1)
Smoke status, n(%)						
Never	179194 (55.1)	22900 (45.4)	138226 (55.9)	17089 (61.6)	535 (39.6)	177673 (54.8)
Previous	113804 (34.9)	16768 (33.3)	88619 (35.8)	9432 (33.9)	433 (32.0)	114357 (35.3)
Current	32358 (10.0)	10746 (21.3)	20365 (8.2)	1247 (4.5)	386 (28.5)	31972 (9.9)
Body mass index, kg/m^2	27.2±4.5	29.3±5.6	26.8±4.2	26.0±3.6	30.5±6.6	27.1±4.5
Appendicular lean soft tissue, kg	22.3±5.3	23.1±5.7	22.2±5.2	22.1±5.1	23.2±5.8	22.3±5.3
ALST/$height^2$, kg/m^2	7.7±1.3	8.1±1.4	7.7±1.2	7.7±1.2	8.2±1.6	7.7±1.3
Systolic blood pressure, mmHg	138.0±18.6	140.6±18.8	137.8±18.6	134.5±18.0	139.9±19.9	138.0±18.6
Diastolic blood pressure, mmHg	82.2±10.1	83.6±10.4	82.1±10.1	80.7±9.8	82.3±11.0	82.2±10.1
Comorbidities, n(%)						
Hypertension	172816 (53.1)	31841 (63.2)	129114 (52.2)	11861 (42.8)	933 (68.9)	171883 (53.1)
Angina	9638 (3.0)	2201 (4.4)	6879 (2.8)	558 (2.0)	113 (8.3)	9529 (2.9)
Heart failure	434 (0.1)	116 (0.2)	289 (0.1)	29 (0.1)	10 (0.7)	424 (0.1)
Myocardial infarction	6919 (2.1)	1539 (3.1)	4957 (2.0)	423 (1.5)	78 (5.8)	6841 (2.1)
Stroke	4187 (1.3)	1011 (2.0)	2930 (1.2)	246 (0.9)	54 (4.0)	4133 (1.3)
Diabetes	15298 (4.7)	4096 (8.1)	10485 (4.2)	717 (2.6)	201 (14.8)	15097 (4.7)
Thyroid disease	18235 (5.6)	3774 (7.5)	13120 (5.3)	1341 (4.8)	149 (11.0)	18086 (5.6)

Asthma	36614 (11.3)	6338 (12.6)	27208 (11.0)	3068 (11.1)	189 (14.0)	36425 (11.2)
Chronic bronchitis	3919 (1.2)	1130 (2.2)	2593 (1.1)	196 (0.7)	57 (4.2)	3862 (1.2)
COPD	899 (0.3)	289 (0.6)	581 (0.2)	29 (0.1)	19 (1.4)	880 (0.3)
Cancer	27717 (8.5)	5100 (10.1)	20648 (8.4)	1969 (7.1)	196 (14.6)	27521 (8.5)
On-use medicine, n(%)						
Statin	50569 (15.5)	9963 (19.8)	37238 (15.1)	3368 (12.1)	384 (28.4)	50185 (15.5)
TMP	336 (0.1)	36 (0.1)	236 (0.1)	64 (0.2)	3 (0.2)	333 (0.1)
Cimetidine	236 (0.1)	44 (0.1)	164 (0.1)	28 (0.1)	2 (0.2)	234 (0.1)
Fibrates	819 (0.3)	146 (0.3)	608 (0.3)	65 (0.2)	3 (0.2)	816 (0.3)
Steroids	1691 (0.5)	632 (1.3)	1002 (0.4)	57 (0.2)	55 (4.1)	1636 (0.5)
C-reactive protein, mg/L	1.3 (0.6, 2.6)	2.2 (1.1, 4.3)	1.2 (0.6, 2.4)	0.9 (0.5, 1.7)	3.5 (1.6, 7.6)	1.3 (0.6, 2.6)
Neutrophil-to-lymphocyte ratio	2.3±1.2	2.4±1.6	2.3±1.1	2.3±1.0	2.9±2.3	2.3±1.2
Serum creatinine, mg/dL	0.81±0.19	0.77±0.14	0.81±0.19	0.95±0.16	0.81±0.21	0.82±0.19
Cystatin C, mg/L	0.9±0.2	1.1±0.1	0.9±0.1	0.8±0.1	1.4±0.3	0.9±0.2
HDL cholesterol, mg/dL	56.4±14.9	51.3±13.6	57.1±14.8	59.8±15.0	46.2±12.9	56.5±14.8
LDL cholesterol, mg/dL	137.5±33.3	138.8±35.1	137.7±33.1	133.9±32.0	128.8±37.4	137.6±33.3
Triglycerides, mg/dL	152.1±88.7	179.8±100.0	148.4±86.1	134.7±79.8	191.4±104.6	151.9±88.6
Urine albumin-creatinine ratio	6.0 (3.7, 10.0)	6.4 (3.9, 10.9)	6.0 (3.7, 10.0)	5.0 (3.1, 8.3)	7.9 (4.6, 16.8)	6.0 (3.7, 10.0)
Uric acid, mg/dL	5.2±1.3	5.5±1.3	5.1±1.3	5.2±1.3	6.0±1.5	5.2±1.3
CKD-EPI eGFR equation[b]						
$eGFR_{cr}$, ml/min/1.73m^2	90.9±13.1	94.5±10.6	91.5±13.1	79.2±11.8	90.6±15.6	90.9±13.1
$eGFR_{cys}$, ml/min/1.73m^2	88.9±15.8	72.4±11.5	90.9±14.6	101.4±11.0	49.4±9.8	89.1±15.6
eGFR absolute difference, ml/min/1.73m^2	-1.6 (-10.4, 6.2)	-20.5 (-25.2, -17.4)	-0.5 (-6.7, 5.3)	20.2 (17.2, 25.0)	-40.5 (-45.4, -36.6)	-1.5 (-10.3, 6.3)
$eGFR_{cys}/eGFR_{cr}$	1.0±0.2	0.8±0.1	1.0±0.1	1.3±0.1	0.5±0.1	1.0±0.2
EKFC eGFR equation						
$eGFR_{cr}$, ml/min/1.73m^2	86.6±12.9	89.2±10.7	87.2±13.0	77.0±11.9	84.9±14.0	86.7±12.9
$eGFR_{cys}$, ml/min/1.73m^2	86.3±12.9	73.9±10.7	87.9±12.0	95.4±9.23	53.2±9.5	86.5±12.8
eGFR absolute difference, ml/min/1.73m^2	-0.5 (-6.1, 5.3)	-14.6 (-18.8, -10.7)	0.1 (-3.6, 4.5)	17.8 (13.5, 22.7)	-30.7 (-36.3, -26.4)	-0.4 (-6.1, 5.1)

$eGFR_{cys}/eGFR_{cr}$	1.0±0.1	0.8±0.1	1.0±0.1	1.3±0.1	0.6±0.1	1.0±0.1
Weekly physical activity, MET	1850 (840, 3759)	1653 (720, 3612)	1866 (864, 3759)	2013 (967, 3848)	1386 (660, 3096)	1853 (840, 3759)
Townsend deprivation index	-2.2 (-3.7, 0.4)	-1.4 (-3.3, 1.6)	-2.3 (-3.7, 0.2)	-2.4 (-3.8, -0.1)	-0.3 (-2.8, 2.8)	-2.2 (-3.7, 0.4)

[a]eGFR absolute difference was calculated as $eGFR_{cys}$ - $eGFR_{cr}$.

[b]$eGFR_{cr}$ was calculated using the CKD-EPI 2009 race-independent equation; $eGFR_{cys}$ was calculated using the CKD-EPI 2012 equation.

Lab SI units conversions: serum creatinine: multiply the value in mg/dL by 88.4 to convert to µmol/L; HDL cholesterol and LDL cholesterol: multiply the value in mg/dL by 0.0259 to convert to mmol/L; triglycerides: multiply the value in mg/dL by 0.0113 to convert to mmol/L; uric acid: multiply the value in mg/dL by 59.48 to convert to µmol/L

ALST, Appendicular lean soft tissue; BMI, Body mass index, CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; COPD, Chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; EKFC, European Kidney Function Consortium; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; TMP, Trimethoprim

Using both CKD-EPI or EKFC equations, individuals with a lower $eGFR_{cys}$ or lower $eGFR_{cys}$ ratio tended to be older, male, and with a higher prevalence of current smokers. They also exhibited higher fat-free mass, higher blood pressures, and a higher prevalence of comorbidities. Additionally, these individuals had higher concentrations of CRP, NLR, triglycerides, UACR, and uric acid (Supplementary Table S2).

Association of eGFR discordance with all-cause mortality

With a median follow-up of 13.7 years, a total of 26,465 deaths were observed. Mortality varied significantly across groups of eGFR discordance. Using CKD-EPI equations, at baseline, 50,414 (15.5%) participants had lower $eGFR_{cys}$, and 27,732 (8.5%) had higher $eGFR_{cys}$. Using EKFC equations, the number of participants with lower $eGFR_{cys}$ was reduced to 24,076 (7.4%), and those with higher $eGFR_{cys}$ was slightly reduced to 23,836 (7.3%). Regardless of eGFR equations, mortality rate per 1,000 patient years was substantially higher in individuals with lower discordant $eGFR_{cys}$ than in those with concordant or higher $eGFR_{cys}$ (Table 7-2).

Table 7-2 Mortality rate of the study population, stratified by eGFR discordance categories.

	Population size (%)	**Mortality case (%)**	**Mortality rate, per 1000 person-years**
Overall	325356 (100)	26465 (8.1)	6.06
CKD-EPI equation[a]			
eGFR absolute difference, mL/min/1.73m^2			
< -15	50414 (15.5)	7216 (14.3)	10.93
-15 to 15	247210 (76.0)	18068 (7.3)	5.42
≥ 15	27732 (8.5)	1181 (4.3)	3.14
$eGFR_{cys}/eGFR_{cr}$			
< 0.6	1354 (0.4)	445 (32.9)	27.64
≥ 0.6	324002 (99.6)	26020 (8.0)	5.98

EKFC equation			
eGFR absolute difference, mL/min/1.73m^2			
< -15	24076 (7.4)	3770 (15.7)	12.03
-15 to 15	277444 (85.3)	21620 (7.8)	5.79
≥ 15	23836 (7.3)	1075 (4.5)	3.33
$eGFR_{cys}/eGFR_{cr}$			
< 0.6	324 (0.1)	131 (40.4)	35.78
≥ 0.6	325189 (99.9)	26344 (8.1)	6.03

[a]$eGFR_{cr}$ was calculated using the CKD-EPI 2009 race-dependent equation; $eGFR_{cys}$ was calculated using the CKD-EPI 2012 equation. eGFR absolute difference was calculated as $eGFR_{cys}$ - $eGFR_{cr}$.

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; EKFC, European Kidney Function Consortium

There were consistent significant associations between eGFR discordance and mortality in all the four models, regardless of CKD-EPI or EKFC equations. Using CKD-EPI equations, in Model 4, those with lower $eGFR_{cys}$ had a 53% higher risk of mortality (HR=1.53, 95%CI: 1.48, 1.57) compared to those with concordant eGFR. Those with higher $eGFR_{cys}$ had a 30% lower risk of mortality (HR=0.70, 95%CI: 0.66, 0.75). Higher absolute difference in eGFR was associated with 24% lower mortality risk per one SD (HR=0.76, 95%CI: 0.75, 0.77). Those with $eGFR_{cys}$ < 60% $eGFR_{cr}$, had more than double the risk of mortality compared to those with higher $eGFR_{cys}$ (HR=2.25, 95%CI: 2.04, 2.47). The associations between eGFR discordance identified through EKFC equations and mortality had similar effect sizes as those using the CKD-EPI equations (Table 7-3, Figure 7-1).

Table 7-3 Linear association of eGFR discordance with all-cause mortality

	Model 1		Model 2		Model 3		Model 4	
	HR (95%CI)	**P-value**	**HR (95%CI)**	**P-value**	**HR (95%CI)**	**P-value**	**HR (95%CI)**	**P-value**
CKD-EPI equation[a]								
eGFR absolute difference, mL/min/1.73m^2								
< -15	1.58 (1.54, 1.63)	P<0.001	1.58 (1.54, 1.63)	P<0.001	1.53 (1.48, 1.57)	P<0.001	1.53 (1.48, 1.57)	P<0.001
-15 to 15	Ref.		Ref.		Ref.		Ref.	
≥ 15	0.68 (0.64, 0.72)	P<0.001	0.68 (0.64, 0.72)	P<0.001	0.70 (0.66, 0.75)	P<0.001	0.70 (0.66, 0.75)	P<0.001
Continuous: per +1 SD	0.75 (0.73, 0.76)	P<0.001	0.75 (0.73, 0.76)	P<0.001	0.76 (0.75, 0.77)	P<0.001	0.76 (0.75, 0.77)	P<0.001
$eGFR_{cys}/eGFR_{cr}$								
< 0.6	2.48 (2.26, 2.73)	P<0.001	2.48 (2.26, 2.73)	P<0.001	2.24 (2.04, 2.47)	P<0.001	2.25 (2.04, 2.47)	P<0.001
≥ 0.6	Ref.		Ref.		Ref.		Ref.	
EKFC equation								
eGFR absolute difference, mL/min/1.73m^2								
< -15	1.82 (1.75, 1.88)	P<0.001	1.81 (1.75, 1.88)	P<0.001	1.73 (1.67, 1.80)	P<0.001	1.73 (1.67, 1.80)	P<0.001
-15 to 15	Ref.		Ref.		Ref.		Ref.	
≥ 15	0.73 (0.68, 0.78)	P<0.001	0.73 (0.68, 0.78)	P<0.001	0.75 (0.71, 0.81)	P<0.001	0.75 (0.71, 0.81)	P<0.001
Continuous: per +1 SD	0.71 (0.70, 0.72)	P<0.001	0.71 (0.70, 0.72)	P<0.001	0.73 (0.71, 0.74)	P<0.001	0.73 (0.71, 0.74)	P<0.001
$eGFR_{cys}/eGFR_{cr}$								
< 0.6	3.41 (2.87, 4.06)	P<0.001	3.41 (2.87, 4.05)	P<0.001	3.05 (2.57, 3.63)	P<0.001	3.05 (2.57, 3.63)	P<0.001
≥ 0.6	Ref.		Ref.		Ref.		Ref.	

[a]$eGFR_{cr}$ was calculated using the CKD-EPI 2009 race-dependent equation; $eGFR_{cys}$ was calculated using the CKD-EPI 2012 equation. eGFR absolute difference was calculated as $eGFR_{cys}$ - $eGFR_{cr}$.

Model 1 was adjusted for age, sex, race, Townsend deprivation index, smoking status, Body Mass Index, systolic blood pressure, diastolic blood pressure, hypertension, angina, heart failure, myocardial infarction, stroke, diabetes, asthma, chronic bronchitis, chronic obstructive pulmonary disease, cancer, HDL-cholesterol, LDL-cholesterol, triglyceride, neutrophile to lymphocyte ratio, baseline eGFRcr, urine albumin-creatinine ratio, weekly physical activity. Model 2 was adjusted for confounders in Model 1 and factors that affect SCr, namely appendicular lean soft tissue mass/Height^2, the use of cimetidine, co-trimoxazole, fibrate, and trimethoprim. Model 3 was adjusted for confounders in Model 1 and factors that affect CysC, namely thyroid disease, C-reactive protein, use of steroids, and uric acid. Model 4 was the final model which was adjusted for all the confounding factors in Model 1, 2, and 3.

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; EKFC, European Kidney Function Consortium

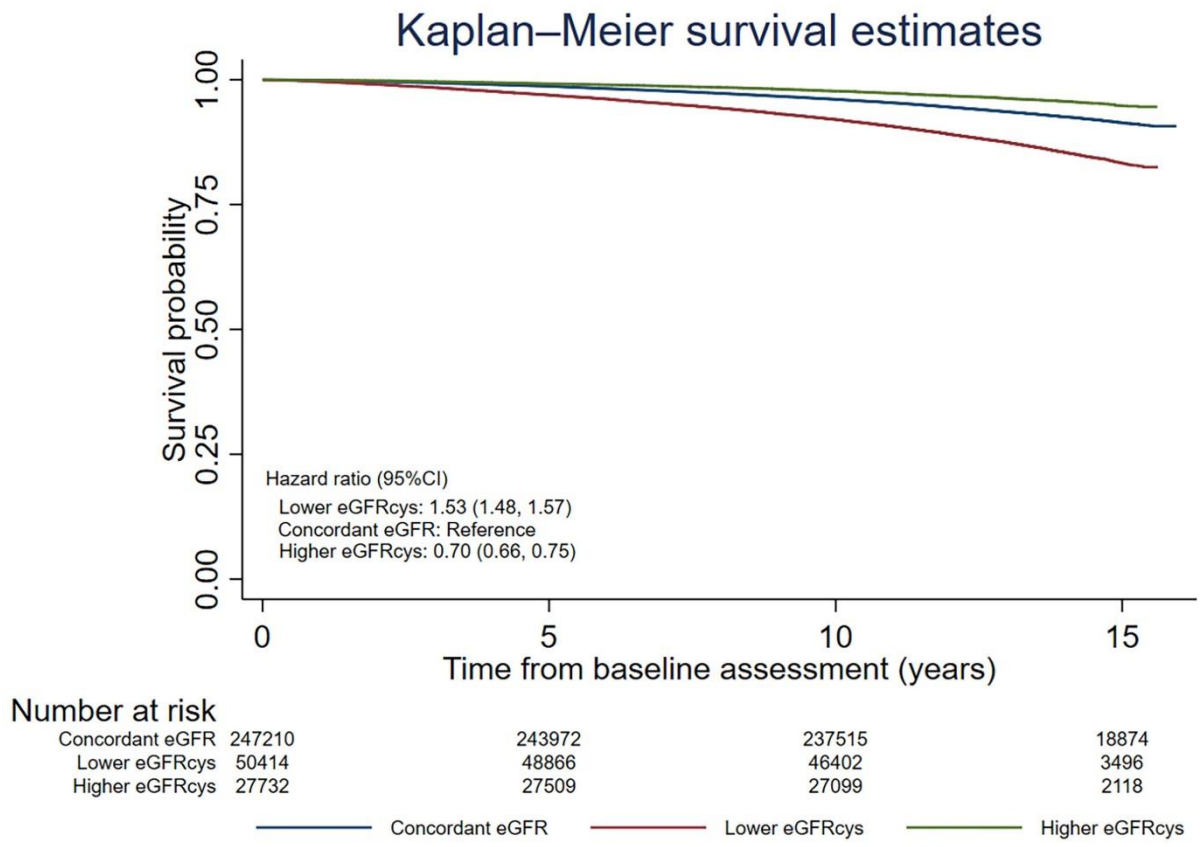


Figure 7-1. Kaplan–Meier survival estimates graph.

Note: Stratified by eGFR discordance categories identified using the CKD-EPI 2009 race-independent $eGFR_{cr}$ equation and the CKD-EPI 2012 $eGFR_{cys}$ equation. Concordant eGFR: $eGFR_{cys} - eGFR_{cr}$ is between −15 and 15 mL/min/1.73 m^2. Lower eGFRcys: $eGFR_{cys} - eGFR_{cr} < -15$ mL/min/1.73 m^2. Higher eGFRcys: $eGFR_{cys} - eGFR_{cr} \geq 15$ mL/min/1.73 m^2.

Stratified analyses

Using data from CKD-EPI equations, there were significant interaction effects of age (cutoff: 65 years) and BMI (cutoff: 30 kg/m^2) regardless of how eGFR discordance was evaluated. The association of eGFR discordance with mortality in those aged 65 years or above was attenuated, compared to their younger counterparts. The same phenomenon was observed in participants with BMI below and over 30kg/m^2. The strong (*P* for interaction<0.001) interaction effect of ALST/$Height^2$ and baseline CKD stages was observed only on the association of eGFR absolute difference with mortality. The magnitude of the studied association was attenuated in those with a higher ALST/$height^2$, and enhanced in those with an advanced stage of baseline CKD stage. No strong

interaction of discordance with mortality was observed by CRP (cutoff: 2mg/L). No significant interactions were by sex or ethnicity (Supplementary Table S3).

Sensitivity analyses

In the two-year landmark analysis, the association between eGFR discordance and mortality was still strongly significant using both CKD-EPI and EKFC equations (Supplementary Table S4).

7.5. Discussion

This study found that eGFR discordance, with lower $eGFR_{cys}$, has a prevalence of 7.4~15.5% in the UK Biobank general population study. At baseline, participants with lower $eGFR_{cys}$ (i.e., $eGFR_{cys}$ - $eGFR_{cr}$<-15 ml/min/1.73m^2 or $eGFR_{cys}$< 60% $eGFR_{cr}$) had elevated mortality risk: an association that persisted after adjustment for a range of common confounders. Conversely, those with $eGFR_{cys}$ - $eGFR_{cr}$≥ 15 ml/min/1.73m^2 or $eGFR_{cys}$ ≥60% eGFRcr had lower mortality. Consistent interaction was identified by age and BMI. Sensitivity analyses showed results similar to the primary analysis.

Our research findings expand those of previous studies targeting specific patient groups or post-surgical populations, generalizing the finding to a large unrestricted general population, and subgroups, even after adjusting for inflammation and anthropometric measurements. [20, 32-34] One study involving 3,993 patients who underwent elective cardiac surgery found that patients with $eGFR_{cys}$< 60% $eGFR_{cr}$ have a 96% higher risk of mortality (HR=1.96, 95%CI: 1.63, 2.36).[34] Another longitudinal study of participants with diabetes conducted in China, the United States, and the United Kingdom showed a lower risk of mortality when $eGFR_{cys}$ - $eGFR_{cr}$ was increased.[32]

The occurrence of discordantly low $eGFR_{cys}$ may be due to an increase in CysC levels resulting in a decreased $eGFR_{cys}$, a decrease in SCr levels leading to an increased $eGFR_{cr}$, or a combination of both. Known factors that can cause elevated CysC concentrations include obesity and inflammation, while reduced muscle mass, decreased physical activity, and dietary changes (e.g., reduced meat dietary intake) can lead to lower SCr concentrations.[2] All the factors above have been linked to mortality.[35-37]

Participants with BMI≥30 kg/m^2 showed a smaller magnitude of association, and obesity had a significant interaction (*P* for interaction=0.001). This is similar to the findings of He et al., although they did not observe the interaction effect of obesity.[32] In terms of skeletal muscle mass, the interaction effect of ALST/$Height^2$ was observed in eGFR absolute differences. As ALST/$Height^2$ increases, the effect size was attenuated towards the null in participants with an eGFR absolute difference of <−15 ml/min. To be specific, HR decreased from 1.62 (95%CI 1.51, 1.75) to 1.39 (95%CI: 1.30, 1.49) as ALST/$Height^2$ increases. No clear trends were observed in those with higher $eGFR_{cys}$. Because serum creatinine is almost solely produced from muscle, an increase in muscle mass can lead to a reduction in $eGFR_{cr}$ but it does not indicate kidney impairment. Therefore, the negative discordance between eGFR measurements and the elevated mortality risk may not be solely attributed to kidney-related factors. Instead, non-kidney-related factors, such as muscle mass, may play a significant role.

Another cause of eGFR discordance may be the reduction in the diameter of the pores on the glomerular membrane. This reduction blocks the filtration of larger molecules like CysC, resulting in increased CysC concentrations in the body, while smaller molecules like creatinine remain unaffected. Consequently, this results in a decrease in $eGFR_{cys}$ while maintaining $eGFR_{cr}$, causing the observed eGFR discordance. However, the mechanism linking this pathological phenomenon to adverse outcomes remains unclear. Notably, a study that used both eGFR and iohexol-measured mGFR found that a lower $eGFR_{cys}$/$eGFR_{cr}$ ratio, but not mGFR, was a risk factor for all-cause mortality.[11] This further suggests that eGFR discordance may reflect the influence of non-GFR factors, rather than changes in the kidneys.

An enhancement in the magnitude of the association between negative eGFR absolute difference and mortality was observed across baseline CKD stages. The higher the CKD stage, the stronger the association. It may reflect the unstable and unhealthy status of the kidneys at advanced CKD stages.

Although there is no unified standard for defining eGFR discordance using the relative difference, this study, like most other studies, adopted $eGFR_{cys}$ < 60% $eGFR_{cr}$ as the criterion. However, less than 1% of this study population met the criterion. Conversely, the

proportion of $eGFR_{cys}$ - $eGFR_{cr}$<-15 ml/min/1.73m^2 was 15.5%. This indicated that using $eGFR_{cys}$ < 60% $eGFR_{cr}$ as the sole criterion may seriously underestimate eGFR discordance. Among those with $eGFR_{cys}$ - $eGFR_{cr}$<-15 ml/min/1.73m² in this study, the mean (SD) of $eGFR_{cys}$/$eGFR_{cr}$ was 0.76 (0.07), and this group covered 99.6% (1,348 out of 1,354) individuals with $eGFR_{cys}$ < 60% $eGFR_{cr}$. In the group with -15 ml/min/1.73m^2≤eGFR absolute difference<15 ml/min/1.73m^2, the mean (SD) of $eGFR_{cys}$/$eGFR_{cr}$ was 0.99 (0.09). Therefore, we suggest that determining the specific cut-off value for $eGFR_{cys}$/$eGFR_{cr}$ should be the focus of future research.

This was a very large and well-phenotyped population study, with systematically collected creatinine and cystatin-C measured at a central laboratory. Using UK Biobank allows additional adjustment for variables that are not included in all other datasets. We have found that the association persists even when factors that influence values of SCr and Cys C were accounted for. As most participants had an eGFR above 90 ml/min/1.73m^2, current clinical guidelines do not necessitate regular kidney function tests in this group. Consequently, eGFR discordance may be used as a screening tool to indicate patients who are at high risk of adverse outcomes.

However, several limitations should be noted. The UK Biobank did not collect measured GFR, preventing researchers from distinguishing between truly normal and diseased kidney populations. Given that eGFR and mGFR can differ by up to 30%, this study cannot address whether eGFR discordance indicates parenchymal kidney disease. The prevalence and implications of eGFR discordance may differ in more diverse populations. Future studies with more ethnically and demographically varied cohorts are warranted to better understand these patterns. Due to collinearity, we have not adjusted for $eGFR_{cr}$ and $eGFR_{cys}$ simultaneously. Therefore, we could not tell if the findings can be solely attributed to eGFR difference. In addition, as we used the EKFC and race-dependent CKD-EPI equations which are recommended for European populations, our findings may not fully reflect patterns observed when using the 2021 CKD-EPI equation without race, which has been increasingly adopted in North America. Future studies in North American populations applying race-free equations are warranted to assess the generalizability of our findings.

This study found that nearly one fifth of the general population of UK Biobank has discordantly lower $eGFR_{cys}$. More importantly, there was a significant association between substantial eGFR discordance (i.e., $eGFR_{cys}$ - $eGFR_{cr}$ < -15 ml/min/1.73m^2 or $eGFR_{cys}$ <60% $eGFR_{cr}$) and higher all-cause mortality in the general population. The study also highlighted that age and BMI≥30 kg/m^2 significantly influenced the association between eGFR discordance and mortality. Our results suggest that, considering the high prevalence of eGFR discordance and its strong association with mortality, eGFR discordance may be a potential indicator for risk stratification.

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7.7. Supplementary tables and figures for Chapter 7

Supplementary Table S1 Identification code of drugs in the UK Biobank

Type	ID	Product name
Cimetidine		
	1140865426	cimetidine
	1140879402	cimetidine+alginate
	1140909500	tagamet 100 tablet
Fibrate		
	1140861858	lopid 300 capsule
	1140861924	bezafibrate
	1140861926	bezalip 200mg tablet
	1140861928	bezalip-mono 400mg m/r tablet
	1140861944	clofibrate
	1140861954	fenofibrate
	1140862026	ciprofibrate
	1141157260	bezafibrate product
Statin		
	1140861848	colestid 5g/sachet granules
	1140861868	nicotinic acid product
	1140861884	maxepa 1g capsule
	1140861892	acipimox
	1140861894	olbetam 250mg capsule
	1140861936	questran 4g/sachet powder
	1140861942	cholestyramine+aspartame 4g/sachet powder
	1140861958	simvastatin
	1140864592	lescol 20mg capsule
	1140865576	cholestyramine
	1140881748	zocor 10mg tablet
	1140888590	colestipol
	1140888594	fluvastatin
	1140888648	pravastatin
	1141146138	lipitor 10mg tablet
	1141146234	atorvastatin

	1141157416	cholestyramine product
	1141162544	lipantil micro 67mg capsule
	1141172214	supralip 160mg m/r tablet
	1141181868	omacor 1g capsule
	1141188546	niaspan 500mg m/r tablet
	1141192414	crestor 10mg tablet
	1141192736	ezetimibe
	1141192740	ezetrol 10mg tablet
	1141200040	zocor heart-pro 10mg tablet
Steroids		
	1140874930	prednisolone
	1140874976	methylprednisolone
	1140883026	methylprednisolone+neomycin
	1141157402	prednisolone product
Trimethoprim and co-trimoxazole		
	1140857180	bactrim paediatric 120mg tablet
	1140857182	bactrim 960mg/3ml intramuscular injection
	1140857184	bactrim 480mg/5ml intravenous infusion
	1140873780	co-trimoxazole
	1140873798	bactrim 480mg tablet
	1140873856	bactrim 480mg dispersible tablet
	1140873858	bactrim 960mg tablet
	1140873860	bactrim adult suspension
	1140873862	bactrim paediatric s/f syrup
	1140873876	monotrim 100mg tablet
	1140873878	monotrim 200mg tablet
	1140873880	monotrim 50mg/5ml s/f suspension
	1140873882	monotrim 100mg/5ml injection
	1140873966	trimethoprim

Supplementary Table S2 Baseline characteristics of the study population, stratified by EKFC eGFR discordance categories.

Characteristics	eGFR absolute difference[a]			$eGFR_{cys}/eGFR_{cr}$	
	Lower $eGFR_{cysc}$	Concordant eGFR	Higher $eGFR_{cysc}$	<0.6	≥ 0.6
Size, n(row%)	24076 (7.4)	277444 (85.3)	23836 (7.3)	324 (0.1)	325032 (99.9)
Age, year	55.7±7.9	56.9±8.1	54.2±8.5	58.4±7.5	56.6±8.1
Male, n(%)	17046 (70.8)	129789 (46.8)	5734 (24.1)	223 (68.8)	152346 (46.9)
Race, n(%)					
White	22460 (93.3)	265277 (95.6)	21741 (91.2)	306 (94.4)	309172 (95.1)
Black	118 (0.5)	3119 (1.1)	1381 (5.8)	0 (0.0)	4618 (1.4)
South Asian	980 (4.1)	3446 (1.2)	139 (0.6)	14 (4.3)	4551 (1.4)
Other	518 (2.2)	5602 (2.0)	575 (2.4)	4 (1.2)	6691 (2.1)
Smoke status, n(%)					
Never	10007 (41.6)	154283 (55.6)	14904 (62.5)	113 (34.9)	179081 (55.1)
Previous	7384 (30.7)	98612 (35.5)	7808 (32.8)	104 (32.1)	113700 (35.0)
Current	6685 (27.8)	24549 (8.8)	1124 (4.7)	107 (33.0)	32251 (9.9)
Body mass index, kg/m^2	29.8±5.8	27.0±4.4	26.2±3.9	29.6±6.6	27.2±4.5
Appendicular lean soft tissue, kg	25.4±5.7	22.2±5.2	20.5±4.5	24.9±5.9	22.3±5.3
ALST/$height^2$, kg/m^2	8.6±1.4	7.7±1.2	7.3±1.1	8.5±1.7	7.7±1.3
Systolic blood pressure, mmHg	140.0±18.2	138.2±18.6	133.4±18.3	137.7±18.9	138.0±18.6
Diastolic blood pressure, mmHg	84.4±10.5	82.2±10.1	80.1±9.8	81.6±11.8	82.2±10.1
Comorbidities, n(%)					
Hypertension	15073 (62.6)	147822 (53.3)	9921 (41.6)	198 (61.1)	172618 (53.1)
Angina	1067 (4.4)	8101 (2.9)	470 (2.0)	25 (7.7)	9613 (3.0)
Heart failure	68 (0.3)	334 (0.1)	32 (0.1)	1 (0.3)	433 (0.1)
Myocardial infarction	843 (3.5)	5759 (2.1)	317 (1.3)	26 (8.0)	6893 (2.1)
Stroke	482 (2.0)	3478 (1.3)	227 (1.0)	10 (3.1)	4177 (1.3)
Diabetes	2176 (9.0)	12542 (4.5)	580 (2.4)	50 (15.4)	15248 (4.7)
Thyroid disease	1373 (5.7)	15276 (5.5)	1586 (6.7)	30 (9.3)	18205 (5.6)
Asthma	3004 (12.5)	30947 (11.2)	2663 (11.2)	40 (12.3)	36574 (11.3)
Chronic bronchitis	572 (2.4)	3160 (1.1)	187 (0.8)	17 (5.2)	3902 (1.2)
COPD	154 (0.6)	719 (0.3)	26 (0.1)	6 (1.9)	893 (0.3)
Cancer	2035 (8.5)	23749 (8.6)	1933 (8.1)	46 (14.2)	27671 (8.5)
On-use medicine, n(%)					
Statin	4689 (19.5)	43180 (15.6)	2700 (11.3)	79 (24.4)	50490 (15.5)
TMP	20 (0.1)	247 (0.1)	69 (0.3)	1 (0.3)	335 (0.1)
Cimetidine	17 (0.1)	189 (0.1)	30 (0.1)	1 (0.3)	235 (0.1)
Fibrates	71 (0.3)	692 (0.2)	56 (0.2)	0 (0.0)	819 (0.3)
Steroids	329 (1.4)	1298 (0.5)	64 (0.3)	15 (4.6)	1676 (0.5)
C-reactive protein, mg/L	1.1 (1.0, 1.1)	0.9 (0.8, 1.0)	0.8 (0.8, 0.9)	1.5 (1.4, 1.6)	0.9 (0.8, 1.0)
Neutrophil-to-lymphocyte ratio	2.5±1.6	2.3±1.2	2.3±1.0	3.0±2.8	2.3±1.2
Serum creatinine, mg/dL	0.80±0.13	0.81±0.19	0.96±0.17	0.81±0.19	0.82±0.19
Cystatin C, mg/L	1.1±0.1	0.9±0.2	0.8±0.1	1.6±0.4	0.9±0.2
HDL cholesterol, mg/dL	47.8±12.5	56.8±14.7	61.3±15.1	42.9±12.2	56.4±14.8
LDL cholesterol, mg/dL	137.1±34.7	137.9±33.3	133.9±32.2	124.8±36.9	137.6±33.3
Triglycerides, mg/dL	193.8±108.9	150.4±86.8	130.2±74.9	192.2±102.3	152.1±88.7
Urine albumin-creatinine ratio	5.9 (3.6, 10.5)	6.1 (3.7, 10.1)	5.3 (3.3, 8.7)	7.9 (4.4, 17.5)	6.0 (3.7, 9.9)
Uric acid, mg/dL	5.8±1.3	5.1±1.3	5.0±1.2	6.1±1.4	5.2±1.3

CKD-EPI eGFR equation[b]					
$eGFR_{cr}$, ml/min/1.73m^2	97.0±9.9	91.6±12.5	74.7±11.3	94.0±16.3	90.9±13.1
$eGFR_{cys}$, ml/min/1.73m^2	71.1±12.0	89.8±15.3	96.0±12.3	44.7±10.9	88.9±15.8
eGFR absolute difference, ml/min/1.73m^2	-25.0 (-29.5, -21.0)	-1.6 (-8.9, 5.0)	20.2 (15.5, 25.9)	-47.9 (-53.6, -43.9)	-1.6 (-10.4, 6.2)
$eGFR_{cys}/eGFR_{cr}$	0.7±0.1	1.0±0.1	1.3±0.1	0.5±0.1	1.0±0.2
EKFC eGFR equation					
$eGFR_{cr}$, ml/min/1.73m^2	92.6±10.3	87.4±12.4	72.3±11.2	89.0±14.6	86.6±12.9
$eGFR_{cys}$, ml/min/1.73m^2	72.0±10.8	87.0±12.4	93.3±10.7	48.3±10.0	86.4±12.9
eGFR absolute difference, ml/min/1.73m^2	-19.1 (-22.8, -16.8)	-0.5 (-5.0, 4.1)	19.6 (17.0, 23.7)	-39.9 (-44.8, -35.6)	-0.5 (-6.1, 5.3)
$eGFR_{cys}/eGFR_{cr}$	0.8±0.1	1.0±0.1	1.3±0.1	0.5±0.1	1.0±0.1
Weekly physical activity, MET	1653 (695, 3750)	1864 (855, 3759)	1935 (942, 3726)	1427 (693, 3566)	1851 (840, 3759)
Townsend deprivation index	-1.1 (-3.1, 2.1)	-2.3 (-3.7, 0.3)	-2.3 (-3.7, 0.1)	-0.1 (-2.9, 2.9)	-2.3 (-3.7, 0.4)

[a]eGFR absolute difference was calculated as $eGFR_{cys}$ - $eGFR_{cr}$.

[b]$eGFR_{cr}$ was calculated using the CKD-EPI 2009 race-independent equation; $eGFR_{cys}$ was calculated using the CKD-EPI 2012 equation.

Lab SI units conversions: serum creatinine: multiply the value in mg/dL by 88.4 to convert to µmol/L; HDL cholesterol and LDL cholesterol: multiply the value in mg/dL by 0.0259 to convert to mmol/L; triglycerides: multiply the value in mg/dL by 0.0113 to convert to mmol/L; uric acid: multiply the value in mg/dL by 59.48 to convert to µmol/L

ALST, Appendicular lean soft tissue; BMI, Body mass index, CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; COPD, Chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; EKFC, European Kidney Function Consortium; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; TMP, Trimethoprim

Supplementary Table S3 Subgroup analysis of the association of CKD-EPI eGFR discordance with mortality

	Male	**Female**	**CRP< 2mg/L**	**CRP≥2mg/L**
	HR (95%CI), P-value	**HR (95%CI), P-value**	**HR (95%CI), P-value**	**HR (95%CI), P-value**
Sample size	152569	172787	108467	216889
eGFR absolute difference, mL/min/1.73m^2				
< -15	1.58 (1.51, 1.63), P<0.001	1.46 (1.40, 1.54), P<0.001	1.48 (1.42, 1.55), P<0.001	1.52 (1.46, 1.58), P<0.001
-15 to 15	Ref.	Ref.	Ref.	Ref.
≥ 15	0.70 (0.65, 0.76), P<0.001	0.70 (0.63, 0.76), P<0.001	0.74 (0.69, 0.79), P<0.001	0.70 (0.63, 0.78), P<0.001
P for interaction	0.05		0.59	
$eGFR_{cys}/eGFR_{cr}$				
< 0.6	2.37 (2.10, 2.67), P<0.001	1.96 (1.67, 2.28), P<0.001	2.52 (2.11, 3.02), P<0.001	2.11 (1.88, 2.36), P<0.001
≥ 0.6	Ref.	Ref.	Ref.	Ref.
P for interaction	0.17		0.04	

	Age<65 years	**Age≥65 years**	**BMI<30 kg/m^2**	**BMI≥30 kg/m^2**
	HR (95%CI), P-value	**HR (95%CI), P-value**	**HR (95%CI), P-value**	**HR (95%CI), P-value**
Sample size	262360	62996	251916	73440
eGFR absolute difference, mL/min/1.73m^2				
< -15	1.62 (1.56, 1.69), P<0.001	1.40 (1.34, 1.47), P<0.001	1.55 (1.50, 1.61), P<0.001	1.42 (1.35, 1.49), P<0.001
-15 to 15	Ref.	Ref.	Ref.	Ref.
≥ 15	0.70 (0.65, 0.76), P<0.001	0.74 (0.67, 0.82), P<0.001	0.71 (0.67, 0.76), P<0.001	0.74 (0.65, 0.85), P<0.001
P for interaction	<0.001		0.001	
$eGFR_{cys}/eGFR_{cr}$				
< 0.6	2.23 (1.96, 2.54), P<0.001	2.20 (1.91, 2.53), P<0.001	2.48 (2.18, 2.81), P<0.001	1.86 (1.60, 2.15), P<0.001
≥ 0.6	Ref.	Ref.	Ref.	Ref.
P for interaction	<0.001		<0.001	

	Black	**Non-black**
	HR (95%CI), P-value	**HR (95%CI), P-value**
Sample size	320738	4618
eGFR absolute difference, mL/min/1.73m^2		
< -15	1.44 (1.04, 1.99), P=0.03	1.53 (1.48, 1.57), P<0.001
-15 to 15	Ref.	Ref.
≥ 15	0.71 (0.41, 1.22), P=0.22	0.70 (0.66, 0.75), P<0.001
P for interaction	0.99	
$eGFR_{cys}/eGFR_{cr}$		
< 0.6	1.78 (0.81, 3.91), P=0.15	2.30 (2.08, 2.53), P<0.001
≥ 0.6	Ref.	Ref.
P for interaction	0.88	

	CKD Stage 1 HR (95%CI), P-value	CKD Stage 2 HR (95%CI), P-value	CKD Stage 3a HR (95%CI), P-value	CKD Stage 3b/4/5 HR (95%CI), P-value
Sample size	201658	117825	4951	922
eGFR absolute difference, mL/min/1.73m^2				
< -15	1.51 (1.45, 1.57), P<0.001	1.57 (1.49, 1.65), P<0.001	2.06 (1.66, 2.55), P<0.001	2.24 (0.88, 5.71), P=0.09
-15 to 15	Ref.	Ref.	Ref.	Ref.
≥ 15	0.78 (0.66, 0.92), P=0.003	0.73 (0.68, 0.78), P=0.003	0.65 (0.53, 0.79), P=0.003	0.36 (0.20, 0.64), P<0.001
P for interaction			0.01	
$eGFR_{cys}/eGFR_{cr}$				
< 0.6	2.07 (1.82, 2.37), P<0.001	2.31 (1.98, 2.69), P<0.001	2.14 (1.42, 3.23), P<0.001	2.30 (1.14, 4.68), P=0.02
≥ 0.6	Ref.	Ref.	Ref.	Ref.
P for interaction			0.89	

	ALST/Height^2 <1SD HR (95%CI), P-value	ALST/Height^2 ±1SD HR (95%CI), P-value	ALST/Height^2 >1SD HR (95%CI), P-value
Sample size	41151	238428	45777
eGFR absolute difference, mL/min/1.73m^2			
< -15	1.62 (1.51, 1.75), P<0.001	1.49 (1.44, 1.55), P<0.001	1.39 (1.30, 1.49), P<0.001
-15 to 15	Ref.	Ref.	Ref.
≥ 15	0.73 (0.62, 0.86), P<0.001	0.70 (0.65, 0.75), P<0.001	0.83 (0.70, 0.98), P=0.003
P for interaction		<0.001	
$eGFR_{cys}/eGFR_{cr}$			
< 0.6	2.05 (1.64, 2.56), P<0.001	2.22 (1.94, 2.54), P<0.001	2.02 (1.70, 2.41), P<0.001
≥ 0.6	Ref.	Ref.	Ref.
P for interaction		0.11	

$eGFR_{cr}$ was calculated using the CKD-EPI 2009 race-dependent equation; $eGFR_{cys}$ was calculated using the CKD-EPI 2012 equation. eGFR absolute difference was calculated as $eGFR_{cys}$ - $eGFR_{cr}$. ALST, appendicular lean soft tissue; BMI, body mass index

Supplementary Table S4 Two-year landmark analysis on the linear association of eGFR discordance with mortality (N=321681).

	HR (95%CI), P-value
CKD-EPI equation[a]	
eGFR absolute difference, mL/min/1.73m^2	
< -15	1.48 (1.43, 1.53), P<0.001
-15 to 15	Ref.
≥ 15	0.71 (0.66, 0.75), P<0.001
Continuous: per +1 SD	0.77 (0.76, 0.78), P<0.001
$eGFR_{cys}/eGFR_{cr}$	
< 0.6	2.12 (1.90, 2.36), P<0.001
≥ 0.6	Ref.
EKFC equation	
eGFR absolute difference, mL/min/1.73m^2	
< -15	1.66 (1.60, 1.73), P<0.001
-15 to 15	Ref.
≥ 15	0.76 (0.71, 0.81), P<0.001
Continuous: per +1 SD	0.74 (0.73, 0.75), P<0.001
$eGFR_{cys}/eGFR_{cr}$	
< 0.6	2.96 (2.43, 3.61), P<0.001
≥ 0.6	Ref.

[a]$eGFR_{cr}$ was calculated using the CKD-EPI 2009 race-dependent equation; $eGFR_{cys}$ was calculated using the CKD-EPI 2012 equation. eGFR absolute difference was calculated as $eGFR_{cys}$ - $eGFR_{cr}$.

Models was adjusted for age, sex, race, Townsend deprivation index, smoking status, Body Mass Index, systolic blood pressure, diastolic blood pressure, hypertension, angina, heart failure, myocardial infarction, stroke, diabetes, asthma, chronic bronchitis, chronic obstructive pulmonary disease, cancer, HDL-cholesterol, LDL-cholesterol, triglyceride, neutrophile to lymphocyte ratio, baseline eGFRcr, urine albumin-creatinine ratio, weekly

physical activity, appendicular lean soft tissue mass/height^2, use of cimetidine, use of co-trimoxazole, use of fibrate, use of trimethoprim, thyroid disease, C-reactive protein, use of steroids, and uric acid.

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; EKFC, European Kidney Function Consortium

8. Chapter 8 – Fixed-dose vs loose-dose combination antidiabetic therapy and cardiorenal outcomes in type 2 diabetes: A nationwide comparative effectiveness study

The original article of this chapter has been presented in the form of late-breaking abstract #31 at the 61st EASD Annual Meeting on September 19, 2025.

The original article of this chapter has been published in Cardiovascular Diabetology on September 23, 2025.

Liu Q, Welsh P, Celis-Morales C, Lees JS, Mark PB, Pazzagli L. Fixed-dose vs loose-dose combination antidiabetic therapy and cardiorenal outcomes in type 2 diabetes: a nationwide comparative effectiveness study. Cardiovasc Diabetol. 2025 Sep 23;24(1):365. doi: 10.1186/s12933-025-02936-w.

8.1. Abstract

Background

Combination therapy is gaining attention in type 2 diabetes management due to its potential to reach glucose goals within a shorter period. However, the long-term comparative cardiorenal effectiveness of fixed- versus loose-dose combinations remains unclear. This study aimed to assess whether oral antidiabetic fixed-dose combination (FDC) therapy is associated with improved cardiorenal outcomes in adults with type 2 diabetes compared with loose-dose combination (LDC) therapy. A secondary objective was to evaluate the mediating role of medication adherence in these associations.

Methods

This population-based, new-user, active-comparator cohort study used Swedish national registers. Propensity score matching without replacement was applied. Study outcomes included acute myocardial infarction, atrial fibrillation, unstable angina, heart failure, ischaemic stroke, and eGFR<30 ml/min/1.73m^2. Associations with cardiorenal outcomes were assessed using Cox regression. Adherence was defined as the proportion of days covered>80% during the first year.

Results

The median follow-up time was 4.0 years for cardiovascular outcomes and 3.8 years for kidney outcomes. In the matched cohort (mean age 62 years; 67% male), FDC users had higher treatment adherence (68.6% vs. 46.5%). FDC was associated with a lower rate of heart failure (HR=0.88; 95%CI: 0.79, 0.99), with adherence mediating 47% of this association. In people aged ≥65 years, FDC was associated with a lower rate of heart failure (HR=0.79; 95%CI: 0.69, 0.91). The observed association was attenuated with further matching for diabetes duration or when drugs were matched at the ATC code level. No associations between FDC use and other outcomes were identified.

Conclusions

FDC therapy in people with type 2 diabetes was associated with a lower rate of heart failure, particularly in older adults. Higher medication adherence appeared to mediate nearly half of this association.

8.2. Background

The 2024 and 2025 American Diabetes Association (ADA) Standards of Care in Diabetes recommend early combination therapy for type 2 diabetes patients. [1, 2] This approach is preferred over stepwise addition, as it offers faster attainment of glycaemic goals and longer durability of the glycaemic effect. [1, 2] However, while the guidelines promote early combination therapy, they do not specify the optimal formulation, i.e. fixed-dose combination (FDC) versus loose-dose combination (LDC). This study aims to address this research gap by comparing the two formulations.

As a form of combination therapy, FDC refers to combining two or more active ingredients into a single dosage form, while LDC refers to administering the same agents as separate pills. FDC may offer several advantages over LDC, not only by improving medication adherence and reducing the risk of adverse events [3-6], but also by lowering pill burden and simplifying treatment regimens. Additionally, FDC may benefit patients by reducing overall treatment costs and improving dose flexibility, which are important factors influencing treatment satisfaction and long-term outcomes.[5, 7, 8] These benefits make FDC a compelling option for long-term diabetes management, especially for patients undergoing polypharmacy. However, LDC may be preferred in certain cases, such as when flexible dose titration is needed or when patients experience side effects from one component of the combination. The choice between FDC and LDC needs to be individualized based on patient characteristics, treatment goals, and tolerability.

Since 2010, the number of approved FDCs has increased, most of which are metformin-based dual-agent combinations. [9] In Sweden, 14 non-insulin oral antidiabetic FDCs are currently available, with 71% (10 out of 14) being metformin-based. [10] Among these, four combine metformin with dipeptidyl peptidase 4 inhibitors (DPP4i), four are metformin with sodium–glucose cotransporter 2 inhibitors (SGLT2i), and the remaining two combine metformin with thiazolidinedione (TZD).

In Sweden, FDCs account for 4.2% of all prescribed non-insulin antidiabetic drugs filled at pharmacies as of 2024. [11] Among patients receiving combination therapy, FDC use reaches 37%~45% in Japan and 54% in the United States. [4, 12]

Current research on FDCs has largely focused on short-term glucose-lowering effects, demonstrating greater reductions in haemoglobin A1c (HbA1c) and fasting plasma glucose. [13, 14] However, the focus of diabetes management has extended from glycaemic control to cardiorenal protection. Hence, the associations of FDCs with long-term cardiorenal outcomes remain unexplored.

The objective of this study is to investigate whether, in adults with type 2 diabetes receiving routine clinical care, FDCs compared with LDCs are associated with lower risks of cardiovascular and kidney outcomes, using a propensity score matched cohort study design.

8.3. Methods

Study design and population

This study used a new-user active-comparator study design. The study population included individuals with type 2 diabetes who initiated metformin monotherapy between July 1, 2005, and September 30, 2021. Information on filled prescriptions was extracted from the Swedish Prescribed Drug Register (PDR) [15]. The PDR register contains nationwide information on all filled prescriptions in Sweden from July 1, 2005. Prescribed drugs were identified through Anatomical Therapeutic Chemical (ATC) codes (eTable 1). Initiation of metformin therapy was identified by the first dispensed prescription of metformin. The diagnosis of type 2 diabetes was cross-checked using primary diagnosis records from the Swedish National Patient Register (NPR).[16] (Figure 8-1)

To ensure a history of metformin monotherapy, a 1-year washout period from the PDR launch was implemented. Patients with medication history of SGLT2i, DPP4i, or TZD within one year prior to the initiation of metformin monotherapy were excluded.

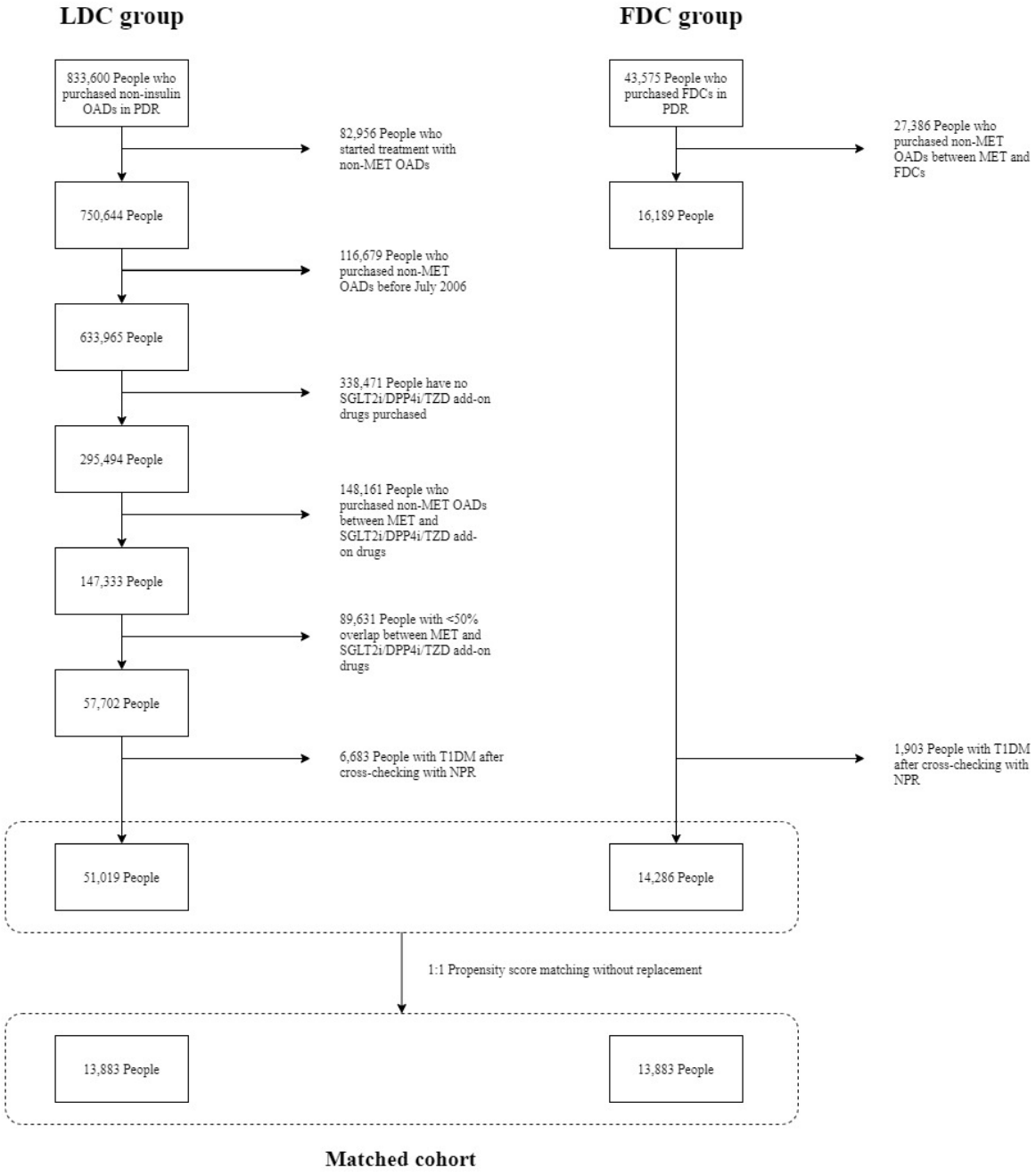

Figure 8-1. Study population selection flowchart

PDR, Swedish Prescribed Drug Register; MET, metformin; OAD, oral antidiabetic drug; SGLT2i, sodium-glucose cotransporter 2 inhibitor; DPP4i, dipeptidyl peptidase 4 inhibitor; TZD, thiazolidinedione; T1DM, Type 1 Diabetes Mellitus; NPR, the Swedish National Patient Register; FDC, fixed-dose combination; LDC, loose-dose combination

Comparator groups

The study exposure was the initiation of FDC drugs, defined as the first filled prescription of the FDC. FDCs were restricted to combinations of metformin with either an SGLT2i, a DPP4i, or a TZD.

The comparator group of LDCs included individuals receiving metformin and an add-on drug concurrently to emulate the pharmacological exposure of FDC users. Concurrent use was defined as a ≥50% overlap time between the dispensing duration of metformin and that of a subsequent add-on drug. The dispensing duration was calculated as the number of packages dispensed multiplied by the number of defined daily dose (DDD) contained in each package. The second agent did not need to be started within a fixed number of days after the purchase of metformin to be considered part of LDC therapy. The relative criterion of 50% provided greater flexibility in identifying LDC therapy. This is because the overlap between metformin and the second agent may occur at the beginning, in the middle, or in the second half of the metformin dispensing duration. A fixed-value criterion would have likely misclassified valid LDC exposed individuals. By applying an intention-to-treat approach, subsequent changes to antidiabetic regimens (additions, discontinuations, or switches) did not alter exposure definition; i.e. follow-up continued regardless.

Study outcomes

The study outcome was the first incidence of cardiovascular or kidney outcomes during follow-up. The outcomes were selected based on prior studies on SGLT2i/DPP4i/TZD and clinical knowledge. [17-22]

The primary outcome included several cardiovascular outcomes, identified from the primary diagnosis in inpatient and specialised outpatient care in the NPR register using the International Classification of Diseases, 10th Revision (ICD-10) code, including unstable angina, myocardial infarction, atrial fibrillation, heart failure, and ischaemic stroke. (eTable 2)

The secondary outcome was kidney impairment, defined as a creatinine-based estimated glomerular filtration rate (eGFRcr) less than 30 ml/min/1.73m^2. The creatinine-based eGFR was collected from the Swedish National Diabetes Register (NDR). [23] In NDR,

creatinine measurements are collected from patient records during routine clinical visits at specialist clinics and primary care centres. The average interval between creatinine measurements in our study population is 6.7±4.1 months. A lag time of 90 days from the index date was used to avoid reverse causation bias and only events occurring after 90 days were considered. To avoid potential immortal time bias, a follow-up time of at least three months from the index treatment was imposed for all individuals in the study population and person time prior this was not included in the analysis.

Covariates

A total of 45 baseline covariates were selected, including sociodemographic characteristics [24], comorbidities, and medication history within one year prior to cohort entry, and were extracted from the NPR, PDR, and the Total Population Register (TPR).[25] Additionally, calendar year of cohort entry, cardiovascular or renal events within one year prior to cohort entry [26] and the drug class of the add-on drug/FDC (i.e., SGLT2i/DPP4i/TZD) were included. A detailed list of covariate definitions and data sources can be found in eTable 3. The directed acyclic graph for this study can be found in eFigure 1.

Calculation of medication adherence

A proxy for medication adherence was assessed using the proportion of days covered (PDC) by medications within 365 days from the index date. Individuals with a PDC greater than 80% were regarded as adherent. The index date was defined as the first day of purchasing the first add-on drug or the FDC. For the FDC group, the numerator was the number of days with the FDC available during the observation period. For the LDC group, the PDC needed to reflect the concurrent use of metformin and the add-on drug; thus, the numerator was the number of days with the add-on drug available during the observation period. Early refills and stockpiling were managed using a carry-over approach, in which overlapping days were shifted forward and the additional supply was appended to the end of the prior dispensing duration. If a refill occurred within the PDC observation window but its dispensing duration extended beyond the window, the coverage was truncated at the end of the window to avoid overestimation of adherence.

Main analysis

To adjust for confounding and minimise baseline differences between the comparator groups, a 1:1 propensity score matching without replacement was performed, including all covariates. The caliper width was set to 0.20 of the standard deviation of the logit of the propensity score, following published recommendations.[27] Individuals with propensity scores outside the common support region were excluded. A standardized percentage bias of less than 10% after matching was considered a good balance between the two groups.

For the matched cohort, Cox proportional hazards regression models were used to explore the associations between the use of FDC versus LDC and each cardiovascular and renal outcome. Models were fitted with robust standard errors clustered on matched pairs to account for the dependence induced by matching. The proportional-hazards assumption was evaluated using Schoenfeld residuals and no violation was found. Follow-up continued until the earliest occurrence of death, first incidence of the outcome, emigration from Sweden, or end of data availability (September 30, 2022).

Additional analyses

The mediating role of medication adherence was analysed using the STATA "med4way" package [28] and was presented as the proportion mediated. Stratified analyses were pre-specified for age (≥65 years or not), sex (male or female), and drug class (SGLT2i, DPP4i, or TZD), with interaction effects examined.

Additionally, the study population was also stratified by the drug class (SGLT2i/DPP4i/TZD) of the add-on/FDC drugs first, followed by matching and regression analyses within each stratum.

For sensitivity analyses, first, the cohort was matched on the duration of type 2 diabetes by cohort entry (obtained from the NDR) in addition to all covariates. The duration of diabetes was defined as the time interval between the first recorded diagnosis of type 2 diabetes and the index date of the treatment. Second, the cohort was matched on paired ATC codes instead of the drug class. Specifically, an FDC (e.g., A10BD15: metformin and dapagliflozin) was paired with its corresponding LDC (e.g., A10BK01: dapagliflozin) (eTable 4). Third, to assess the robustness of the results to residual immortal time bias the

lag time was removed and all the events occurring during the 90-day lag time were included as outcomes, and the primary analysis was repeated. Fourth, the primary analysis was repeated in a subset population without history of chronic ischaemic heart disease, myocardial infarction, and unstable angina.

8.4. Results

Baseline population characteristics

In the unmatched population, the LDC group had 51,019 people, and the FDC group had 14,286 people. Individuals with a filled prescription for SGLT2i, whether in FDC or LDC form, had a higher prevalence of angina, ischaemic heart disease, myocardial infarction, and recent CVD events (13~14%) compared to other drug classes (3~7%) (eTable 5). Each year, approximately 80% of the study population was in the LDC group (eTable 6). The use of SGLT2i has increased over time, and the proportion of DPP4i usage has initially increased but has slightly declined in recent years, whereas the use of TZD drugs has consistently decreased and has become less common in recent years (eTable 7).

After 1:1 propensity score matching, both groups included 13,883 individuals, with a total matched cohort of 27,766 people. The mean±standard deviation (SD) of age was 61.8 ± 11.9 years in the LDC group and 62.0 ± 11.7 years in the FDC group. The proportion of males was 67.0% in both groups (Table 8-1).

Table 8-1. Unmatched and matched major baseline characteristics of the study population.

	Unmatched population		Matched population		Standardized
	LDC	FDC	LDC	FDC	percentage bias
N (row%)	51,019 (78.1)	14,286 (21.9)	13,883 (50.0)	13,883 (50.0)	NA
Age in year, mean (SD)	63.1 (11.8)	61.9 (11.8)	61.8 (11.9)	62.0 (11.7)	2.0
Male, N (%)	32,854 (64.4)	9,570 (67.0)	9,295 (67.0)	9,296 (67.0)	0.0
PDC, median (IQR)	0.70 (0.49, 1.00)	0.98 (0.70, 1.00)	0.72 (0.47, 1.00)	0.98 (0.71, 1.00)	NA
Adherence, N (%)	23,417 (45.9)	9,750 (68.3)	6,452 (46.5)	9,519 (68.6)	NA
Antidiabetic drug class, N (%)					
SGLT2i	20,136 (39.5)	5,625 (39.4)	4,722 (34.0)	5,583 (40.2)	12.7
DPP4i	28,537 (55.9)	7,191 (50.3)	7,724 (55.6)	6,863 (49.4)	-12.4
TZD	2,346 (4.6)	1,470 (10.3)	1,437 (10.4)	1,437 (10.4)	0.0
Comorbidities, N (%)					
Atrial fibrillation	3,599 (7.1)	819 (5.7)	774 (5.6)	815 (5.9)	1.2
Angina	5,047 (9.9)	1,449 (10.1)	1,383 (10.0)	1,441 (10.4)	1.4
Atherosclerosis	631 (1.2)	156 (1.1)	157 (1.1)	156 (1.1)	-0.1
Cardiomyopathy	438 (0.9)	108 (0.8)	102 (0.7)	106 (0.8)	0.3
Heart failure	2,044 (4.0)	439 (3.1)	376 (2.7)	436 (3.1)	2.3

Ischaemic heart disease	4,799 (9.4)	1,448 (10.1)	1,320 (9.5)	1,441 (10.4)	2.9
Myocardial infarction	4,708 (9.2)	1,284 (9.0)	1,159 (8.3)	1,278 (9.2)	3.0
Ischaemic stroke	2,323 (4.6)	530 (3.7)	491 (3.5)	529 (3.8)	1.4
Hemorrhage stroke	530 (1.0)	115 (0.8)	110 (0.8)	115 (0.8)	0.4
Hypertension	4,803 (9.4)	1,213 (8.5)	1,217 (8.8)	1,207 (8.7)	-0.3
Acute kidney injury	208 (0.4)	41 (0.3)	34 (0.2)	41 (0.3)	0.9
CKD Stage 1	23 (<0.1)	5 (<0.1)	5 (<0.1)	5 (<0.1)	0.0
CKD Stage 2	43 (0.1)	10 (0.1)	15 (0.1)	10 (0.1)	-1.3
CKD Stage 3	85 (0.2)	12 (0.1)	11 (0.1)	12 (0.1)	0.2
Proteinuria	56 (0.1)	<5 (<0.1)	<5 (<0.1)	<5 (<0.1)	0.0
Diabetic nephrology	140 (0.3)	26 (0.2)	26 (0.2)	26 (0.2)	0.0
Diabetic neuropathy	215 (0.4)	55 (0.4)	55 (0.4)	55 (0.4)	0.0
Diabetic retinopathy	1,983 (3.9)	497 (3.5)	488 (3.5)	496 (3.6)	0.3
Anemia	734 (1.4)	159 (1.1)	131 (0.9)	159 (1.1)	1.8
Alcoholic liver disease	102 (0.2)	23 (0.2)	22 (0.2)	23 (0.2)	0.2
Cirrhosis	94 (0.2)	36 (0.3)	35 (0.3)	35 (0.3)	0.0
Chronic hepatitis	443 (0.9)	104 (0.7)	101 (0.7)	101 (0.7)	0.0
Hyperthyroidism	703 (1.4)	158 (1.1)	142 (1.0)	157 (1.1)	1.0
Hypoparathyroidism	265 (0.5)	59 (0.4)	51 (0.4)	59 (0.4)	0.8

Asthma	1,280 (2.5)	332 (2.3)	340 (2.4)	330 (2.4)	-0.5
Smoking	86 (0.2)	22 (0.2)	18 (0.1)	22 (0.2)	0.7
Drinking	1,241 (2.4)	292 (2.0)	290 (2.1)	291 (2.1)	0.0
Dyslipidemia	557 (1.1)	234 (1.6)	225 (1.6)	234 (1.7)	0.6
Obesity	1,552 (3.0)	441 (3.1)	446 (3.2)	438 (3.2)	-0.3
Recent CVD events [a], N (%)	4,659 (9.1)	1,124 (7.9)	1,016 (7.3)	1,114 (8.0)	2.5
Recent renal events [b], N (%)	186 (0.4)	28 (0.2)	34 (0.2)	28 (0.2)	-0.8
Drug, N (%)					
Antihypertensives	35,474 (69.5)	9,642 (67.5)	9,343 (67.3)	9,482 (68.3)	2.2
Beta blocker	23,856 (46.8)	6,436 (45.1)	6,224 (44.8)	6,353 (45.8)	1.9
Calcium channel blocker	20,557 (40.3)	5,464 (38.2)	5,326 (38.4)	5,396 (38.9)	1.0
Diuretics	17,748 (34.8)	4,496 (31.5)	4,382 (31.6)	4,454 (32.1)	1.1
Lipid regulators	38,087 (74.7)	10,247 (71.7)	9,860 (71.0)	10,067 (72.5)	3.4
Antithrombotics	24,571 (48.2)	6,645 (46.5)	6,399 (46.1)	6,532 (47.1)	1.9
Corticosteroids	13,150 (25.8)	3,503 (24.5)	3,458 (24.9)	3,479 (25.1)	0.3
NSAIDs	34,176 (67.0)	9,120 (63.8)	8,994 (64.8)	9,037 (65.1)	0.7
Marital status					
Unmarried	10,968 (21.7)	2,863 (20.6)	2,875 (20.7)	2,863 (20.6)	-0.2
Married	26,069 (51.6)	7,617 (54.8)	7,632 (55.0)	7,613 (54.8)	-0.3

Separated	9,619 (19.0)	2,533 (18.2)	2,522 (18.2)	2,533 (18.2)	0.2
Widowed	3,864 (7.6)	874 (6.3)	854 (6.2)	874 (6.3)	0.6

[a]Recent CVD events: Angina, atherosclerosis, atrial fibrillation, cardiomyopathy, chronic ischaemic heart disease, heart failure, myocardial infarction, and stroke, occurring within 1 year prior to the cohort entry.

[b]Recent kidney events: Acute kidney injury, CKD Stage 1~3, and proteinuria occurring within 1 year prior to the cohort entry.

SGLT2i, sodium-glucose cotransporter-2 inhibitor; DPP4i, dipeptidyl peptidase-4 inhibitor; TZD, thiazolidinedione; CKD, chronic kidney disease; CVD, cardiovascular disease; NSAID, nonsteroidal anti-inflammatory drug; LDC, loose-dose combination; FDC, fixed-dose combination; PDC, proportion of days covered; IQR, interquartile range.

Numbers smaller than five were presented as “<5” in accordance with data anonymization standards to minimize the risk of re-identification.

Matched baseline characteristics were well balanced for most covariates, with a standardized percentage of bias between -10.0% and 10.0% (e.g., age: 2.0%, sex: 0.0%, comorbidities: -1.3% to 3.0%, current medication: 0.3% to 3.4%). Minor imbalances persisted in the distribution of antidiabetic drug classes and geographic regions (Table 8-1, eTable 8). No meaningful imbalances were observed for key demographic or clinical characteristics.

Angina was the most prevalent comorbidity in the matched groups (10.0% FDC and 10.4% LDC), followed by ischaemic heart disease (9.5% and 10.4%, respectively). The use of lipid-lowering agents was found in more than 70% of the people in both groups, whereas more than 60% of the people in both groups used antihypertensive drugs or non-steroidal anti-inflammatory drugs. The FDC group showed higher PDC (median [IQR]: 0.98 [0.71, 1.00] vs 0.72 [0.47, 1.00]) and better medication adherence (68.6% vs 46.5%) than the LDC group during the first year after the index treatment (Table 8-1).

Cardiovascular outcome analyses

In both the unmatched and matched populations, heart failure was the most common cardiovascular outcome, followed by unstable angina and acute myocardial infarction. In the matched population, the proportion of outcomes was generally similar between groups, except for heart failure, which was more common in the LDC group (eTable 9). Heart failure had the highest incidence rate of 8.7 cases per 1,000 person-years (95% Confidence Interval [CI]: 8.2, 9.2), while unstable angina had the lowest, 2.8 cases per 1,000 person-years (95%CI: 2.5, 3.0) (Table 8-2).

Table 8-2. Hazard ratios in the overall population after propensity score matching

	Acute MI	Atrial fibrillation	Unstable angina	Heart failure	Ischaemic stroke	eGFRcr <30 ml/min/1.73m^2
Events during follow-up, N	934	732	404	1,250	732	185
Follow-up time in years, Median (IQR)	4.0 (1.9, 7.3)	4.0 (1.9, 7.3)	4.0 (1.9, 7.4)	4.0 (1.9, 7.3)	4.0 (1.9, 7.3)	3.8 (1.9, 6.3)
Incident rate per 1000 person-years, (95%CI)						
Overall	6.5 (6.1, 6.9)	5.0 (4.7, 5.4)	2.8 (2.5, 3.0)	8.7 (8.2, 9.2)	5.0 (4.7, 5.4)	2.8 (2.5, 3.3)
LDC	6.6 (6.0, 7.2)	5.1 (4.6, 5.6)	2.9 (2.5, 3.3)	9.2 (8.5, 9.9)	4.9 (4.4, 5.4)	3.0 (2.5, 3.7)
FDC	6.4 (5.8, 7.0)	5.0 (4.5, 5.5)	2.7 (2.3, 3.1)	8.1 (7.5, 8.8)	5.2 (4.7, 5.8)	2.6 (2.1, 3.3)
Hazard Ratio (95%CI)	0.98 (0.86, 1.11)	0.98 (0.85, 1.14)	0.93 (0.77, 1.13)	0.88 (0.79, 0.99)	1.07 (0.92, 1.24)	0.89 (0.67, 1.19)

Sample size for cardiovascular outcomes is 27,766, for kidney outcome, the size is 13,728.

MI, myocardial infarction; eGFR, estimated glomerular filtration rate

In the matched cohort, the median follow-up duration was four years for heart failure and was highly consistent across all other cardiovascular outcomes. A total of 1,250 heart failure cases were observed during follow-up. The incidence rate of heart failure was lower in the FDC group (8.1 vs 9.2 per 1000 person-years) (Table 2). The FDC group had a lower rate of heart failure (Hazard Ratio [HR] = 0.88; 95%CI: 0.79, 0.99). Medication adherence mediated 47% (95%CI: 5%, 90%) of this association. The absolute risk differences in heart failure between FDC and LDC users aged 65 years or above at 3, 5, and 10 years are -1.0%, -1.6%, and -3.1%, respectively. When assessing the mediating effect of continuous PDC on the association between FDC and heart failure incidence, the result was consistent, with continuous PDC mediating 48% (95%CI: 0%, 97%) of the association. The Kaplan–Meier survival graph showed paralleled curves between groups. No differences were observed in other cardiovascular outcomes (Table 8-2, Figure 8-2A-E).

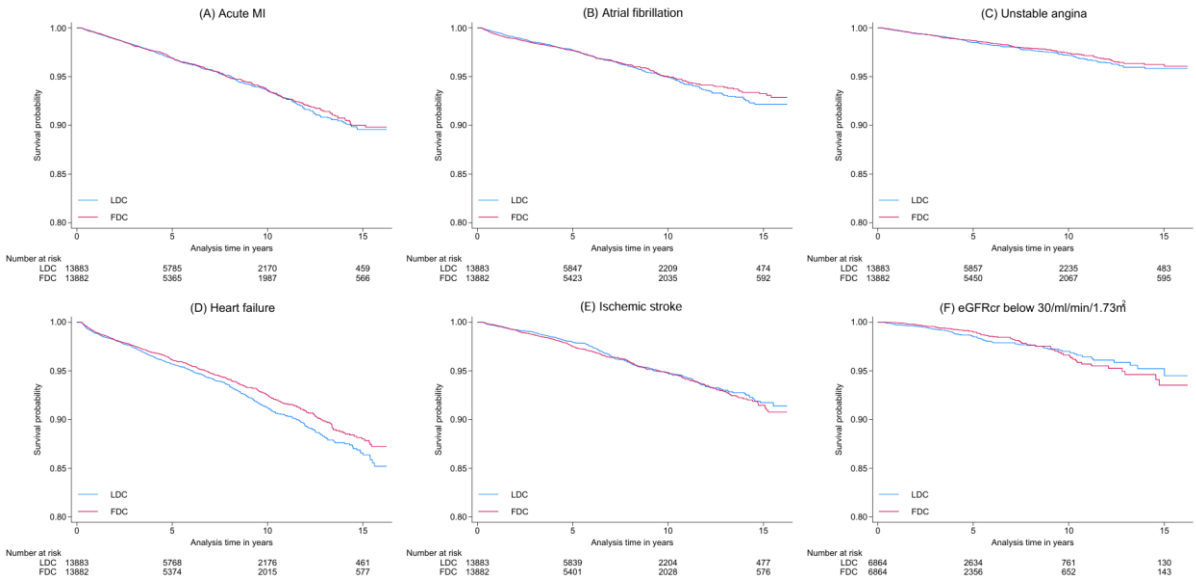

Figure 8-2. Kaplan-Maier survival curves for outcomes

(A) Acute MI; (B) Atrial fibrillation; (C) Unstable angina; (D) Heart failure; (E) Ischaemic stroke; (F) eGFRcr below 30 ml/min/1.73m^2

FDC, fixed-dose combination; LDC, loose-dose combination; MI, myocardial infarction; eGFR, estimated glomerular filtration rate

In the stratified analysis, in people aged 65 years and older, FDC was associated with a lower rate of heart failure (HR = 0.79; 95%CI: 0.69, 0.91), with evidence of interaction

effect (P=0.03). There was no evidence of a sex interaction (P=0.73). Female FDC initiators also had a lower rate of acute myocardial infarction (HR=0.78; 95%CI: 0.60, 1.00) (Table 8-3).

Table 8-3. Hazard ratios for the stratified analyses after propensity score matching

	Sample size	Acute MI	Atrial fibrillation	Unstable angina	Heart failure	Ischaemic stroke	Sample size[a]	eGFRcr<30 ml/min/1.73m^{2a}
Hazard Ratio (95%CI)								
Age								
Below 65 years	15,668	1.06 (0.88, 1.28)	1.01 (0.80, 1.29)	1.02 (0.77, 1.34)	1.03 (0.86, 1.25)	1.11 (0.88, 1.39)	7,321	1.37 (0.76, 2.44)
65 years and older	12,098	0.89 (0.74, 1.06)	0.95 (0.79, 1.14)	0.84 (0.64, 1.11)	0.79 (0.69, 0.91)	1.02 (0.85, 1.23)	6,407	0.74 (0.53, 1.04)
Sex								
Male	18,591	1.06 (0.91, 1.23)	1.02 (0.86, 1.21)	0.97 (0.78, 1.21)	0.90 (0.78, 1.02)	1.15 (0.96, 1.37)	9,195	0.89 (0.59, 1.33)
Female	9,175	0.78 (0.60, 1.00)	0.90 (0.68, 1.18)	0.81 (0.53, 1.24)	0.86 (0.70, 1.06)	0.92 (0.71, 1.19)	4,533	0.89 (0.59, 1.36)
LDC/FDC Drug class								

SGLT2i	10,305	1.10 (0.80, 1.50)	1.20 (0.88, 1.63)	1.18 (0.78, 1.77)	0.87 (0.69, 1.10)	1.12 (0.75, 1.66)	5,622	5.17 (0.60, 44.60)
DPP4i	14,587	0.95 (0.80, 1.13)	0.92 (0.76, 1.11)	0.91 (0.70, 1.18)	0.87 (0.75, 1.01)	1.04 (0.86, 1.25)	7,341	0.85 (0.60, 1.19)
TZD	2,874	0.91 (0.71, 1.18)	0.91 (0.63, 1.30)	0.76 (0.49, 1.16)	0.89 (0.70, 1.13)	1.08 (0.82, 1.42)	765	0.90 (0.48, 1.67)

[a]Sample size of this column is for the outcome eGFRcr<30 ml/min/1.73m^2 only.

SGLT2i, sodium-glucose cotransporter-2 inhibitor; DPP4i, dipeptidyl peptidase-4 inhibitor; TZD, thiazolidinedione; MI, myocardial infarction; eGFR, estimated glomerular filtration rate; FDC, fixed-dose combination; LDC, loose-dose combination

When stratified by drug class after matching, FDC users of metformin plus DPP4i did not have a significantly different risk of heart failure compared to LDC users, with a CI crossing the null (HR=0.87; 95%CI: 0.75, 1.01). No differences were observed in other cardiovascular outcomes (Table 8-3).

A summary table of sensitivity analyses can be found in the supplementary material (eTable 11). Additional matching on diabetes duration reduced the study population by 34.3%, owing to the reduced population coverage of the NDR (the source of duration data), leaving 18,250 individuals. The average duration of type 2 diabetes was 6.6±5.4 and 6.6±5.6 years in the LDC and FDC groups, respectively. No differences were observed in any CVD outcomes. (eTable 12).

After matching at ATC level, the matched population was as well balanced as the population matched at the drug class level, with minor imbalances remaining in three counties. The association of FDC use with heart failure was not significantly lower in the unstratified population (HR=0.91; 95%CI: 0.80, 1.02) (eTable 13). Stratified analyses on heart failure outcomes showed that individuals aged 65 years and older had a lower rate of heart failure (HR=0.82; 95%CI: 0.71, 0.94) when initiating FDCs (eTable 14). The results were consistent using outcomes occurring at any time after the index date (eTable 15), and in people without history of chronic ischaemic heart disease, myocardial infarction, and unstable angina (eTable 16, eTable 17)

Kidney outcome analysis

In the unmatched 65,305 people, 33,849 people (51.8%) did not have creatinine data available. The missing rate was similar in LDC and FDC groups (52.7% and 47.3% respectively). In the matched population of 13,728 people, the mean baseline eGFRcr was 91±25 ml/min/1.73m^2 and 91±24 ml/min/1.73m^2 in the LDC and FDC groups, respectively. With a median follow-up of 3.8 years (95%CI: 1.9, 6.3), the incidence rate was 2.8 cases per 1000 person-years (95%CI: 2.5, 3.3). A total of 185 cases was identified, with 83 in the FDC group and 102 in the LDC group. No associations between FDC and kidney events were observed. (Figure 8-2F, Table 8-3, eTable 10,12,13, 15).

8.5. Discussion

In this real-world cohort study of people with type 2 diabetes who initiated treatment with metformin, the use of FDCs was associated with a modestly lower rate of heart failure than the LDC regimen was, and 47% of this was mediated by medication adherence. This association was also observed in people aged 65 years and older, but was attenuated with further matching for diabetes duration or when drugs were matched at ATC code level instead of drug classes. No associations were observed for myocardial infarction, atrial fibrillation, unstable angina, stroke, or kidney impairment.

The observed cardiovascular protection of FDC therapy on heart failure could be partly explained by differences in medication adherence, since poor adherence could lead to an increased risk of CVD events.[29] The FDC initiators in this study had higher adherence. FDCs reduce pill burden and simplify treatment, which may improve patients' consistency in taking medications as prescribed.

The larger association of FDCs with heart failure in people aged 65 years and older, could be explained by age-related alterations in pharmacokinetics and pharmacodynamics. [30] Older adults more frequently experience declined kidney function, leading to reduced drug clearance and prolonged plasma drug concentrations. Additionally, the higher prevalence of multimorbidity and polypharmacy in older adults may further influence drug absorption and excretion, potentially leading to increased drug exposure when taking FDCs.

Analysis stratified on drug class suggested that FDCs of metformin with DPP4i may be associated with a lower rate of heart failure. One trial has shown metformin and sitagliptin combination associated with improved glucose control compared to metformin monotherapy, which may indirectly reduce the risk of complications. [31] However, another study reported that the addition of saxagliptin to existing antidiabetic therapy was associated with an increased risk of hospitalization for heart failure (HR=1.27; 95%CI: 1.07, 1.51).[32] Notably, that study did not include a direct comparison with metformin monotherapy, although metformin accounted for approximately 70% of concomitant antidiabetic use throughout the trial. Another important concern is around the 40% concomitant use of sulfonylureas, as sulfonylureas have been associated with elevated cardiovascular risk. Finally, saxagliptin in that study was not administered as an FDC.

Therefore, it was an LDC-versus-LDC study. Consequently, the study provides only limited direct inference for our findings.

In analyses stratified on drug class, the observed differences may be attributable to factors inherent to the FDC itself. FDCs are designed to control drug release kinetics and enhance the physical and chemical stability of each component within the gastrointestinal environment. FDCs often incorporate specialized excipients or matrix systems designed to prevent the degradation of labile compounds in the presence of others, a limitation seen in LDCs. [33] This approach is useful, as several antidiabetic agents in this study, such as empagliflozin and saxagliptin, are labile under hydrolytic or oxidative stress conditions.[34, 35] Additionally, co-granulation, modified release layers, or enteric coatings can yield measurable differences in bioavailability and therapeutic performance. However, since our study did not include pharmacokinetic or bioavailability data, formulation-related mechanisms should be considered speculative in explaining our findings. Finally, in additional analyses in populations with stratification by drug classes pre-matching, no associations were observed.

There were no differences in acute myocardial infarction, ischaemic stroke, unstable angina, or atrial fibrillation between the FDC and LDC treatment groups. To date, only a few SGLT2i (e.g., canagliflozin) have been recommended by regulatory agencies to reduce the risk of major adverse cardiovascular events in people with known atherosclerotic CVD, but not in people at risk of those diseases.[36] In terms of cardiovascular events, the major protective effect of SGLT2i observed is on heart failure. [36] However, current research has not shown evidence of beneficial cardiovascular effects of DPP4i.[37]

As yet, SGLT2i has shown kidney-protective effects, but there is insufficient evidence supporting the use of DPP4i.[37] This study has observed no associations between FDC and the kidney outcome (eGFRcr<30 ml/min/1.73m^2). This may be due to the suboptimal capture of kidney events, the short follow-up duration (median: 3.8 years), and the generally healthy status of the kidneys at baseline in both groups, resulting in low power. Because only 185 renal events accrued, statistical power was limited. Using Schoenfeld’s approximation (two-sided α =0.05, 1:1 allocation), with 185 events the minimum detectable effect at 80% power was around HR=0.66. Thus, the null finding for the renal

outcome likely reflects limited power and not necessarily absence of effect. Further studies on people with impaired kidney function and longer follow-up periods are encouraged.

Despite stratification and matching, baseline cardiovascular disease differed across drug classes, especially among SGLT2i users, indicating prescribing bias by cardiovascular risk. This may cause confounding by indications, potentially masking the true benefits of FDC in high-risk patients, which could explain part of the attenuation in associations observed in the analyses stratified by drug class and with matching at ATC level.

This study contributes to the clinical management of type 2 diabetes by examining the role of FDCs in combination therapy, thereby clarifying a previously ambiguous area within current guidelines. To our knowledge, this is the first study that has specifically examined cardiorenal endpoints for FDC versus LDC therapy in type 2 diabetes using real-world data.

This study has several strengths, such as the large, real-world cohort drawn from routine clinical practice, which enhances the generalizability of the findings to broader populations. Additionally, the use of an active comparator provides a rigorous reference group, and a wide range of baseline characteristics and comorbidities were adjusted for to minimize confounding.

Despite these strengths, several limitations must be acknowledged. First, the exposure definition relied on prescription fill records, which do not provide information on the actual duration and doses. Second, there were imbalances in drug classes after matching, which could have biased the results of the main analysis. However, stratified analysis on each drug class showed possible negative associations in DPP4i FDC users. Third, the sensitivity analysis using matching at ATC code level showed similar trends as those of the primary findings but an attenuation in associations. While matching at the ATC code level may offer a greater control over potential confounding by indication, matching at the drug class level is more aligned with the original aim of the study to compare the form of combination therapies (FDC versus LDC), independently from individual drug effect. Additionally, current clinical guidelines [1] recommend combination therapies based on drug classes (e.g., metformin in combination with SGLT2i) rather than specific drugs (e.g., dapagliflozin). Therefore, drug class-level matching enhances the clinical generalizability

of our findings. Fourth, the proportion of days covered is a proxy for adherence, and this is only an approximation of true medication-taking behaviour. [38] Fifth, the follow-up time, although sufficient to observe differences in heart failure, may not have been long enough to detect divergences in outcomes such as chronic kidney disease progression or hard atherosclerotic events, which develop over many years. Sixth, the intention-to-treat approach did not reflect the drug-switching during the follow-up duration. In the matched population, 25.6% of FDC users switched to LDC or non-metformin monotherapy, compared to 8.5% of LDC users. There were also users who switched to a different treatment group and later returned to their original treatment. This imbalance in switching may dilute the differences between FDC and LDC therapies, potentially underestimating the true comparative effectiveness of FDC. However, the mediation analysis has partially offset this disadvantage, showing that being adherent is an important mediator. Seventh, GLP-1 receptor agonists were not included in this study because no FDCs containing GLP-1 RAs were available on the market at the time of data collection. Future research on GLP-1 RAs is required. Eighth, potential informative censoring like death and emigration may bias the results. However, the direction of the effect is unknown. All-cause mortality during follow-up was 8.0% in FDC users and 8.6% in LDC users. The emigration rate was 0.94% and 0.68% for FDC and LDC users respectively. Given these small and similar rates, any differential informative censoring is expected to be modest and unlikely to explain the results. Finally, this study was conducted within a specific healthcare system. Differences in FDC availability, prescribing practices, and government subsidies may limit the generalizability of the findings to other countries.

These findings suggest that the use of FDCs is associated with a lower rate of heart failure in people with type 2 diabetes, but not with a reduced risk of other cardiovascular outcomes. The association is partially mediated by improved adherence. This strategy could be particularly relevant for older adults or those facing polypharmacy. In health care systems with low FDC uptake, FDCs may serve as a practical tool to simplify treatment, improve adherence, and reduce heart-failure burden. Priority should be given to clinically equivalent FDCs for adults with type 2 diabetes who require dual oral therapy, especially older patients and those on polypharmacy, with parity (or better) reimbursement to reduce access barriers. Due to the neutral results for non-heart failure outcomes, policy implementation should proceed via evidence-guided pilots with prospective monitoring.

While the results highlight a potential clinical advantage of FDC use, especially in routine clinical care settings, further studies are encouraged to focus on pragmatic trial designs, long-term kidney outcomes, and high-risk groups such as patients with impaired kidney function.

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8.7. Supplementary tables and figures for Chapter 8

eTable 1 Identified antidiabetic SGLT2i/DPP4i/TZD drugs in the study population

ATC code	Drug class	Drug name
A10BD03	Biguanides+TZD	metformin and rosiglitazone
A10BD05	Biguanides+TZD	metformin and pioglitazone
A10BD07	Biguanides+DPP4i	metformin and sitagliptin
A10BD08	Biguanides+DPP4i	metformin and vildagliptin
A10BD10	Biguanides+DPP4i	metformin and saxagliptin
A10BD11	Biguanides+DPP4i	metformin and linagliptin
A10BD15	Biguanides+SGLT2i	metformin and dapagliflozin
A10BD20	Biguanides+SGLT2i	metformin and empagliflozin
A10BD23	Biguanides+SGLT2i	metformin and ertugliflozin
A10BG02	TZD	rosiglitazone
A10BG03	TZD	pioglitazone
A10BH01	DPP4i	sitagliptin
A10BH02	DPP4i	vildagliptin
A10BH03	DPP4i	saxagliptin
A10BH05	DPP4i	linagliptin
A10BK01	SGLT2i	dapagliflozin
A10BK03	SGLT2i	empagliflozin
A10BK04	SGLT2i	ertugliflozin

TZD, Thiazolidinedione; DPP4i, Dipeptidyl Peptidase-4 Inhibitor; SGLT2i, Sodium-Glucose Cotransporter 2 Inhibitor; ATC, Anatomical Therapeutic Chemical.

eTable 2 Study outcomes and their ICD-10 codes

	ICD-10 codes
Unstable angina	I20.0
Myocardial infarction	I21.*
Atrial fibrillation	I48.0~2
Heart failure	I50.*
Ischemic stroke	I63.*

eTable 3 List of covariates, their definitions, and data source

	Source	Definition
Age	TPR	Age in years by cohort entry
Sex	TPR	Male and female
Antidiabetic drug class		
SGLT2i	PDR	ATC codes: A10BD15, A10BD20, A10BD23, A10BK01, A10BK03, A10BK04
DPP4i	PDR	ATC codes: A10BD07, A10BD08, A10BD10, A10BD11, A10BH01, A10BH02, A10BH03, A10BH05
TZD	PDR	ATC codes: A10BD03, A10BD05, A10BG02, A10BG03
Comorbidities		
Atrial fibrillation	NPR	ICD-10 codes: I48.0~2
Angina	NPR	ICD-10 codes: I20
Atherosclerosis	NPR	ICD-10 codes: I70
Cardiomyopathy	NPR	ICD-10 codes: I42
Heart failure	NPR	ICD-10 codes: I50
Chronic ischemic heart disease	NPR	ICD-10 codes: I25
Myocardial infarction	NPR	ICD-10 codes: I21
Ischemic stroke	NPR	ICD-10 codes: I63
Hemorrhage stroke	NPR	ICD-10 codes: I60~I62
Hypertension	NPR	ICD-10 codes: I10~I13, I15
Acute kidney injury	NPR	ICD-10 codes: N17
CKD Stage 1	NPR	ICD-10 codes: N18.1
CKD Stage 2	NPR	ICD-10 codes: N18.2
CKD Stage 3	NPR	ICD-10 codes: N18.3
Proteinuria	NPR	ICD-10 codes: R80
Diabetic nephrology	NPR	ICD-10 codes: E11.2
Diabetic neuropathy	NPR	ICD-10 codes: E11.4
Diabetic retinopathy	NPR	ICD-10 codes: E11.3

Anemia	NPR	ICD-10 codes: D50~D53, D55~D59
Alcoholic liver disease	NPR	ICD-10 codes: K70
Cirrhosis	NPR	ICD-10 codes: K74.0
Chronic hepatitis	NPR	ICD-10 codes: B18, K73
Hyperthyroidism	NPR	ICD-10 codes: E03, E05
Hypoparathyroidism	NPR	ICD-10 codes: E20, E21
Asthma	NPR	ICD-10 codes: J45
Smoking	NPR	ICD-10 codes: F17, Z72.0
Drinking	NPR	ICD-10 codes: F10
Dyslipidemia	NPR	ICD-10 codes: E78
Obesity	NPR	ICD-10 codes: E66
Recent CVD events[a]	NPR	ICD-10 codes: I20, I21, I25, I42, I48.0~2, I50, I60~I63, I70
Recent renal events[a]	NPR	ICD-10 codes: N17, N18.1~3, R80
Medication History		
Antihypertensives	PDR	ATC codes: C09
Beta blocker	PDR	ATC codes: C07
Calcium channel blocker	PDR	ATC codes: C08
Diuretics	PDR	ATC codes: C03
Lipid regulators	PDR	ATC codes: C10A
Antithrombotics	PDR	ATC codes: B01A
Corticosteroids	PDR	ATC codes: H02
NSAIDs	PDR	ATC codes: M01A
Marital status	TPR	Unmarried, married, separated, widowed.
Place of residence	TPR	21 counties of Sweden

[a]Recent event: Event occurred within 1 year prior to the cohort entry.

ATC, Anatomical Therapeutic Chemical; ICD, International Classification of Disease; SGLT2i, sodium-glucose cotransporter 2 inhibitor; DPP4i, dipeptidyl peptidase 4 inhibitor; TZD, thiazolidinedione; CKD, chronic kidney disease; CVD, cardiovascular disease; NSAIDs, non-steroidal anti-inflammatory drugs; NPR, Swedish National Patient Register; PDR, Swedish Prescribed Drug Register; NDR, Swedish National Diabetes Register; TPR, Total Population Register.

eTable 4 Paired ATC groups for propensity score matching at ATC code level

LDC	Paired FDC
A10BG02 (rosiglitazone)	A10BD03 (metformin and rosiglitazone)
A10BG03 (pioglitazone)	A10BD05 (metformin and pioglitazone)
A10BH01 (sitagliptin)	A10BD07 (metformin and sitagliptin)
A10BH02 (vildagliptin)	A10BD08 (metformin and vildagliptin)
A10BH03 (saxagliptin)	A10BD10 (metformin and saxagliptin)
A10BH05 (linagliptin)	A10BD11 (metformin and linagliptin)
A10BK01 (dapagliflozin)	A10BD15 (metformin and dapagliflozin)
A10BK03 (empagliflozin)	A10BD20 (metformin and empagliflozin)
A10BK04 (ertugliflozin)	A10BD23 (metformin and ertugliflozin)

LDC, loose-dose combination; FDC, fixed-dose combination; ATC, Anatomical Therapeutic Chemical.

eTable 5 Baseline characteristics by drug class of the add-on antidiabetic drug in the unmatched population

	Drug class of the add-on antidiabetic drug		
	SGLT2i	DPP4i	TZD
N (row%)	25,761 (39.4)	35,728 (54.7)	3,816 (5.8)
Age in years, mean (SD)	63.0 (11.5)	63.0 (12.1)	59.9 (11.2)
Male, N (%)	17,755 (68.9)	22,285 (62.4)	2,384 (62.5)
Comorbidities, N (%)			
Atrial fibrillation	2,156 (8.4)	2,147 (6.0)	115 (3.0)
Angina	3,334 (12.9)	2,947 (8.2)	215 (5.6)
Atherosclerosis	333 (1.3)	423 (1.2)	31 (0.8)
Cardiomyopathy	310 (1.2)	221 (0.6)	15 (0.4)
Heart failure	1,375 (5.3)	1,061 (3.0)	47 (1.2)
Chronic ischemic heart disease	3,719 (14.4)	2,404 (6.7)	124 (3.2)
Myocardial infarction	3,551 (13.8)	2,320 (6.5)	121 (3.2)
Ischemic stroke	1,277 (5.0)	1,502 (4.2)	74 (1.9)
Hemorrhage stroke	303 (1.2)	327 (0.9)	15 (0.4)
Hypertension	2,697 (10.5)	3,113 (8.7)	206 (5.4)
Acute kidney injury	94 (0.4)	150 (0.4)	5 (0.1)
CKD Stage 1	16 (0.1)	12 (0.0)	0 (0.0)
CKD Stage 2	27 (0.1)	25 (0.1)	<5 (<0.1)
CKD Stage 3	42 (0.1)	55 (0.2)	0 (0.0)
Proteinuria	26 (0.1)	32 (0.1)	0 (0.0)
Diabetic nephrology	48 (0.2)	104 (0.3)	14 (0.4)
Diabetic neuropathy	96 (0.4)	160 (0.4)	14 (0.4)
Diabetic retinopathy	1,062 (4.1)	1,301 (3.6)	117 (3.1)
Anemia	415 (1.6)	447 (1.3)	31 (0.8)
Alcoholic liver disease	51 (0.2)	69 (0.2)	5 (0.1)
Cirrhosis	70 (0.3)	58 (0.2)	<5 (<0.1)
Chronic hepatitis	232 (0.9)	289 (0.8)	26 (0.7)
Hyperthyroidism	381 (1.5)	460 (1.3)	20 (0.5)

Hypoparathyroidism	131 (0.5)	182 (0.5)	11 (0.3)
Asthma	711 (2.8)	832 (2.3)	69 (1.8)
Smoking	57 (0.2)	50 (0.1)	<5 (<0.1)
Drinking	704 (2.7)	759 (2.1)	70 (1.8)
Dyslipidemia	419 (1.6)	353 (1.0)	19 (0.5)
Obesity	936 (3.6)	951 (2.7)	106 (2.8)
Recent CVD events, N (%)[a]	3,401 (13.2)	2,237 (6.3)	145 (3.8)
Recent renal events, N (%)[b]	75 (0.3)	138 (0.4)	<5 (<0.1)
Drug, N (%)			
Antihypertensives	18,929 (73.5)	23,915 (66.9)	2,272 (59.5)
Beta blocker	13,283 (51.6)	15,641 (43.8)	1,368 (35.8)
Calcium channel blocker	11,227 (43.6)	13,769 (38.5)	1,025 (26.9)
Diuretics	9,253 (35.9)	11,924 (33.4)	1,067 (28.0)
Lipid regulators	20,290 (78.8)	25,715 (72.0)	2,329 (61.0)
Antithrombotics	13,544 (52.6)	16,118 (45.1)	1,554 (40.7)
Corticosteroids	7,598 (29.5)	8,584 (24.0)	471 (12.3)
NSAIDs	18,330 (71.2)	23,219 (65.0)	1,747 (45.8)

[a]Recent CVD events: Angina, atherosclerosis, atrial fibrillation, cardiomyopathy, chronic ischemic heart disease, heart failure, myocardial infarction, and stroke, occurring within 1 year prior to the cohort entry.

[b]Recent kidney events: Acute kidney injury, CKD Stage 1~3, and proteinuria occurring within 1 year prior to the cohort entry.

SGLT2i, sodium-glucose cotransporter-2 inhibitor; DPP4i, dipeptidyl peptidase-4 inhibitor; TZD, thiazolidinedione; CKD, chronic kidney disease; CVD, cardiovascular disease; NSAIDs, nonsteroidal anti-inflammatory drug; SD, standard deviation; LDC, loose-dose combination; FDC, fixed-dose combination.

Numbers smaller than five were presented as "<5" in accordance with data anonymization standards to minimize the risk of re-identification.

eTable 6 Time trend of the proportion of initiators of an LDC and an FDC drug in the unmatched population

	LDC	FDC
Index year, N (row%)		
2006	313 (45.5)	375 (54.5)
2007	732 (52.4)	665 (47.6)
2008	1,065 (80.0)	267 (20.0)
2009	906 (68.9)	409 (31.1)
2010	1,032 (75.5)	336 (24.5)
2011	1,202 (77.5)	349 (22.5)
2012	1,328 (79.6)	341 (20.4)
2013	1,647 (80.8)	392 (19.2)
2014	2,045 (78.6)	558 (21.4)
2015	2,904 (80.4)	708 (19.6)
2016	3,752 (80.4)	919 (19.6)
2017	4,914 (80.1)	1,228 (19.9)
2018	6,013 (81.4)	1,374 (18.6)
2019	6,604 (81.1)	1,540 (18.9)
2020	6,688 (78.0)	1,891 (22.0)
2021	7,719 (77.2)	2,292 (22.8)
2022	2,155 (77.1)	642 (22.9)

LDC, loose-dose combination; FDC, fixed-dose combination.

eTable 7 Time trend of proportions of antidiabetic drug filled prescriptions by drug class and combination types in the unmatched population

	Unmatched, LDC group			
	SGLT2i	DPP4i	TZD	Total
N, row (%)	20,136 (39.5)	28,537 (55.9)	2,346 (4.6)	51,019 (100.0)
Year, N (%)				
2006	0 (0.0)	0 (0.0)	313 (13.3)	313 (0.6)
2007	0 (0.0)	238 (0.8)	494 (21.1)	732 (1.4)
2008	0 (0.0)	791 (2.8)	274 (11.7)	1,065 (2.1)
2009	0 (0.0)	729 (2.6)	177 (7.5)	906 (1.8)
2010	0 (0.0)	909 (3.2)	123 (5.2)	1,032 (2.0)
2011	0 (0.0)	1,091 (3.8)	111 (4.7)	1,202 (2.4)
2012	0 (0.0)	1,278 (4.5)	50 (2.1)	1,328 (2.6)
2013	63 (0.3)	1,523 (5.3)	61 (2.6)	1,647 (3.2)
2014	251 (1.2)	1,762 (6.2)	32 (1.4)	2,045 (4.0)
2015	458 (2.3)	2,407 (8.4)	39 (1.7)	2,904 (5.7)
2016	767 (3.8)	2,935 (10.3)	50 (2.1)	3,752 (7.4)
2017	1,389 (6.9)	3,428 (12.0)	97 (4.1)	4,914 (9.6)
2018	2,641 (13.1)	3,265 (11.4)	107 (4.6)	6,013 (11.8)
2019	3,328 (16.5)	3,140 (11.0)	136 (5.8)	6,604 (12.9)
2020	4,128 (20.5)	2,449 (8.6)	111 (4.7)	6,688 (13.1)
2021	5,426 (26.9)	2,163 (7.6)	130 (5.5)	7,719 (15.1)
2022	1,685 (8.4)	429 (1.5)	41 (1.7)	2,155 (4.2)

	Unmatched, FDC group			
	Metformin+SGLT2i	Metformin+DPP4i	Metformin+TZD	Total
N, row				14,286
(%)	5,625 (39.4)	7,191 (50.3)	1,470 (10.3)	(100.0)
Year, N (%)				
2006	0 (0.0)	0 (0.0)	375 (25.5)	375 (2.6)
2007	0 (0.0)	0 (0.0)	665 (45.2)	665 (4.7)
2008	0 (0.0)	50 (0.7)	217 (14.8)	267 (1.9)
2009	0 (0.0)	302 (4.2)	107 (7.3)	409 (2.9)
2010	0 (0.0)	260 (3.6)	76 (5.2)	336 (2.4)
2011	0 (0.0)	342 (4.8)	7 (0.5)	349 (2.4)
2012	0 (0.0)	338 (4.7)	<5 (<0.3)	341 (2.4)
2013	0 (0.0)	389 (5.4)	<5 (<0.3)	392 (2.7)
2014	14 (0.2)	543 (7.6)	<5 (<0.3)	558 (3.9)
2015	13 (0.2)	694 (9.7)	<5 (<0.3)	708 (5.0)
2016	136 (2.4)	780 (10.8)	<5 (<0.3)	919 (6.4)
2017	326 (5.8)	900 (12.5)	<5 (<0.3)	1,228 (8.6)
2018	611 (10.9)	762 (10.6)	<5 (<0.3)	1,374 (9.6)
2019	800 (14.2)	737 (10.2)	<5 (<0.3)	1,540 (10.8)
2020	1,318 (23.4)	570 (7.9)	<5 (<0.3)	1,891 (13.2)
2021	1,845 (32.8)	444 (6.2)	<5 (<0.3)	2,292 (16.0)
2022	562 (10.0)	80 (1.1)	0 (0.0)	642 (4.5)

LDC, loose-dose combination; FDC, fixed-dose combination.

SGLT2i, sodium-glucose cotransporter-2 inhibitor; DPP4i, dipeptidyl peptidase-4 inhibitor; TZD, thiazolidinedione.

Numbers smaller than five were presented as "<5" in accordance with data anonymization standards to minimize the risk of re-identification.

eTable 8 Propensity score matching results of index year, place of residence, and ATC codes

Index year:

	Before matching		After matching		
	LDC group	FDC group	LDC group	FDC group	Standardized percentage bias
Index year, N (%)					
2006	313 (0.6)	375 (2.6)	304 (2.2)	367 (2.6)	3.6
2007	732 (1.4)	665 (4.7)	685 (4.9)	656 (4.7)	-1.2
2008	1,065 (2.1)	267 (1.9)	280 (2.0)	259 (1.9)	-1.1
2009	906 (1.8)	409 (2.9)	501 (3.6)	406 (2.9)	-4.5
2010	1,032 (2.0)	336 (2.4)	362 (2.6)	326 (2.3)	-1.8
2011	1,202 (2.4)	349 (2.4)	408 (2.9)	340 (2.4)	-3.2
2012	1,328 (2.6)	341 (2.4)	394 (2.8)	333 (2.4)	-2.8
2013	1,647 (3.2)	392 (2.7)	426 (3.1)	369 (2.7)	-2.4
2014	2,045 (4.0)	558 (3.9)	578 (4.2)	538 (3.9)	-1.5
2015	2,904 (5.7)	708 (5.0)	727 (5.2)	677 (4.9)	-1.6
2016	3,752 (7.4)	919 (6.4)	925 (6.7)	878 (6.3)	-1.3
2017	4,914 (9.6)	1,228 (8.6)	1,249 (9.0)	1,179 (8.5)	-1.8
2018	6,013 (11.8)	1,374 (9.6)	1,364 (9.8)	1,320 (9.5)	-1.0
2019	6,604 (12.9)	154 (10.8)	1,472 (10.6)	1,495 (10.8)	0.5
2020	6,688 (13.1)	1,891 (13.2)	1,660 (12.0)	1,850 (13.3)	4.0
2021	7,719 (15.1)	2,292 (16.0)	2,008 (14.5)	2,251 (16.2)	4.8
2022	2,155 (4.2)	642 (4.5)	540 (3.9)	639 (4.6)	3.5

Places of residence:

	Before matching		After matching		
	LDC group	FDC group	LDC group	FDC group	Standardized percentage bias
County, N (%)					
Stockholm	7,233 (14.3)	1,936 (13.9)	2,005 (14.4)	1,936 (13.9)	-1.4
Uppsala	1,723 (3.4)	349 (2.5)	364 (2.6)	349 (2.5)	-0.6
Södermanland	1,906 (3.8)	346 (2.5)	352 (2.5)	346 (2.5)	-0.2
Östergötland	1,501 (3.0)	2,573 (18.5)	1,498 (10.8)	2,569 (18.5)	25.7
Jönköping	1,854 (3.7)	896 (6.5)	1,384 (10.0)	896 (6.5)	-16.1
Kronoberg	1,038 (2.1)	103 (0.7)	92 (0.7)	103 (0.7)	0.7
Kalmar	1,259 (2.5)	259 (1.9)	274 (2.0)	259 (1.9)	-0.7
Gotland	340 (0.7)	44 (0.3)	46 (0.3)	44 (0.3)	-0.2
Blekinge	1,027 (2.0)	91 (0.7)	96 (0.7)	91 (0.7)	-0.3
Skåne	7,349 (14.5)	1,517 (10.9)	1,467 (10.6)	1,517 (10.9)	1.1
Hallands	1,909 (3.8)	410 (3.0)	428 (3.1)	410 (3.0)	-0.7
Västra Götaland	8,506 (16.8)	1,167 (8.4)	1,212 (8.7)	1,167 (8.4)	-1.0
Värmland	2,407 (4.8)	560 (4.0)	563 (4.1)	560 (4.0)	-0.1
Örebro	2,103 (4.2)	183 (1.3)	158 (1.1)	183 (1.3)	1.1
Västmanland	1,579 (3.1)	373 (2.7)	382 (2.8)	373 (2.7)	-0.4
Dalarna	1,954 (3.9)	469 (3.4)	494 (3.6)	469 (3.4)	-1.0
Gävleborg	2,035 (4.0)	372 (2.7)	385 (2.8)	372 (2.7)	-0.5
Västernorrland	1,677 (3.3)	312 (2.2)	309 (2.2)	312 (2.2)	0.1
Jämtland	779 (1.5)	259 (1.9)	332 (2.4)	259 (1.9)	-4.1
Västerbotten	117 (2.3)	1,122 (8.1)	1,168 (8.4)	1,122 (8.1)	-1.5
Norrbotten	1,171 (2.3)	546 (3.9)	874 (6.3)	546 (3.9)	-13.6

ATC codes:

	Before matching		After matching		
	LDC group	FDC group	LDC group	FDC group	Standardized percentage bias
ATC pairs, N (row %)					
Metformin + Dapagliflozin / Dapagliflozin	3,029 (94.4)	182 (5.6)	220 (56.2)	172 (43.8)	-1.9
Metformin + Emapgliflozin / Empagliflozin	16,791 (75.7)	5,402 (24.3)	4,532 (45.8)	5,373 (54.2)	12.6
Metformin + Ertugliflozin / Ertugliflozin	31 (45.6)	37 (54.4)	31 (47.7)	34 (52.3)	0.6
Metformin + Sitagliptin / Sitagliptin	24,876 (78.7)	6,761 (21.3)	7,664 (54.1)	6,501 (45.9)	-16.8
Metformin + Vildagliptin / Vildagliptin	222 (49.6)	226 (50.4)	199 (54.2)	168 (45.8)	-2.5
Metformin + Saxagliptin / Saxagliptin	596 (90.9)	60 (9.1)	65 (54.6)	54 (45.4)	-0.9
Metformin + Linagliptin / Linagliptin	3,091 (95.5)	148 (4.5)	251 (63.9)	142 (36.1)	-4.3
Metformin + Rosiglitazone / Rosiglitazone	869 (37.9)	1,424 (62.1)	855 (38.0)	1,395 (62.0)	16.9
Metformin + Pioglitazone / Pioglitazone	1,514 (97.1)	46 (2.9)	66 (60.0)	44 (40.0)	-1.3

LDC, loose-dose combination; FDC, fixed-dose combination; ATC, Anatomical Therapeutic Chemical.

eTable 9 Distribution of outcomes in the unmatched and matched populations

	Unmatched population		Matched population	
	LDC	FDC	LDC	FDC
N (row%)	51,019 (78.1)	14,286 (21.9)	13,883 (50.0)	13,883 (50.0)
Cardiovascular outcomes, N (%)				
Acute MI	1,451 (2.8)	459 (3.2)	485 (3.5)	449 (3.2)
Atrial fibrillation	1,350 (2.6)	358 (2.5)	378 (2.7)	354 (2.5)
Unstable angina	1,687 (3.3)	578 (4.0)	553 (4.0)	564 (4.1)
Heart failure	2,414 (4.7)	587 (4.1)	677 (4.9)	573 (4.1)
Stroke	1,304 (2.6)	374 (2.6)	364 (2.6)	368 (2.7)

	Unmatched population		Matched population	
	LDC	FDC	LDC	FDC
N (row%)	51,019 (78.1)	14,286 (21.9)	6,864 (50.0)	6,864 (50.0)
Kidney outcome, N (%)				
eGFRcr<30 ml/min/1.73m^2	873 (1.7)	196 (1.4)	102 (1.5)	83 (1.2)

MI, myocardial infarction; LDC, loose-dose combination; FDC, fixed-dose combination; eGFR, estimated glomerular filtration rate.

eTable 10 Hazard ratios (HR) following propensity score matching, with pre-matching stratification by drug class

	SGLT2i	DPP4i	TZD
Sample size, N	11,150	13,725	2,866
Cardiovascular outcomes, HR (95%CI)			
Acute MI	1.24 (0.90, 1.71)	1.05 (0.88, 1.25)	0.94 (0.73, 1.20)
Atrial fibrillation	1.19 (0.89, 1.61)	1.00 (0.82, 1.21)	0.88 (0.62, 1.24)
Unstable angina	1.20 (0.80, 1.80)	0.91 (0.69, 1.19)	0.84 (0.55, 1.28)
Heart failure	0.94 (0.75, 1.18)	0.92 (0.78, 1.07)	0.86 (0.68, 1.09)
Ischemic stroke	0.95 (0.65, 1.38)	1.02 (0.84, 1.24)	1.04 (0.80, 1.36)
Sample size, N	6,150	6,844	738
Kidney outcome, HR (95%CI)			
eGFRcr<30 ml/min/1.73m^2	1.73 (0.41, 7.23)	0.78 (0.55, 1.09)	1.09 (0.58, 2.06)

MI, myocardial infarction; LDC, loose-dose combination; FDC, fixed-dose combination; eGFR, estimated glomerular filtration rate; SGLT2i, sodium-glucose cotransporter-2 inhibitor; DPP4i, dipeptidyl peptidase-4 inhibitor; TZD, thiazolidinedione.

eTable 11 Summary table of sensitivity analyses

	Extra matched on the diabetes duration	Replaced drug class with paired ATC codes	No 90-day lag on outcomes	In population without history of chronic IHD, MI, and unstable angina
Sample size	18,250	27,766	27,766	22,979
Cardiovascular outcomes				
Acute MI	0.93 (0.78, 1.10)	1.00 (0.88, 1.14)	0.94 (0.82, 1.06)	0.96 (0.82, 1.12)
Atrial fibrillation	0.94 (0.77, 1.13)	1.04 (0.90, 1.21)	0.95 (0.82, 1.09)	1.04 (0.88, 1.23)
Unstable angina	0.87 (0.67, 1.12)	0.88 (0.72, 1.06)	0.88 (0.73, 1.07)	0.86 (0.67, 1.11)
Heart failure	0.92 (0.79, 1.07)	0.91 (0.80, 1.02)	0.84 (0.75, 0.94)	0.92 (0.80, 1.05)
Ischemic stroke	0.97 (0.80, 1.17)	0.99 (0.85, 1.14)	1.00 (0.86, 1.15)	0.99 (0.84, 1.17)
Sample size	13,728	13,728	13,728	11,188
Kidney outcome				
eGFRcr<30 ml/min/1.73m^2	0.89 (0.66, 1.19)	0.84 (0.63, 1.12)	0.84 (0.63, 1.12)	0.96 (0.69, 1.33)

IHD, ischaemic heart disease, MI, myocardial infarction; eGFR, estimated glomerular filtration rate.

eTable 12 Hazard ratios (HR) following propensity score matching on all covariates and diabetes duration

	HR (95%CI)
Cardiovascular outcomes	
Acute MI	0.93 (0.78, 1.10)
Atrial fibrillation	0.94 (0.77, 1.13)
Unstable angina	0.87 (0.67, 1.12)
Heart failure	0.92 (0.79, 1.07)
Ischemic stroke	0.97 (0.80, 1.17)
Kidney outcome	
eGFRcr<30 ml/min/1.73m^2	0.89 (0.66, 1.19)

Sample size for cardiovascular outcomes is 18,250, for kidney outcome, the size is 13,728.

MI, myocardial infarction; eGFR, estimated glomerular filtration rate.

eTable 13 Hazard ratios (HR) following propensity score matching on all covariates in which drug class was replaced by ATC codes

	HR (95%CI)
Cardiovascular outcomes	
Acute MI	1.00 (0.88, 1.14)
Atrial fibrillation	1.04 (0.90, 1.21)
Unstable angina	0.88 (0.72, 1.06)
Heart failure	0.91 (0.80, 1.02)
Ischemic stroke	0.99 (0.85, 1.14)
Kidney outcome	
eGFRcr<30 ml/min/1.73m^2	0.84 (0.63, 1.12)

Sample size for cardiovascular outcomes is 27,766, for kidney outcome, the size is 13,728.

MI, myocardial infarction; eGFR, estimated glomerular filtration rate.

eTable 14 Hazard ratios (HR) for the stratified analyses following propensity score matching on all covariates in which drug class was replaced by ATC codes, for heart failure outcome only

	Sample size	HR (95%CI)
Age		
Below 65 years	15,668	1.06 (0.87, 1.28)
65 years and older	12,098	0.82 (0.71, 0.94)
Sex		
Male	18,591	0.91 (0.80, 1.04)
Female	9,175	0.90 (0.78, 1.11)

eTable 15 Hazard ratios (HR) following propensity score matching, all outcomes after the index date were used (removing 90-day lag time)

	Acute MI	Atrial fibrillation	Unstable angina	Heart failure	Ischemic stroke	eGFRcr <30 ml/min/1.73m^2
Events during follow-up, n	954	746	416	1,283	757	193
Follow-up time in years, Median (IQR)	4.0 (1.9, 7.2)	4.0 (1.9, 7.3)	4.0 (1.9, 7.4)	4.0 (1.9, 7.3)	4.0 (1.9, 7.3)	3.8 (1.9, 6.3)
Incident rate per 1000 person-years, (95%CI)	6.6 (6.2, 7.1)	5.1 (4.8, 5.5)	2.8 (2.6, 3.1)	8.9 (8.4, 9.4)	5.2 (4.9, 5.6)	3.0 (2.6, 3.4)
Hazard Ratio (95%CI)	0.94 (0.82, 1.06)	0.95 (0.82, 1.09)	0.88 (0.73, 1.07)	0.84 (0.75, 0.94)	1.00 (0.86, 1.15)	0.84 (0.63, 1.12)

Sample size for cardiovascular outcomes is 27,766, for kidney outcome, the size is 13,728.

MI, myocardial infarction; eGFR, estimated glomerular filtration rate.

eTable 16 Hazard ratios (HR) following propensity score matching, in population without history of ischaemic heart disease

	HR (95%CI)
Cardiovascular outcomes	
Acute MI	0.96 (0.82, 1.12)
Atrial fibrillation	1.04 (0.88, 1.23)
Unstable angina	0.86 (0.67, 1.11)
Heart failure	0.92 (0.80, 1.05)
Ischemic stroke	0.99 (0.84, 1.17)
Kidney outcome	
eGFRcr<30 ml/min/1.73m^2	0.96 (0.69, 1.33)

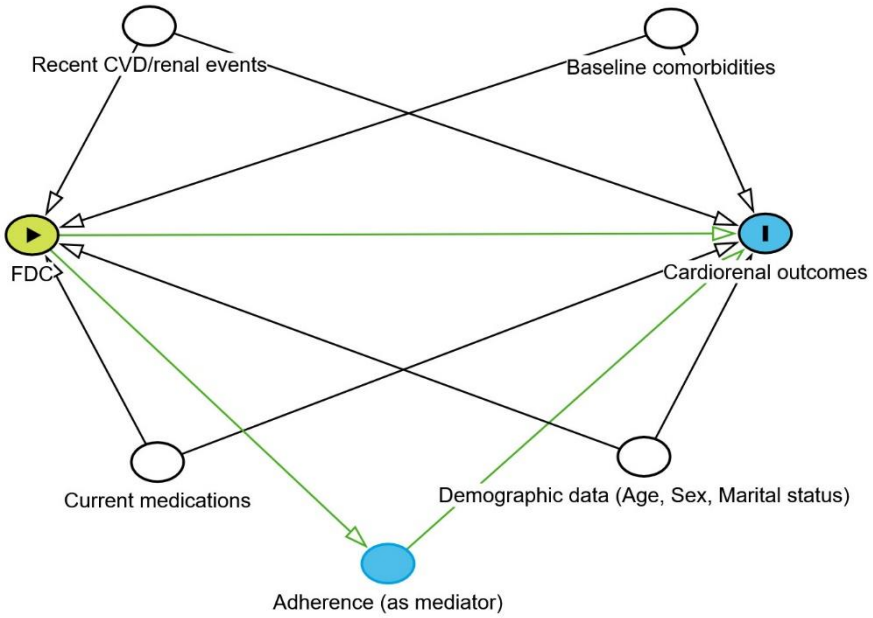
Sample size for cardiovascular outcomes is 22,979, for kidney outcome, the size is 11,188.

MI, myocardial infarction; eGFR, estimated glomerular filtration rate.

eTable 17 Hazard ratios (HR) for the stratified analyses following propensity score matching, in population without history of ischaemic heart disease, for heart failure outcome only

	Sample size	HR (95%CI)
Age		
Below 65 years	13,982	1.01 (0.88, 1.37)
65 years and older	8,997	0.78 (0.66, 0.93)
Sex		
Male	14,812	0.91 (0.77, 1.08)
Female	8,167	0.93 (0.73, 1.18)

eFigure 1 Directed acyclic graph for this study



Solid green circle, exposure; solid blue circle without character “I”, mediator; solid blue circle with character “I”, outcome; solid blank circle, adjusted confounding factors; solid black line, confounding path; solid green line, causal path of interest; arrow, direction.

9. Chapter 9 – Discussion

9.1. Summary of key findings

This thesis explored the intricate association between the kidneys and health outcomes across multiple populations, drawing on both systematic reviews and original research. Five studies were conducted to examine physical activity, eGFR discordance, and pharmacological treatment, and their associations with kidney and cardiovascular health.

The first part of this thesis examined the role of physical activity in kidney biomarkers. Chapter 4 presents a systematic review that showed increasing physical activity was associated with improved kidney biomarker profiles in the general population, but identified important gaps in the literature, including limited studies of generally small size. Building on these findings, Chapter 5 uses UK Biobank data to demonstrate that increasing levels of physical activity were independently associated with improved kidney health, as assessed by eGFR and albuminuria. These results fulfil the first objective of the thesis by reinforcing the role of physical activity as a modifiable factor in maintaining kidney health and support its integration into preventive public health measures.

The second part of the thesis centres on how kidney health is measured, particularly the intraindividual discrepancy between creatinine-based and cystatin C–based eGFR. Chapter 6 synthesizes existing evidence and shows that such discordance is prevalent in patients with type 2 diabetes or chronic kidney disease. The discordance often associates with higher rates of mortality, cardiovascular disease, and kidney disease progression. By highlighting the status quo of research on eGFR discordance, this chapter meets the second objective of this thesis. Chapter 7 follows up with original study in a large UK cohort, finding that people with lower eGFRcys relative to eGFRcr had significantly elevated mortality risk. These findings imply a dual-marker approach in clinical assessments, warning that relying solely on one measure may miss vulnerable populations thus meeting the third objective. Of note, the results in Chapter 7 have caused extensive debate in routine biochemistry units across the UK (including Core Biochemical Assay Laboratory (CBAL) at Cambridge (Personal communication, Dr. Ian Halsall), and was the subject of a journal club for the publishing journal Clinical Chemistry. Additionally, the Lab Medicine at Queen Elizabeth University Hospital, Glasgow are now preparing to measure cystatin-C as part of kidney function work-up in selected patient groups.

Finally, Chapter 8 shifts focus to pharmacological management in individuals with type 2 diabetes. Using data from Swedish national registers, the study evaluated fixed-dose combination oral antidiabetic therapies. The study shows that, beyond glycaemic control, fixed-dose combination drugs may offer cardiovascular benefits, thereby highlighting their potential role in integrated cardiorenal protection strategies, thus meeting the fourth objective. This research may be of great importance by filling the gap in current guidelines of diabetes management.

Taken together, these studies show that kidney phenotypes, including creatinine-cystatin C discordance, carry independent and clinically relevant information for mortality and cardiovascular risk. The thesis moves from measurement to management by clarifying when dual-marker eGFR adds value, demonstrating that discordance can refine risk beyond eGFR and albuminuria, and showing that treatment strategy, such as fixed-dose combinations, can improve heart failure outcomes partly through better adherence. These findings provide a practical basis for more individualised prevention and offer concrete signals for future guideline updates.

9.2. Comparison with existing research

Physical activity and the kidneys

The earlier chapters of this thesis discussed the association between physical activity and kidney health. It was observed that among healthy individuals, a reduction in physical activity was associated with a higher rate of rapid decline in eGFRcr, whereas an increase in physical activity was not significantly associated with this outcome. In contrast, when kidney function was assessed using cystatin C, a reduction in physical activity was not associated with eGFRcys rapid decline, but an increase in physical activity was associated with a lower rate of rapid decline in eGFRcys. These findings not only suggest a dose–response association between physical activity and kidney health but also highlight the importance of considering the choice of biomarker in studies using kidney function as an outcome, as different biomarkers may lead to different interpretations.

The dose-response relationship between physical activity and health outcomes has been reported in previous studies. A meta-analysis examining leisure-time physical activity demonstrated a negative nonlinear association with the risk of developing metabolic syndrome. Specifically, engaging in 150 minutes of moderate-intensity physical activity

per week (equivalent to 10 MET hours/week) was associated with a 10% risk reduction. This risk reduction reached 53% with 70 MET hours/week, when compared to individuals who were physically inactive.[1]

However, it is important to note that simply being active (from inactive) may not necessarily be associated with health benefits. This thesis demonstrated that for every 1,000 MET minutes/week change in physical activity, the associated change in kidney outcome risk was merely around 2–4%. One thousand MET minutes/week is equivalent to approximately 3 hours and 20 minutes of moderate-intensity physical activity per week (e.g., cycling at ≥15 km/h). Therefore, it seems that only physical activity with a certain intensity is associated with significant kidney benefits.

A similar finding was reported in a structured, two-year, moderate-intensity exercise intervention study published in November 2023, which focused on 1,381 healthy older adults aged 70–89.[2] No significant change in kidney health biomarkers was observed after the intervention. That said, in observational analysis on the intervention data when time-updated step count was used as the study exposure, higher time-updated step counts (i.e., remaining highly active at every repeated assessment) were significantly associated with improved biomarker profiles. For example, urine albumin was reduced by 0.22 (95%CI: 0.06, 0.37) mg/g and NGAL by 0.16 (95%CI: 0.07, 0.24) pg/mL per mg/dL of urine creatinine after intervention. This suggests that maintaining a consistently high level of physical activity is key to deriving kidney health benefits. However, it should be noted that the significant results were achieved through comparing quartile 4 over quartile 1, which implies possibly huge between-group differences. The study also provided results using per SD elevation of steps (1,487 steps), which are similar to those of using quartiles.[2]

In future, research may focus on identifying the optimal intensity, duration, and frequency of physical activity required to maximize kidney benefits specific to various populations. Studies exploring the different health implications of eGFRcr and eGFRcys could support individualized risk prevention or classification in the context of precision medicine.

eGFR discordance and health outcomes

This thesis first reviewed the current research on eGFR discordance, summarizing that most published studies have overly focused on patients with kidney disease or diabetes,

while neglecting the general population. Based on this, the thesis then investigated the association between eGFR discordance and mortality in a large general UK population. The study shows that people with low eGFRcys and high eGFRcr have a higher risk of mortality, and that age 65 or older and BMI ≥ 30 kg/m^2 show interaction effects.

For the population aged 65 and above, a study found that individuals with eGFRcys < 70% eGFRcr have a higher mortality risk (HR = 1.43, 95%CI: 1.12, 1.82). This result is lower than the finding in this thesis for the same age group (HR = 2.20, 95%CI: 1.91, 2.53), though this may be due to the lower threshold for eGFRcys used in this study (<60% eGFRcr).[3]

As for the population with BMI ≥ 30 kg/m^2, this thesis showed that their mortality risk is lower compared to those with lower BMI. This is consistent with some published research[4], but contradicts the conclusion of a meta-analysis.[5] However, the latter study's conclusion is limited by the studies selected for inclusion, as selected studies only considered patients with diabetes and had very small sample sizes in each subgroup. Therefore, the role of eGFR discordance in the obese population may warrant further investigation.

Other studies have focused on specific clinical populations, such as post-organ transplant patients. A study of 47 post-solid organ transplant patients receiving tacrolimus showed that the median bias between eGFRcys calculated using CKD-EPI equations and eGFRcr was -22 ml/min/1.73m^2, or 40% bias, which exceeded the 30% tolerance limits.[6, 7] If eGFRcys is used to measure kidney function, there is a risk of misclassifying individuals into a more advanced CKD stage. If eGFRcr is used, there is a risk of underestimating the CKD stage. The discrepancy between eGFRcys and eGFRcr increases when eGFRcr is ≥60 ml/min/1.73m^2. Using eGFRcr-cys provides a more accurate classification of CKD stage.[6] This study further reflects that the applicability of different formulas and kidney biomarkers varies across populations.

However, neither the findings of this thesis nor other studies have defined a clear criterion for eGFR discordance and the corresponding kidney function equations. From the author's perspective, using the eGFRcr-cys as an assessment of kidney function seems prudent in most situations. Further research is needed to define eGFR discordance, establish

measurement standards, and investigate the causes and health implications of this condition across populations.

Fixed-dose combination drugs and cardiorenal outcomes

In the last part of this thesis, the author explored the association of oral antidiabetic fixed-dose combination drugs (FDC) with cardiorenal outcomes in comparison to loose-dose combination drugs (LDC).

Research on the application of oral FDC antidiabetic drugs has gradually increased since around 2007. However, the majority of FDC studies focus on glycaemic goals and safety issues in comparison with LDC users. One meta-analysis suggested that FDC, compared to co-administered dual therapy, achieved significantly greater HbA1c reduction, and the FDC population had relatively higher medication adherence.[8]

Furthermore, some studies compared the effects of different FDCs (alogliptin + metformin vs. vildagliptin + metformin), and the results showed that both FDCs had similar effects on blood glucose control and safety [9] As there are several FDCs on the market with combinations of three drugs, some studies have compared the efficacy of dual-drug FDCs and triple-drug FDCs (e.g., dapagliflozin + sitagliptin + metformin vs. sitagliptin + metformin).[10] However, triple-drug FDCs are newly developed and not yet widely available (e.g., Trijardy XR: empagliflozin + linagliptin + metformin).[11] The novelty of studies on triple-drug FDCs is commendable, but their clinical utility remains debatable.

The novelty of this thesis lies in the fact that, under the current clinical guidelines that emphasize protecting the heart and kidneys of people with diabetes, the research used real-world data to focus on patients' cardiorenal outcomes rather than the widely explored glucose-lowering effects. The study also investigates the mediating effect of medication adherence. The results show that patients using dual-drug FDCs had better cardiovascular outcomes compared to co-administered dual LDC users, and medication adherence mediated about half of the cardiovascular benefits of using FDCs. This study covers the three most common FDC drug categories, SGLT2i + metformin, DPP4i + metformin, and TZD + metformin, demonstrating good applicability.

9.3. Implications of findings in clinical context

Firstly, this thesis showed the complexity of the association between physical activity and kidney health, which shows completely different directions depending on the kidney function biomarker used (SCr or Cys C). This suggests that alternative analyses using a second biomarker should be performed when analysing kidney function to get a holistic view. Furthermore, this thesis showed a significant association between eGFR discordance and mortality in a general population. This finding may directly influence clinicians' assessment of patient risk. When a significant discrepancy exists, clinicians should be vigilant, as this discrepancy may indicate that the patient's true kidney function can be overestimated (most of the time) or underestimated. The evaluation of the risk of adverse outcomes can be misleading. In risk stratification in the general population, reliance on only one eGFR biomarker should be approached with caution.

It is worth noting that some studies have shown that the latest race-independent CKD-EPI eGFR equation modestly overestimated eGFR in European populations and shifted CKD stages to a better stage than the race-adjusted 2009 equation.[12] The race-independent equation is also less accurate than the EKFC equation developed specifically for European populations.[13] Given that the US National Kidney Foundation and the American Society of Nephrology are promoting the race-independent eGFR equation [14], this thesis reminds clinicians to be aware of the potential limitations of these equations when dealing with European patients. It is recommended that international guidelines provide more detailed explanations of the applicability and limitations of eGFR equations in different populations.

In addition to the above, another clinical significance of this thesis lies in providing insights for the selection of combination therapy for patients with type 2 diabetes. Current clinical guidelines recommend early combination therapy, but they do not suggest specific combination patterns. This study, for the first time, compared the effects of FDC and LDC treatments on cardiorenal outcomes using real-world data, providing strong evidence for clinicians to choose FDC drugs over LDC. The study found that FDC treatment demonstrates potential advantages in heart failure risk management. The mediating role of adherence in the association between FDC treatment and heart failure further emphasizes the importance of selecting a treatment regimen with higher adherence. For elderly patient populations, the benefits of FDC are particularly evident, as they often have multiple

comorbidities and complex medication regimens. Simplifying the treatment plan can be crucial to improving treatment adherence and outcomes.

9.4. Strengths and limitations

This thesis possesses several strengths. Firstly, each original study in this thesis is based on a systematic review conducted prior to the research. Through this review, the author identified gaps in existing studies and used these insights to design targeted research. This approach ensures that the research is novel and directly addresses current issues, rather than being speculative or unsubstantiated. Secondly, the data for this thesis are sourced from the UK Biobank and the Swedish national registers. These databases are rigorously managed, with strict standards for sampling and data management, ensuring high data quality. With sample sizes ranging from hundreds of thousands to millions, the large sample size and data quality provide a solid foundation for the reliability of the study's conclusions.

However, like all research, this thesis has its limitations. Firstly, all the studies conducted are observational, meaning they can only show associations between exposure and outcome, but cannot establish causality. Much of the kidney–CVD association likely reflects shared upstream vascular disease and endothelial dysfunction, with renal pathways acting as amplifiers rather than sole causes. Secondly, some covariates, such as self-reported comorbidities, may be prone to recall errors by participants, which could introduce bias. Thirdly, while the data sources used in this thesis covered a broad range of covariates, not all the confounders were provided, leading to remaining confounding effects. Fourthly, participants of the UK Biobank do not fully reflect the UK national population. Therefore, the generalizability of our findings to the entire UK population may be limited. While this means that prevalences (such as prevalence of discordance) cannot be taken as true reflections of the wider populations, exposure-outcome associations should remain valid in the absence of any unidentified interactions with population demographics. Fifthly, the kidney function in this thesis was assessed using eGFR rather than mGFR. Existing research suggests that mGFR and eGFR can differ by around 30%.[15] Since this difference is a systematic bias, using mGFR in place of eGFR could result in numerical changes in the findings, although the direction of the conclusions should remain consistent.

9.5. Final conclusions

This thesis shows that measurement choices in kidney function have meaningful clinical consequences. Creatinine and cystatin C provide complementary information; discordance between their eGFR estimates is common and prognostically meaningful; and treatment strategies that support adherence can improve cardiorenal outcomes. These findings justify targeted use of cystatin C, routine consideration of discordance when decisions matter, and the use of combination therapy where appropriate, helping to move from better measurement to better management.

9.6. Future works

Based on the scope of this thesis and the current research trends, several future works may be suggested:

Standardization of eGFR discordance:

A key question is which equation should be used to calculate eGFR. Should a single equation be universally applied, or should population-specific equations be used for better accuracy? The standardization of eGFR discordance remains an important unresolved issue. Future work should evaluate alternative thresholds and definitions of eGFR discordance using data-driven approaches. A comparative methodological study could be conducted using Youden index, inflection point, or other cut-off derivation techniques to determine thresholds. These candidate thresholds could then be validated across multiple cohorts, especially in the US and European populations. A matrix constructed by various eGFR equations and target populations and filled by corresponding thresholds could be presented.

Aetiologies of eGFR discordance:

A prevailing physiological hypothesis suggests that eGFR discordance arises due to a reduction in the pore size of the glomerular filtration barrier, selectively impeding the filtration of cystatin C while allowing creatinine to pass. However, it is highly unlikely that this is the sole mechanism.[16] Notably, accurate assessment of pore size requires electron microscopy, which is impractical for large-scale clinical screening. Therefore, to what extent is discordance attributable to structural changes in the filtration barrier versus changes in biomarker-influencing factors? Among people identified as having eGFR discordance, what the proportion of each of these contributors (structural change /

biomarker-influencing factors) is? Furthermore, how do these distinct aetiologies differentially affect clinical outcomes? These are questions requiring further investigation.

Livers, kidneys, and pharmacological treatments for type 2 diabetes:
The 2025 ADA guidelines introduced a new subsection in the pharmacological approaches addressing the mitigation of metabolic dysfunction-associated steatotic liver disease (MASLD) and metabolic dysfunction-associated steatohepatitis (MASH), positioned after recommendations for mitigating cardiovascular and kidney risk.[17] Among classical antihyperglycemic agents, pioglitazone is frequently used to manage MASLD/MASH risks. However, it is well-documented that thiazolidinediones may exacerbate cardiac load due to fluid retention.[18] Another recommended agent is GLP-1 RAs, which are known for their cardio and renal protective effects. A third recommended approach is the combination of GLP-1 RAs with pioglitazone. However, the optimal timing for initiating treatment remains unclear. Furthermore, the fluid retention may, paradoxically, compromise the heart and the kidneys. Thus, how to balance the treatment and adverse effects represents an important area for further research. Additionally, given the high prevalence of chronic liver disease and diabetic kidney disease, their interrelationship may warrant exploration.

A pragmatic comparative effectiveness study could evaluate whether GLP-1 RAs + pioglitazone provides additional cardiorenal or hepatic benefits beyond GLP-1 RA monotherapy in diabetic patients with MASLD/MASH risks. This would address the current uncertainty around treatment sequencing and safety trade-offs (e.g., thiazolidinedione-induced fluid retention).

A new-user, active-comparator design in large electronic health record datasets (e.g., UK CPRD, TriNetX) can be applied. Primary outcomes can be heart failure incidence and decline in eGFR, while histologically informed MASH regression or ALT/AST normalisation can be secondary outcomes.

9.7. References for Chapter 9

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