Chapter 1

General introduction and outline of this thesis
Metformin

The biguanide metformin (1,1-dimethylbiguanide hydrochloride) is the recommended first-line oral antihyperglycemic therapy to treat patients with type 2 diabetes mellitus(1). It is estimated that globally half a billion individuals have diabetes mellitus, a number that will increase considerably in the coming decades(2). Extrapolating data from the worldwide observational DISCOVER study, up to 70% of these patients might use metformin, which can be explained because it is a widely available and extremely cheap drug with a long-standing track record of safety and effectiveness(3-5). For centuries, the guanidine-rich plant *Galega Officinalis*, also known as Goat’s rue, French lilac, or Italian Fitch, was used as a traditional herbal medicine to relieve symptoms of a disease that is now known as diabetes mellitus(6). After discovering and synthesizing guanidine derivatives, such as the biguanides metformin, buformin, and phenformin, the former was introduced as a therapy to treat diabetes by the French physician Jean Sterne in 1957.

Remarkably, there is still no definitive consensus on the exact antihyperglycemic mechanism of action of metformin(7). Most agree that its main effect is suppression of lactate conversion into glucose within the liver, known as gluconeogenesis. However, secondary mechanisms are subject to debate, including enhanced glucose uptake by peripheral tissues, upregulation of several signaling pathways, and local effects on the intestinal wall and its microbiome(7-9). A unique property of metformin is mild but specific inhibition of complex I of the mitochondrial electron transport system(10, 11). This interference with mitochondrial metabolism most probably underlies the pleiotropic effects of the drug(7, 8).

Metformin-associated lactic acidosis

In the case of metformin intoxication, either accidental or intentional, gluconeogenesis is overly inhibited by metformin, causing an increase in blood lactate levels (Figure 1). This mechanism, which is part of the Cori cycle, is a critical pathway that clears systemic lactic acid production(12-17). Besides reducing hepatic and renal lactate clearance, systemic lactate production is also increased since toxic metformin levels increase anaerobic metabolism due to systemic mitochondrial respiratory suppression(16-23). Consequently, severe metabolic acidosis develops, known as metformin-associated lactic acidosis (MALA). This is a condition that requires emergency hemodialysis in the intensive care unit (ICU) and has been associated with a high mortality rate, ranging from 20 to 45%(24-28).
Definition

Numerous researchers have investigated the association of metformin use with the development of lactic acidosis. Prospective trials and population-based observational cohort studies report an incidence of lactic acidosis of approximately 3 to 10 per 100,000 person-years among metformin users, which is an incidence that may be difficult to distinguish from the background rate of lactic acidosis in the overall population with diabetes(29-34). Clinically, however, MALA has been considered a entity on its own being associated with a lactate level that is often higher than 10 mmol/L. Indeed, this has been underlined by detailed information provided in case reports, case series, and smaller cohort studies(24-28). Thus, the difference in perception of whether metformin is associated with lactic acidosis can be brought back to the fact that most studies estimating the incidence of MALA are by defi nition quantitative, whereas the issue actually is qualitative (i.e., to which extent does metformin or another condition contribute to the event’s nature and its outcome)(12).

Recently, a set of defi nitions on the relation of metformin with lactic acidosis has been proposed(12). Depending on the presence of other pathophysiological conditions and the blood metformin concentration, severe lactic acidosis (often defined as pH <7.20 and lactate >5 mmol/L) among metformin users can be
regarded as being unrelated to, associated with, or induced by metformin therapy(12). Metformin-unrelated lactic acidosis obviously occurs quite often since many critically ill patients with other primary causes of hyperlactatemia (e.g., sepsis or out-of-hospital cardiac arrest) use metformin. In case of toxic metformin levels, defined as metformin blood concentration >5 mg/L, it is difficult to distinguish lactic acidosis caused by metformin alone (i.e., metformin-induced lactic acidosis) or mainly by metformin.

**Aim of this thesis**

The aim of this thesis is to better understand the pharmacokinetic and pharmacodynamic properties of metformin in order to identify individuals at risk of developing MALA to ultimately prevent this severe complication requiring aggressive therapy in the ICU.

**Effects on renal function**

Metformin is an unusual hydrophilic drug that exists in its protonated form (i.e., cation) under all (patho-)physiological circumstances. Due to these physicochemical characteristics, passive diffusion across cell membranes will be minimal, and its transport is, therefore, primarily carrier-mediated(35, 36). The oral bioavailability of metformin is approximately 50%, and the drug is neither metabolized nor bound to plasma proteins(35).

Because the primary mode of elimination is the excretion of unchanged drug in urine, patients with renal insufficiency are at risk of accumulating metformin and subsequently developing MALA(36). Renal clearance of metformin is approximately four times higher than the glomerular filtration rate, indicating extensive secretion of the drug(35). In the proximal tubules, metformin is primarily taken up by organic cation transporter (OCT) 2 through facilitated transport down an electrochemical gradient. Subsequently, metformin is luminally excreted by two cation-proton antiporters: multidrug and toxin extrusion transporter (MATE) 1 and 2K(37).

Although indirect, the mediation of transcellular movement against gradients requires ATP. Abundant mitochondria in the proximal tubule’s epithelial cells, estimated as 30-40% of the total volume, support the extraordinarily high energy demand of these cells(38). Organic cation transport, in general, is an energy-dependent process(39, 40). In **Chapter 2**, we hypothesize that metformin may inhibit its own energy-consuming renal transport due to inhibition of mitochondria when exposed to toxic metformin concentrations. In this chapter, we investigate whether the addition of metformin before or during *ex vivo* isolated normothermic machine perfusion of porcine and rat kidneys affects its elimination. Therefore, we measured the metformin concentration in plasma and urine using liquid
chromatography-tandem mass spectrometry to calculate the amount of metformin that is secreted. Moreover, parameters of renal function, renal metabolism, and organic cation transporters expression were assessed.

Kidney transplantation is the treatment of choice for patients with end-stage renal disease(41). However, ischemia-reperfusion injury can negatively impact kidney graft function, affecting both short- and long-term outcomes(42). Because it has been postulated that metformin may attenuate ischemia-reperfusion injury beyond its glucose-lowering actions(43), we investigate in Chapter 3 the effects of pre- and postconditioning with metformin on markers of renal injury and function, respectively, using the same experimental setup as used in Chapter 2.

Likewise, it is thought that metformin upregulates protective pathways during cardiac ischemia-reperfusion injury, which might improve left ventricular function after myocardial infarction(44, 45). The Glycometabolic Intervention as Adjunct to Primary Percutaneous Coronary Intervention in ST-Segment Elevation Myocardial Infarction (GIPS) III trial showed that initiation of metformin in patients without diabetes who presented with ST-segment elevation myocardial infarction did not improve left ventricular function or cardiovascular outcomes(46, 47). Approximately 15% of these patients developed contrast-induced acute kidney injury (AKI), a condition that is associated with increased morbidity and mortality, respectively(48, 49). Because impaired renal function is also associated with an increased risk of MALA, we aimed to verify that metformin in itself might impair renal function in a population at risk to develop AKI(29, 50). Besides that, as stated before, there is some evidence that metformin could act as a renoprotective agent(43, 51). Hence, as a substudy of the GIPS-III trial, we investigate in Chapter 4 whether metformin treatment initiated shortly after myocardial infarction and subsequent iodinated contrast exposure affects renal function in patients without known diabetes and pre-existing renal dysfunction.

**Effects on lactate metabolism**

Lactate is the most robust early routine laboratory marker for outcome in critically ill patients(52, 53). In general, lactate levels are elevated as part of the stress response due to lactate overproduction or impaired lactate utilization, respectively(15, 36). Therefore, lactate is increasingly measured to stratify risk and to monitor the course of critical illness(15). Because metformin is known to interfere with both lactate production and metabolism, it may act as lactate-generation amplifier and thus cause increased lactate levels among metformin users without the patient being as severely ill as metformin nonusers with similar lactate levels. Indeed, compared to critically ill patients not using metformin, some studies reported that metformin users have higher lactate levels at admission.
without a concomitant increase in mortality(54-57). However, others could not corroborate these findings(58-62). In Chapter 5, we study a large cohort of patients admitted to ICUs in northern Denmark to determine how the association of early lactate level with mortality is different in metformin users compared with metformin nonusers.

Using a similar dataset, we investigate in Chapter 6 the longitudinal changes of lactate over time for metformin users and nonusers. Subsequently, we stratify the cohort based on preadmission renal function and acute kidney injury within the first day of ICU admission to determine whether chronic or acute renal impairment modifies this association.

Metformin disposition
After multiple oral dosages, the volume of distribution is around 300 liters when correcting for bioavailability, indicating considerable tissue uptake(35). Normally, metformin shows two-compartment pharmacokinetics with a terminal half-life of 20 hours, suggesting the existence of a deeper compartment(35). The gastrointestinal wall has been proposed as a site of metformin accumulation(63, 64), but it has also been shown that metformin considerably accumulates in the kidney and liver after oral administration to mice(64, 65). Although there are some reports regarding metformin tissue accumulation after intentional intoxication, metformin disposition after accidental intoxication is unknown(66, 67). In Chapter 7, we report metformin levels measured in various tissues of a patient admitted to the ICU with accidental metformin intoxication.

Hypothesis
After extensively reviewing reports on metformin pharmacokinetics and presenting a detailed case report of a patient treated for MALA at our ICU, we propose a hypothesis in Chapter 8 that comprises that several potential mechanisms are required to occur in parallel for a patient to develop MALA. These mechanisms are subsequently illustrated using pharmacokinetic simulations. To lift a corner of the veil, we propose the hypothesis that the amount of metformin present in the body during presentation with toxic metformin levels may be remarkably small. Consequently, in MALA, metformin’s volume of distribution may be decreased as well, accelerating the development of toxic metformin concentrations when renal function is impaired. Combining these elements, we believe that acidosis may be a key factor that contributes at different levels to the chain of events ultimately leading to MALA.
References


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