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Gluconeogenesis and oxidative stress in renal proximal tubular cells

A non-systematic review.

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Foreword

Work on this thesis has provided an opportunity for a highly rewarding investigation of some of the most fundamental and interesting topics of our biology and how they interact. This process has given me knowledge and insights that I shall cherish dearly. I am grateful to my supervisor Lars M. Ytrebø for trusting me to an adventurous exploration.

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1 Summary

This thesis explores the hypothesis that renal gluconeogenesis has a protective role against oxidative stress in proximal tubular cells. Oxidative stress is a primary driver of pathophysiology in many diseases. This thesis reviews the fundamental factors that drive oxidative stress through electron leakage in mitochondria, highlighting tissue metabolic rate and tissue oxygenation as important driving factors. The proximal tubules of the kidneys are emphasized as being highly exposed to these driving factors, making them predisposed for electron leakage and therefore reliant on mitigation of oxidative stress through antioxidative defenses. It is known that glycolysis supports antioxidative systems, but proximal tubular cells run gluconeogenesis instead of glycolysis and a possible role of gluconeogenesis in protecting against oxidative stress has not received attention. This thesis employs a non-systematic literature review to explore a hypothesis involving several interconnected topics, attempting to synthesize existing knowledge to inform the generation of new ideas, testable hypotheses and future experiments. The primary result of this review is the finding that there is conceptual support for the hypothesis and that it has some plausibility, encouraging further exploration. However, several topics have been omitted due to scope limitations that could be important for the hypothesis in question. Possible interpretations and future directions are discussed.

2 Introduction

Oxidative stress mediated through the generation of radical oxygen species (ROS) has in recent times become a topic of great interest as a driver of pathology in wide variety of diseases responsible for a large proportion of global morbidity and mortality (1, 2). The bulk of ROS is generated due to electron leakage in mitochondria (3), and the rate of ROS generation is highly driven by the tissue specific metabolic rate (K_i) and the partial pressure of oxygen in mitochondria ($mitoPO_2$)(4). The specific metabolic rate varies enormously between tissues(5) and $mitoPO_2$ increases with increased local tissue oxygenation relative to local oxygen consumption (6). A high propensity for electron leakage is therefore maintained if a high $mitoPO_2$ coincides with a high K_i. In general, this condition does not occur in most tissues of the body, as a low K_i is typically maintained in highly oxygenated tissues such as lung parenchyma or arterial endothelium (5), and tissue oxygenation is limited in organs

with a high K_i by autoregulation of blood flow such that oxygen delivery does not greatly exceed oxygen demand. The renal cortex is highly overperfused (7), proximal tubular cells (PTCs) of the kidneys have an extremely high Ki (8), and they cannot autoregulate blood-supply based on oxygen demand (9). This combination of factors indicate an increased propensity towards electron leakage in PTCs compared to most other tissues. Mitochondrial dysfunction and oxidative stress have emerged as important drivers of acute kidney injury and chronic kidney disease (CKD) (10) with PTCs being heavily involved. Due to their high propensity to generate ROS, PTCs are highly reliant on antioxidative defenses to mitigate oxidative stress, evident by their heavily oxidized internal state and increased levels of glutathione (GSH) (11). It is well known that glycolysis is a key supporting pillar of antioxidative functions in most cells of the body through NADPH regeneration via the pentose phosphate pathway (PPP) and a failure of the PPP typically leads to severe cellular dysfunction or death due to oxidative stress (12). PTCs do not run glycolysis during normal conditions of health and instead run gluconeogenesis (13), so PPP in PTCs cannot be fed glycolytically under normal conditions. The PPP can theoretically be fed via gluconeogenesis just as well as via glycolysis since gluconeogenesis also generates glucose-6-phosphate (G6P) for the glucose-6-phosphate dehydrogenase (G6PD) enzyme, the starting enzyme of the PPP. The antioxidative systems of PTCs can therefore be supported by gluconeogenesis via the PPP. It is notable that decreased capacity for gluconeogenesis in PTCs is often associated with damage to proximal tubules and reduced kidney function in acute kidney injury (AKI)(14), in sepsis associated acute kidney injury (SA-AKI)(15, 16), and is a key feature of worsening chronic kidney disease (CKD)(17, 18). Renal gluconeogenesis might have a role in supporting proximal tubular health as a component of redox homeostasis in PTCs. The interplay between renal gluconeogenesis and redox homeostasis in PTCs has received very little attention. This thesis explores the hypothesis that renal gluconeogenesis has a protective role against oxidative stress in PTCs with the goal of informing future research directions, aiming to improve fundamental understanding of the interplay between metabolism and oxidative stress.

3 Materials and methods

3.1 Description of Literature Search

This thesis explores questions that interconnect many complex topics. For this purpose, a non-systematic literature review was chosen, allowing exploration of a complex and interconnected literature in a broad fashion while allowing freedom to go into topics in depth as needed to clarify uncertainties or answer key questions. The order of chapters in the result section approximates the order in which this review was conducted in terms of topic investigation, although there was overlap at times.

3.1.1 Search Strategy

The primary source for literature was PubMed, selected for its comprehensive repository of medical research. To complement PubMed searches, google scholar was used at times when sources could not be found on PubMed. ChatGPT4 was employed iteratively to refine search strategies and clarify uncertainties in interpretation, thus enhancing search efficiency and focus. This process involved an initial exploration of literature, note-taking, and subsequent query refinement facilitated by interactive discussions with ChatGPT4 to clarify complex topics, leading to new search directions.

3.1.2 Search topics

Searches were made for sources covering a broad range of both general and specific topics. Topics included, but were not limited to: Oxidative stress, role of oxidative stress in disease (both in general and in relation to several specific diseases), reactive oxygen species, reductive stress, redox balance, redox homeostasis, oxidative phosphorylation, electron transport, mitochondrial function, sources of reactive oxygen species, electron leakage, factors driving electron leakage, modelling of mitochondrial electron leakage, electron carrier couples, mitochondrial oxygen tension, oxygen tensions in various tissues and compartments, metabolism, specific metabolic rate across organs and various tissues, renal oxygen delivery, renal metabolism, antioxidant systems, glutathione, antioxidative upkeep, NADPH, antioxidative systems and glycolysis, G6PD, pentose phosphate pathway, kidney physiology, renal glucose handling, renal glucose metabolism, gluconeogenesis and antioxidative systems, gluconeogenesis and oxidative stress, renal gluconeogenesis

connections with other renal functions. For each single topic, several searches were typically conducted using various combinations of different keywords relevant to the topic.

3.1.3 Inclusion and Exclusion Criteria

The selection favoured review articles published after 2010 to strive for current information. Articles were favoured based on the level of analysis of their narrative and relevance to the topic areas. In cases where single sources were found that covered several relevant research topics at an adequate level they were selected in favor of using several separate sources, reducing reference count. Original research was included regardless of publication date if it provided important insights into specific questions related to the hypothesis being formulated. Certain older sources were included for historical perspective.

3.2 Selection Process

3.2.1 Screening and Selection

Articles were initially screened by title and abstract for relevance. Number of sources evaluated by initial screening was not tracked but is estimated to be roughly one order of magnitude higher than number of sources included in this step. The selection was based on intuitive judgment of the content's applicability to the thesis topics. This non-systematic approach was necessary due to the exploratory nature of the review and wide array of topics. 359 Sources were included in an EndNote research library after initial screening. After inclusion in the research library, they were sorted into groups according to topic and a secondary screening was performed where they were further evaluated. 273 sources were excluded after secondary screening and 86 sources were selected for inclusion and in depth review.

3.2.2 Critical Appraisal

Given the constraints of time and the broad scope of the review, a systematic critical appraisal was not conducted. Instead, emphasis was placed on the interpretative analysis of the literature, assessing the credibility and contribution of each source based on its content.

3.2.3 Data Extraction and Synthesis

This review did not involve traditional quantitative data extraction but instead focused on the qualitative integration of findings from various sources. The goal was synthesis, aiming

to weave together existing knowledge and insights gained during the research process to formulate and explore novel hypotheses.

3.2.4 Reflexivity and Bias

The researcher's background in engineering informed the analytical approach to biological systems, viewing them through the lens of design and functionality. This influenced the framing of questions and the interpretation of biological complexity as analogous to engineered systems.

3.3 Ethical Considerations

No ethical issues were pertinent to the conduct of this literature review, as the study did not involve primary data collection or intervention with human and animal subjects.

4 Results

This thesis explores the hypothesis that renal gluconeogenesis supports redox homeostasis in PTCs. Here is a summary of the structure of this review: Sections 4.1-4-6 lays the groundwork by investigating key points relevant to the hypothesis. These topics were covered briefly in the introduction and are explored in greater detail here. Section 4.7 then explores the hypothesis itself more directly.

"4.1: Redox balance and oxidative stress" - The groundwork for this thesis begins with an introductory review of basic concepts of redox balance, redox homeostasis and oxidative stress and covering the importance of electron leakage as a source of oxidative stress.

"4.2 Electron leakage" – Due to its importance as the biggest source of oxidative stress, factors affecting electron leakage are investigated. Fundamental factors inside the mitochondria driving electron leakage are described and connected to factors at the cellular and at the tissue/organ level. Metabolic rate and tissue oxygenation are highlighted as key drivers of electron leakage.

"4.3 Tissue metabolic rate, mitochondrial density and oxygen delivery" – Due to the importance of metabolic rate and tissue oxygenation as drivers of electron leakage, these factors are compared across various organs and tissues in the body. PTCs are revealed as being exposed to a unique combination of elevated driving factors of electron leakage,

making them highly reliant on antioxidative defenses. Mitochondrial density is also discussed.

“4.4 Antioxidative systems” - Due to their importance in mitigating oxidative stress, antioxidative defenses are reviewed. Enzymatic and catalytic antioxidants are highlighted as critical in the context of mitigating oxidative stress due to electron leakage in mitochondria. The central importance of GSH and NADPH is covered, and they are connected to glucose metabolism.

“4.5 Glucose metabolism and antioxidative defenses” - Connections between glucose metabolism and antioxidative systems covered in 4.4 are explored, showing how glucose metabolism underpins antioxidative systems via NADPH regeneration and contributing to GSH synthesis.

“4.6 Glucose metabolism and transport in PTCs” - Gluconeogenesis in PTCs is discussed along with glucose reabsorption and transport.

“4.7 Does gluconeogenesis protect against oxidative stress in PTCs?” The main hypothesis of this thesis is presented in full.

4.1 Redox balance and oxidative stress

Biological redox reactions involve the transfer of electrons between molecules in a paired reaction, where one molecule is oxidized (loses electrons) and another is reduced (gains electrons), driving fundamental processes of life such as cellular respiration and metabolism (3). The concept of redox balance is concerned with the maintenance of equilibrium between oxidation and reduction reactions within biological systems and maintaining a healthy internal redox state(19).

Redox balance is typically expressed in terms of the redox state of certain coupled redox pairs, typically between nicotinamide adenine dinucleotide (NAD) in its oxidized or reduced form (NAD^+/ NADH), between nicotinamide adenine dinucleotide phosphate (NADP) in its oxidized or reduced form ($NADP^+$/ NADPH), or between glutathione (GSH) and glutathione disulfide (GSSG)(3). The redox state of these couples can be described by their ratios or by their redox potential described by the Nernst equation(19). NAD^+/ NADH is typically

maintained at a highly oxidized state in cytosol, critical for catabolic processes that depend on high quantities of NAD^+ as an electron acceptor. The $NADP^+$/ NADPH and pair on the other hand is vital for anabolic processes as well as antioxidative defense alongside GSH/GSSG, and these two pairs are maintained in a highly reduced state(19). These coupled redox pairs are the central hubs of redox biology and their ratios are maintained at fairly stable levels, thereby maintaining redox balance(19). These redox pairs are also coupled against each other in such a way that catabolic and anabolic metabolic processes are kept in balance with overall energy expenditure and the redox state of the internal milieu (19).

A central task of our redox biology is to utilize oxygen with its strong electronegativity as a final electron acceptor in catabolic metabolism for the generation of adenosine triphosphate (ATP) and other interconvertible energy currencies (19), all the while limiting the potentially harmful effects of oxygen on our biomolecular structures. Oxygen is toxic in high concentrations as it spontaneously reacts in water to form radical species such as superoxide anions ($O2^-$) and hydrogen peroxide (H_2O_2). These chemicals have extremely high reactivity and short half-life in vivo, where they can cause damage by oxidizing essential biomolecules such as DNA, proteins, and lipids, leading to mutations, protein dysfunction, and cell membrane disruption (3). ROS also lead to the formation of reactive nitrogen species (RNS) leading to nitrosative stress (3). Balancing the utility of consuming oxygen vs mitigating its harmful effects are therefore thought of as one of the most primary challenges of evolution, especially for vertebrate life on land (20).

Generation of a certain amount of ROS is inevitable as it happens spontaneously with oxygen in water at a low rate (19), and at a higher rate in mitochondria where high flux of redox chemistry is maintained (3). Since they are potentially harmful and inevitable, life has evolved beneficial ways to utilize ROS. Phagocytic immune cells utilize the oxidative toxicity of ROS as a highly effective weapon for killing pathogens through oxidative bursts (21). ROS play crucial roles in normal physiology by participating in cell signaling, immune response, regulation of vascular tone, cell differentiation, apoptosis, and maintaining cellular redox balance (2) (22, 23).

Oxidative stress occurs when reactive species are generated at rates and in quantities higher than what can be mitigated by antioxidative defenses causing reactive species and oxidative

damage of biomolecules to accumulate above normophysiologic levels. The concept of oxidative stress stems from Selye's concept of general stress (1) and is crucially integrated into adaptive mechanisms evolved to maintain homeostasis. Manageable oxidative stress in conditions of health trigger an array of adaptive mechanisms designed to strengthen the organism against the stressor. As an example, oxidative stress plays a central role in driving the signal cascade responsible for adaptations to several major forms of exercise (24). Oxidative stress can therefore have a hormetic effect which is sometimes beneficial as long as stress levels don't exceed the hormetic zone, i.e. if the level of stress does not exceed the capacity of the organism to repair and beneficially adapt to the stressor (25). As in toxicology, the dose makes the poison. It is important to note that oxidative stress involves a loss of balance between factors that drive production of reactive species and antioxidative factors that mitigate reactive species and their effects as they are produced (26). This highlights the importance of antioxidative systems, of which GSH/GSSH is the most versatile and important protective redox buffer (27).

Disruptions in redox balance towards either a more oxidized or reduced state can both lead to oxidative stress. It is generally considered beneficial for the organism to maintain a highly reduced intracellular milieu as a shift towards and oxidized intracellular state is typically characterized by an overproduction of ROS and (3), however disrupting redox balance too far towards a reduced state can cause reductive stress, which is also associated with overproduction of ROS (28). Both oxidative and reductive deviations from redox balance thus leads to a pathologic overproduction of ROS and RNS and are ultimately oxidative in their mechanism of damage(28).

In the short-term, excessive oxidative stress triggers a cascade of cellular responses resulting in acute inflammation and tissue damage, potentially overwhelming the cell and leading to apoptosis or necrosis (29, 30). In sepsis for instance, metabolic dysregulation, mitochondrial dysfunction and oxidative stress has recently emerged as key drivers of cellular damage, organ dysfunction and mortality (31-34). Over the long term, sustained high levels of ROS and RNS contribute to the pathogenesis of chronic diseases, primarily through the persistent oxidative modification of DNA, proteins, and lipids. Oxidative stress has for instance been associated as an important driver of the development and progression of cancer (35),

various common neurodegenerative diseases (36), cardiovascular diseases (37), diabetes (38), and is even suggested as a key driver of the aging process (25, 35).

There are many potential sources of ROS, both endogenous and exogenous. The most important endogenous sources of ROS are the mitochondrial electron transport chain, NADPH oxidases (NOX enzymes), and enzymatic reactions such as those involving xanthine oxidase and cytochrome P450(3, 26). The most significant exogenous sources include ultraviolet and ionizing radiation, environmental pollutants, heavy metals, and certain drugs and toxins (26). The largest source of ROS by far is electron leakage within mitochondria (3, 26, 39), making factors driving mitochondrial electron leakage essential to understand.

4.2 Electron leakage

The primary source of ROS in mitochondria is electron leakage in the respiratory chain (3, 39). Electron leakage in mitochondria refers to the escape of electrons from the normal sequence of electron transfers in the electron transport chain (ETC), leading to the formation of ROS. There is a wide array of reactive species but the two ROS of primary concern and impact are superoxide and hydrogen peroxide and most other reactive species are downstream of these (3, 26, 39). Electron carriers that can participate in this reaction include NADH, $FADH_2$, ubiquinol, cytochromes and iron-sulfur proteins within the complexes (3). There are 11 known sites in mitochondria where electron leakage can occur however the bulk of electron leakage happens at 5 sites, namely complex I and III, 2-oxoglutarate dehydrogenase (OGDH), pyruvate dehydrogenase (PDH), and Succinate dehydrogenase (SDH) (3). Each site of ROS generation has a wide variety of specific factors that can affect the rate of ROS generation at the site in question (3, 39), and a complete treatment of these site-specific factors is beyond the scope of this review. Most important for this thesis are the major intramitochondrial factors driving electron leakage which are common across all sites of ROS generation, and how these relate to intracellular and tissue/organ level factors.

The tendency towards electron leakage in the mitochondrial compartment is driven by the redox potential for each specific leakage reaction, described in the Nernst equation (40). From this it is given that two factors, the concentration of reduced electron carriers [NADH] and the mitoPO_2, greatly affect the tendency for electron leakage across all leakage sites. A highly reduced state of NADH/NAD can promote leakage as more electrons are available for

unintended transfer to oxygen, and conversely, elevated oxygen levels leave more oxygen available to accept electrons.

MitoPO2 depends on an oxygen gradient between local circulation and the mitochondrial compartment and is dictated by the balance between tissue oxygenation and oxygen consumption. Maintaining mitoPO$_2$ levels depends on continual supply of oxygenated blood (6) that is balanced against oxygen consumption via autoregulation of blood supply on the tissue level and via intracellular redox balancing on the cellular level (19). As mitoPO2 is reduced, this can drive down electron leakage as long as mitoPO2 does not fall below the threshold needed to fully sustain oxidative phosphorylation. This can be seen in exercising skeletal muscle that actually reduce mitochondrial ROS generation compared to resting conditions as mitoPO2 is reduced due to increased oxygen consumption relative to delivery (24), (although ROS generation from other sources than mitochondria increase in this particular example, leading to a sum increase of ROS in exercising muscle). Elevated mitoPO2 increases the rate of electron leakage since more molecular oxygen is available to accept leaked electrons and from the perspective of managing oxidative stress it is therefore beneficial to keep it low. MitoPO2 has been cited within a range of approximately 0.5-7.5 mmHg, reflecting the highly controlled redox environment within mitochondria (41), however, there is a sparsity of historical data on this and recent advancements in measurement techniques have indicated that mitoPO$_2$ might be higher than previously thought (42).

Concentrations of saturated electron carriers such as NADH in mitochondria depend on several factors. On the influx side, NADH concentration is driven by cellular metabolic rate which provides continual supply of electrons to saturate NAD+. Overall flux of reactants is more important than moment to moment concentrations, making metabolic rate the biggest factor driving electron leakage besides oxygen concentration (26). A high metabolic rate involves a higher electron flux through the ETC, which inherently increases the probability of electron leakage since it scales with the level of electron flux (4). The bulk of cells in the human body maintain a very low metabolic rate (43), thus keeping oxidative stress low while simultaneously sparing precious calories.

Mitochondrial density and composition also have a major impact on mitochondrial electron carrier concentration, as mitochondrial density varies greatly between cell types and the total NADH flux in a cell is divided between the available mitochondrial mass. Increased mitochondrial density means a given electron carrier flux is divided across a greater mass, resulting in lower intramitochondrial electron carrier concentrations and lower oxidative load. Mitochondrial composition varies greatly between cell types, modulating the effect of mitochondrial density, with some cell types containing a higher content of oxidative phosphorylation (oxphos) proteins and others possessing a more diverse array of metabolic proteins (44).

Another factor which affects electron carrier concentrations is the mitochondrial membrane potential (Δψm). A high membrane potential can increase the probability of electron leakage because a high Δψm can slow down the transfer of electrons through the electron transport chain (ETC), leading to accumulation of saturated electron carriers and increased opportunities for them to react with oxygen (4, 39). Δψm is greatly determined by the balance between the main driver of the proton gradient (catabolism) and utilizer (ATP consumption). The balance between these two processes is crucial to maintain redox balance within mitochondria.

If catabolism outpaces ATP consumption, this causes Δψm to increase, causing the ETC to become overwhelmed with an excess supply of reduced electron carriers. This "backing up" of the electron chain leads to exponentially increasing electron leakage(45). Uncoupling mechanisms have evolved which counter this phenomenon in mitochondria (39). When the ETC becomes saturated, this triggers the activation of uncoupling proteins (UCPs) that dissipate the proton gradient, bypassing complex V (46). This controlled leakage of protons reduces the electrochemical gradient, which diminishes the driving force for electron transport and decreases the likelihood of electron leakage from the ETC. The heat generated from this uncoupling process also plays a major role in thermogenesis and is responsible for a considerable portion of basal metabolic rate (BMR) (47).

In summary, there are a large number of modulating factors which affect rates of electron leakage, and these vary between sites and reactions. In summary, the two primary drivers of electron leakage across leakage sites in mitochondria are mitoPO_2 which is driven by tissue

oxygenation, and mitochondrial concentrations of saturated electron carriers which is driven by specific metabolic rate distributed over mitochondrial density.

4.3 Tissue metabolic rate, mitochondrial density and oxygen delivery.

There are 4 organs in the mammalian body that maintains a constant high rate of metabolism during healthy resting conditions, measured in kcal/kg/day, these are the liver with 200, the brain with 240, the heart with 440 and the kidneys with 440 (5, 43). Combined, these 4 organs represent roughly 80% of total oxygen consumption (48). Skeletal muscle is the fifth highest energy consumer and maintains between 10-20 kcal/kg/day at rest, with remaining tissues maintaining a rate between 4-20 kcal/kg/day. A large portion of the basal metabolic rate (BMR) is taken up by cellular housekeeping functions such as maintaining solute gradients across membranes and thermogenesis (49). Cardiac and skeletal muscle has tremendous capacity for increasing oxygen consumption and specific metabolic rate during activity, driving up total body VO2 from 3-3.5 ml/kg/min at rest in untrained subjects to as high as 45. Elite athletes can increase their VO2 nearly 25-fold (48), represented nearly entirely by an increase in cardiac and skeletal muscle oxygen consumption from resting baseline. Skeletal muscle can increase oxygen consumption roughly 50-100-fold (48).

High metabolic rates and oxygen consumption necessitate high mitochondrial densities to support energy turnover. Brain, liver, kidney, heart and skeletal muscle thus all have high mitochondrial densities compared to all other tissues in the body. Mitochondria in different tissues also have different ratios of proteins responsible for energy turnover via oxidative phosphorylation (oxphos proteins) vs proteins responsible for metabolic functions such as substrate synthesis for anabolism or metabolite conversion. Kidney and liver mitochondria have a fairly even split between metabolic and oxphos proteins, while heart and muscle mitochondria are dominated by oxphos proteins (44). Skeletal muscle and the heart are unique in the sense that in order to enable vast increases in energy expenditure during times of intense physical activity they must maintain a mitochondrial density far in excess of what is needed to support the otherwise much lower resting metabolic rates in these organs. This means that while in the resting state, the excess mitochondrial density has beneficial implications for the tendency towards electron leakage. This is of extra importance in the heart since the metabolic rate of the heart is still extremely high even at rest, although it has

the capacity to increase its metabolic rate up to sixfold during max exertion, indicating a high level of excess mitochondrial density and oxphos composition during rest which helps to limit electron leakage and oxidative stress. Mitochondrial density is highest in the heart (50) and heart mitochondria have a much higher composition of oxphos proteins than kidney and liver (44).

Oxygen delivery and availability varies greatly between various cells in the body. Cells that are immediate to and proximally downstream of gas exchange, such as lung parenchyma and arterial endothelium, are constantly exposed to high partial pressures of oxygen and have an oxygen supply far in excess of oxygen demand (51). Contrastingly, cells in areas that are poorly vascularized, such as joint cartilage and cells in the lens of the eye, have low oxygen delivery. Cells deep in the renal medulla are highly vascularized but poorly oxygenated due to a steep oxygen gradient from the renal cortex to the medulla (9). Organs that maintain metabolic rates that are constantly high, such as the liver, brain and heart, autoregulate their blood supply such that they overall do not receive oxygen far in excess of what is needed on a continual basis (51). Muscle tissue autoregulates blood supply to a very high degree, and muscle VO2 capacity is limited primarily by oxygen delivery (48).

Autoregulation of blood supply is a fundamental process by which tissues control their own blood flow to match their metabolic demands. This is primarily governed by the smooth muscle cells in the arterioles, which adjust their constriction and dilation in response to various local factors such as oxygen levels, carbon dioxide levels, pH, and metabolic byproducts (51). When tissue oxygen demand increases, vasodilation occurs, enhancing blood flow and oxygen delivery. Conversely, when the demand decreases, vasoconstriction reduces blood flow, limiting oxygen supply. Freshly oxygenated blood from the arteries typically has a high partial pressure of oxygen, but as blood travels through the capillaries and into the tissues, oxygen diffuses out of the blood and into cells where it is utilized in the mitochondria. This diffusion process creates a gradient, with PO_2 decreasing progressively from the arterial blood to the cellular and ultimately mitochondrial environments. This establishment of an oxygen gradient from the vascular bed to the mitochondria is crucial for maintaining mitoPO_2 at an optimal level, affecting mitochondrial functioning and electron leakage (41). Kidneys do not autoregulate blood flow in response to tissue oxygen demand, instead, kidney perfusion is regulated to maintain glomerular filtration rate (GFR) (9, 52).

It is notable that most cells that are exposed to chronically high oxygen levels maintain low metabolic rates. This makes sense from the perspective of oxidative stress being related to mitoPO2 and metabolic rate. Conversely, most organs that maintain chronically high metabolic rates, i.e. the brain, liver and heart, autoregulate their blood supply such that oxygen is not delivered far in excess of demand. The cells of the renal cortex are a special case that deviate from this pattern.

Kidneys have a very particular vascular arrangement imposed due to their primary physiological function of filtration, reabsorption and osmoregulation, and a very high degree of perfusion compared to organ size, which creates an oxygen gradient from the renal cortex to the medulla. Kidneys have a low degree of arterial-venous oxygen extraction and are overperfused (9). Kidneys extract only 10-15% of the oxygen supplied (9), indicating massive overperfusion. There is a steep oxygen gradient from the renal cortex to the medulla, with a tissue PO2 as high as 60mm Hg in the outer cortex (52). This rapidly falls towards 20mmHg towards the inner cortex and tissue PO2 in most of renal parenchyma is much lower than would be expected from the oxygen extraction ratio. Tissue perfusion in the renal parenchyma is highly heterogeneous, with low oxygen tensions dominating in most of the kidney tissue, particularly in the renal medulla, and dispersed areas in the outer cortex that are markedly over-perfused (52). Proximal tubular cells, despite overperfusion of the renal cortex, typically experience moderate oxygen tension, but cannot autoregulate down their blood supply to limit oxygen tension if it gets high or vice versa (52). Renal blood flow regulation, unlike blood flow regulation in other organs, is organized to respond to factors like filtration rate and blood pressure, not to match renal oxygen demand(52).

4.3.1 PTC function, physiology and metabolic rate

Kidneys are exposed to the unique challenge of high metabolic rates in combination with overperfusion and a lack of autoregulation of blood supply vs oxygen demand. Not an ideal combination from the perspective of oxidative stress. Out of all the cells in the kidney, the PTCs have the highest metabolic rate and reside in the area of the kidney with the highest partial pressures of oxygen. The PTCs are some of the most important cells in the kidney, responsible for the bulk of reabsorption of a high number of essential substances from glomerular filtrate. Key electrolytes such as sodium (Na+), potassium (K+), calcium (Ca2+),

magnesium (Mg2+), and chloride (Cl-) are extensively reabsorbed in this segment of the nephron(51). Sodium reabsorption is particularly significant as it drives the reabsorption of many other solutes and water through coupled transport. Under normal physiological conditions, glucose and amino acids are nearly completely reabsorbed in the proximal tubule via active transport mechanisms (51). Nearly all bicarbonate (HCO3-), a critical buffer in blood pH regulation, is filtered by the glomeruli is reabsorbed in the proximal tubule. A large proportion of the water filtered by the glomeruli is reabsorbed in the proximal tubules through osmosis, facilitated by the reabsorption of solutes.

The reabsorption of all these substances requires a vast expenditure of ATP relative to the small length of tubule in which these processes take place. Specific metabolic rates for individual cell types in the human body are not available, however it is clear that PTCs due to their high workload have the highest specific metabolic rate of all cells in the kidney (8). This means PTCs must have a specific metabolic rate significantly higher than the total organ average of the kidney of 440 kcal/kg/day, possibly giving them the highest resting metabolic rate of any cell in the body. Energy expenditure of proximal tubular cells, like many of the cells along the nephron, also scales with perfusion rate. Higher perfusion equates to more filtrate from which metabolites must be reabsorbed, so reported metabolic rates are related to average GFR (7, 49). In most tissues, PO_2 typically rises and falls in tandem with perfusion, but in kidneys there is a much lower association between perfusion and PO_2 since metabolic demand scales with GFR.

From the perspective of electron leakage being a prime source of oxidative stress, the PTCs of the kidney are in a uniquely awkward position out of all cells in the body since they are burdened with a chronically high oxygen tension in combination with chronically high metabolic rate (53). PTCs are known to be very susceptible to oxidative stress (54). Oxidative damage in PTCs is a key driver of pathology in AKI (55). PTCs have a much more oxidized redox status than other cells along the nephron and maintain highly increased antioxidant levels compared to other renal segments (11). Since oxidative stress results from an imbalance of ROS generation rates vs mitigation by antioxidative systems, and PTCs are uniquely exposed to a combination of key factors that drive electron leakage, understanding the antioxidant systems is crucial to understand oxidative stress in these cells.

4.4 Antioxidative systems

The antioxidative defenses of the body are a network of enzymes, molecules, and processes that work together to neutralize harmful free radicals and prevent oxidative damage to cells and tissues. The highest turnover of antioxidative processes occurs in mitochondria where ROS are predominantly generated, employing enzymatic and non-enzymatic antioxidant (NEA) defenses. Enzymatic antioxidants such as superoxide dismutases (SODs), catalase, and glutathione peroxidases (GPxs) are pivotal in the detoxification of ROS within mitochondria, cytosol, and peroxisomes (56). Non-enzymatic antioxidants, including glutathione, vitamin E, and vitamin C, further supplement these defenses, directly scavenging free radicals and repairing oxidative damage. A key challenge of ROS mitigation is the short half-life of these chemicals, making antioxidants with high affinity and high reaction rates essential (57). Enzymatic antioxidative systems have by far the highest turnover rates and capacity for rapidly neutralizing ROS, making them the most important protective system mitigating redox stress in mitochondria. By comparison, non-enzymatic antioxidants have a supportive role within the mitochondria with orders of magnitude lower turnover rate than enzymatic antioxidants, but function as scavengers of reactive species in the rest of the body, so while important in their own right they are not a focus of this review.

4.4.1 Superoxide dismutase

Superoxide Dismutase (SOD) enzyme is the first line of defense against O_2^- and it catalyses the dismutation of O_2^- into oxygen and H_2O_2 at a very high rate(3, 58, 59). $2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$. The exact turnover rate can vary among the different types of SOD (e.g., Cu,Zn-SOD found in the cytosol and Mn-SOD found in mitochondria), but all types exhibit high efficiency in catalyzing the conversion of superoxide radicals. These enzymes utilize the superoxide radical as substrate, converting it into hydrogen peroxide and oxygen without the need for external electron donors like NADPH. The hydrogen peroxide produced is then typically dealt with by catalase (CAT) or glutathione peroxidase (GPx)

4.4.2 Catalase

CAT exhibits one of the highest turnover rates among antioxidant enzymes, capable of converting millions of molecules of hydrogen peroxide (H_2O_2) into water and oxygen each second. This high turnover rate makes catalase exceptionally effective at protecting cells

from the oxidative damage caused by H_2O_2 accumulation. Catalase decomposes hydrogen peroxide (H_2O_2) into water and oxygen. $2H_2O_2 \rightarrow 2H_2O + O_2$. This reaction does not require NADPH or any other external electron donor, however NADPH tightly binds to catalase and in this way protects the enzyme from oxidative degradation (58, 60-62).

4.4.3 Glutathione peroxidase

GPx catalyses the reduction of H_2O_2 or organic hydroperoxides (ROOH) to water (H_2O) or corresponding alcohols (ROH), using glutathione GSH as a reducing agent. In these reactions, two molecules of GSH are oxidized to form glutathione disulfide (GSSG), while the peroxide is reduced to water or an alcohol. For hydrogen peroxide: $2GSH+H_2O_2 \rightarrow GSSG+2H_2$. For organic hydroperoxide: $2GSH+ROOH \rightarrow GSSG+ROH+H_2O$. (3, 58)

4.4.4 Glutathione reductase

GSSG can be reduced back to GSH by the enzyme glutathione reductase (GR), thus maintaining the GSH pool within the cell. This step requires NADPH as an electron donor: $GSSG+NADPH+H+ \rightarrow 2GSH+NADP+$ (3). The continual regeneration of GSH by GR is one of the most crucial processes in the whole antioxidative network do to the central importance of GSH and its associated enzymes.

4.4.5 Glutathione S-transferases and glutaredoxins

Glutathione S-transferases (GSTs) play a direct role in facilitating the addition of glutathione to cysteine residues on proteins. This typically occurs under conditions of oxidative stress where increased ROS can lead to the oxidation of cysteine thiol groups to sulfenic acids. GSTs help catalyze the formation of a disulfide bond between the cysteine's sulfenic acid and the thiol group of GSH, resulting in S-glutathionylation. Under severe oxidative conditions, protein cysteine residues can react directly with glutathione without the aid of enzymes. This often occurs through thiol-disulfide exchange reactions, where an oxidized protein thiol (forming a disulfide bond with another protein cysteine or with a small molecule thiol) reacts with GSH to form a mixed disulfide with glutathione. Glutaredoxins (Grxs) catalyze the removal of glutathione from proteins, a process called deglutathionylation. This is crucial for restoring proteins to their normal functional state. Glutaredoxins use the reducing power of free GSH to cleave the disulfide bond between the protein cysteine and GSH. The reaction typically yields the reduced form of the protein thiol and glutathione disulfide (GSSG). When

proteins are damaged due to oxidative stress, the cycle of glutathionylation and deglutathionylation is a primary mechanism by which the body halts the damage process before oxidation becomes irreversible and restores proteins to their normal state (63). Oxidative stress leads to protein cysteine oxidation, then either enzymatically by GSTs or non-enzymatically, GSH is added to oxidized cysteines. Grxs remove GSH from proteins, restoring their function and lastly glutathione reductase recycles GSSG back to GSH, using NADPH. Critically, this cycle is essential to maintain other components of the antioxidative defense because SOD and CAT would degenerate due to oxidative stress without a mechanism to counteract protein cysteine oxidation, making glutathione essential to maintain the other antioxidative enzymes(3, 12, 27, 58).

4.4.6 Glutathione regeneration of non-enzymatic antioxidants

Once vitamin C (ascorbic acid) is oxidized, it turns into dehydroascorbic acid (DHA). Glutathione plays a crucial role in regenerating vitamin C by reducing dehydroascorbic acid back to ascorbic acid (3). This reaction typically involves two molecules of glutathione (GSH), which convert into glutathione disulfide (GSSG) as they donate electrons to DHA, reducing it back to ascorbic acid. The enzyme glutaredoxin or other similar thioltransferases can facilitate this reaction. Vitamin C not only gets regenerated by glutathione but also helps in regenerating vitamin E (3). When vitamin E (alpha-tocopherol) is oxidized after interacting with free radicals, it forms tocopheroxyl radical. Vitamin C can reduce this radical back to its active form, alpha-tocopherol, thus preventing the propagation of lipid peroxidation in cell membranes.

4.4.7 Glutathione

Glutathione (GSH) is a tripeptide composed of three amino acids: glutamate, cysteine, and glycine. It is a critical antioxidant in cell, acting as the substrate for the GPx and GST anzymatic systems and through direct scavenging of ROS. It is continually restored through GR, a process which requires NADPH (3). GSH also participaties in detoxification by conjugating with various toxins and byproducts of metabolism via the action of glutathione S-transferase enzymes, making them more water-soluble and thus easier to excrete. The rate of GSH synthesis can increase in response to oxidative stress or exposure to toxins, as

these conditions elevate the demand for antioxidant capacity. The availability of cysteine is a limiting factor in GSH synthesis since it is less abundant than glutamate and glycine (64).

4.4.8 The thioredoxin-peroxiredoxin system

The thioredoxin (Trx) and peroxiredoxin (Prx) pathway is an important part of the cellular antioxidant defense system. This pathway depends on the redox-active protein thioredoxin and its ability to reduce disulfide bonds in substrate proteins (65). Thioredoxin itself is reduced by thioredoxin reductase (TrxR), a NADPH-dependent enzyme. Peroxiredoxins is a family of peroxidases that catalyse the reduction of peroxides such as hydrogen peroxide H_2O_2 and organic hydroperoxides (ROOH). These enzymes react with peroxides to form a sulfenic acid intermediate (66). This intermediate can form a disulfide bond with a second cysteine residue, either from the same molecule or from another Prx molecule, or it can be directly reduced by Trx. The reduction cycle of Prxs is thereby completed by Trx, which itself is regenerated by TrxR, linking the peroxidase activity of Prxs to the reducing power of NADPH. NADPH thus serves as the primary electron donor in the Trx-Prx system (65, 66).

4.4.9 Summary of enzymatic antioxidants

SOD and CAT serve as the first line of defense against ROS, efficiently neutralizing superoxide and hydrogen peroxide. However, the glutathione system is the cornerstone of antioxidative defense due to its versatility and regenerative capabilities (3, 27). GPx reduces peroxides using GSH, while GR regenerates GSH from its oxidized form. GSTs and Grxs manage oxidative damage to proteins, and GSH also regenerates key non-enzymatic antioxidants like vitamins C and E. This comprehensive and adaptable system underpins and sustains the broader antioxidative network, making it arguably the most critical component in protecting cells from oxidative stress. As mentioned, the continual regeneration of GSH by GR relies on NADPH as a reducing agent and similarly for the Trx-Prx system, making NADPH the primary “fuel” powering the enzymatic antioxidative systems (12).

4.4.10 NADPH regeneration

The body generates NADPH, a crucial cofactor for biosynthetic reactions, antioxidant defense, and detoxification processes, through several pathways. NADPH provides the reducing power needed for the synthesis of fatty acids, cholesterol, neurotransmitters, and

nucleotides, and is continually required for maintaining the reduced state of glutathione(67). NADPH can also participate in ROS generation through NADPH oxidase(67). Once NADPH has spent its reducing power and lost its proton to become NADP, it must be regenerated from NADP to NADPH. The whole antioxidative defense system therefore depends on the continual regeneration of NADPH from NADP(3, 12, 68). The known ways through which the body generates NADPH are the pentose phosphate pathway, the malic enzyme pathway, isocitrate dehydrogenase, glutamate dehydrogenase, one carbon metabolism and transhydrogenase.

NADPH regeneration via the pentose phosphate pathway (PPP)

The PPP, also known as the hexose monophosphate shunt, is by far the primary major source of NADPH (12). The general purposes of the PPP are generation of NADPH for biosynthetic reactions and oxidative stress protection, production of ribose-5-phosphate for nucleotide synthesis, and participation in metabolic flexibility through sugar interconversion. It operates in two phases, an oxidative and a non-oxidative phase. In the oxidative phase glucose-6-phosphate is oxidized and decarboxylated to generate ribulose-5-phosphate, producing 2 molecules of NADPH per glucose-6-phosphate molecule processed. In the non-oxidative phase ribulose-5-phosphate can be converted into intermediates that re-enter glycolysis or be utilized for nucleotide synthesis(67, 68).

NADPH regeneration via the malic enzyme pathway

The malic enzyme catalyzes the decarboxylation of malate to pyruvate in both cytosol (ME1) and mitochondria (ME2 & ME3), producing NAD(P)H in the process. There are two forms of the enzyme, one using NADP+ and the other using NAD+ as a cofactor. The primary function of malic enzyme and NADPH produced through it is biosynthesis of lipids and cholesterol (69).

NADPH regeneration via isocitrate dehydrogenase pathways

IDH enzymes, including IDH1, IDH2, and IDH3, are critical for cellular metabolism by catalyzing the oxidative decarboxylation of isocitrate to α-ketoglutarate. IDH1 and IDH2 are NADP+-dependent enzymes found in the cytoplasm and mitochondria, respectively, playing roles in biosynthesis and antioxidative defense (67, 70), while IDH3 is an NAD+-dependent

enzyme located in the mitochondrial matrix, essential for the citric acid cycle and energy production.

NADPH regeneration via glutamate dehydrogenase

Glutamate dehydrogenase (GDH) catalyses the reversible oxidative deamination of glutamate to α-ketoglutarate and ammonia in mitochondria, while reducing NAD(P)+ to NAD(P)H. This reaction is central to nitrogen metabolism in the liver and links amino acid metabolism to the citric acid (Krebs) cycle (71). The enzyme can utilize either NAD+ or NADP+ as a cofactor for the deamination reaction, depending on the isoform and tissue type. Generated α-ketoglutarate can be further catabolized for energy production, thus making glutamate a fuel source alternative.

NADPH regeneration via folates in One-Carbon Metabolism

One-carbon metabolism involves the folate cycle, where folate derivatives facilitate the transfer of single carbon units needed in various biological processes. This cycle is linked to the regeneration of methionine from homocysteine (72). Methionine is an essential amino acid that plays a role in the synthesis of glutathione. Methionine contributes to the production of S-adenosylmethionine (SAM), a critical molecule in methylation processes. SAM can be further metabolized to generate homocysteine, which can be converted back into methionine or used to synthesize cysteine. Cysteine is a limiting precursor to glutathione (64). One-carbon metabolism also contributes to NADPH production. Methylenetetrahydrofolate Dehydrogenase (MTHFD) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5,10-methenyltetrahydrofolate, and subsequently to 10-formyltetrahydrofolate. Importantly, MTHFD1 has a bifunctional domain that also reduces NADP+ to NADPH during the conversion of 5,10-methenyltetrahydrofolate to 5,10-methenyltetrahydrofolate (72). There are several forms of MTHFD, cytosolic MTHFD1 and mitochondrial MTHFD2 and MTHFD2L. 5,10-methylenetetrahydrofolate is formed with the transfer of a one-carbon group to tetrahydrofolate (THF) by the enzyme serine hydroxymethyltransferase (SHMT) which catalyzes the conversion of serine to glycine. Serine is synthesized from glucose metabolism via the intermediate 3-phosphoglycerate. NADPH regeneration and glutathione synthesis is therefore linked to glucose metabolism via the role of serine in the folate cycle.

NADPH regeneration via Transhydrogenase

Nicotinamide Nucleotide Transhydrogenase (NNT): Located in the inner mitochondrial membrane, this enzyme transfers hydride ions from NADH to NADP+, regenerating NADPH while oxidizing NADH (73, 74). NNT is a key facilitator of electron carrier pool balancing. For the NAD+/NADH pool, the ratio is maintained highly in favor of NAD+ in the cytosol, ensuring a high oxidative capacity necessary for driving catabolic reactions and suppressing NADH coupled electron leakage. In contrast, the NADP+/NADPH pool maintains a reduced ratio highly in favor of NADPH (75). Exchange of electrons between NADH/NADPH with NNT as a fulcrum balances the catabolic redox stress associated with NADH with the availability of antioxidative reducing equivalents.

Summary of NADPH regeneration

NADPH regeneration is essential for maintaining redox defense and is closely linked to glucose metabolism. NADPH provides the reducing power needed for biosynthetic reactions, antioxidant defense, and detoxification processes. It is continually required to keep GSH in its reduced form, critical for the antioxidative defense system. The primary source of NADPH is the PPP, which branches out from glucose metabolism. In the PPP, glucose-6-phosphate is oxidized to produce ribulose-5-phosphate and NADPH. Other pathways contributing to NADPH regeneration include the malic enzyme pathway, isocitrate dehydrogenase pathways, and glutamate dehydrogenase, linking directly to the Krebs cycle and amino acid metabolism. Additionally, one-carbon metabolism via folate cycles also contributes to NADPH production, once again tying back to glucose metabolism through serine synthesis from glycolytic intermediates. NNT in the mitochondria also plays a crucial role by transferring hydride ions from NADH to NADP+, further linking energy metabolism with redox balance. Sources of NADPH other than those provided through glucose metabolism are typically insufficient to support necessary NADPH production in the absence of glucose metabolism (12).

4.5 Glucose metabolism and antioxidative defenses

Glycolysis and gluconeogenesis are the two opposing pathways that make up glucose metabolism (76). Glycolysis, which breaks down glucose into lactate, operates efficiently in

the cytoplasm of most cells and is particularly vital in tissues with high energy demands, such as muscle and brain cells. This pathway is versatile, functioning under both aerobic and anaerobic conditions, allowing for flexible ATP production independent from oxygen availability. Gluconeogenesis, the process of synthesizing glucose from non-carbohydrate precursors, predominantly occurs in the liver and kidneys (13, 76, 77). It ensures a continuous glucose supply during fasting, intense exercise, or starvation, maintaining blood glucose levels crucial for tissues like the brain and red blood cells.

Regulation of glucose levels in circulation is controlled by hormonal signals, primarily insulin and glucagon(76). In the fed state, elevated blood glucose levels trigger insulin secretion, which facilitates cellular glucose uptake and promotes glycogen storage in the liver and muscles. Insulin also stimulates glycolysis in peripheral tissues, ensuring that excess glucose is efficiently utilized or stored. In contrast, during the fasted state, low blood glucose levels induce glucagon secretion. Glucagon acts on the liver to stimulate glycogenolysis and enhances gluconeogenesis, increasing glucose output into the bloodstream to maintain glucose availability for vital organs, especially the brain. Glycolysis and gluconeogenesis are inherently opposing pathways. Glycolysis breaks down glucose to produce energy, while gluconeogenesis synthesizes glucose to ensure a continuous supply (78). Individual cells rarely switch between these pathways; instead, they primarily commit to one direction based on their differentiated role and metabolic needs. For instance, gluconeogenic cells in the liver and kidneys remain net producers of glucose in both fed and fasted states (13). The regulation by insulin and glucagon adjusts the rates of these processes rather than their direction. In times of glucose excess, the liver and kidneys continue to produce glucose, albeit at a reduced rate due to insulin signaling, which stimulates glucose clearance in peripheral tissues. Conversely, when more glucose is needed, glucagon increases gluconeogenesis rates in the liver. This regulatory mechanism ensures that the total fluxes of glycolysis and gluconeogenesis are balanced, maintaining circulatory glucose levels. By fine-tuning the turnover rates rather than switching pathways, the body efficiently manages energy production and glucose supply to adapt to varying metabolic demands and dietary conditions, ensuring glucose homeostasis.

Of the two pathways, it is known that glycolysis is important to support antioxidative systems (12), most importantly via the PPP which branches out from glucose metabolism at

the glucose-6-phosphate (G6P) node. Flux through the PPP is therefore directly limited by the flux of glucose metabolism. The enzyme glucose-6-phosphate dehydrogenase (G6PD) catalyzes the first and rate-limiting step of the PPP, which regenerates NADPH, essential for reductive biosynthesis and antioxidative defense. This is the most important source of NADPH regeneration for fueling antioxidative systems (12).

Serine synthesis also branches off from glucose metabolism and serine is another molecule that supports antioxidative systems via its role in one-carbon metabolism. Serine can be interconverted with glycine, and both molecules are primary sources of one-carbon units that are vital for the synthesis of nucleotides and methylation reactions (72). The folate-mediated one-carbon metabolism is crucial for DNA synthesis and repair, as well as epigenetic modifications. Methionine synthase uses 5-methyl-THF (derived from the folate cycle) to regenerate methionine from homocysteine, which in turn is used to produce S-adenosylmethionine (SAM), a major methyl donor. Methionine is necessary for the synthesis of cysteine, the thiol-bearing central component of glutathione (72). Serine can be reversibly converted to glycine via hydroxymethyltransferase (SHMT)(72) and glycine is one of the three amino acids needed to synthesize GSH. Serine synthesis begins from 3-phosphoglycerate, an intermediate of glucose metabolism. (PHGDH) catalyzes the conversion of 3-phosphoglycerate into 3-phosphohydroxypyruvate. 3-Phosphoglycerate dehydrogenase (PHGDH) is the first enzyme in the serine biosynthesis pathway and it branches off from glucose metabolism at the 3-phosphoglycerate node. The synthesis of serine from a glycolytic intermediate links glucose metabolism to glutathione synthesis and to a secondary source of NADPH regeneration through one carbon metabolism (72). It has recently been discovered that kidneys are the primary organ which produces excess serine and glycine releasing these metabolites into circulation (77), possibly connecting glucose metabolism to antioxidative systems in kidneys via high levels of serine synthesis.

Glucose metabolism is critical for supporting the antioxidative systems due to its role in driving the bulk of NADPH regeneration via PPP(12) and secondarily via serine synthesis and its central role in one-carbon metabolism (72). Additionally, it supports GSH synthesis directly through serine and glycine synthesis, and indirectly via serine and glycine driving cysteine synthesis (72), the latter being a limiting substrate for glutathione. Two out of three components of GSH trace their de novo synthesis pathways back to PHGDH and glucose

metabolism. Due to these connections, glycolysis has received attention as a supporter of antioxidant systems (12). Although glycolysis gets the attention for its role as a supporter of antioxidative systems, The PPP and PHGDH pathways can in principle be driven both via glycolysis and via gluconeogenesis, since the starting substrates for PPP and PHGDH are intermediates in both glycolytic and gluconeogenic pathways.

4.6 Glucose metabolism in PTCs

Hepatic gluconeogenesis was proposed in the late 20's and later confirmed in detail by the Coris (79). Before the 1960s, the liver was primarily recognized as the main site of gluconeogenesis but in the early 60's it was discovered that renal gluconeogenesis was considerable (80). Key studies by Krebs and colleagues demonstrated that kidney cortical tissue could produce substantial amounts of glucose from various substrates (81), revealing that the kidney's gluconeogenic capacity was comparable to the liver. Today, it's recognized that renal gluconeogenesis constitutes roughly 40-45% of total body glucose production in fasted states and 10-20% in fed states (13, 77, 82). Glucose metabolism in the kidneys differs between the cortex and the medulla and gluconeogenesis happens almost exclusively in PTCs while cells in the medulla run glycolysis (82). PTCs run much higher rates of gluconeogenesis than hepatocytes when accounting for output vs weight (83). PTCs almost do not run glycolysis at all during normal conditions and it has been suggested that due to the role of PTCs in glucose reabsorption it could be metabolically detrimental if they burned glucose (83). Given that they run constant gluconeogenesis at a higher rate than any other cell, it makes sense to forego glycolytic activity as simultaneous activity of both pathways would lead to accumulation of intermediates and futile cycling. PTCs are simultaneously responsible for the bulk of glucose reabsorption as well as virtually all renal gluconeogenesis (82, 84).

Gluconeogenesis produces a significant portion of total CO_2 in PTCs thus being an important contributor to intrarenal PCO_2, supporting sodium reabsorption. Sodium is reabsorbed in tubules via sodium-hydrogen exchange, requiring H+ ions. H+ flux is dependent on PCO_2, and so optimal reabsorption of sodium is partially dependent on elevated CO_2 concentrations (85). PCO_2 in proximal tubules and other segments of the nephron is much higher than in

both arterial and venous circulation(85), reflecting significant CO_2 production and retention in kidneys.

Gluconeogenesis can support renal pH regulation by providing an outlet for glutamine metabolism. Renal pH regulation requires large quantities of ammonia (NH_3)(85). Glutamine is deaminated, releasing ammonia. This ammonia can bind to hydrogen ions to form ammonium NH_4^+which is then excreted in the urine, effectively removing H^+ from the body. Kidneys excrete approximately 50mmol of $NH4^+$ each day to get rid of H^+ ions (85). The remaining carbon skeleton of glutamine can then enter the gluconeogenic pathway as alpha-ketoglutarate.

4.7 Does gluconeogenesis protect against oxidative stress in PTCs?

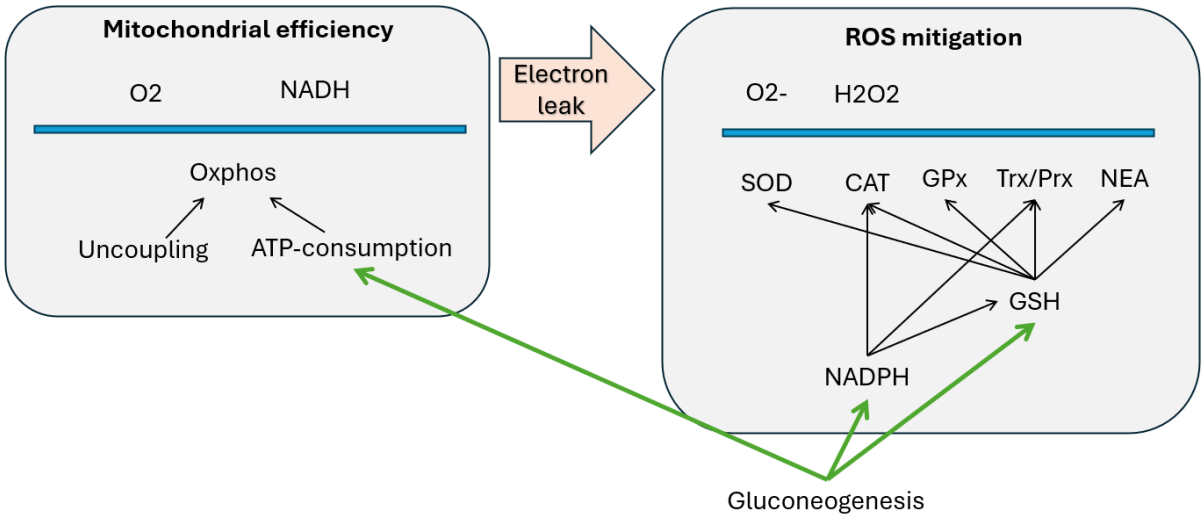

Figure 1 – Possible mechanisms of gluconeogenesis supporting redox balance

Mitochondrial efficiency relies on a balance between the influx of saturated electron carriers (NADH) and O2 on one side and rate of oxidative phosphorylation on the other. Ideally these two rates are balanced such that concentrations of O2 and NADH in mitochondria do not rise to drive excess electron leakage. Coupled oxidative phosphorylation drives ATP-production and energy sinks for continual ATP consumption are needed for balance. If ATP-consumption fails to pull oxphos rates, uncoupling provides a relief valve that allows for dissipation of the proton gradient. ROS produced via electron leak, most importantly O_2^- and H_2O_2, must be mitigated via the antioxidative system. O_2^- is neutralized by SOD, and H_2O_2 is neutralized primarily by CAT which is assisted by GPx, Trx/Prx, and non-enzymatic antioxidants (NEAs). GSH underpins the entire system, as a substrate for GPx which directly neutralize ROS, as a substrate for GSTs which protect other proteins by reducing oxidized cysteine thiol groups, and by directly reducing NEAs. NADPH fuels recycling of GSH and Trx/Prx, and directly binds to proteins like CAT protecting them from oxidation. The entire antioxidative system is therefore crucially reliant on robust continual NADPH regeneration. Gluconeogenesis supports oxphos by providing an energy sink. Gluconeogenesis potentially helps regenerate NADPH by providing substrate for PPP. Gluconeogenesis can also provide substrate for serine synthesis which is linked to secondary NADPH regeneration via its central role in one-carbon metabolism. Serine synthesis is closely connected to GSH biosynthesis pathways via glycine and cysteine.

The hypothesis proposed in this thesis is that gluconeogenesis has a protective role against oxidative stress in PTCs. The basis from which this hypothesis emerged was summarized in the introduction and has been explored in greater detail in the preceding chapters of the results. The proposed mechanisms for gluconeogenic protection against oxidative stress are summarized in *Figure 1*.

		Alveolar cells & Arterial endothelium	Skeletal muscle	Cardiomyocytes	Hepatocytes	CNS	PTCs
Tendency for ROS generation via electron leakage	PO2	Proximal to gas exchange. High PO2.	Autoregulated perfusion. Moderate PO2	Autoregulated perfusion. Moderate PO2	Autoregulated perfusion. Moderate PO2	Autoregulated perfusion. Moderate PO2	Not autoregulated. Typically moderate PO2
	Mitochondrial oxphos density.	low	High. Extreme overcapacity at rest.	Very high. Overcapacity at rest.	High	High	High
	Metabolic rate	Very low	Low at rest. Intermittently high.	Very high at rest. Intermittently extremely high.	High	High	Chronically very high
Mitigating generated ROS	Glucose metabolic flux, supporting antioxidants	Low glycolysis	Low glycolysis	Low glycolysis	High gluconeogensis	High glycolysis	High gluconeogenesis

Table 1 – comparing electron leakage drivers and glucose metabolic flux across cells

The hypothesis is in part based on the observation the challenge of redox balance manifests differently in different organs and and tissues, depending on factors like tissue oxygenataion, specific metabolic rate and mitochondrial density and composition. *Table 1* summarizes this. Cells proximal to gas exchange such as alveolar cells and arterial endothelium cannot limit oxygen tension through autoregulation of blood supply but these cells maintain low metabolic rates thereby limiting oxidative stress from electron leakage. Skeletal muscle and cardiomyocytes have excess capacity of mitochondrial density at rest due to the need for the ability to greatly increase energy turnover in these tissues during strenuous activity. This excess capacity of mitochondrial density at rest distributes metabolic and oxidative load. Most organs such as skeletal muscle, heart, liver, and CNS autoregulate

their blood-supply such that most O2 delivered is consumed, thereby limiting mitochondrial PO2 and oxidative stress due to electron leakage. All organs and tissues maintain low metabolic rates, except for the big four, the heart, liver, CNS and kidneys which have high metabolic rates. The renal cortex is overperfused due to the vascular physiology inherent to renal function and cannot autoregulate blood flow in relation to oxygen demand. Kidneys are therefore in a unique position with regards to exposure to factors driving electron leakage, with PTCs being by far the most extreme case. The combination of *figure 1* and *table 1* summarize why the hypothesis is of particular relevance to PTCs. They require high levels of GSH and other antioxidative systems and continual regeneration of NADPH, but do not run glycolysis which is considered the most important source of NADPH regeneration as well as supporting GSH synthesis. This raises questions about metabolic support for antioxidative systems in PTCs, and the proposed hypothesis attempts to address this.

5 Discussion

Living organisms are composed of a large number of interconnected systems, of which the systems involved in redox biology are some of the most fundamental. Despite this, the field of redox biology is quite young compared with other scientific disciplines (1), and there are many aspects of it we are still trying to find the right conceptual models to understand (19). It is becoming clear however that concepts like redox balance and oxidative stress will receive increasing focus, both as an avenue of increasing our fundamental understanding of biology and medicine, and for their roles in health and disease. Modern techniques for detecting and measuring biomolecular structures and solutes have led to the discovery of a vast array of enzymes and substances involved in redox biology that interconnect in a staggering level of complexity but seeming to operate on a few basic principles (19). We are grappling with trying to understand these basic principles, because without them it is very difficult to conceptualize the hierarchical levels of organization of redox biology and figuring out which things are more important and which things are secondary or tertiary details of the system. Decisions relating to choice of direction and omissions in this thesis have been aimed at trying to grasp for and ask questions about the fundamentals.

Omissions

The breath of topics covered in this review and the limitations of scope have necessitated some deliberate omissions to maintain narrative focus. Firstly, the choice to focus on electron leakage as the primary source of ROS and a deep dive into mechanisms of electron leakage has come at the expense of similar in-depth treatment of other sources of ROS that are of interest. NADPH-oxidases as a source of ROS are connected to several topics in this thesis but were omitted due to scope constraints. Secondly, given the importance and central role of NADPH regeneration, one could argue for a more thorough investigation into the IDH, GDH and NNT pathways in relation to the hypothesis than what is included in section 4.4.10. Again, the decision was made deliberately to keep it short to prevent narrative divergence.

Methodology

This thesis explores questions about fundamental connections between redox biology, metabolism and physiology, exploring a wide range of interconnected topics. This ambitious scope is both a strength and a weakness. While the broad aims have allowed for a wide-ranging review of literature and the generation and exploration of a novel hypothesis, they also mean that some areas may not have been explored with the necessary depth and rigor. The non-systematic nature of this literature review is acknowledged. The selection and appraisal of sources may therefore be subject to bias, and important sources might have been overlooked. The thesis focuses on qualitative synthesis rather than quantitative data extraction and analysis. This limits the ability to statistically validate the hypotheses proposed. The reliance on narrative synthesis means that the connections drawn are not empirically tested within the scope of this work. The key strength of the approach relative to this thesis is that it has allowed for crucial flexibility in exploring diverse topics, enabling narrative synthesis.

Implications

This thesis focuses on a possible role of gluconeogenesis in supporting redox balance in PTCs, but most of the underlying connections that point to this hypothesis in PTCs are also present in hepatocytes, except that they have a lower metabolic rate and can better

autoregulate their blood supply. The hypothesis therefore raises questions about gluconeogenesis and hepatic redox balance. Renal glucose metabolism is heavily disrupted in several disease states. Notably, as CKD progresses through stages of severity, gluconeogenesis falls of proportionally with fall in kidney function(17, 18). Gluconeogenesis is drastically reduced in sepsis and PTCs change to a glycolytic expression (86), and this represents a dramatic disruption of kidney glucose metabolism compared to the fluctuations normally seen between fed and fasted states (13). The mechanisms by which these changes in renal glucose metabolism occur in disease states are not well understood and implications for pathophysiology and treatment are unclear.

Falsification and future directions

Attempts have been made to falsify the hypothesis within the chosen methodology. Sources of NADPH in PTCs is a key question, and direct measurement of sources and sinks of NADPH in gluconeogenic cells in various cellular compartments could potentially falsify the primary claim of the hypothesis. Several searches have been made to investigate if such measurements have been performed and no sources have been found. There are complex methodological challenges associated with measuring sources and sinks of electrons for specific electron carrier couples. Citrate is a possible candidate for an alternate source of NADPH in PTCs. Kidneys have recently been found to absorb citrate and burn it as the only major mammalian organ that does this (77). A possible explanation for this could be that low quantities of citrate are released by the cells in the rest of the body and absorbed by the kidneys as a mechanism to subsidize renal NADPH regeneration via IDH. Citrate seems to account for 20% of kidney TCA turnover and this seems to be happening in proximal tubular cells (54), with little citrate consumption in other parts of the kidney. Investigating citrate metabolism in PTCs could be a viable line of inquiry regarding NADPH regeneration outside of PPP. Another possible avenue of investigating sources of NADPH in PTCs is via genomic and proteomic analysis regarding enzymes in alternate NADPH-generating pathways, described in section 4.4.104.4.10, and how these are expressed compared to other cell types. A viable route of falsification of the hypothesis is to measure the contributions from gluconeogenic precursors to downstream metabolites of PPP in gluconeogenic cells using a omics approach with radiolabelled isotope tracing. This could provide strong indirect

evidence for or against the hypothesis. Such measurements might be feasible, but no sources have been found that perform such an investigation.

6 Conclusion

This thesis explores the hypothesis that renal gluconeogenesis has a protective role against oxidative stress in PTC using a non-systematic literature review focusing on narrative synthesis. This hypothesis addresses questions of fundamental connections between metabolism, physiology and redox biology. This review finds some conceptual support for this hypothesis in terms of possible mechanisms and a rationale within the examined context, encouraging further exploration of this topic. The methodology used has weakness in terms of ability to rigorously test the hypothesis. Possible avenues of falsification have been included.

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