Chapter 9
Summary, general discussion and future perspectives
The biguanide metformin is the recommended first-line oral therapy to treat type 2 diabetes mellitus due to its antihyperglycemic action with the proven ability to reduce cardiovascular comorbidities with a favorable safety profile(1-4). Because the primary mode of elimination is the excretion of unchanged drug in urine, some patients with renal dysfunction can accumulate metformin and subsequently develop severe lactic acidosis (metformin-associated lactic acidosis, MALA). This condition is caused by impairment of (hepatic) gluconeogenesis and excessive systemic mitochondrial inhibition(5-8). Therefore, metformin is currently contraindicated in patients with severe chronic kidney disease. Moreover, it is recommended to be used with caution when conditions are present that may reduce renal function(4).

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 intensive care unit (ICU). While generating evidence that proved or refuted certain hypotheses, new research questions arose that were worthwhile investigating. Based on these findings, a perspective of the landscape will be provided, encompassing research on metformin pharmacokinetics and its toxic effects that can serve as a foundation for future research.

In Chapter 2, we investigated whether the stepwise increase of metformin concentrations before or during ex vivo isolated normothermic machine perfusion (NMP) of porcine and rat kidneys affects its elimination. In rat experiments, metformin clearance decreased when the kidneys were exposed to higher metformin concentrations (30 mg/L or 300 mg/L, compared to oral metformin pretreatment only). In contrast, creatinine clearance was not different between treatment groups. For the first 90 minutes of normothermic perfusion in a porcine kidney study, metformin perfusion fluid concentration remained within the therapeutic range. After that, the metformin perfusion fluid concentration was increased rapidly until a mean concentration of 179 mg/L was reached at the end of the experiment. In porcine kidneys, the metformin clearance peaked at 90 minutes of perfusion and declined thereafter. The creatinine clearance remained relatively stable throughout the experiment. Although one interpretation was that metformin would negatively impact its own elimination, this observation can also be explained by saturation of metformin transporters with a Michaelis-Menten constant of 23.0 mg/L. Interestingly, fractional sodium excretion, an indicator for tubular function in this model, was not different between metformin treatment groups and controls, irrespective of the metformin concentration. In rat kidneys exposed to 300 mg/L metformin, oxygen consumption was decreased and lactate production was increased, respectively, indicating a shift towards anaerobic metabolism presumably by inhibition of mitochondrial respiration(9).
The same experimental setup was used in Chapter 3. In this study, we examined the effects of preconditioning and postconditioning with metformin on ischemia-reperfusion injury. The most important finding for this thesis is that exposure to massive doses of metformin (up to a concentration of 300 mg/L) was neither associated with increased kidney injury markers, structural damage nor was it detrimental to kidney function. Of note, the overall function of the kidneys used in this model, and possibly thus also tubular function, can be considered poor.

In conclusion, metformin excretion decreased under increasing metformin concentrations, which can be explained by the saturation of renal metformin transporters, although we cannot entirely exclude a self-inhibitory effect. Moreover, both preconditioning and postconditioning with metformin had minor effects on kidney injury or function, respectively, during NMP of rat and porcine kidneys. Remarkably, metformin concentrations that are highly toxic in vivo did not negatively impact tubular function. In contrast, preconditioning with metformin improved hepatobiliary function and reduced markers of liver injury during NMP and subsequent orthotopic transplantation of rat livers, underlining the fact that metformin affects the liver's metabolism and thus ischemia-reperfusion injury differently than in kidneys(10). It would be interesting to determine which cellular mechanism underlies the protective effects of metformin pretreatment of organ donors. For example, both AMP-activated protein kinase (AMPK) activation and alterations of the redox state have been described to be the mode of action of metformin, which could be tested within the isolated perfused liver or kidney setup by adding AMPK stimulator AICAR, by normalizing the cytosolic redox state by infusion of methylene blue, or by using $^{13}$C positional isotopomer tracer analyses, respectively(9, 11-13).

Interestingly, preconditioning with low-dose metformin dramatically protects gentamycin-induced nephrotoxicity in rats by correcting mitochondrial alterations(14). Moreover, it has been suggested that organic cation transporter (OCT) 2 overexpression might cause an increase in gentamycin-induced nephrotoxicity, which could be attenuated by co-administration of metformin(15). It is unknown whether metformin reduces gentamycin-induced nephrotoxicity within our isolated perfused porcine kidney model and whether co-administration of OCT-2 inhibitor cimetidine can exert a similar effect.

Chapter 4 describes the results of a substudy of the GIPS-III trial. In this trial, patients without diabetes, who were treated with primary percutaneous coronary intervention for ST-segment elevation myocardial infarction (STEMI), were randomized to receive metformin or placebo for four months. Compared with baseline, the change in estimated glomerular filtration rate (eGFR) four months after randomization did not differ between the metformin (-5.9 ± 0.8 mL/
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min/1.73 m²) and control group (-7.1 ± 0.8 mL/min/1.73 m², P = 0.27 for overall interaction between treatment groups). Furthermore, after adjustment for covariates, metformin treatment was also not associated with the development of contrast-induced acute kidney injury (AKI). Thus, initiation of metformin shortly after primary percutaneous coronary intervention in patients presenting with STEMI without diabetes or prior renal impairment had no adverse effect on renal function. Moreover, no hospitalizations due to lactic acidosis were observed during the study period.

Previously, metformin use has been associated with mixed but, in all cases, minor effects on renal function(16-23). Despite extensive efforts to control for bias using numerous techniques in these observational studies, the association of metformin use with either improvement or decline in renal function might be influenced by confounding by indication or other methodological limitations(24, 25). A large randomized placebo-controlled randomized study could provide a final answer regarding the effects of metformin on renal function. However, because metformin is currently the recommended first-line oral antihyperglycemic therapy to treat patients with type 2 diabetes mellitus, such a trial would lack clinical equipoise because it would withhold patients the standard of care. To maintain clinical equipoise, patients could be randomized to receive standard renal function adjusted dosages vs. higher dosages of metformin before a second-line antihyperglycemic therapy is administered. However, such a study would require a considerable sample size to have enough power to detect differences in renal function. On the other hand, performing a study that randomizes patients without diabetes who undergo an elective surgical procedure with a high risk of postoperative renal dysfunction to either metformin or placebo pre-operatively might be hampered due to deeply entrenched sentiments about the risk of developing MALA. Therefore, administering metformin to a population at risk for developing AKI is more justifiable when this occurs in a highly controlled environment with frequent monitoring of glucose and lactate levels using blood gas analysis, like the ICU.

Indeed, several smaller studies have shown that administration of metformin to critically ill patients is safe and, in most cases, effective in treating hyperglycemia, but large randomized controlled trials are lacking(26-30). Due to predisposing factors such as high body mass index and diabetes combined with administration of high-dose corticosteroids, marked insulin resistance frequently occurs in critically ill patients with coronavirus disease 2019(31, 32). In our experience, metformin administration as compassionate use clearly reduced insulin resistance in this patient group, but its efficacy and safety have not been formally established yet(31).

Because smaller cohort studies have shown that metformin users have higher lactate levels at ICU admission without a concomitant increase in mortality, we
determined in Chapter 5 whether the association between lactate levels and mortality around ICU admission is different in metformin users compared with metformin nonusers. We studied 37,293 patients admitted to ICUs in northern Denmark with any circulating lactate measured 12 hours before until 6 hours after ICU admission between January 2010 and August 2017. Of those, 9% used metformin. Lactate levels were strongly associated with mortality for both metformin users and nonusers. However, the association of lactate with mortality was different for metformin users, with a lower mortality rate in metformin users than in nonusers when admitted with similar lactate levels. This was observed over the whole range of lactate levels, and consequently, the relation of lactate with mortality was shifted rightwards for metformin users. Lactate levels around ICU admission should therefore be interpreted according to metformin use.

Using a similar dataset to the previous chapter, we report in Chapter 6 the longitudinal changes of lactate over time for metformin users and nonusers. Metformin users had 0.61 (95% confidence interval, 0.45-0.77) mmol/L higher lactate levels than nonusers in the early phase of critical illness, which disappeared within 24 hours of ICU admission. Importantly, the difference in lactate levels between metformin users and nonusers was higher in patients with AKI stage 2 or 3. In contrast, the difference was marginal in patients without renal dysfunction or AKI stage 1. Thus, the association of metformin use with increased lactate levels is more pronounced in critically ill patients with more severe AKI.

A limitation of both studies is that the metformin blood concentration was not routinely measured to determine a dose-response relationship. Clinically, the difference between therapeutic levels (i.e., <5 mg/L) and toxic levels of at least >10 mg/L might prevent many subclinical cases for developing frank MALA(33). Measuring metformin levels in a prospective cohort of acutely admitted critically ill metformin users could provide insight whether the pharmacodynamic effects of the drug on lactate metabolism as studied in Chapters 5 and 6 are only reserved for patients with a significant intoxication, or that subclinical metformin intoxications are more common than often thought. However, it will be challenging to create an unbiased dose-response curve between metformin concentration and lactate level because patients with an unrecognized metformin intoxication might also be the most severely ill at risk of developing hyperlactatemia due to various other reasons. Nevertheless, from a clinical point of view, establishing the prevalence of metformin intoxication (with or without lactic acidosis) in this population would provide insight into the a priori chances that metformin contributes to hyperlactatemia in a critically ill patient. Furthermore, only sparse data is available regarding metformin concentration in a more general population of individuals with diabetes when stratified according to the eGFR(34, 35).
We report the clinical course and autopsy of a patient with accidental metformin intoxication in Chapter 7. Several days after the onset of gastrointestinal symptoms, the patient was admitted to the ICU with acute renal failure and severe lactic acidosis. In this case, lactic acidosis can be explained by a combination of metformin intoxication, septic shock, and liver failure. Indeed, the initial plasma metformin concentration was 24.6 mg/L, which is a toxic concentration. Despite renal replacement therapy and supportive care, the patient remained anuric and ultimately died from multi-organ failure 39 hours after ICU admission. During the autopsy, hepatic steatosis and signs of a shock liver as well as renal tubular necrosis were observed. The metformin level in homogenized cortical kidney tissue of 36.8 mg/kg was eightfold higher than the last plasma concentration measured within 3 hours before the patient died. In other organs, including the liver, however, the metformin level approximated the last measured plasma concentration, which declined over time due to prolonged renal replacement therapy.

Renal secretory and subsequent drainage mechanisms were thus overwhelmed. They were neither able to eliminate metformin into urine sufficiently nor redistribute metformin through the circulation to other compartments, despite the presence of a concentration gradient after removing the drug from the circulation by renal replacement therapy. Although OCTs are known to transport cations in a bidirectional fashion, the kidney was evidently unable to equalize with the metformin concentration in the blood(36). Acute tubular necrosis was histologically confirmed during autopsy, which most probably was preceded by a state of reduced organic cation transport capacity due to OCT and multidrug and toxin extrusion (MATE) transporter protein expression downregulation, functional and organizational mitochondrial alterations, and apoptosis in order to minimize the energy-consuming activities that might aggravate cellular injury(37-41).

In line with our observation of metformin retention, it is generally accepted that efflux is the rate-limiting step in renal transcellular transport of metformin, which is further exemplified by the accumulation in renal tissue after oral and intravenous administration to animals, respectively, and after metformin intoxication in humans(42-44). This is further underlined by a higher affinity of metformin for OCT-2 than the MATE transporters(36). Subsequently, metformin accumulation in blood occurs when the intracellular metformin concentration increases to such an extent that it impairs the driving force for influx by OCT-2(45). Possibly, intracellular accumulation can lead to further mitochondrial inhibition by metformin, which might reduce tubular transport capacity even more(46, 47). However, it is unknown whether renal secretion of metformin is influenced by changes in cellular energy or by mitochondrial inhibition specifically, which could be tested in vitro using precision-cut human kidney slices because in these structures cellular integrity including influx and efflux transporters remain intact(48).
In Chapter 8, we provide an overview of metformin's pharmacokinetic properties, and, subsequently, we sketch a hypothesis of the chain of events that ultimately might lead to the development of MALA. In our experience, patients develop MALA remarkably suddenly, but an all-encompassing theory of why this happens at such a fast pace is lacking. This chapter describes metformin toxicokinetics of a patient admitted to the ICU with MALA (maximum metformin blood concentration 22.6 mg/L) and found that only a daily metformin dose (1769 mg) was eliminated during recovery. This small amount of metformin being present in the body indicates an accordingly low volume of distribution (Vd) when developing MALA. Under normal circumstances, the Vd is estimated to be approximately 300 liters(49). Dynamic Vd's are characteristic for actively transported drugs, like metformin(50). In addition, some studies suggest that the Vd of metformin is related to renal clearance, which could accelerate the rise in circulating metformin(49, 51, 52). When we simulated in a pharmacokinetic model a situation in which a diminished metformin clearance (30 mL/min) was combined with a decreased Vd (75 L), toxic concentrations were reached within days. Acidosis may be the key factor that affects uptake, transporter-mediated tissue distribution, and renal clearance instead of merely being a consequence of metformin intoxication. Concluding, in Chapter 8, we propose that MALA is an interplay of progressive changes in absorption, Vd, and tubular secretion, for which acidosis might be the common denominator. Combined, these factors may contribute to the sudden development of MALA. The fact that most metformin users do not develop MALA even during transient renal impairment might be explained by the unusual combination of events that has to happen until this severe adverse effect of metformin occurs.

It is intriguing that most described cases, including the cases described in Chapters 7 and 8, have a history of gastrointestinal complaints preceding the onset of MALA(53). Apart from the fact that severe lactic acidosis combined with gastrointestinal complaints should always lead to the immediate consideration of enteral ischemia, these symptoms may also indicate that the intestinal function has temporarily changed, leading to an abnormally high bioavailability of metformin. Further studies on enteral permeability markers during MALA may indicate whether altered permeability is relevant in its pathogenesis. For example, increased blood levels of intestinal fatty-acid binding protein or D-lactate, the stereoisomer of L-lactate that is only produced by bacteria residing in the gastrointestinal tract, could be indicative of a compromised gut barrier function(54).

Because metformin might affect thiamine absorption through competitive inhibition, it has been suggested that these gastrointestinal symptoms could also be explained by a relatively under-recognized form of thiamine deficiency associated with severe hyperlactatemia, known as gastrointestinal beriberi(55-57). Future studies should investigate whether thiamine deficiency is common among metformin users and specifically in patients with MALA.
Currently, clinical research into the pathogenesis of MALA is limited by its low incidence, different definitions used, and because the determination of the metformin concentration in blood is not readily available (58). However, there is also no established preclinical model mimicking the development of MALA. Indeed, it is very challenging to experimentally induce a prolonged state of severe acidosis (pH ≤ 7.0) that does not rapidly kill the animal, making it difficult to reproduce the impact of severe lactic acidosis on the marked contraction of metformin's Vd. The impact of more moderate acidosis on the biodistribution of other drugs, such as bupivacaine and thiopental, has been studied. However, such studies could only induce a pH of 7.25 for 30 minutes (59). Hence, the regularly used preclinical metformin intoxication model resembles an intentional intoxication, which is clinically associated with different etiology and outcomes (6, 60). It would be interesting to determine the Vd after oral or intravenously metformin administration when severe septic shock is induced in a large animal model (61, 62).

The clinical take-home message of this thesis is that in patients who are known to use metformin and present with renal impairment accompanied by severe lactic acidosis of uncertain origin, MALA should, in all cases, be considered until proven otherwise. Therefore, if MALA is considered, immediate hemodialysis is mandatory irrespective of the availability of a blood metformin concentration at that time. Hyperlactatemia caused by other conditions, such as sepsis or liver failure, is typically considered a consequence of that specific critical illness and not a cause (63). Accordingly, administration of sodium bicarbonate to increase the arterial pH is only reluctantly used in these conditions. However, it improved survival in patients with severe acute kidney injury and concomitant metabolic acidosis (64, 65). We argue that treatment with sodium bicarbonate may be much more rational because, according to our hypothesis, sodium bicarbonate administration may partly correct the metabolic acidosis and, consequently, increase metformin's Vd by redistribution into peripheral tissues. Therefore, while waiting for the initiation of hemodialysis, aggressive bicarbonate treatment may be the cornerstone in the initial resuscitation of a critically ill patient with a clinical suspicion of MALA.
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in vitro studies confirm that inhibition of the renal apical efflux transporter multidrug and toxin extrusion (MATE) 1, and not altered absorption, underlies the increased metformin exposure observed in clinical interactions with cimetidine, trimethoprim or pyrimethamine. Pharmacol Res Perspect. 2017;5(5):e00357.


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