



Metformin & Chronic Kidney Disease (CKD) : a reassessment of the benefit/risk ratio

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Thèse de Doctorat

Biologie Santé spécialité Néphrologie - Toxicité urémique

présentée à *l'Ecole Doctorale en Sciences Technologie et Santé (ED 585)*

de l'Université de Picardie Jules Verne

par

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pour obtenir le grade de Docteur de l'Université de Picardie Jules Verne

***Metformine et insuffisance rénale :
réévaluation du rapport bénéfices/risques***

Soutenue le 29 octobre 2015 après avis des rapporteurs, devant le jury d'examen :

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***METFORMIN & CHRONIC KIDNEY DISEASE (CKD):
A REASSESSMENT OF THE BENEFIT/RISK RATIO***

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❖ ARTICLES

- Briet C, Saraval-Gross M, **Kajbaf F**, Fournier A, Hary L, Lalau JD. Erythrocyte metformin levels in patients with type 2 diabetes and varying severity of chronic kidney disease. *Clin Kidney J*. 2012 Feb;5(1):65-67.
- **Kajbaf F**, Lalau JD. The criteria for metformin-associated lactic acidosis: the quality of reporting in a large pharmacovigilance database. *Diabet Med*. 2013 Mar;30(3):345-8.
- **Kajbaf F**, Lalau JD. The prognostic value of blood pH and lactate and metformin concentrations in severe metformin-associated lactic acidosis. *BMC Pharmacol Toxicol*. 2013 Apr 12; 14(1):22.
- **Kajbaf F**, Arnouts P, De Broe M, Lalau JD. Metformin therapy and kidney disease: a review of guidelines and proposals for metformin withdrawal around the world. *Pharmacoepidemiol Drug Saf*. 2013 Oct;22(10):1027-35.
- **Kajbaf F**, Fendri S, Basille-Fantinato A, Diouf M, Rose D, Jounieaux V, Lalau JD. The relationship between metformin therapy and sleep quantity and quality in Type 2 diabetic patients referred for potential sleep disorders. *Diabet Med*. 2014 May;31(5):577-80.
- Lalau JD, Azzoug ML, **Kajbaf F**, Briet C, Desailoud R. Metformin accumulation without hyperlactataemia and metformin-induced hyperlactataemia without metformin accumulation. *Diabetes Metab*. 2014 Jun;40(3):220-3.
- Lalau JD, **Kajbaf F**. Mortality rate in so-called "metformin-associated lactic acidosis": a review of the data since the 1960s. *Pharmacoepidemiol Drug Saf*. 2014 Nov;23(11):1123-7.
- **Kajbaf F**, Mentaverri R, Diouf M, Fournier A, Kamel S, Lalau JD. The Association between 25-Hydroxyvitamin D and Hemoglobin A1c Levels in Patients with Type 2 Diabetes and Stage 1–5 Chronic Kidney Diseases. *Int J Endocrinol*. Volume 2014. Doi: 10.1155/2014/142468.
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- **Kajbaf F**, De Broe ME, Lalau JD. "Therapeutic concentrations" of metformin: a systematic review. *Clin. Pharmacokinet*. 2015 Sep 2. [Epub ahead of print].

❖ LETTERS TO EDITOR

- Lalau JD, **Kajbaf F**, Arnouts P, De Broe M. Mortality and metformin use in patients with advanced chronic kidney disease. *Lancet Diabetes Endocrinol*. 2015 Sep;3(9):680-1.
- Lalau JD, **Kajbaf F**. Metformin poisoning: a debate on recommendations for extracorporeal treatment. *Crit Care Med* (accepted for publication).

❖ ORAL PRESENTATIONS

- **Kajbaf F**, Oprisiu R, Henry L, Lemaire-Hurtel AS, Bouvier J, Boitte F, Brazier M, Diouf M, Lalau JD, Fournier A. Higher serum 25OH Vit D levels are associated with lower HbA1C levels in Type 2 diabetic patients with various stages of chronic kidney disease and treated with metformin. 20th meeting of the Balkan Clinical Laboratory Federation, Belgrade, 18-22 Sept. 2012.
- **Kajbaf F**, Lalau JD, Azzoug M, Lemaire-Hurtel AS, De Broe ME. Metformin Therapy at Different Stages of Chronic Kidney Disease. American Society of Nephrology - Kidney Week, Philadelphia USA, 11–16 Nov. 2014 (Journal of the American Society of Nephrology, November 2014, Volume 25, Abstract Edition; page 76A).

❖ POSTERS

- **Kajbaf F**, Lalau JD. Acidose lactique associée à la metformine : la metformine pourrait-elle être protectrice ? Congrès Annuel Francophone de Diabète SFD, Nice, 20-23 mars 2012 (Diabetes & Metabolism. 2012 38;(S2) A102).
- **Kajbaf F**, Lalau JD. Can metformin improve survival in severe metformin-associated lactic acidosis? 48th Annual Meeting of the European Association for the Study of Diabetes Berlin, 2-5 Oct. 2012 (Diabetologia. 2012 55;(S1) S325).
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- Lalau JD, **Kajbaf F**. Accumulation de la metformine: que pouvons-nous déduire des corrélations entre le pH artériel et la lactacidémie et les taux sanguins de metformine? Congrès Annuel Francophone de Diabète SFD, Montpellier, 26-29 mars 2013 (Diabetes & Metabolism. 2013 39;(S1) A57).
- Lalau JD, **Kajbaf F**, Basille-Fantinato A, Rose D, Fendri S. Le traitement par metformine est associé à un sommeil de meilleure qualité. Congrès Annuel Francophone de Diabète SFD, Montpellier, 26-29 mars 2013 (Diabetes & Metabolism. 2013 39;(S1) A57).
- Lalau JD, **Kajbaf F**, Rahmani A, Fournier A. La metformine peut-elle être utilisée dans l'insuffisance rénale? Mise en place d'un protocole. Congrès Annuel Francophone de Diabète SFD, Montpellier, 26-29 mars 2013 (Diabetes & Metabolism. 2013 39;(S1) A57).
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- Lalau JD, Azzoug ML, **Kajbaf F**, Briet C, Desailoud R. Hypo- et hypersensibilité aux effets métaboliques de la metformine : un concept nouveau. Congrès Annuel Francophone de Diabète SFD, Paris, 11-14 mars 2014 (Diabetes & Metabolism. 2014 40;(S1) A109).

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- Lalau JD, **Kajbaf F**, Azzoug M, Lemaire-Hurtel AS, De Broe ME. Metformin therapy at different stages of chronic kidney disease. The International Society of Nephrology (ISN) World Congress of Nephrology. Cape Town 13-17 March 2015 (<http://www.posters2view.eu/wcn2015/view.php?nu=2728>).
- Lalau JD, **Kajbaf F**. Connaît-on les taux thérapeutiques de metformine ? Congrès Annuel Francophone de Diabète SFD, Bordeaux, 24-27 mars 2015 (Diabetes & Metabolism. 2014 41;(S1) A118-9).
- Lalau JD, **Kajbaf F**, Azzoug M, Lemaire-Hurtel AS, De Broe ME. Traitement par metformine dans l'insuffisance rénale. Congrès Annuel Francophone de Diabète SFD, Bordeaux, 24-27 mars 2015 (Diabetes & Metabolism. 2014 41;(S1) A113-4).
- Lalau JD, Bennis Y, **Kajbaf F**. Étude de l'élimination de la metformine accumulée: le raisonnement selon la demi-vie plasmatique (courte) est faux ! Congrès Annuel Francophone de Diabète SFD, Bordeaux, 24-27 mars 2015 (Diabetes & Metabolism. 2014 41;(S1) A114).

PART I. Introduction

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BACKGROUND

Metformin is acknowledged worldwide as having a pivotal role in the primary treatment of type II diabetes mellitus (T2DM). However, there is still a debate concerning the drug's beneficial and adverse effects – particularly because of the high and increasing proportion of patients presenting with established or suspected kidney disease. Even though a critical review of the literature in this field has questioned the claimed danger of lactic acidosis in metformin-treated patients, the latter context still influences treatment strategies. Moreover, the relationship between metformin and lactic acidosis is not a simple one in which accumulation of the drug inevitably leads to the adverse event of lactic acidosis. Indeed, lactic acidosis in a metformin-treated patient is not necessarily accompanied by metformin accumulation, nor does metformin accumulation necessarily lead to lactic acidosis.

Given that metformin is eliminated (unmodified) by the kidneys, it is logically contraindicated in severe kidney disease. Most countries, however, do not have precisely defined treatment guidelines in kidney disease. The existing national guidelines differ significantly in terms of both the parameters used and the recommended values for these parameters.

In this introduction, the following aspects are successively considered: the “protagonists” in the debate (metformin, lactic acidosis, and chronic kidney disease (CKD)), the pathophysiology of lactic acidosis in metformin-treated patients (i.e. lactic acidosis that is *unrelated* to metformin, lactic acidosis *induced* by metformin, and the intermediate (and more complex) situation referred to as “metformin-associated lactic acidosis” (MALA)), the debate over the therapeutic concentrations of metformin, and the debate over metformin contraindications in patients with CKD.

The present work is divided into three parts. The first part consists of a critical examination of the reporting of MALA, the criteria for MALA and the mortality rate in this context. The second part deals with the advantages and limitations of measuring the metformin concentrations in blood when screening for metformin accumulation and when assessing that latter's extent and consequences. In the third and last part, the results of a prospective study conducted in metformin-treated patients with CKD (at all CKD stages) are examined.

The goal of the present work is to develop a practical guide for metformin therapy in CKD, with regards to the following paradox: metformin should be prescribed less widely because of the increasing frequency of contraindications, whereas it should also be prescribed more widely because of its impressive, beneficial effects.

The protagonists: metformin, lactic acidosis and CKD

Metformin

Discovery

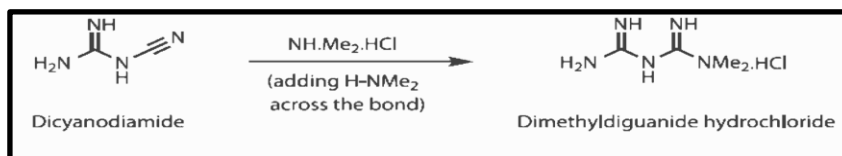
Galega officinalis (a perennial herb with the common names of “goat’s rue”, “French lilac”, “Spanish sanfoin” or “false indigo”) was used as an herbal medicine in medieval Europe. Studies conducted before the 19th century showed that *G. officinalis* was rich in guanidine. Studies in the 1910s-1930s then demonstrated that guanidines possessed hypoglycemic activity in animals [Dronsfield 2011].

Although several glucose-lowering biguanides were synthesized in the 1920s, it was in 1922 that Emil Werner and James Bell (chemists from Dublin) first synthesized metformin. Hesse and Taubmann described the compound’s hypoglycemic effect on animals in 1929. The French physician and clinical pharmacologist Jean Sterne (1909-1997) first named the dimethylbiguanide compound as “Glucophage” (i.e. a “glucose eater”) and published the first clinical trial results in 1957 [Bailey 2004]

Chemistry

In 1922, Werner and Bell used dicyanodiamide as a precursor in the synthesis of guanidine derivatives. Reaction of dicyanodiamide with dimethylammonium chloride in acidic conditions forms metformin in 3–4 hours.

Figure 1.1. Chemical synthesis of metformin.



Metformin has hydrophilic properties, with acid dissociation constant (pKa) values of 2.8 and 11.5. Consequently, more than 99.9% of the molecules exist as cations at physiological pH [Dronsfield 2011].

Pharmacology

Metabolic effects

Many clinical studies (including double-blind, placebo-controlled, randomized clinical trials) have shown that metformin can substantially lower blood glucose and glycosylated hemoglobin levels in diabetic patients [Cavallo-Perin 1989, Stumvoll 1995, Avilés-Santa 1999, DeFronzo 1995]. Metformin inhibits hepatic gluconeogenesis and increases the action of insulin in several target organs (muscle

and fat, to be precise). Metformin increases the sensitivity of both hepatic and peripheral tissues to insulin [Viollet 2012].

Metformin's effects on glycemic control and the oxidative disposal of glucose do not modify lactate metabolism in muscle [Stumvoll 1995]. One does not expect a significant increase in plasma lactate in patients on well-tolerated metformin therapy because of the conversion of lactate to glucose in the liver (via the Cori cycle). However, in animal models, metformin increases extrahepatic splanchnic lactate production by stimulating anaerobic glucose metabolism [Bailey 1992].

In the postprandial state, metformin increases the plasma lactate level due to lactate production by the small intestine [Bailey 1992] and defective lactate uptake by the liver cells [Radziuk 1997]; however, this hyperlactatemic effect is weak at the recommended dosage of metformin [Radziuk 1977]. In the intestine, metformin inhibits lactate conversion through gluconeogenesis and augments lactate production through accelerating glycolysis and shifting the intracellular redox potential from aerobic to anaerobic glucose metabolism [Radziuk 1977, Arieff 1980].

Mechanisms of action in glucose metabolism

Metformin's positively charged guanidinium group (responsible for cellular uptake) means that the concentration of the drug in the mitochondrial matrix may be 100-fold higher than in plasma under physiological conditions [Wilcock 1991]. However, suprapharmacological metformin concentrations are needed to reduce glucose production through inhibition of the complex 1 respiratory chain in mitochondria and activation of AMP-activated protein kinase [He 2015, El-Mir 2000, Owen 2000]. In fact, inhibition of complex 1 is followed by an increase in AMP levels, which (i) blocks the cAMP-PKA pathway via the inhibition of the adenylyl cyclase activity (leading to a reduction in hepatic glucose output via antagonism of glucagon) and (ii) activates AMPK, which is known to be the key "fuel gauge" that protects cells under energy-restricted conditions [Cao 2014, He 2014].

Pharmacologic concentrations of metformin (i.e. those achievable in the portal vein after the administration of usual therapeutic doses) can activate AMPK, which inhibits gluconeogenic gene expression by phosphorylation of CBP and CRTC2. Likewise, pharmacologic metformin concentrations inhibit mitochondrial glycerol 3-phosphate dehydrogenase; this results in an increase in cytosolic NADH levels, prevention of lactate consumption and reduction in gluconeogenesis [Madiraju 2014, Miller 2013].

Effects unrelated to glucose metabolism

Metformin also have many beneficial effects unrelated to glucose metabolism. It has notably been shown to prevent diabetes itself, the micro- and macrovascular complications of diabetes, major events in patients with heart failure, apoptotic neuron death, cancer, osteopenia, and polycystic

ovary syndrome [Forouzandeh 2014, Kourelis 2012, Jeyabalan2012, Johnson 2014]. Taken as a whole, these various aspects give to metformin an amazing disease-preventing profile.

Pharmacokinetics and pharmacodynamics

Pharmacokinetics

Metformin's bioavailability after oral intake is around 50–60%. After absorption by the small intestine, metformin distributes quickly to many different tissues. The luminal concentration in the gastrointestinal tract remains high, whereas binding to plasma proteins is negligible. Metformin's volume of distribution after single intravenous administrations of 250 mg to 1000 mg varied from 63 to 276 L. Likewise, the apparent volume of distribution after a single oral intake reportedly ranges from 230 L to 380 L for doses of 850 and 1000 mg and is 600 L with a daily dose of 2000 mg [Graham 2011].

After oral administration of metformin, the peak plasma concentration (1 to 2 mg/l) is reached after ~3 h for a 1.5 g dose and after 1 to 2 hours for a dose of 500 mg to 1000 mg. Metformin is eliminated unchanged by the kidneys by filtration and active tubular secretion [Lipska 2011], with a clearance rate four to five times higher than that of creatinine. Renal clearance of metformin decreases in parallel with the reduction in renal function [Graham 2011]. Thus, the occurrence of metformin accumulation is necessarily linked to either severe kidney failure or overdose.

After intravenous administration of metformin, most of the drug (80–100%) is rapidly eliminated in an unchanged form in the urine. After oral administration of metformin in subjects with normal kidney function, the renal clearance rate is 510 ± 130 ml/min [for a review, see Graham 2011]. The apparent total clearance (CL/F) of metformin was estimated to be 1140 ± 330 mL/min. The elimination of metformin from plasma follows a bi-exponential or even a tri-exponential concentration–time curve. On the basis of the excretion rate in urine after the cessation of multiple dosage regimens, the terminal half-life ($t_{1/2}$) is around 20 hours. This long elimination time indicates the existence of a deep compartment for metformin. In a study evaluating the oral administration of a single dose of metformin in healthy subjects, $t_{1/2}$ for elimination from erythrocytes was 23.4 hours, compared with 2.7 hours for elimination from plasma [Graham 2011].

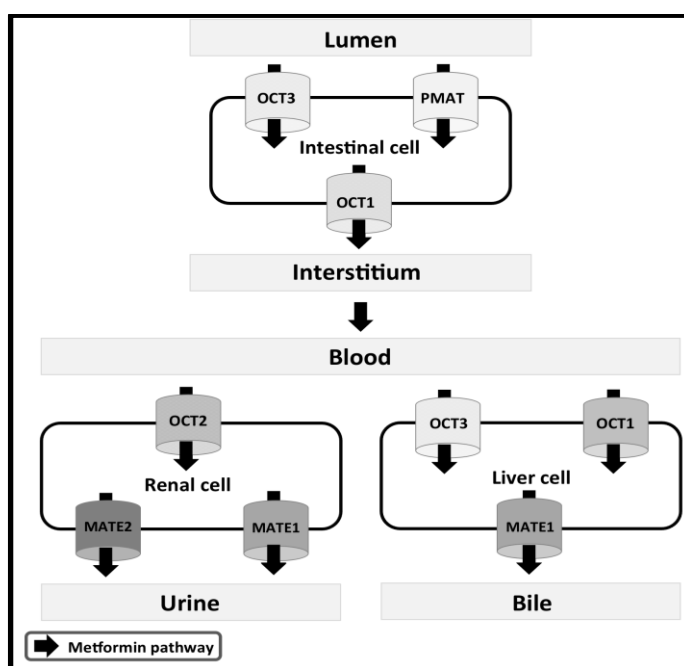
There are few pharmacokinetics data available for metformin in a context of CKD. A small series of patients with moderate CKD (creatinine clearance rate=31-60 ml/min, n=4) and severe CKD (creatinine clearance=10-30 ml/min, n=6), had a higher plasma concentration and longer blood half-life of (relative to normal subjects of various ages) after administration of a single dose of 850 mg metformin. The renal clearance of metformin fell in proportion to the decrease in the creatinine clearance rate. Metformin's mean \pm standard error (SE) plasma half-life was around 7 ± 4 h in healthy,

young adults, 16 ± 7 h in patients with moderate CKD and 17 ± 9 h in patients with severe CKD [Sambol 1995]. In the latter study, metformin clearance in the patients with moderate CKD was 74%-78% lower than in normal subjects [Sambol 1995]. The maximum concentration (C_{max}) and the area under the concentration-time curve (AUC) were higher in subjects with moderate to severe CKD than in patients with mild CKD or normal renal function. Likewise, in another small series ($n=5$) of CKD patients (creatinine clearance rates: 20-45 ml/min), the mean $t_{1/2}$ was 4.94 ± 1.11 h (vs. 1.52 ± 0.3 h in normal subjects) after 1 g of intravenous metformin [Sirtori 1978].

The role of metformin transporters

Metformin is a hydrophilic drug that is mostly present as a cation *in vivo*. As a consequence, metformin transfer across the cell membrane is mediated by several carriers (e.g. the organic cation transporters (OCTs) and the plasma membrane monoamine transporters (PMATs)) [Todd 2014]. The PMATs located on the luminal side of the enterocytes are the main transporter for the intestinal absorption of metformin, whereas OCT1 (expressed on the enterocyte's basolateral membrane) is responsible for transporting metformin into the interstitial fluid. Likewise, the OCT1 and OCT3 expressed on the basolateral membrane of hepatocytes mediate metformin uptake by the liver. The OCT2 expressed on the basolateral membrane of the kidneys enables the uptake of metformin into renal epithelial cells [Tzvetkov 2009]. The multidrug and toxin extrusion 1 and 2 (MATE1 and MATE2K) proteins (located in the luminal membrane of the kidney and liver) mediate the elimination of metformin into the bile and the urine [Wang 2014].

Figure 1.2. Transporters involved in metformin absorption, hepatic uptake and urinary excretion.



Pharmacogenomics

Age, renal function and pathophysiological status can affect the pharmacokinetics and pharmacodynamics of metformin [Sambol 1995]. Furthermore, there is inter-individual variability in the response to metformin. This heterogeneity may be due (to some extent) by genetic factors. The clinical consequences of this heterogeneity may range from (i) the absence of an expected therapeutic effect to (ii) an adverse drug reaction [Todd 2014].

Genetic variations and polymorphisms of the aforementioned transporters can influence metformin's pharmacodynamics and pharmacokinetics. Most current studies have focused on genes involved in the pharmacokinetics of metformin, whereas there are very few data on the pharmacogenomics-pharmacodynamics relationships.

Several polymorphisms of the OCT1 gene (SLC22A1) have been described. In healthy subjects, the presence of at least one of four reduced-function variants of this gene (R61C, G401S, 420del and G465R) attenuates metformin's effect on glucose tolerance tests. Likewise, a higher AUC for the plasma metformin concentration, a higher C_{max} and a lower volume of distribution were observed in individuals with these reduced-function variants [Tzvetkov 2009].

OCT2 has fewer functional variations than OCT1. Contradictory results have been found in studies of the effects of OCT2 variants on renal clearance and C_{max}. However, no association between OCT2 variants and clinical outcomes has yet been demonstrated [Tzvetkov 2009].

Six non-synonymous OCT3 variants have been detected by sequence analysis. Three of these (rs68187715/T44M, rs8187717/A116S and rs8187725/T400I) altered metformin uptake in *in vitro* studies. The impact of genetic differences on intestinal absorption seems to be low, whereas the variations have significant effects on the hepatic processing of metformin and on the therapeutic response to metformin in glucose tolerance tests [Todd 2014].

MATE1 and MATE2-K have a very low frequency of functional variations. It has been shown that the single nucleotide polymorphism (SNP) rs2289669 (G>A) in the SLC47A1 gene (encoding the MATE1 transporter) influences the glucose-lowering effect of metformin and is associated with a reduction in A1C level and MATE1 transporter activity [Becker 2009].

Plasma clearance of metformin is highly correlated with the clearance of creatinine ($r=0.85$, $p<0.001$). In a pharmacokinetic study of a single 500 mg oral dose of metformin performed in 103 healthy subjects, the renal clearance of metformin varied up to 3.8-fold as a function of the OCT1 polymorphism, in addition to variations related to age and the creatinine clearance rate [Tzvetkov 2009]. Furthermore, promoter variants of MATE1 and MATE2 are important for renal secretory clearance of metformin [Stocker 2013]. The impact of these polymorphisms has been confirmed in patients taking 1 g of metformin twice daily over a 24-month period. Strikingly, an 80-fold interindividual variation in trough steady-state plasma concentrations of metformin was

documented [Christensen 2011]. In view of these genotype variations, the conventional assumption whereby the plasma metformin concentration depends on the glomerular filtration rate (GFR) is being challenged [Adam 2014].

Lactic acidosis

Lactic acidosis is commonly defined as a serum lactate level >5 mmol/L and a serum pH <7.35 . It is often associated with a high anion gap [Luft 1983]. An increase in the anion gap is sometimes considered to be a determinant of lactic acidosis. Nevertheless, the anion gap is a relatively insensitive indicator of abnormal lactate metabolism - even in patients with hyperlactatemia [Stacpoole 1993, Iberti 1990].

Lactic acidosis is conventionally divided into Type A (anaerobic, with impaired tissue oxygenation) and Type B (aerobic, with maintenance of tissue oxygenation). Type A is a sequel of tissue hypoxia (e.g. a state of shock) that results in the anaerobic production of lactic acid. In Type B, tissue hypoxia is not a cornerstone feature, whereas other systemic disorders (such as liver disease, glycogen storage disease, seizures, neoplastic conditions, diabetes, drugs and toxins) are considered to be precipitating or triggering factors [Cohen 1976, Cohen 1983, DuBose 1977]. However, this distinction has become obsolete because a restricted oxygen supply and metabolic factors often act simultaneously [Lalau2010].

It is important to draw another distinction between predisposing and precipitating factors for lactic acidosis. Diabetes itself may be considered as both a predisposing and precipitating factor [Williamson 1967, Arieff 1976, Moorhouse 1964]. Indeed, diabetes is a micro- and a macrovascular disease; dehydration is frequent during decompensation phases and many drugs (e.g. diuretics, angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, and non-steroidal anti-inflammatory drugs) may alter renal function - particular in the elderly [Prikis 2007].

Lactate metabolism

Lactate is metabolized mainly by the liver (50%), the kidney (20%) and (to a lesser extent) the striated muscles, the heart and the brain. The liver is an important organ in glucose production and lactate clearance. In the fasting state, pyruvate and lactate are converted to glucose via gluconeogenesis, with an increase in the activity of the mitochondrial enzyme pyruvate carboxylase. In the fed state, lactate is removed via the oxidation of pyruvate to acetyl coenzyme A; the rates of these reactions are governed by the activity of the mitochondrial pyruvate dehydrogenase enzyme complex [Stacpoole 1993].

Under physiological conditions with a normal oxygen supply, cells metabolize glucose to pyruvate through glycolysis in the cytoplasm. Pyruvate may either be converted to lactate (pyruvate + NADH + H⁺ ⇌ lactate + NAD⁺) or oxidized into carbon dioxide and water through the Krebs cycle and oxidative phosphorylation in mitochondria.

In oxidative phosphorylation, electron transfer in the respiratory chain coincides with proton extrusion and generation of a mitochondrial membrane potential. Protons subsequently re-enter the mitochondria through the pore of ATP synthase and ATP is produced by the enzyme's catalytic subunit. Through energy consumption during cell work, ATP hydrolysis produces proton ions, which are used again in mitochondrial ATP production; this results in the prevention of proton buildup and maintains an internal acid–base balance.

Lactate is not toxic *per se*. It can even substitute for glucose as a metabolic substrate through entry into the tricarboxylic acid cycle via pyruvate. It can then be used as a gluconeogenic substrate, oxidized or transaminated into alanine. Intravenous lactate infusion prevents brain dysfunction during hypoglycemia in healthy volunteers [King 1997] and in diabetic subjects [King 1998]. Lactate is therefore no longer considered to be as a waste product; on the contrary, its production could be considered as an adaptive response to a lack of energy. When mitochondrial function is impaired for some reason, cells can gain energy and survive for a short period of time by producing lactate [Brooks 2009]. Moreover, acidosis itself should be considered as an adaptive response to insufficient energy provision and might prolong cell viability [Gores 1989].

Lactate during anaerobiosis/aerobiosis

In hypoxic situations (in which mitochondria are unable to produce ATP), the rate of glycolysis increases to provide additional ATP. This results in excess pyruvate production and in a decrease in the lactate-to-pyruvate ratio. Next, lactate is released from the cell into the bloodstream by lactate-proton co-transporters. Nevertheless (and in contrast to the action of mitochondria), glycolysis cannot bind the protons released by ATP hydrolysis. As a consequence, the increase in the proton concentration causes intracellular acidosis [Vernon 2010].

Hyperlactatemia can also result from increased aerobic glycolysis and may thus depend on factors other than tissue hypoxia. In fact, any condition interrupting normal pyruvate metabolism and increasing pyruvate availability (e.g. pyruvate dehydrogenase inhibition by toxin or any pathologic deficit or toxin affecting normal mitochondrial functions) could lead to hyperlactatemia through conversion of pyruvate to lactate.

Lactic acidosis may also occur in the absence of cellular hypoxia. For example, liver failure leads to an increase in blood lactate levels mainly because of impaired elimination [Kreisberg 1980]. Acidosis also aggravates hyperlactatemia because it hampers the hepatic clearance of lactate. In severe

acidosis, the liver becomes a lactate-producing organ [Cohen 1976]. Acute kidney failure may reduce the metabolism of lactate by the kidney, which metabolizes up to 30% of the lactate load when hepatic gluconeogenesis and lactate uptake by the liver are impaired [Yudkin 1975, Cohen 1975].

From hyperlactatemia to acidosis

The correlation between hyperlactatemia and metabolic acidosis has also been described according to Stewart's acid-base classification, in which three independent variables control the pH: a strong ion difference (SID), pCO₂, and the total amounts of weak acids and proteins in plasma. An increase in the lactate level leads to a reduction in SID, which has an acidifying effect. Nonetheless, in Stewart's model, an increase in lactate levels does not necessarily result in acidosis, since concurrent modifications in SID, changes in the quantity of weak acids and proteins lead to fluctuations in pCO₂, which in turn can impact the pH [Jansen 2009].

Although the term "lactic acidosis" is used very commonly, a clinically relevant relationship between lactate and pH or excess base has never been observed. Furthermore, a cause-effect relationship between production of lactate and acidotic status has never been demonstrated in an animal model, (at least in muscle tissue) [Kravitz 2013].

Although hyperlactatemia has often been linked to the occurrence of metabolic acidosis, there is no straightforward relationship between lactate production and acidosis. In contrast, lactate production can slow down the process of acidosis by consuming the proton ions in the cytosol [Lalau 2015]. In fact, a significant relationship between lactate and pH might only exist at high lactate levels.

As noted above, no protons are produced during the metabolism of glucose or pyruvate to lactate. In fact, the protons originate from the hydrolysis of ATP to adenosine diphosphate (ADP) and inorganic phosphate (Pi). If these protons cannot be recycled within the mitochondria (i.e. under anaerobic conditions), acidosis may coincide with hyperlactatemia [Lalau 2015].

Outcomes

In the context of lactic acidosis, mortality rates of up to 80% have been reported [Smith 2011, Stacpoole 1994]. The prognostic value of the lactate level is however subject to debate. In some large, retrospective studies, lactate levels failed to accurately predict hospital deaths [Pal 2006]. In contrast, a prospective cohort study of 877 patients found a mortality rate of around 5% in patients with a lactate level between 0 and 2.5 mmol/L, 9.0% for 2.5 to 4.0 mmol/L, and 38 % for ≥ 4.0 mmol/L [Shapiro 2005]. With a view to reconciling these contradictory results, it should firstly be noted that the prognosis of lactic acidosis is not related to the severity of hyperlactatemia *per se* but depends on the underlying mechanism. In fact, the prognosis is poor when lactic acidosis is due to prolonged hypoxia [Vecchio 2014] or sepsis [Kraut 2014]. Secondly, the change over time in the

lactate level has greater prognostic value than the lactate level *per se* [Nguyen 2004]. Indeed, as mentioned above, lactate should be viewed as energy substrate rather than a toxic substance [Leverve 1999]. Lactate substitutes directly for glucose as a metabolic substrate through entry via pyruvate into the tricarboxylic acid cycle and can be either used as a gluconeogenic substrate, oxidized or transaminated into alanine. Intravenous lactate can prevent brain dysfunction during hypoglycemia in insulin-dependent diabetes [King 1998].

Chronic kidney disease

CKD is characterized by a progressive, irreversible loss of renal function [Jha 2013]. The kidney has a pivotal role in homeostasis of the body via the urinary excretion of endogenous substances and toxins and maintenance of the balance between water and solute inputs and outputs [Eckardt 2013]. Thus, the coordinated procedure of glomerular filtration, tubular secretion and tubular reabsorption in each nephron (the kidney's functional unit) maintains homeostasis.

In developed countries, over 10% of the adult populations present some degree of CKD. Healthcare systems are being affected by the rising incidence of CKD (8% annually), which consumes up to 2% of overall healthcare expenditure [López-Novoa 2010].

Definition and criteria

All disorders affecting the kidney's structure and functions are referred to as kidney diseases, and are categorized as either acute (time since onset ≤ 3 months) or chronic (time since onset > 3 months). Acute kidney injury is a type of acute kidney disease in which alterations in kidney function appear within 1 week [Kidney Int 2012].

Formerly, it was assumed that a drop in the GFR of less than 15% was not clinically important [Eckardt 2013]. This viewpoint led to the inappropriate classification of patients into two groups: those with so-called "end-stage renal disease" and those with "pre-end-stage renal disease" (also referred to as "pre-dialysis patients"), with a slighter degree of renal impairment. This categorization was further complicated by the use of various ambiguous terms (e.g. "renal insufficiency" or "pre-uremia") or terms describing the etiology of the kidney disease (e.g. diabetic nephropathy, glomerulonephritis etc.) [Eckardt 2013].

In 2002, a consistent, clear definition of CKD (based on kidney function measures and disregarding the cause of impaired kidney function) and a staging system were at last agreed by the medical community [Eckardt 2013]. The corresponding guidelines focused on estimation of the GFR from serum creatinine and the quantification of markers of kidney damage (primarily albuminuria).

The relevance of this staging system was emphasized by the fact that several large studies demonstrated that even early-stage disease is associated with more frequent and more severe

adverse events and poor outcomes. These globally accepted glossary and definitions prompted better investigation of the degree of CKD affecting patient care and public health policies.

In 2013, the worldwide “Kidney Disease: Improving Global Outcomes” (KDIGO) initiative updated the definition of CKD as follows: “CKD is defined as abnormalities of kidney structure or function, present for more than 3 months, with implications for health”.

Table 1.1. Definition of CKD.

Criteria for CKD (either of the following present for > 3 months)	
Markers of kidney damage (one or more)	Albuminuria (AER \geq 30 mg/24 hours; ACR \geq 30 mg/g [\geq 3 mg/mmol]) Urine sediment abnormalities Electrolyte and other abnormalities due to tubular disorders Abnormalities detected by histology Structural abnormalities detected by imaging History of kidney transplantation
Decreased GFR	GFR $<$ 60 ml/min/1.73 m ² (GFR categories G3a-G5)
Abbreviations: CKD, chronic kidney disease; GFR, glomerular filtration rate.	

CKD is classified on the basis of cause, GFR category and albuminuria category, as follows:

- Assign the cause of CKD based on the presence or absence of systemic disease and the location within the kidney of observed or presumed pathologic-anatomic findings;
- Assign GFR and albuminuria categories as follows:

Table 1.2. Staging of CKD.

GFR categories in CKD		
GFR category	GFR (ml/min/1.73 m ²)	Terms
G1	\geq 90	Normal or high
G2	60–89	Mildly decreased*
G3a	45–59	Mildly to moderately decreased
G3b	30–44	Moderately to severely decreased
G4	15–29	Severely decreased
G5	$<$ 15	Kidney failure
Abbreviations: CKD, chronic kidney disease; GFR, glomerular filtration rate.		
*Relative to young adult level		
In the absence of evidence of kidney damage, neither GFR category G1 nor G2 fulfill the criteria for CKD.		

Table 1.3. Staging of albuminuria in CKD.

Albuminuria categories in CKD				
Category	AER (mg/24 hours)	ACR (approximate equivalent)		Terms
		(mg/mmol)	(mg/g)	
A1	$<$ 30	$<$ 3	$<$ 30	Normal to mildly increased
A2	30–300	3–30	30–300	Moderately increased*
A3	$>$ 300	$>$ 30	$>$ 300	Severely increased**
Abbreviations: AER, albumin excretion rate; ACR, albumin-to-creatinine ratio; CKD, chronic kidney disease.				
*Relative to young adult level.				
**Including nephrotic syndrome (albumin excretion usually $>$ 2200 mg/24 hours [ACR $>$ 2220 mg/g; $>$ 220 mg/mmol]).				

[Kidney Int 2013].

Glucose metabolism in CKD

The kidneys participate in glucose homeostasis by producing glucose through gluconeogenesis, promoting glucose uptake from the circulation, and by reabsorbing glucose from the glomerular filtrate [Mitrakou 2013]. Accordingly, glucose metabolism is often impaired in CKD patients – mainly as a result of altered renal gluconeogenesis [Mak 2000].

End-stage renal disease (ESRD) is typically characterized by an insulin-resistant state [DeFronzo 1987]. Defective insulin secretion is also frequent in ESRD [Allegra 1990]. In contrast, it is less well known that insulin resistance may also be observed in less severe grades of CKD [Chen 2003, Menon 2005].

Nevertheless, insulin resistance should be taken into consideration because of its well-known association with endothelial dysfunction, renin-angiotensin-aldosterone system activation, increased formation of reactive oxygen species, dyslipidemia, and systemic inflammation - leading ultimately to an increase in cardiovascular risk [de Boer 2008].

Diabetic nephropathy

Diabetic nephropathy is the most common cause of glomerulopathy and ESRD in the USA and Europe. This condition can occur in insulin- and non-insulin-dependent diabetes, and is characterized by the occurrence of persistent microalbuminuria. A high proportion of diabetic patients suffer from CKD of varying severity [Tuttle 2014].

Although kidney disease occurring in diabetic patients is referred to as “diabetic kidney disease”, CKD may have an origin other than diabetes. Indeed, the majority of diabetic patients have hypertension, which is a well-known triggering factor for diabetic nephropathy. Genetic status is an important determinant for the development of nephropathy in a context of diabetes. More than one-third of patients with T2DM and only around one-third of those with T1DM develop diabetic nephropathy, regardless of their degree of glycemic control [Kidney Int 1995, López-Novoa 2010].

Diabetic nephropathy develops over a long period of time. During the initial stages of the disease, the patient displays hyperfiltration (characterized by high GFR values and the occasional occurrence of microalbuminuria). These abnormalities can be observed for around 5 years. For the following 20 years or so, GFR may decline progressively as microalbuminuria progresses to moderate proteinuria. In the final stages, severe proteinuria appears (in the presence or absence of nephrotic syndrome) and chronic renal insufficiency gradually turns into ESRD [Schena 2005].

During the course of the disease, any acute hypovolemic event (such as dehydration, sepsis, hemorrhage, etc.) may trigger a decline in renal function [López-Novoa 2010].

Pharmacotherapy in CKD

Glomerular blood flow/filtration, tubular secretion/reabsorption, renal bio-activation and metabolism are altered in CKD patients. Given the presence of proteinuria and the other homeostatic impairments that characterize CKD, drug-protein binding, volume distribution, and even non-renal metabolism may be altered. The common, cautious approach logically involves the withdrawal of any drug eliminated by the kidney when the GFR falls below 60 mg/l (i.e. patients with stage ≥ 3 CKD). Depending on the nature of the drug, withdrawal will prevent either a further deterioration in renal function or toxic effects due to drug accumulation [Hartmann 2010, Scheen 2014].

However, another strategy involves adjusting the dose to match the renal function; this allows one to maintain the therapeutic effect while avoiding the risk of drug accumulation and, ultimately, toxic effects [Hartmann 2010].

In the late 1950s, the first systematic analysis of the prolongation of drug half-lives in severe CKD and was carried out by Kunin *et al*, who suggested adjusting the drug dose according to the level of renal function [Kunin 1959]. More recently, it has been shown that anticancer drugs eliminated by the kidney may be administered to dialysis patients if the dose is lowered by 20-60% [Boesler 2005].

When renal function declines and elimination half-lives increase, doses of drugs eliminated by the kidneys can be adjusted either by reducing the dosage size (D), and/or increasing the time interval between the doses, as set out in Dettli's rule [Dettli 1976]:

Figure 1.3. Dettli's rule for dose adjustment in CKD.

$$\frac{D}{\tau} = \frac{D_{norm}}{\tau_{norm}} \cdot \frac{T_{1/2norm}}{T_{1/2}}$$

This, however, is a general approach. In practice, dose reduction and augmentation of the dose interval should be applied differently depending on the exact nature of the kidney failure. During acute events (e.g. administration of antibiotics to treat a septic episode in a patient with severe CKD), a high loading dose should be administered with an extended time interval between doses. For chronic drug treatment (as discussed below for metformin in CKD) in non-terminal renal disease, only the daily dose should be reduced for maintenance of therapeutic levels.

Lactic acidosis in metformin therapy

Epidemiology

Initial reports

Severe metabolic acidosis in the absence of ketosis in biguanide-treated diabetic patients was first described in 1959 [Walker 1959]. In 1969, a case of lactic acidosis was linked to metformin, although the dose was only 150 mg/day (indicating that phenformin was actually the problematic drug) [Assan 1969]. Between 1969 and 1972, no other cases of lactic acidosis have been reported in relation to metformin therapy. In 1972, a fatal case of lactic acidosis during metformin therapy was reported for the first time. Again, a causative role of metformin can be questioned because it was administered for a very short period (only 3 days) [Lebacqz 1972]. In 1974, several cases of lactic acidosis were reported as being linked to metformin treatment [Harding 1974].

Incidence

The following Table summarizes literature reports of lactic acidosis in metformin-treated patients. Most of the reports were case reports from pharmacovigilance series.

Table 1.4. Case reports of lactic acidosis in metformin-treated patients.

Years (Period)	Country	No. of cases	Incidence (patient-years)	Reference	Type of study
1963-1997 (34 years)	UK	31	–	[Chan 1999]	Case reports to UK Committee on Safety of Medicines and UK Medicines Control Agency
1976-1986 (10 years)	UK	–	0.027/1000	[Bailey 1988]	Survey data reported
1972-1977 (5 years)	Switzerland	2	0.067/1000	[Bailey 1988]	Survey data reported
1972-1981 (9 years)	Sweden	–	0.084/1000	[Bailey 1988]	Survey data reported
1977-1991 (14 years)	Sweden	16 cases	0.15/1000 (1977-1981) 0.024/1000 (1987-1991)	[Wiholm 1993]	Voluntary reporting of suspected to Swedish Adverse Drug Reactions Advisory Committee
1972-1983 (11 years)	Canada	No case in 56,000 patients-year	-	[Lucis 1983]	Report of a monitoring study of LA case in metformin therapy to health protection branch of Health and Welfare Canada
1980-1995 (15 years)	Canada	-	9/100,000	[Stang 1999]	Historical cohort from the Saskatchewan Health administrative databases.
1984-1992 (8 years)	France	73 cases in 2,476,061 patients-years	-	[Chan 1999]	Not précised
1995-1996 (1 year)	USA	47 cases	3/100,000	[Misbin 1998, DeFronzo 1995]	Cases reported to Food and Drug Administration (FDA)
2005	USA	No case in 7,227 patients		[Cryer 2005]	1-year, randomized, open label, parallel-group, multicenter, clinical trial
1994-2005 (11 years)	UK	-	3.3/100,000	[Bodmer 2008]	nested case-control analysis using the U.K. based General Practice Research Database

1995-2003 (8 years)	Germany	14 cases		[von Mach 2004]	inquiries concerning metformin in a poison center
2001-2005 (4 years)	Spain	13 cases	2004: 1.38/1000 2005: 0.76/1000	[Almirall 2008]	Retrospective analysis of all cases defined by hospital discharge with a diagnosis of metabolic acidosis
1999-2006 (7 years)	USA	14 cases		[Wills 2010]	Retrospective chart review of metformin overdoses cases of the two Poison Centers
2000- 2008 (8 years)	Netherlands	16 cases	47/100,000	[van Berlo-van de Laar 2011]	Review of the medical records of patients with metformin serum concentrations in a referral hospital
2010	Global study		4.3/100,000	[Salpeter 2010]	Pooled data from 347 comparative trials and cohort studies comprising 70,490 patient-years
2007-2012 (5 years)	UK	35 cases	10.37/100,000*	[Richy 2014]	retrospective analysis of U.K. patient records from the Clinical Practice Research Datalink database comprising 77601 patients
2004-2012 (8 years)	UK		7.4/100,000** (Incidence of LA or lactate concentrations >5mmol/L)	[Eppenga 2014]	A cohort of 223,968 metformin users identified from the Clinical Practice Research Datalink (CPRD)
1993-2013 (20 years)	Australia	5 cases in metformin overdoses >3g		[McNamara 2015]	Retrospective case series of acute metformin overdoses (> 3 g) admitted to a toxicology unit
1971-2014 (43 years)	Australia	152 cases of suspected MALA	2.3/100,000	[Huang 2015]	Review of lactic acidosis cases associated with metformin and reported to Australian Therapeutic Goods Administration
<p>* By ignoring the cases with other potential proximal causes for lactic acidosis, the incidence rate reduced by 5.23 per 100,000 patient-years in moderately reduced kidney function and 2.90 per 100,000 person-years in patients who had mildly reduced renal function.</p> <p>** The authors mentioned that "inclusion of patients with a lactate concentration >5 mmol/L (26% of the cases) may cause an overestimate compared with lactic acidosis during metformin use alone, since elevated lactate concentrations do not necessarily signify a diagnosis of lactic acidosis.</p>					

As evidenced by the above table, the incidence of lactic acidosis in metformin-treated patients varies significantly (from 2.4 to 138 per 100,000 patient-years) but averages 3 to 10 per 100,000 patient-years. The variability is accounted for by the heterogeneity of the demographic characteristics (the duration of diabetes, the duration of metformin therapy, metformin intoxication, in- or outpatients, etc.). More importantly, information about the clinical setting was very scarce - in particular regarding associated pathologies such as sepsis, cardiovascular failure, etc. As a consequence (at least in these series), metformin's responsibility in the lactic acidotic event has certainly been overestimated [Salpeter 2006].

As mentioned above, diabetes itself (through its vascular complications) is a predisposing or even triggering factor for lactic acidosis. The following question then arises: does metformin therapy augment the incidence of lactic acidosis? An assessment of the incidence of lactic acidosis prior to the introduction of metformin in the US (9.7-16.9 events per 100 000 person-years) provides an answer [Brown 1998]. This value is similar to those mentioned in the above table, meaning that metformin therapy is not associated with an increased risk of lactic acidosis.

Studying the link between metformin and lactic acidosis

Premises in the literature

A review published in 1973 contained the following sentence: “*Metformin, commonly used biguanide, has been recorded as associated (we underlined) with lactic acidosis on one occasion only*” [LANCET 1973] referring to a case-report published a year earlier [Lebacqz 1972]. The first occurrence of the wording “Metformin-associated lactic acidosis” appeared in a short letter published in January 1977 (entitled “Biguanides and lactic acidosis”) in the following terms: “*In France there were 68 cases of phenformin-associated and 4 of metformin-associated lactic acidosis reported to mid-1975*” [Phillips Brit Med J 1977].

At the same time, Assan *et al.* published an article entitled “Metformin-Induced Lactic Acidosis in the Presence of Acute Renal Failure” [Assan 1977]. In 1978, Phillips then published a paper entitled “Metformin Associated lactic Acidosis” [Phillips 1978].

Coining the terms MULA, MILA, and MALA

At this stage, it possible (and indeed essential) to draw a distinction between three situations

1. *Metformin-unrelated lactic acidosis* (MULA). In this situation, there is no metformin accumulation. This is especially the case when blood metformin levels are low or even undetectable.
2. *Metformin-induced lactic acidosis* (MILA). This refers to lactic acidosis due solely to metformin, i.e. in the absence of any associated pathology [Lalau 2001]. The typical case is that of isolated metformin intoxication (which is rare because intoxication usually concerns several drugs). A somewhat similar case is that of acute metformin accumulation due to acute kidney failure; nevertheless kidney failure *per se* could be considered as a pathology, and may also result from systemic disease. In other words, the kidney can never be “healthy” in kidney failure.
3. *Metformin-associated lactic acidosis* (MALA). This is the most common and most complex case. The word “associated” means that both metformin accumulation and one or more associated pathologies are responsible for inducing lactic acidosis, to variable relative extents. The term “MALA” may, however, create confusion between lactic acidosis following metformin accumulation and lactic acidosis in a patient who happens to have been treated with metformin and in whom the drug has not accumulated.

In summary, metformin accumulation may be a precipitating factor for lactic acidosis (e.g. in metformin intoxication), an underlying factor (e.g. sepsis due to chronic metformin accumulation in CKD), or an associated factor (e.g. an acute pathology inducing acute kidney failure and therefore metformin accumulation if metformin is not withdrawn in time) [Lalau 2000, 2010,

2015]. Conversely, a pathological condition may be either an underlying factor (e.g. hepatic failure), an associated factor (as mentioned above), or a precipitating factor (e.g. sepsis or hemorrhage).

Pathophysiology

MULA

This first scenario is independent of metformin. We shall refer therefore to lactic acidosis in general.

MILA

As noted above (in the section entitled *Metabolic effects of metformin*), there is no significant increase in lactate levels during treatment with usual doses of metformin. However metformin overdose can trigger lactic acidosis even in the absence of associated intoxications or pathology [Chang 2009, Gjedde 2003, Wills 2010, Nisse 2003, Dell'Aglio 2009]. In animal studies, lactate concentrations started to increase when plasma metformin concentrations reached 20 mg/L during metformin infusion [Wang 2003]. Acute overdoses of metformin in pigs (6 ± 2 g, leading to a mean final serum concentration of 77 ± 45 mg/L) were able to trigger lactic acidosis, whereas a lower concentration (i.e. 3 mg/L) was not associated with lactic acidosis [Protti 2012].

In fact, once metformin accumulates (due to intoxication, acute kidney failure, and acidosis impairing uptake of organic cations [Urakami 1988]), MILA may result from the enhancement of metformin's various effects on lactate metabolism (i.e. anaerobic stimulation of lactate production in intestinal and liver cells, and defective lactate elimination by the liver and the kidney – both of which result from alteration of the mitochondrial respiratory chain complex I pathway).

Clinical data show that relative to safe levels of metformin, elevated levels of metformin accentuate the inhibition of hepatic gluconeogenesis and the increase in hepatocyte anaerobic metabolism, leading to impaired lactate elimination and a further decline in lactate uptake [von Mach 2004, Chu 2003]. Indeed, high metformin levels have a strong inhibitory action on gluconeogenesis - mainly due to a primary inhibition of hepatic lactate uptake [Radziuk 1997]. Metformin inhibits hepatic gluconeogenesis, possibly via a decrease in the cytosolic adenosine triphosphate/adenosine diphosphate (ATP/ADP) ratio, which results in a reduction in lactate use [Schäfer 1972].

Inhibition of mitochondrial respiration is ultimately considered to be the crucial factor in the pathogenesis of MILA, with its consequences on both cellular lactate overproduction and impaired lactate elimination [Vecchio 2014]. *In vitro* studies have showed that high concentrations of metformin (12.9-1290 mg/l) inhibit the mitochondrial respiratory chain complex I and mitochondrial electron transport, leading to an augmentation of anaerobic metabolism [El-Mir 2000, Owen 2000] and a reduction in gluconeogenesis.

In patients with metformin intoxication and severe lactic acidosis ($\text{pH}=6.93\pm0.20$ and $\text{lactate}=18\pm6$ mM on admission), oxygen consumption is very low. This decrease is probably due to a combination of hepatic and extra-hepatic inhibition of mitochondrial respiration [Protti 2010]. Indeed, dysfunction of kidney mitochondrial complex 1 has been also observed in animals and in patients with metformin intoxication [Guo 2006, Rifkin 2011].

MALA

The pathophysiology of MALA differs from that of MULA and MILA. Indeed, the generation of lactic acidosis (either independently of metformin or due to metformin) is a biological phenomenon, whereas MALA concerns the causal role of metformin (among other contributors) to lactic acidosis. In other terms, to what extent is metformin responsible?

One can consider the presence or absence of a correlation between blood metformin levels on one hand and pH and lactate levels on the other [Prikis 2007, Peters 2008]. This matter is subject to debate in the literature, although most of the series are rather small [Lalau 1995, Stades 2004, Lalau 1998A, Vecchio 2014]. We are currently studying a large series ($n=130$) in which metformin levels appear to be well correlated with both pH and lactate [Lalau, work in preparation]. One must however remain cautious in this respect because (i) a rather strong correlation does not rule out huge variations in pH and lactate for a given plasma metformin concentration and (ii) the strength of the correlation might be due to a few, very high plasma metformin concentrations). On this basis, it is at least possible to suggest that metformin has a role in the development of lactic acidosis.

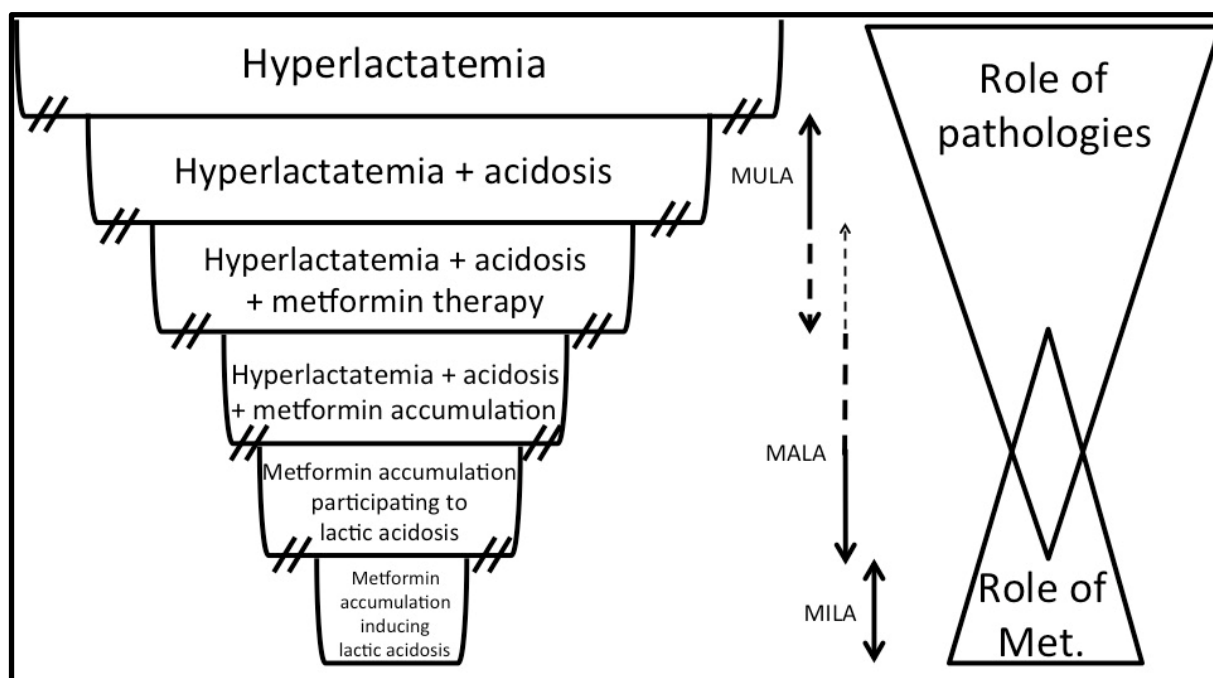
The problem is however much more complex: (i) even in well-tolerated metformin therapy, an enormous (80-fold) variation in trough steady-state plasma concentrations of metformin has been reported in a large cohort of type 2 diabetes patients taking 1 g of metformin twice daily over a 24-month period [Christensen 2011]; (ii) the temporal relationship between metformin therapy, metformin accumulation, and the metabolic consequences of metformin accumulation has not been taken into consideration in the literature. Let us illustrate this point by the following case: “a 62-year-old patient who developed anuria (serum creatinine level of 4 mg/dL [350 mmol/L]) and lactic acidosis (blood lactate 16.3 mmol/L, pH 7.09). As metformin therapy had not been withdrawn, a high plasma metformin concentration would have been expected and would have prompted the conclusion that lactic acidosis occurred as a result of drug accumulation. In fact, the plasma metformin concentration (0.4 mg/L) was well within the therapeutic range (0.6 – 0.5 mg/L) due to the recent occurrence of kidney failure following cardiogenic shock. [Lalau 2010]”. This means that metformin accumulation would have occurred in this case only if metformin had not been withdrawn - despite the fact that oligianuria is a formal contraindication to metformin therapy. However, once

metformin accumulates, the patient's lactic acidosis status will have changed by the time the metformin assay is performed [Lalau 2014A].

To further complicate the debate, it must be considered that metformin accumulation (even when major) is not necessarily accompanied by (simultaneous) hyperlactatemia [Lalau 2000]. Reciprocally, low levels of metformin may induce hyperlactatemia and metformin-induced hyperlactatemia does not necessarily lead to acidosis [Lalau 2014B].

An initial attempt to address this difficult issue is presented in Figure 1.4, which shows the respective role of metformin accumulation and associated pathologies in the generation of MALA.

Figure 1.4. Schematic differences of MULA, MILA and MALA and role of underlying pathologies and metformin in these issues.



Moreover, the term “mixed” (to emphasize the combination of metformin accumulation and pathologies) indicates the partial link between metformin and lactic acidosis more clearly than “associated” does. Until an exact causal role of metformin in lactic acidosis is proven, lactic acidosis should only be vaguely defined as “lactic acidosis occurring in metformin-treated patients”, i.e. without an allusion to a causal role of metformin.

However, if we further refine the wording and consider the fact that hyperlactatemia does not necessarily lead to acidosis, MALA should be renamed as “metformin-associated hyperlactatemia-associated acidosis” or even “hyperlactatemia-associated acidosis occurring in metformin-treated patients”.

Ultimately, this critical analysis weakens a quantitative approach to so-called MALA. This explains why the intraobserver agreement on the causal role of metformin in MALA is extremely low - even within an expert panel [Stades 2004].

At this stage, therefore, one thing is clear: MALA is not a single entity.

A debate concerning the therapeutic concentration of metformin

Metformin has been available since 1957. Over fifty years later, one can legitimately question whether a clear definition of the "therapeutic concentrations" is available. We therefore looked at whether or not there is a literature consensus on the "therapeutic concentrations" of metformin. We searched the scientific literature from January 1957 to November 2014 with the keywords "metformin", "therapeutic concentration", "therapeutic level" and "therapeutic range". When the suggested values were defined by citing a literature reference, the types of studies in cited references and the concordance of data between the citations and their sources were studied. One hundred and twenty documents were identified. These documents reported or cited 65 different "therapeutic" plasma metformin concentrations or ranges. The values ranged from 0.129 to 90 mg/l but most were between 0.1 and 4 mg/l. When considering concentration ranges, the lowest and highest boundaries were 0 and 1800 mg/l. The narrowest range was 0.000225-0.003 mg/l and the broadest was 150-1800 mg/l. Only four original research studies (3.3%) directly determined a "therapeutic concentration". Fifty-four publications (45%) cited previous studies as defining the therapeutic concentrations, whereas 62 publications (51.7%) mentioned "therapeutic concentrations" but did not even cite a supporting reference. The supporting references were mostly reviews, pharmacokinetic studies and *in vitro* studies (none of which was performed with the specific objective of defining the therapeutic concentrations for metformin). In the 54 publications that cited references, concordance between the wording of the citation and the true nature of the source data was observed in only 23 cases (42.6%).

Table 1.5. Therapeutic concentrations of metformin: summary of the available literature.

Number of publications	Number of original research studies	Number of publication citing a supporting reference	Number of publications not citing a supporting reference	For publications citing a supporting reference (n=54), concordance of data between the publication and the source	
				Yes	No
120 (100%)	4 (3.3%)	54 (45%)	62 (51.7%)	23 (42.6%)	31 (57.4%)

In summary: (i) 120 documents reported or cited 65 different "therapeutic plasma metformin concentrations" or ranges; (ii) although metformin has been available for over fifty years, major methodological and/or conceptual errors have confounded the literature concerning its therapeutic concentrations. *(full data presented in Appendix I)*

A dose-efficacy study with measurement of the corresponding plasma metformin concentrations is therefore needed to define the therapeutic concentration window for metformin.

A debate concerning guidelines and proposals from around the world

Metformin is recognized around the world as having a pivotal role in the primary treatment of T2DM. However, there is still debate as to the drug's beneficial and adverse effects – particularly in terms of the high proportion of patients presenting with established or suspected kidney disease. Even though a critical review of the literature in this field has questioned the claimed danger of lactic acidosis in metformin-treated patients, the latter context still influences treatment strategies.

Official and literature-based guidelines on the metformin treatment of type II diabetes mellitus patients with CKD were therefore compared and contrasted, and the selected criteria for drug withdrawal were critically analyzed. The objective was to help physicians to refine their analysis of the available evidence before deciding whether to continue or withdraw metformin therapy in CKD patients (in compliance with their locally applicable regulatory and legislative framework).

The systematic search for metformin contraindications was performed from January 1990 and December 2014 in (i) official documents from the world's 20 most populated countries and the 20 most scientifically productive countries in the field of diabetology and (ii) publications referenced in electronic databases from 1990 onwards. We identified 3 international guidelines, 31 national guidelines, and 20 proposals in the scientific literature. The criteria for metformin withdrawal were (i) mainly qualitative in the most populated countries, (ii) mainly quantitative in the most scientifically productive countries (with, in all cases, a suggested threshold for withdrawing metformin) and (iii) quantitative in all but one of the literature proposals, with a threshold for withdrawal in most cases (n=17) and/or adjustment of the metformin dose as a function of renal status (n=8). There was a good degree of consensus on serum creatinine thresholds, whereas guidelines based on estimated GRF (eGFR) thresholds varied from 60 mL/min/1.73 m² up to stage 5 CKD. Only one of the proposals has been tested in a prospective study.

Table 1.6. Summary of the available data according to the nature of the source and the criteria for withdrawing metformin.

Criteria		Guidelines from 20 most populated countries	Guidelines from 20 most scientifically productive countries	Proposals from scientific literature (n=20)	
Qualitative		19	6	1*	
Quantitative	Threshold	Serum creatinine	14	16	1
		Creatinine clearance or eGFR	2	9	16**
	Adjustment	Serum creatinine	—	—	—
		Creatinine clearance or eGFR	—	—	7**

* Plus a proposal for adjustment of the metformin dose according to the CKD stage

** A single proposal may include a threshold and/or a dose adjustment.

In summary, (i) the criteria for metformin withdrawal are mainly qualitative in the most populated countries; (ii) quantitative criteria for metformin withdrawal are mostly based on serum creatinine values; (iii) the current contraindications for metformin exclude its use in a large proportion of patients with CKD; and (iv) in contrast to recommendations for other drugs excreted by the kidney, guidelines do not involve adjustment of the metformin dose as a function of renal status (in stable patients) (full data presented in Appendices II-IV).

After our analysis, Inzzuchi *et al.* have proposed the following approach:

Table 1.7. Possible approach to metformin prescribing in the setting of CKD.

CKD stage	eGFR, mL/min per 1.73 m ²	Maximal total daily dose, mg	Other recommendations
1	≥90	2550	
2	60 -<90	2550	
3A	45 -<60	2550	Avoid if kidney function is or expected to become unstable Consider more cautious follow-up of kidney function
3B	30 -<45	2550	Do not initiate therapy at this stage but drug may be continued Avoid if kidney function is or expected to become unstable Consider more cautious follow-up of kidney function
4	15 -<30	Do not use	
5	<15	Do not use	

The authors added however that such a “strategy has not been evaluated or validated in a clinical trial; there are no data to support its efficacy, safety, or potential to improve clinical outcomes” [Inzzuchi 2014].

AIMS

1. To perform a critical, qualitative examination of reports of so-called MALA, with a focus on (i) the criteria for the definition of MALA (i.e. pH, lactate, and metformin levels), (ii) the prognostic value of the latter criteria in severe MALA, and (iii) the mortality rate. This approach is justified by the fact that MALA is generally considered to be a single entity in the literature and has therefore only been addressed quantitatively in the past.
2. To examine the advantages and limitations of measuring blood metformin concentrations in blood when screening for metformin accumulation and assessing its extent and consequences. This objective is justified by the insufficient consideration given to the measurement of metformin concentrations in MALA.
3. To conduct a prospective study with metformin therapy in patients at all CKD stages, given the absence of this type of study in the literature.

In view of these aims, the work is structured into three corresponding parts.

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***PART II. A critical analysis of the reporting of the
so-called ‘metformin-associated lactic acidosis’ (MALA)***

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INTRODUCTION

Background

Any debate on MALA must include a critical re-examination of diagnosis and prognosis. 1. When considering the criteria for MALA, it has previously shown that the criteria for establishing a causal link between metformin and lactic acidosis in literature reports of MALA were met in only a few patients (15.3%) [Lalau 2001]. However, this study concerned a total of 26 patients documented in 21 published reports. It may be therefore suspected that reports in pharmacovigilance databases would concern a much larger population. 2. Any debate on the beneficial and harmful effects of metformin must include a critical re-examination of mortality data. At present, the conventional view is still that "metformin-associated lactic acidosis is rare but is still associated with a high mortality rate" [Hermann 1979, Schwarzbeck 1995, Wilhom 1993]. 3. It has have previously shown that neither blood lactate concentration nor plasma metformin concentration was of prognostic value with respect to mortality in MALA [Lalau 1995, Lalau 1999]. However, given that arterial pH and blood lactate concentration may vary (from ≤ 7.34 to 6.4 and from > 5 mmol/L to 35.5 mmol/L, respectively, in the Lalau's report [Lalau 1999]), a focus on severe MALA is needed in order to try to better understand the paradox whereby metformin provides impressive, beneficial effects but is also associated with life-threatening adverse effects.

Aims

The aim of this chapter is threefold: (i) to review all reports stated as "metformin-associated lactic acidosis" in the largest available database in order to know whether or not the criteria are indeed met; (ii) given that metformin was first registered for diabetes treatment in the 1950s [Gottlieb 1962], to determine whether (and to what extent) the mortality rate for MALA has changed significantly since then; and (iii) to analyze the prognostic value of blood pH and lactate and plasma metformin concentration in severe.

Hypotheses

In view of the foregoing, it may be hypothesized as follows: (i) the quality of reporting is poor but nevertheless improves with time (i.e. as knowledge about metformin grows); (ii) the mortality rate should have diminished over time; and (iii) plasma metformin concentrations do not have prognostic value even in severe MALA, that would militate against the metformin's widely assumed, potentially lethal toxicity.

METHODS

Data sources

Merck Serono (the metformin's manufacturer) provided us with access to its pharmacovigilance database on metformin. We systematically searched for and studied cases described as "metformin-associated lactic acidosis" from January 1995 to August 2010. The database is a compilation of all the cases worldwide brought to Merck Serono's attention during the study period. Most entries are indirect, i.e. cases for which information is transmitted to Merck Serono by local or national health authorities. Other cases are documented through spontaneous declarations (from physicians, pharmacists, patients, etc.) or via the medical literature.

The mortality in MALA was also studied in this large database and also in case reports of MALA in the scientific and medical literature. The keywords "metformin", "lactic acidosis", "hyperlactatemia", "lactate", and "mortality" were used to search English-language documents indexed in the MEDLINE, SCOPUS and SCIENCE DIRECT electronic databases between January 1960 and January 2014.

Design

A start date of 1995 was chosen for the study of criteria of MALA for two reasons: (i) metformin was first authorized for sale in the USA in 1995; and (ii) the first report on a series of patients with MALA (n=14) with data on the pH and lactate and plasma metformin concentration was published in the same year [Lalau 1995]. Since then, the publication of several other papers concerning the association between metformin and lactic acidosis [Chan 1999, Lalau 1999, Lalau 2003, Luft 1983, Salpeter 2003, Misbin 2004, Stades 2004], including a larger series of MALA [Lalau 1999], may have influenced the extent to which the criteria for metformin-associated lactic acidosis were met in reports over the 15-year period from 1995 to 2010, i.e. a significant length of time.

The study of the mortality rate in MALA was started from the 1960s, i.e. virtually since the availability of metformin, to establish whether the rate has changed over time. By contrast, a less order date recent was also chosen for the prognostic assessment of severe MALA to circumvent the bias of a better outcome nowadays in severe ill patients than in the past.

Data extraction and selection

The following criteria for MALA were searched from the Merck Serono database: an arterial pH < 7.35, a blood lactate concentration > 5 mmol/l [Luft 1983], and a detectable plasma metformin concentration.

The study of mortality rate in MALA was done by extracting data from data sources in which an outcome was reported. In the scientific literature, rather large series, i.e. series comprising 20 case reports or more, were only considered.

The criteria for study selection in severe MALA were the presence of severe lactic acidosis at admission (defined as an arterial pH < 7.0 and a blood lactate concentration >10 mmol/L) and the availability of data on the plasma metformin concentration (regardless of the assay used) and survival (defined as discharge from the intensive care unit). Comparisons between survivors and non-survivors were made using Student's unpaired t-test for quantitative, demographic variables and a chi-2 test for qualitative variables. Test results with p-values ≤0.05 were considered to be statistically significant. Outcomes were compared with those in Friesecke et al.'s study [Friesecke 2010], in which cases of severe MALA were compared with cases of similarly severe metformin-independent lactic acidosis.

Data presentation

The criteria for MALA were recorded for the study period as a whole and by year. For the purposes of the Figure 1, the number of cases reported in 2010 (from January to August) was extrapolated to a 12-month period.

The literature series of mortality rate in MALA were analyzed by publication year and by study period. Data from the pharmacovigilance database were analyzed year-per-year. However, given the low number of cases recorded between 1995 and 2001, it has been decided to pool the annual data over this period in order to have at least 20 case reports (as for the literature series). From 2002 onwards, the data are presented year by year.

RESULTS

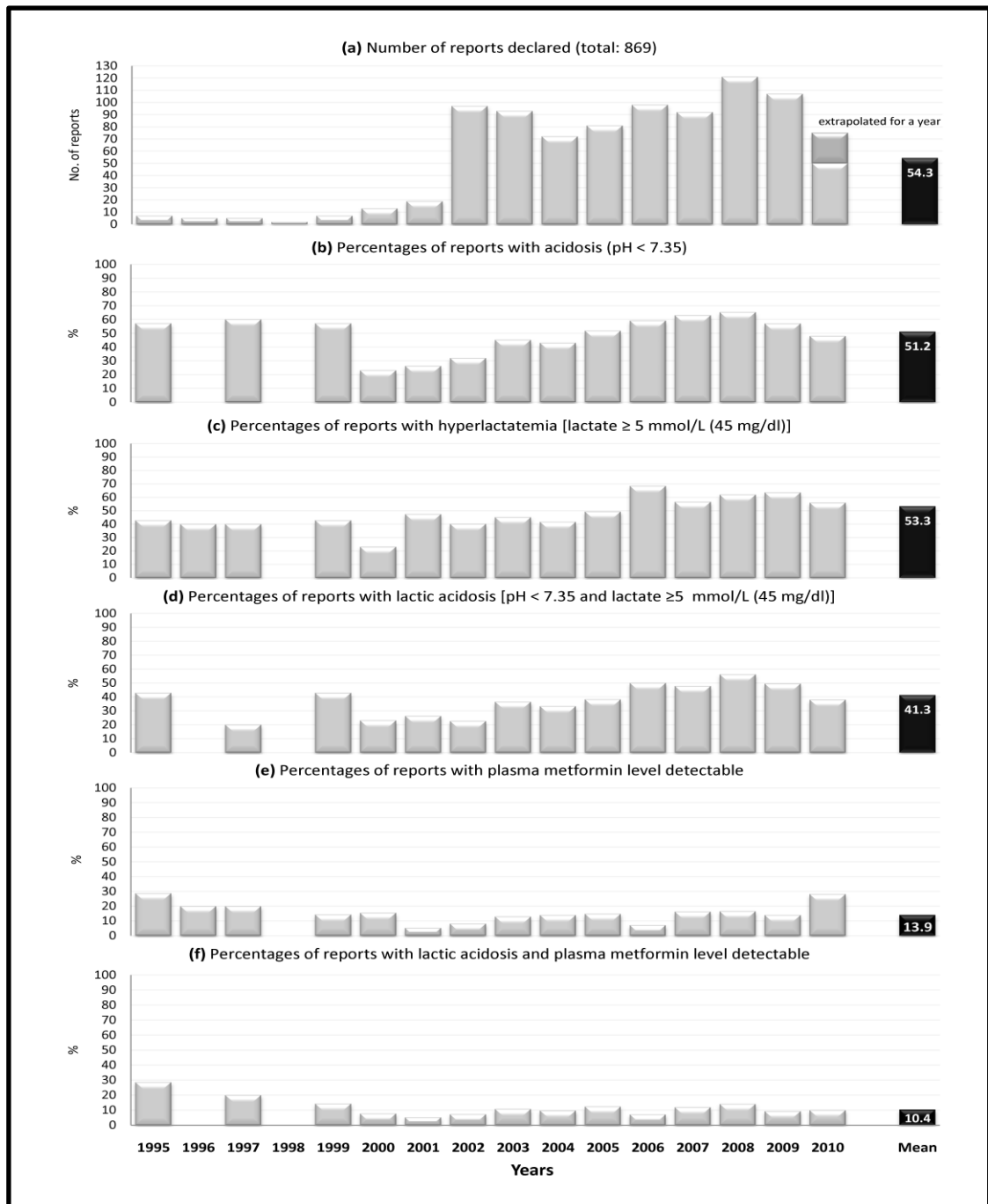
Criteria for MALA

For the period running from January 1995 to August 2010, we identified 869 reports of MALA in the Merck Serono pharmacovigilance database. These reports originated from 32 countries (Europe: 74.7%, North- and South-America 18.9 %, Asia: 3.1%, Oceania: 3.3%).

Figure 1 shows the number of reports per year and the proportion of cases meeting the criteria for MALA. Over the study period, the mean number of reports per year (54.3) masks a ten-fold difference between the first third of the study period and the remaining time. Fewer than 10 cases a year were generally reported from 1995 to 2001, whereas the annual average was around 100 for the subsequent period. As a consequence, the year-by-year analysis of fulfillment of the criteria for MALA has more statistical power in the second period (2002-2010).

The blood pH, lactate and metformin concentration values ranged from 6.05 to 7.9, 0.88 to 60.4 mmol/L and 0 to 188 mg/L, respectively.

Figure 2.1. The number of reports of MALA from 1995 to 2010 and the proportion of cases meeting the accepted criteria. Data are presented per year and as an average. The three criteria for MALA are presented separately and as a combination.



Overall, 41.3% of the patients met the criteria for lactic acidosis and a detectable metformin concentration was recorded in 13.9% of cases. Of the patients with an available metformin measurement, only one had an undetectable concentration. Relative to the proposed peak therapeutic concentration of 2.5 mg/L [Graham 2011], 8.3, 16.5, and 75.2% of the patients had normal concentrations, moderately augmented concentrations (> 2.5 mg/L but < 10 mg/L), and markedly augmented concentrations (> 10 mg/L), respectively.

True acidosis and hyperlactatemia were reported in just over half the reports (51.2% and 53.3%, respectively). A detectable plasma metformin concentration was noted in around 1 patient in 7 (13.9%). All three criteria (i.e. low pH value, high lactate concentration, and detectable plasma metformin concentration) were met in 10.4% of cases.

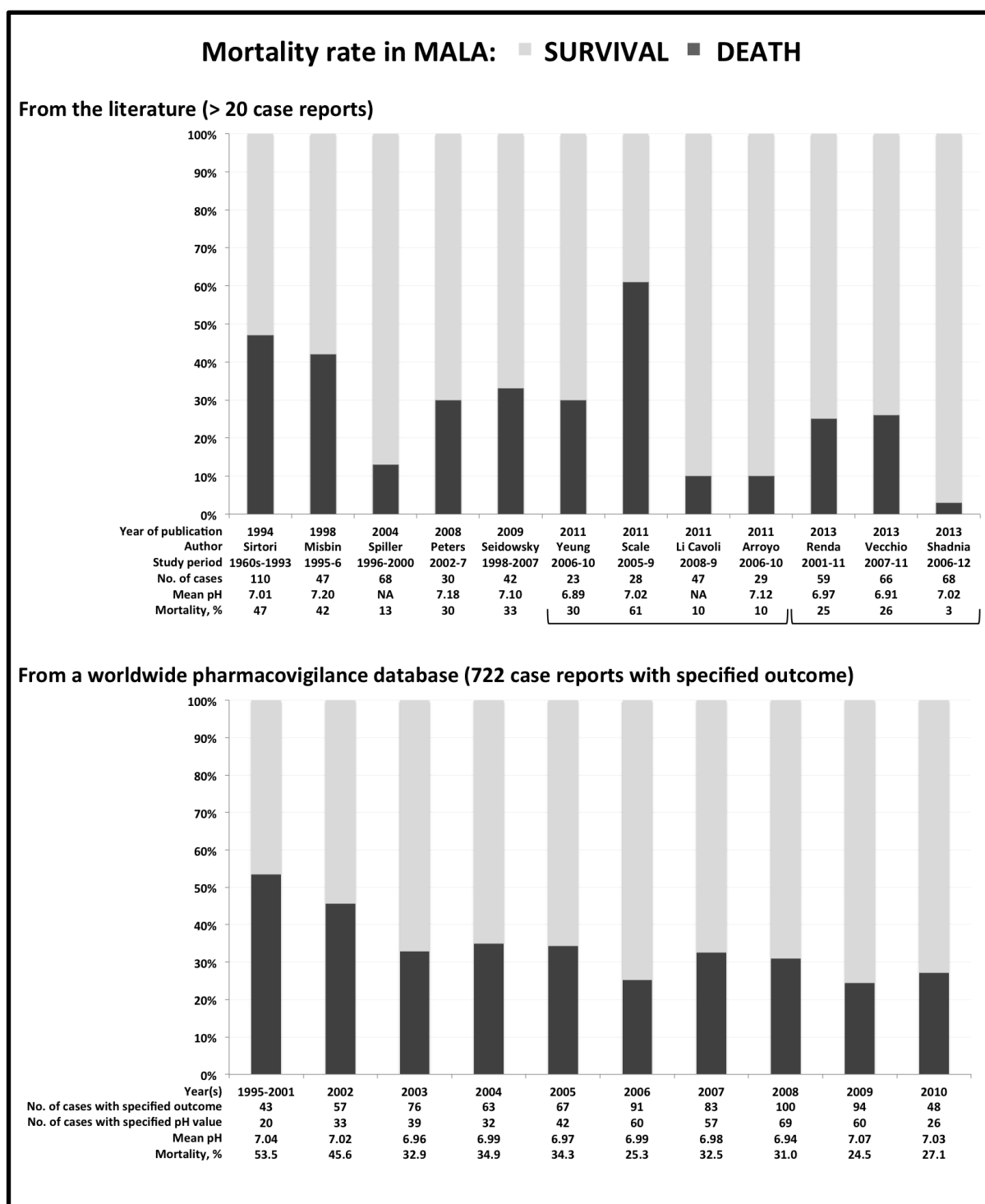
Overall, the year-by-year proportion of cases meeting the criteria for MALA did not change considerably over the study period.

Mortality rate in MALA

Twelve literature series, published between 1994 and 2013 [Sirtori 1994, Misbin 1998, Spiller 2004, Peters 2008, Seidowsky 2009, Yeung 2011, Scale 2011, Li Cavoli 2011, Arroyo 2011, Renda 2013, Vecchio 2014, Shadnia 2013], were identified. The earliest series covered a period running from the 1960s onwards [Sirtori 1994]. In the pharmacovigilance database, 722 case reports had information on the outcome available. None of the literature series or case reports gave data on all the three criteria needed to diagnose MALA (i.e. the blood pH, lactate concentration, and metformin concentration).

In the literature series, the number of cases reports varied from 23 to 110, with a total of 591 and a mean per series of 51.4 ± 24.7

Figure 2.2. Mortality (%) in MALA from two data sources: the scientific literature (top panel), and the worldwide pharmacovigilance database (bottom panel) (□: survivors; ■: non-survivors).



The mortality rate ranged from 3% to 61%, and the mean pH varied from 6.89 to 7.20. Although the highest mortality rate was twenty-fold greater than the lowest rate, the value was $\leq 33\%$ in 9 out of the 12 series and appeared to fall over time (from 47% in the first series (1960s-1993) to $\leq 25\%$ in the most recent series).

In the pharmacovigilance database, the mortality rate fell steadily throughout the study period (from around 50% to around 25%), whereas the mean pH barely varied around a value of 7.0 (range: 6.94-7.07).

Severe MALA

Fifty-six patients (17 males and 39 females) met the selection criteria (out of a series of 869 case reports of MALA from 32 countries). Most cases (74.7%) came from Europe.

Table 2.1. Population's main characteristics (mean \pm SD, [range]).

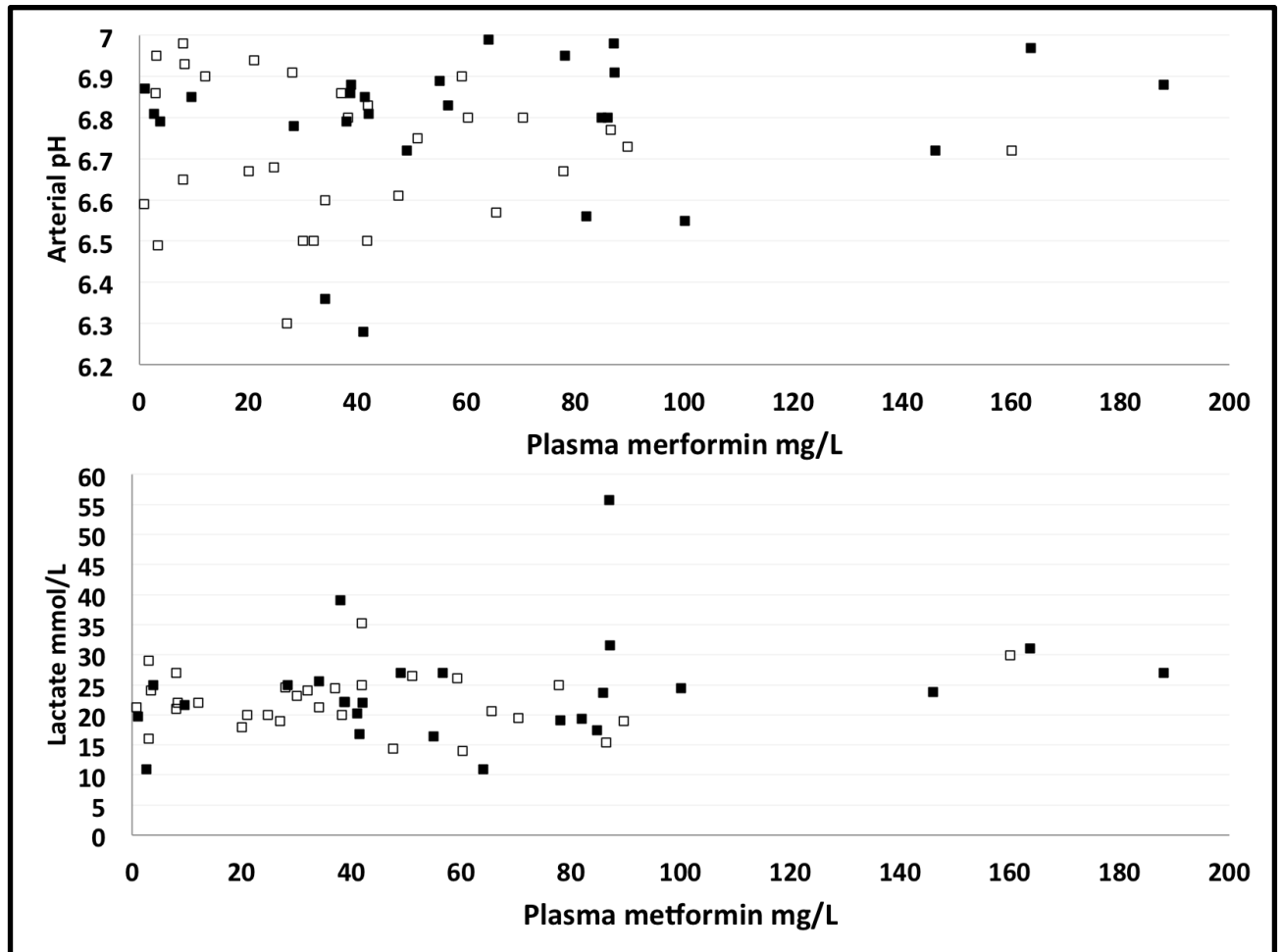
	All patients (n = 56)	Survivors (n = 30)	Non-survivors (n= 26)	p-value for survivors/ non-survivors comparison
Age	62.9 \pm 12.1 [39-83]	61.7 \pm 11.1 [40-81]	64.2 \pm 13.1 [39-83]	0.44
Creatinine, μ mol/L	587 \pm 270 [57-1000]	672 \pm 265 [133-1000]	508 \pm 310 [57-929]	0.09
pH	6.75 \pm 0.17 [6.28-6.99]	6.73 \pm 0.17 [6.30-6.98]	6.79 \pm 0.17 [6.28-6.99]	0.16
Lactate, mmol/L	23.07 \pm 6.94 [10.9-55.7]	22.24 \pm 4.72 [14-35.3]	24.0 \pm 8.9 [10.9-55.7]	0.26
Plasma metformin level, mg/L	48.3 \pm 39.6 [0.8-188]	39.66 \pm 34.22 [0.8-160]	63.3 \pm 47.4 [1.01-188]	0.04

Only 2 patients (3.5%) had a plasma metformin concentration within the therapeutic range (based on the upper limit of 2.5 mg/L recently proposed [Graham 2011]). The majority of patients (73%) displayed marked metformin accumulation, with a value over 25 mg/L.

The overall survival rate in these patients was 53.6% (30 out of 56). This rate did not depend on the date during the study period (data not shown). In other words, the outcome was not more favorable because of more recent observations in survivors. Some patients survived even with pH values as low as 6.5 and lactate and metformin concentrations as high as 35.3 mmol/L and 160 mg/L ($N < 1$ mg/L), respectively.

Survivors and non-survivors did not differ significantly in terms of the mean arterial pH and lactate concentration. Individual patient data for these two parameters are shown in **Figure 2.3**. The mean metformin concentration however was higher in non-survivors.

Figure 2.3. Arterial pH and lactate concentrations as a function of the plasma concentrations and outcome in 56 patients with severe MALA (□: survivors; ■: non-survivors).



The main presumed triggering factors for lactic acidosis are presented in Table 2 and are grouped into four categories: kidney failure, other organ failures, overdose and other conditions. Sepsis and multi-drug intoxication rates were significantly higher in non-survivors than in survivors ($p = 0.007$ and 0.04 , respectively).

Table 2.2. Prognosis according to the nature of the presumed main triggering medical conditions (one condition per patient or more).

Associated condition	Total no.	Survivors no. (%)	Patients who died, no. (%)	p-value for survivors/ non-survivors comparison
<i>Kidney failure</i>				
Total	34	19 (55.9)	15 (44.1)	0.87
Acute failure	21	11	10	0.89
Chronic failure	7	5	2	0.43
Duration unspecified	6	3	3	1.00
<i>Other organ failure</i>				
Total	10	6	4	0.74
Liver	4	2	2	1.00
Cardiovascular system	3	3	0	0.24
Lung	3	1	2	0.59
<i>Overdose</i>				
Total	8	3	5	0.45
Metformin (alone)	4	3	1	0.61
Multi drugs (including metformin)	4	0	4	0.04
<i>Other conditions</i>				
Total	13	3	10	0.02
Sepsis	6	0	6	0.007
Alcohol abuse	3	2	1	1.00
Cancer	1	0	1	0.46
Pancreatitis	1	1	0	1.00
Neurological disorder	1	0	1	0.46
Acute denutrition	1	0	1	0.46
Indetermined	3	2	1	1.00

We also analyzed the number of presumed triggering factors per patient as a function of the outcome.

Table 3.3. Prognosis according to the number of presumed triggering medical conditions.

No. of medical conditions	Total no.	Survivors, no. (%)	Patients who died, no. (%)	p-value for the survivor/ non-survivor comparison
No identified or unspecified	3	2	1	1.00
1	38	25	13	0.01
2 or more	15	3	12	0.005

The presence of only one factor was more frequent in survivors ($p = 0.01$) than in non-survivors; conversely, the presence of two or more combined factors appeared to be significantly more frequent in non-survivors than survivors ($p = 0.005$).

A comparison of our study results with those of Friesecke et al. [Friesecke 2010] shows the same proportion of MALA survivors (around 50%) and the same mean pH (pH 6.75 in both studies). In contrast, Friesecke et al. did not report on any survivors in metformin-independent lactic acidosis, despite the presence of less severe acidosis (mean pH: 6.86).

DISCUSSION

Criteria for MALA

The present study comprised all cases of so-called “metformin-associated lactic acidosis” recorded from January 1995 to August 2010 in a pharmacovigilance database. The present study considered more than ten times more cases (n=869) than previous series (the largest of which concerned 49 patients [Lalau 1999]). Strikingly, the criteria for lactic acidosis were met in 41.3% of the patients. Even though diagnosis of MALA requires the availability of a plasma metformin concentration (in order to study appropriately the link between metformin and lactic acidosis [Lalau 2010, Lalau 2015]), this variable was noted in only 14% of cases. Consequently, a full set of data for MALA (pH and lactate and metformin concentration) were present for only a minority of patients. The year-by-year proportion of cases meeting the criteria for MALA was relatively stable over the study period. Hence, the quality of report had not improved over time - despite greater experience of metformin pharmacovigilance since the drug's introduction in the USA and a growing body of knowledge on MALA in the scientific and medical literature. We suggest that the greater number of reports in the second period (2002-2010) was due to growing experience and thus an increase in the proportion of cases declared, rather than an increase in the absolute number of incidents per year.

However, MALA is a complex issue and cannot be adequately assessed by merely counting the number of cases. Quantitative reasoning (i.e. the proportion of cases of so-called MALA meeting the criteria) is only valid if “metformin-associated lactic acidosis” is a single clinical entity. In fact, MALA itself is an equivocal concept because metformin may either cause lactic acidosis or merely be a coincidental factor. In other terms, lactic acidosis in a patient taking metformin is not necessarily “metformin-associated lactic acidosis” [Lalau 2010]. Similarly, metformin should only be qualified as “associated” with lactic acidosis when its blood concentration has been merely measured and is elevated (rather than just being detectable). Even though determination of a low metformin blood concentration can rule out the involvement of metformin in lactic acidosis, the opposite is not necessarily true; high plasma metformin concentrations are not necessarily accompanied by hyperlactatemia [Lalau 2000].

In the present series, one patient did not even have a detectable plasma metformin concentration and thus was suffering from “metformin-unrelated lactic acidosis”. In contrast, cases of lactic acidosis

with very high plasma metformin concentrations and no other potentially causative etiologies should be referred to as "metformin-induced lactic acidosis". Hence "metformin-associated" should refer to all other situations, i.e. with both an elevated metformin concentration and an associated pathology. However, even in this latter situation, the strength of the link between metformin and lactic acidosis may vary, since the metformin concentration may vary considerably (from the limit of detection to 188 mg/L in this series). When considering not only the conventional criteria for MALA but also the strength of the link between metformin and lactic acidosis (on the basis of an elevated plasma metformin concentration), the drug's role in triggering so-called MALA was correctly assessed in only a very few reports in the Merck Serono database. It is indeed important to draw a distinction between (i) lactic acidosis in which elevated metformin has at least some degree of responsibility and (ii) severe pathology (e.g. sepsis, circulatory failure, etc.) occurring in a metformin-treated patient but in the absence of metformin accumulation.

This discussion is not speculative. The presence or absence or extent of metformin accumulation, conditions the patient's management and his/her vital prognosis [Lalau 2001, Lalau 2000, Prikis 2007].

Mortality rate

The present review is the first to have analyzed the mortality rate in MALA as a function of time. To this end, we searched for (i) all literature series with more than 20 case reports published since the 1960s (soon after metformin was first registered) and (ii) a large, worldwide pharmacovigilance database. We cannot rule out some degree of publication overlap between the scientific literature and the pharmacovigilance database. However, it is noteworthy that the mean pH varied widely in the literature series but only slightly in the pharmacovigilance database.

Our analysis of the mortality rates revealed two contrasting features. On one hand, the mortality rate varied markedly from one literature series to another (from 3% to 61%). On the other, the rate was frequently around 33% and showed a steady decrease over time (from around 50% until the 2000s to 25% by 2013).

There were also contrasting features with regard to pH. The literature series were again characterized by high variability (with means per series ranging from 7.20 to 6.89, i.e. from mild acidosis to severe acidosis), whereas the mean pH in the cases in the pharmacovigilance database was close to 7.0 throughout the study period.

However, the real challenge is to correctly determine the factors with which mortality is associated (rather than determining the mortality rate itself). With reference to the term "metformin-associated lactic acidosis", mortality may be "associated" with (i) metformin, (ii) systemic pathologies and/or (iii) the severity of lactic acidosis. Strikingly, none of the data sources gave information on all three

criteria for MALA (i.e. blood pH < 7.35, a blood lactate concentration >5 mmol/l, and at least a known blood metformin concentration [Kajbaf 2013A], (although the latter is a weaker criterion, since it does imply metformin accumulation).

Even when the above criteria are met (and given that blood pH, lactate and metformin values do not have prognostic value when considering in isolation [Seidowsky 2009, Kajbaf 2013B]), one can consider that the change over time in the mortality rate reflects the change over time in the outcome of systemic pathologies that happen to occur in metformin-treated patients (such as cardiac failure, sepsis, hemorrhage, etc.). Although the term "metformin-associated lactic acidosis" starts with the word "metformin", the drug is not particularly involved in these pathologies (especially when metformin accumulation is not present). Hence, strictly speaking, lactic acidosis is merely an instance of a systemic pathology "associated with metformin therapy".

If metformin nevertheless influences mortality in MALA, it would tend to improve the prognosis. Indeed, the mortality rate of 50% typically reported for MALA nowadays is only valid for severe MALA (i.e. pH < 7.0 and a blood lactate concentration >10 mmol/l) [Kajbaf 2013B]. The reduction over time in the mortality rate in MALA does not indicate that metformin is less dangerous than in the past. In contrast, this decrease may testify to a protective effect metformin, since the mortality rate for severe lactic acidosis in patients not treated with metformin is 100%. Indeed, metformin-treated patients are the only ones able to survive with a pH as low as 6.3 and a blood lactate concentration as high as 35.3 mmol/l [Kajbaf 2013B].

Our study had several limitations. Firstly, the literature series varied in size. Secondly (and more importantly), our data sources lacked information on the blood metformin concentrations. At first sight, it seems difficult to draw any firm conclusions about the "true" mortality rate in MALA. However, the present study presents the information communicated through the literature, i.e. the "real life" reporting of MALA. Moreover, the heterogeneity of the various series is not necessarily a bias because it reflects the respective responsibilities of metformin and systemic diseases in the triggering of lactic acidosis. Even prospective studies may not circumvent these difficulties, since (i) it would be difficult to simultaneously categorize patients in terms of the severity of systemic diseases, the severity of lactic acidosis, and the extent of metformin accumulation; (ii) the severity of hyperlactatemia should not be stratified because is not a critical prognostic factor per se (the change in hyperlactatemia over the first few days is more important [Lee 2008]); and (iii) massive metformin accumulation does not necessarily lead to lactic acidosis (because lactic acidosis can still occur during guideline-compliant metformin therapy) [Lalau 2014]. The only situation in which the impact of metformin on outcome could be judged would be "metformin-induced" (and not metformin-associated) lactic acidosis" (i.e. lactic acidosis unambiguously induced by metformin overdose) [Bonsignore 2013, Duong 2013, Timbrell 2012, Miller 2011].

Severe MALA

To the best of our knowledge, this is the largest series of metformin-treated patients with severe lactic acidosis yet reported. The first striking feature is that the majority of patients (53%) survived, despite a mean arterial pH of 6.73 and a mean lactate concentration of over 20 mmol/L (22.24 mmol/L). In comparison, the mean arterial pH in our previous series of unselected MALA patients was much higher, at 7.04 (arterial lactate concentration: 15.1 mmol/L) [Lalau 1999].

Even though most of the patients survived (independently of age), it is important to know whether survival was related to the severity of lactic acidosis, the extent of metformin accumulation and/or the nature and number of triggering factors. A link with lactic acidosis *per se* can be ruled out, since mean pH and lactate values were similar in survivors and non-survivors. Even though the mean metformin concentration was higher in non-survivors, metformin responsibility can also be ruled out with a good degree of confidence because this concentration difference was due to a very high value (188 mg/L, N < 1 mg/L) in only one non-survivor having combined triggering factors.

Indeed, outcomes appeared to be strongly correlated with (i) the nature of the triggering factors, since some conditions were significantly more frequent in non-survivors (i.e. sepsis and multidrug intoxication, with p values of 0.007 and 0.04, respectively) and (ii) the number of triggering factors, since the presence of just one factor was more frequent in survivors and two or more factors were more frequently observed in non-survivors.

The fact that metformin concentrations may widely vary in MALA complicates analysis of outcomes. Indeed, metformin-treated patients do not necessarily develop lactic acidosis - even in the presence of marked metformin accumulation [Lalau 2011]. It is nevertheless possible to distinguish patients according to their metformin level: (i) undetectable or low, (ii) normal, (iii) slight to moderate elevation or (iv) marked elevation [Lalau 2000]. In our previous report, we noted that 6 of the 49 patients (12.2%) had a plasma metformin concentration at or below the upper limit of the therapeutic range [Lalau 1999]. The homogeneous distribution of metformin concentrations in the present series enabled us to better test for an association between metformin accumulation and outcome. Indeed, only 3.5% of the patients had a low value, whereas the great majority (almost 80%) had marked accumulation, >10 mg/L.

Lastly, we are keen to reconcile the impressively beneficial metabolic and vascular effects of metformin on one hand with the drug's widely assumed, potentially lethal toxicity in MALA on the other – as summed up by a sentence like “metformin-associated lactic acidosis is rare but is still associated with a high mortality rate”.

Indeed, our report on a rather large series of patients with severe MALA and a death rate of almost 50% highlights how severe the complication really is. However, the problem is much more complex

than that, since MALA is not a clearly defined clinical entity [Lalau 2010]. One way to circumvent this difficulty is thus to compare the outcome in MALA with that in metformin-independent lactic acidosis. The data from Friesecke et al.'s [Friesecke 2010] recent study (in which MALA was compared with similarly severe lactic acidosis due to other causes) showed the same proportion of survivors (around 50%) and the same mean pH (6.75) for MALA as in our (much larger) series but, strikingly, did not feature any survivors in metformin-independent lactic acidosis (despite less severe acidosis (mean pH: 6.86)) [Graham 2011]. There are two possible explanations for this striking difference in survival when comparing MALA and metformin-independent lactic acidosis: (i) the presence of less severe, acidosis-triggering co-morbidities in MALA patients (because metformin accumulation is responsible for a proportion of cases of observed acidosis) and/or (ii) a protective effect of metformin (due to its vascular properties [Bouskela 1993] and its action on the respiratory-chain complex [Batandier 2006]).

Whatever the underlying reason, the present report's most remarkable finding is the unexpectedly high proportion of MALA survivors - despite an arterial pH as low as 6.5 (in 5 patients) and a lactate concentration as high as 35.3 mmol/L. However, this observation is coherent with the growing body of preclinical and clinical evidence demonstrating unexpectedly rapid recovery and survival in massive metformin accumulation and/or very severe MALA [Bouskela 1993, Batandier 2006, Gjedde 2003, Lalau 2005, Nyirenda 2006, Seidowsky 2009].

CONCLUSION

The present work leads to several practical and important conclusions. 1. The great majority of cases denoted as "metformin-associated lactic acidosis" in the largest pharmacovigilance database available had missing or incomplete data. Lactic acidosis (i.e. with both true acidosis and true hyperlactatemia) was absent in more than half of the patients. It was impossible to assess whether or not metformin had a putative link with lactic acidosis in 9 out of 10 cases. This type of incomplete reporting emphasizes the greater role that national pharmacovigilance networks should play in receiving, processing and validating spontaneous reports of adverse events and determining the presence of a causal link between events and medications. 2. Overall, the mortality rate in MALA fell from around 50% to around 25% over the study period (from the 1960s to date). This finding does not mean that metformin is less dangerous than before; it suggests (unsurprisingly, for such a long study period) only that patients hospitalized for severe diseases have a better outcome nowadays than in the past. 3. In comparison with common forms of lactic acidosis, severe MALA is particular in that the majority of patients survive – despite a mean pH that is usually thought to be fatal. For this

type of MALA patients, the outcome was related to the nature and number of triggering factors, rather than the severity of lactic acidosis or the extent of metformin accumulation.

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PART III. Blood metformin levels and metformin accumulation

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INTRODUCTION

Knowledge of blood metformin concentrations is essential in several respects: (i) establishing whether or not patients are treatment-compliant, (ii) adjusting the dose as a function of renal status, (iii) evaluating metformin accumulation in overdose situations, and (iv) investigating a putative link between metformin and lactic acidosis. The need for this information was highlighted by a case report featuring very severe lactic acidosis (pH 6.38, with recovery) (Ahmad 2002). The authors identified metformin as the cause of this acidosis, since “toxicology later confirmed the presence of almost twice the therapeutic plasma concentration of metformin”, i.e. 3.4 mg/L. However, it is difficult to draw firm conclusions about a putative causal link between metformin and severe lactic acidosis on the basis of this case report, since the observed metformin concentration was only slightly elevated. Indeed, 70-fold higher concentrations have been observed in our clinic. Hence, the analysis of the risk and extent of metformin accumulation requires further refinement.

Chapter 3.1. Screening for metformin accumulation in CKD

Introduction

Background

On the basis of a novel metformin assay, Frid et al. published clinical recommendations on use of the drug in patients with type 2 diabetes and varying severity of CKD. The researchers stated that a threshold of 20 $\mu\text{mol/l}$ (2.8 mg/l) was a “preliminary upper therapeutic limit” [Frid 2010]. However, the latter work can be questioned for several reasons: (i) the patients with an estimated glomerular filtration rate (eGFR) $>60 \text{ mL/min/1.73 m}^2$ were not given the full therapeutic dose (median: 1500 mg/d); (ii) 22% of the patient population had an eGFR $<60 \text{ mL/min/1.73 m}^2$; (iii) the study reported median metformin concentrations, rather than individual values; (iv) lastly, and most importantly, the metformin concentration was measured in plasma, although the erythrocyte level may provide a better estimation of the risk of accumulation [Lalau 2003]. Indeed, metformin has a longer half-life in erythrocytes than it does in plasma ($23.4 \pm 1.9 \text{ h}$ vs. $2.7 \pm 1.2 \text{ h}$ [Robert 2003]); this makes the interpretation of a single concentration time point less critical if the time of the last metformin intake is not known.

Here, we report on the distribution of erythrocyte metformin levels measured in a larger population with a wider range of eGFRs.

Hypotheses

Two hypotheses were tested: our previous work suggesting that metformin accumulation is not dangerous *per se* (since metformin-associated lactic acidosis is related to the severity of concomitant

pathologies [Lalau 2010]) may have prompted the physicians in our center to continue metformin administration beyond the limit stated in the current guidelines. Secondly, this particular situation offered an opportunity to study the extent to which CKD may lead to significant elevation of the metformin level. Given that metformin clears 4-5 times more quickly than creatinine [Scheen 1996], we did not expect this phenomenon to be significant, provided the metformin dose was reduced proportionally.

Methods

Data source

We systematically reviewed all erythrocyte metformin assay data recorded by our university medical center's pharmacokinetics laboratory in 2008 and 2009. In general, the metformin assays had been requested in order to adjust the dose to the patient's renal status or to screen for metformin accumulation.

Data selection

We then selected all such patients for whom data on renal function and the metformin dose were available. Only one value is shown per patient; if several values were present in our database, we used the earliest one only. We excluded patients with lactic acidosis (arterial lactate > 5 mmol/l and blood pH ≤ 7.35) in a context of acute renal injury or metformin overdose.

Estimation of the glomerular filtration rate

The GFR was estimated with the abbreviated Modification of the Diet in Renal Disease (MDRD) equation, including gender, race, age and serum creatinine parameters. The MDRD equation has 4 variables: $GFR (mL/min/1.73 m^2) = 175 \times (\text{serum creatinine})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African-American})$ (conventional units) [Levey 2006]. Patients were then divided into five CKD stages: >90 mL/min/1.73m² (stage 1), from 90 to 60 mL/min/1.73m² (stage 2), from 60 to 30 mL/min/1.73m² (stage 3), from 30 to 15 mL/min/1.73m² (stage 4) and <15 mL/min/1.73m² (stage 5).

Analytical methods

Creatinine levels were measured in a colorimetric assay. Metformin was isolated from plasma and erythrocytes according to a previously described method [Lacroix]. Solutes were separated on a 10 µm, 4.6 x 250 mm Spherisorb® SCX column (Waters, Saint-Quentin en Yvelines, France) using a mobile phase consisting of 50% acetonitrile and 50% 2 mM NaH₂PO₄ pH 3.8 buffer pumped at a flow rate of 1 mL/min. The eluate was monitored at 230 nm using a diode array detector (Waters). The

retention time for metformin was 8 min. The method showed good linearity ($r>0.997$) over the concentration range 0.10-15.0 mg/L. The lower limits of detection and quantification were 0.05 mg/L and 0.1 mg/L, respectively. The upper limit of quantification was confirmed as 100 mg/L. The precision and accuracy determination were carried out with three different concentrations (0.5, 2 and 8 mg/L) of quality control samples. The intra- and inter-assay precisions (measured as the percent coefficient of variation over the concentrations range of the quality controls) were all <15%. The intra- and inter-assay accuracy (defined as the absolute value of the ratio of the calculated mean values of quality controls to their respective nominal values, expressed as a percentage) ranged from 91% to 109.8%. This method is valuable for the invasive monitoring of diabetic patients treated with this hypoglycaemic agent. All concentration data are quoted with regard to basic metformin. Results are expressed as basic metformin. Detection limit was 0.03 mg/l for erythrocytes.

Statistical analysis

Individual patient metformin levels are presented for each CKD stage. These data were studied with respect to the 95th percentile of the metformin level range observed for the study population as a whole. All statistical analyses were performed with GraphPad Prism 5 software (GraphPad Software, La Jolla, CA).

Results

We identified erythrocyte metformin assay results for 260 patients. We excluded 20 patients because of a lack of information on renal function or the metformin dose. In the remaining 240 patients (males: 64%), the median (range) values for key parameters were as follows; age: 65 (38-88) years; BMI: 32 (20-77) kg/m²; glycated hemoglobin: 8.1 (5.4-15.9); eGFR: 47 (6.8-120) ml/min/1.73m²; metformin daily dose: 2000 (500-4000) mg; metformin level 1.06 mg/l (0.12-3.11).

Table 3.1.1. Characteristics of the study population (median values).

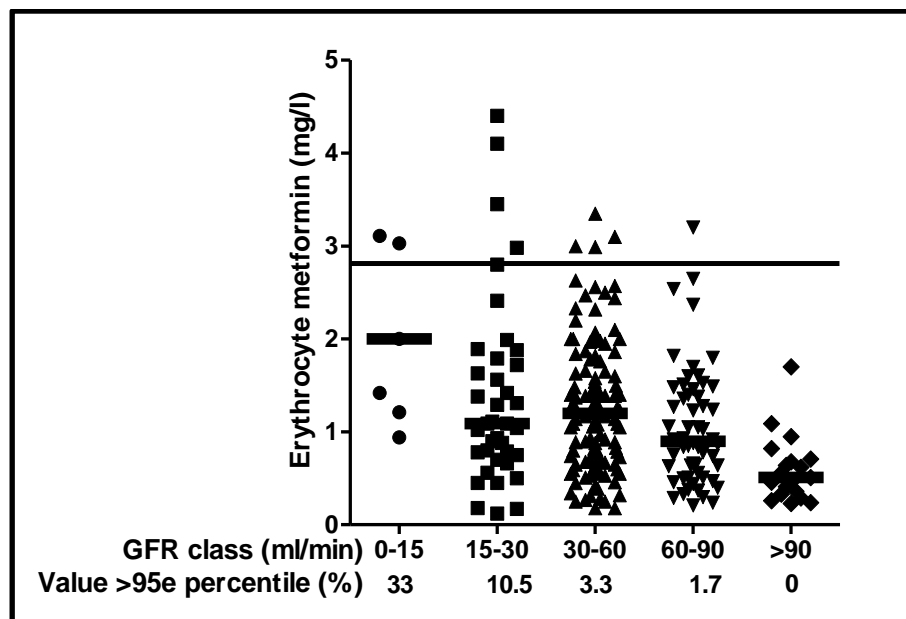
eGFR stage (ml/min)	I (> 90)	II (90-60)	III (60-30)	IV (30-15)	V (< 15)
Number of patients (%)	19 (7.8)	56 (23.2)	121 (50.2)	38 (15.8)	6 (2.5)
Age, years	54.5	66.0	68.0	64.0	59.5
BMI, kg/m ²	33.0	33.0	31.1	32.3	23
HbA1c, %	9.1	8.7	7.6	7.5	6.0
Metformin daily dose, mg	3 000	2 100	2 000	1 000	1350
Metformin level, mg/l	0.54	0.90	1.20	1.09	1.71

Around two-thirds of the patients (68.5%) had an eGFR <60 mL/min/1.73 m². When compared with stage 1 patients, patients with lower eGFR were older and had a lower glycated hemoglobin value

(the more severe the CKD, the lower the glycated hemoglobin value, to be more precise), a lower daily dose of metformin and higher metformin levels. However, stage 5 patients constituted a specific group: they were not obese (and not even overweight), they had a normal glycated hemoglobin value and the median age (59.5) was between the values recorded for stage 2-4 patients (>64.0) and stage 1 patients (54.5). Lastly, the median metformin dose reduction was smaller in stage 5 patients than in stage 4 patients.

For each stage, the distribution of metformin levels and the percentage of levels above the 95th percentile are shown in **Figure 1**. The 95th percentile was 2.86 mg/l. None of the stage 1 patients had a metformin level above this value. In stages 2-4, the percentage of patients with metformin levels above the 95th percentile was relatively small and ranged from 1.7 % to 10.5%; the proportion was higher in the small group of stage 5 patients. Considering individual values, the distribution of metformin levels did not appear to depend on eGFR for stage 2-5 patients.

Figure 3.1.1. Distribution of the individual erythrocyte metformin level (dots) in relation to the patient's eGFR. For each CKD stage, the short horizontal bar represents the median erythrocyte level. The long horizontal bar represents the 95th percentile.



For comparative purposes, the upper limit for a reference group without kidney failure was 1.6 mg/l [Lalau 2003] and the highest ever metformin value reported in the literature was 61 mg/l [Lalau 2011].

Discussion

Here, we report all the individual metformin levels available in our hospital (with the exception of patients with lactic acidosis). The fact that the metformin level was higher on average in patients with CKD is not especially important; the key thing is to be sure that none of the patients had an inappropriate level. A statistically significance difference in mean metformin levels between patients with and without CKD does not necessarily imply that levels of the drug are always elevated in all patients with CKD. Reciprocally, a normal mean level in patients with CKD does not rule out high values in some of these individuals.

Although our non-selective, retrospective study had some disadvantages, it nevertheless reflects “real-life” use of a drug. In this respect, our first hypothesis (i.e. whereby a high proportion of patients with CKD was still being treated with metformin) was confirmed. Indeed, 68.5% of the patients had an eGFR <60 mL/min/1.73 m² and 18.2% even had an eGFR <30 mL/min/1.73 m². This high proportion of patients with a low eGFR is not a source of bias *per se*; metformin assays are requested by physicians because of uncertainty about the existence, extent or stability of CKD and thus the potential for continuing metformin therapy or not. This particular situation offered an opportunity to study the extent to which CKD may lead to significant elevation of the metformin level.

Our second hypothesis (i.e. a low proportion of elevated metformin levels in patients with a low eGFR) was also confirmed. Indeed, given that non-modified metformin is eliminated solely by the kidneys [Lalau 2003], it is striking that there were no significant differences in the distribution of metformin levels between patients in stage 1 of CKD (i.e. eGFR >90 mL/min/1.73 m²) and those with a lower eGFR. However, this can be explained by the fact that median metformin doses were lower in patients with a low eGFR. Compared with stage 1 patients, the median metformin dose was one third lower in stages 2-3, two thirds lower in stage 4 patients and one half lower in stage 5.

In view of the above findings, one should not hastily blame the many physicians who apparently did not comply with the standard contraindications for metformin (i.e. CKD). In fact, common sense dictates that metformin therapy can be continued in kidney failure as long as the metformin dose is adjusted to fit the patient's renal status and thus to prevent a premature switch to other drugs with their own disadvantages.

The above reasoning implies that metformin therapy can be continued in cases of stable CKD. Unfortunately, our study did not include data on the change over time in our patients' renal function. We can nevertheless speculate that stage 2-4 patients had chronic disease (compared with stage 1 patients) because they were older (by at least ten years) and the metformin dose had been reduced

proportionately with the severity of kidney disease. In contrast to stage 1-4 patients, those at stage 5 (i.e. $\text{eGFR} < 15 \text{ mL/min/1.73 m}^2$) constituted a particular group: they were younger, not obese (and not even overweight) and had a normal glycated hemoglobin value. Furthermore, the metformin dose reduction in class 5 patients was smaller than stage 4 patients. Hence, we can speculate that kidney failure was progressing more rapidly in stage 5 patients than in stage 2-4 patients with a more conventional profile of long-standing diabetic nephropathy; this would explain why an appropriate median metformin dose reduction was not applied rapidly enough. In light of this reasoning, it is noteworthy that acute kidney injury appeared to be about three times more frequent than CKD in the largest series of metformin-treated patients with lactic acidosis reported on to date [Lalau 1999].

Conclusion

By applying what was probably a pragmatic metformin dose reduction in our center's low eGFR patients, elevated values were rare and true metformin accumulation was not encountered. Prospective studies are now required to establish whether or not it is possible to continue metformin therapy in CKD, provided that the dose is reduced appropriately. Whereas current guidelines focus on searching for a single "stop/go" eGFR threshold for metformin therapy (amounting to 'the usual dose or not at all') [Jones 2003, McCormack 2005, Herrington 2008, for a review, see Pilmore 2010], we suggest building a nomogram in which the adjusted metformin dose is plotted against eGFR.

Chapter 3.2. Measurements in metformin accumulation

Introduction

Background

It is well known that there is a relationship between metformin levels and lactate metabolism: the higher the blood metformin concentration, the more severe the metabolic disturbance [Prikiš 2007, Peters 2008]. However, the relationship between metformin and lactic acidosis is more complex because (i) lactic acidosis is not necessarily accompanied by metformin accumulation in metformin-treated patients; (ii) metformin accumulation does not necessarily lead to lactic acidosis; and, conversely, (iii) metformin may induce lactic acidosis in the absence of drug accumulation [Lalau 2015].

Studying the relationship between metformin and lactic acidosis therefore involves taking account of putative confounding factors [Gionfriddo 2014], such as the patient profile, the disease context, the assay methods, the type of metformin accumulation (acute vs. chronic), the time of the last metformin administration, the large inter-individual variations in blood metformin levels for a given therapeutic dose and, lastly, inter-individual variations in the metabolic response to metformin accumulation [Christensen 2011, Robert 2003]. Surprisingly, the time frame for determining the status of metformin accumulation has not previously been considered as a confounding factor – even though a delay in measuring blood metformin levels is largely unavoidable in an emergency context in which a metformin assay is not the top priority. Indeed, the late performance of a blood metformin assay may underestimate the level of drug present in the patient on admission (that should be followed by metformin's withdrawal as a result of emergency admission) - especially when the patient is then treated with dialysis and/or the administration of vasoactive drug. Furthermore, the latter procedures may also rapidly modify the blood lactate concentration.

When considering metformin elimination, although metformin is eliminated rapidly and actively by the kidney, once accumulated the drug can accumulate in the tissues and may thus contribute to lactic acidosis. Indeed, the existence of tissue accumulation is suggested by metformin's long terminal half-life ($t_{1/2}$), which was estimated to be around 20 hours on the basis of the rate of excretion in urine after the cessation of multiple-dosage regimens [Graham 2011]. In a study of the oral administration of a single dose of metformin to healthy subjects, we previously estimated the $t_{1/2}$ for elimination from erythrocytes to be 23.4 hours (compared with 2.7 hours in plasma) [Robert 2003]. As a consequence, the measurement of metformin levels in erythrocytes may constitute a readily available indicator of tissue accumulation or elimination [Lalau 2003].

Hence, when blood metformin concentrations are not readily available, two main questions arise: (i) if the time of last administration and metformin's pharmacokinetics are known, is it possible to

retrospectively estimate the extent of metformin accumulation at any time between admission and the blood sample collection?; and (ii) how long after withdrawal does metformin become undetectable in erythrocytes (attesting to its complete elimination from the body)?

Aims

The first aim was to clarify the link between metformin accumulation and its metabolic consequences by taking the time frame for metformin measurement into account. Accordingly, were recorded (i) the proportion of patients for whom the metformin assay was delayed after their admission; (ii) the mean (range) of the time interval between the determination of lactic acidosis on admission and the metformin measurement; and (iii) the impact of the time interval between these two determinations on the assessment of the patient's metabolic status.

The second aim was to study the change over time in plasma and erythrocyte metformin concentrations in patients with metformin accumulation and for whom chronic metformin treatment was withdrawn in an emergency context.

Hypotheses

The following hypotheses were tested: (i) in metformin accumulation, the patient's lactic acidosis status will have changed by the time the metformin assay is performed; (ii) the elimination of accumulated metformin may be prolonged, that may challenge the traditional view according to which the drug clears rapidly because of a short half-life in plasma.

Methods

Data source

All data available in our centre on blood metformin assays were systematically reviewed since the introduction of this technique (from 2003 to 2014). In general, metformin assays had been requested in order to adjust the dose to the patient's renal status or to screen metformin accumulation. We then selected for analysis all patients with metformin accumulation (defined as a plasma metformin concentrations ≥ 5 mg/L, corresponding to 10 times the mean reported therapeutic value [Lalau 2010]) and with available lactic acidosis status on admission.

Data selection

Impact of the time frame on metformin levels

The pH, lactate and the plasma and erythrocyte metformin concentrations were noted on the basis of the reports on patients with metformin accumulation. Patients in whom all these parameters had

been determined at the same time point during hospitalization were selected. The data were then analysed for (i) lactic acidosis (arterial pH < 7.35 and a blood lactate concentration > 5 mmol/L [Luft 1983]) on admission and at the time of the metformin assay and (ii) the extent of metformin accumulation.

Metformin elimination

Patients selected were those selected with metformin accumulation defined as a plasma metformin concentration ≥ 5 mg/L (according to a FDA label stating that “*during controlled clinical trials, which served as the basis of approval for metformin, maximum metformin plasma levels did not exceed 5 mg/l, even at maximum doses.*” [FDA Glucophage leaflet 2013]) and in whom data on both plasma and erythrocyte metformin levels and arterial gas levels were available. On the basis of these reports, a further selection patients was done for retaining those in whom all the above parameters had been determined on admission and for whom metformin concentrations had been remeasured once or more at least five days after admission.

Data presentation

Impact of the time frame on metformin levels

The following data are presented: the clinical setting, blood parameters (arterial pH, arterial lactate, blood metformin levels), and the time interval between admission and subsequent assays.

Metformin elimination

All available metformin blood values were collected in order construct a time-concentration plot (for plasma and erythrocyte concentrations) for each individual. When several metformin assays were performed within a short time interval on the same day, the mean value was calculated. The time course of the individuals' plasma and erythrocyte metformin levels was further analyzed in two ways: (i) calculation of the daily plasma/erythrocyte metformin ratios, and (ii) calculation of the daily reduction in metformin levels (compared with those measured on admission). The rate constant of the last exponential phase (k) was estimated (using linear regression) from the final two quantifiable concentrations on the log concentration-time curve. The terminal half-life ($t_{1/2}$) was calculated as $0.693/k$.

Results

Impact of the time frame on metformin levels

Seventeen eligible reports on 16 patients aged between 44 and 74 were identified. For each individual, the clinical setting, arterial pH, arterial lactate, serum creatinine and blood metformin values and the time interval between admission and subsequent assays are presented in **Table 1**.

All patients had hyperlactatemia (with a minimal value of 3.3 mmol/L) and 10 patients had lactic acidosis. At least one factor for hyperlactatemia associated to metformin accumulation was identified in all but one case. Kidney disease and sepsis appeared to be the main factors associated with metformin accumulation. The serum creatinine concentration was available on admission for 14 cases and was abnormal in 13 of them. Dialysis and/or the administration of vasoactive drugs were initiated prior to blood metformin determination in four and six patients, respectively.

Table 3.2.1 Individual patient data and clinical settings.

Clinical features				Time interval after admission, hours	Biological features on and after admission					
Case	Clinical setting	Dialysis (d) and/or vasoactive drugs (v)	Survival		Serum creatinine $\mu\text{mol/L}$	pH	Arterial lactate mmol/L	Lactic acidosis (LA) status	Plasma metformin mg/L	Erythrocyte metformin mg/L
All study parameters immediately available on admission										
1	Acute aggravation of chronic heart failure	–	Yes	0	564	7.40	3.3	No LA	9.7	4.8
2	No overt failure	–	Yes	0	–	7.40	5.4	No LA	7.38	4.01
3	Sepsis	–	Yes	0	296	7.37	7.6	No LA	7.6	2.53
4	Mesenteric ischemia, CKD	–	Yes	0	738	6.73	8.4	LA	66.8	20.1
Study parameters available partly on admission and completed some hours later										
5*	Multiple drug overdose	d	No	0	187	6.84	27	LA	ND	ND
				3	226	7.15	30	Persistence	56.7	18.6
6	AKF	–	Yes	0	776	7.20	20	LA	ND	ND
				4	745	7.23	18	Persistence	32.5	19
7	Acute aggravation of CKD	v	No	0	653	6.90	16	LA	ND	ND
				4	640	6.80	21	Persistence	43.3	8.7
8	Sepsis	v	No	0	161	6.70	28	LA	ND	ND
				5	160	6.67	26	Persistence	7.1	1.5
9*	Multiple drug overdose	v	Yes	0	68	7.41	6.6	No LA	ND	ND
				7.5	127	7.17	11.4	Appearance	64	19
10	AKF	d	Yes	0	1073	7.26	3.7	No LA	ND	ND
				8.5	486	7.41	1.6	No LA	15.8	19.4
11	Pulmonary embolism and CKD	–	Yes	0	292	7.36	5.2	No LA	ND	ND
				9	305	7.31	4.6	No LA	20.5	13.2
12	AKF	–	Yes	0	ND	7.31	10.5	LA	ND	ND
				11	538	7.14	14.4	Persistence	38	8.7
13	Sepsis	v	Yes	0	1327	7.02	10.8	LA	ND	ND
				11.5	1285	7.21	7.6	Persistence	50.1	18.5
14	Sepsis	–	Yes	0	231	7.45	6	No LA	ND	ND
				17	244	7.39	1.7	No LA	6.3	7.6
15	AKF	–	Yes	0	ND	7.20	5.7	LA	ND	ND
				39	928	ND	4.1	Disappearance	10.4	15.1
16	Sepsis	dv	Yes	0	958	6.91	16	LA	ND	ND
				40	353	7.42	1.7	Disappearance	39.9	16.8
17	Metformin overdose and AKF	dv	Yes	0	1357	6.79	20	LA	ND	ND
				52	ND	7.36	2.1	Disappearance	11.4	11.3
* Cases 5 and 9 concern the same patient										

* Cases 5 and 9 concern the same patient.

Arterial pH and lactate were available for all 16 patients on admission. Eleven of the latter had acidosis. The study parameters were determined simultaneously on admission in only 4 patients. The time interval between the assessment of lactic acidosis status on admission and sample collection for the full set of parameters varied greatly from one patient to another; it was between 0 and 6 hours in eight cases, between 6 and 12 hours in five cases, between 12 and 24 hours in one case, between 1 and 2 days in two cases, and over 3 days (52 hours, in fact) in one case. The mean time interval was 12.5 ± 16 hours. In the 9 cases with lactic acidosis on admission and for whom the sampling for the metformin measurement was performed later, lactic acidosis persisted at the time of such measurement in 6 cases and had resolved in 3 cases. Conversely, one patient (case 9) developed lactic acidosis after admission. Blood metformin levels were high in all reports - even after dialysis and even for the longest time to blood sampling - and ranged from 6.3-66.8 mg/L in plasma (mean \pm SD: 28.7 ± 21.3 ; normal upper limit <1.35) and 1.5-20.1 mg/L in erythrocytes (mean \pm SD: 12.3 ± 6.6 ; normal upper limit <1.65). The plasma metformin levels were higher than the erythrocyte metformin levels whenever the time to blood sampling after admission was below 8 hours and in half the cases with a longer time interval. Lastly, three of the 16 patients (18.8%) failed to survive.

Metformin elimination

A total of 12 reports met our selection criteria. The clinical setting and biochemical data (arterial pH, arterial lactate, serum creatinine and blood metformin values) for each of the 12 individuals are presented in **Table 3.2.2**.

Table 3.2.2. The patients' main clinical and biochemical characteristics.

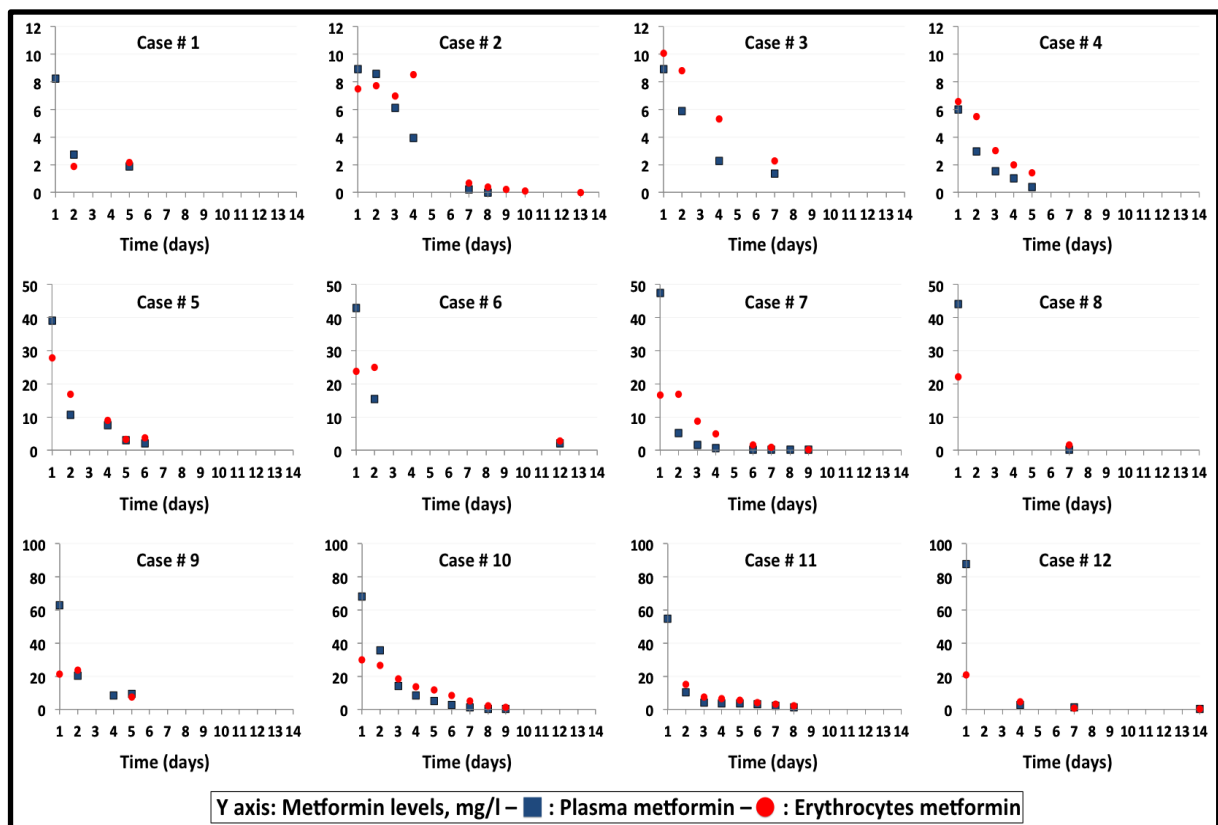
Case	Clinical setting	Characteristics on admission					Dialysis	Last measurement		
		Lactate (mmol/l)	Arterial pH	Serum creatinine (μ mol/l)	Plasma metformin (mg/l)	Erythrocyte metformin (mg/l)		Time interval after first blood sample collection (days)	Plasma metformin (mg/l)	Erythrocyte metformin (mg/l)
1	Isolated anuria	28.0	7.01	703	68.0	29.7	yes	9	0.2	0.2
2	Isolated anuria	13.1	6.95	670	39.1	27.8	yes	6	2.09	3.72
3	Sepsis	11.5	7.07	1091	42.9	23.7	yes	12	1.99	2.83
4	Isolated anuria	14.5	6.72	814	54.6	—	yes	8	1.37	2.11
5	Myocardial infarction, sepsis	11.0	7.35	390	8.9	10.1	no	13	1.33	2.27
6	Acute pulmonary oedema	15.5	7.17	338	6.0	6.59	yes	5	0.36	1.4
7	Multi drugs overdose	2.09	ND*	472	8.9	7.5	yes	7	0.00	0.00
8	Acute renal failure	15	6.81	907	62.9	21.4	yes	5	9.3	7.89
9	Metformin and alcohol overdose, cirrhosis, sepsis	22	6.05	113	8.25	—	yes	5	1.87	2.13
10	Dehydration, sepsis	20	6.59	731	47.5	16.7	yes	9	0.1	0.24
11	Dehydration	16	6.83	679	88.0	21.0	yes	14	0.1	0.1
12	Anuria postsurgery	9.4	7.15	914	44.2	22.2	yes	7	0.1	1.64
Mean \pm SD value		14.84 ± 6.56	6.88 ± 0.35	651.83 ± 278.42	39.94 ± 26.98	18.67 ± 8.19	-	8.33 ± 3.17	1.57 ± 2.57	2.04 ± 2.19

*not determined

The mean \pm SD age was 64.2 ± 8.1 , and the male/female gender ratio was 1:2. Acute renal failure was the most predominant triggering factor for metformin accumulation. Indeed, 11 of the 12 had severe, acute renal failure (with serum creatinine levels ranging from 338 to 1091 $\mu\text{mol/L}$). The cause of the renal failure, however, was not always specified. Furthermore, all 12 patients presented lactic acidosis on admission; this was generally severe, with mean \pm SD pH and lactate values of 6.88 ± 0.35 and 14.8 ± 6.56 mmol/L, respectively. Lastly, the 11 patients suffering from acute renal failure were treated with dialysis. All 12 patients survived.

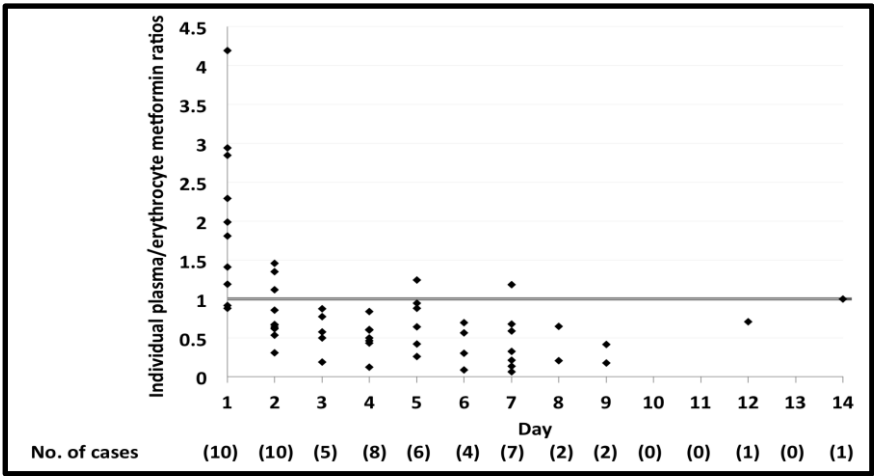
The mean \pm SD initial blood metformin concentrations were 39.9 ± 27.0 mg/L and 18.7 ± 8.2 mg/L in plasma and in erythrocytes, respectively (ranges, 6.0-88 and 6.59-29.7, respectively). The mean time interval between the first and the last blood sample collection for the metformin assay was 8.33 ± 3.17 days (range, 5-14). Metformin remained detectable in the three patients with the longest time intervals between the first and last sample collections (12 or more days, **Figure 3.2.1.**).

Figure 3.2.1. Individual plasma and erythrocyte metformin time-concentration data points in the study patients. Due to the wide range of metformin levels (expressed in mg/L), three scales were used on the concentration axis.



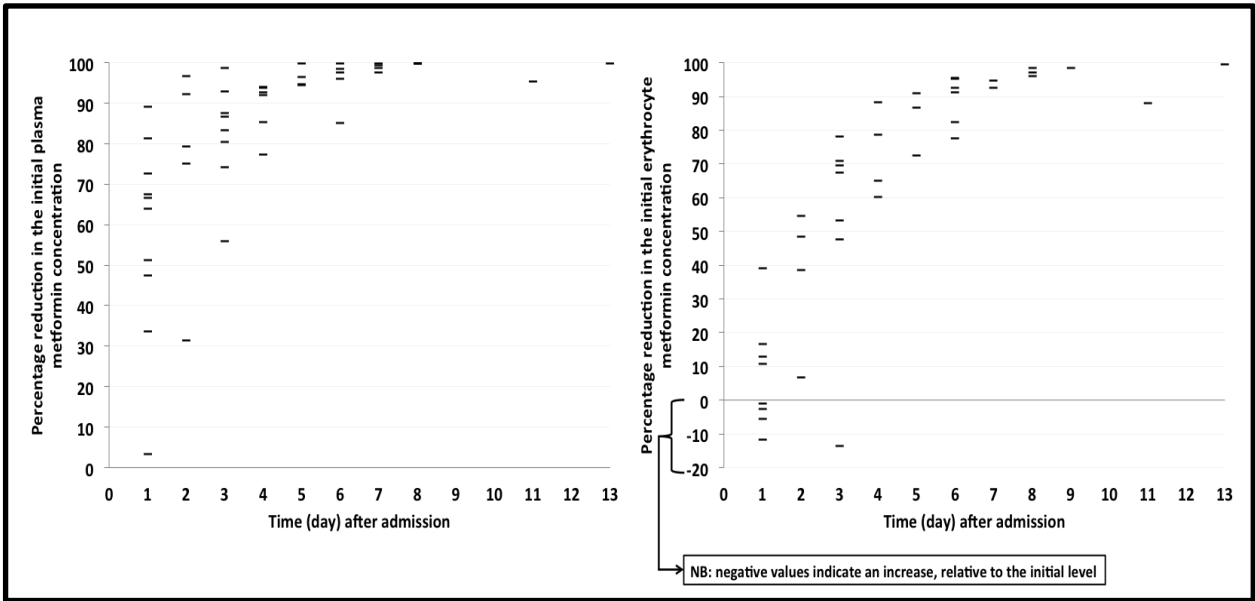
Metformin was eliminated more rapidly from plasma than from erythrocytes. However, as already mentioned above, it was possible to detect metformin in plasma up to day 13 post-admission.

Figure 3.2.2. Change over time in the individual plasma/erythrocyte metformin ratios for each day post-admission.



This figure shows the change over time in the ratios of individual plasma/erythrocyte metformin levels during the study period (including all the available data). Although metformin concentrations were always initially higher in plasma than in erythrocytes, the plasma concentration fell below the erythrocyte concentration as early as day 2. Most of the data were collected during the first seven days post-admission. On day 7, metformin concentrations remained detectable in plasma and in erythrocytes in all patients. In one patient, the plasma concentration was still higher than the erythrocyte concentration at this time point.

Figure 3.2.3. The reduction in individual metformin levels over the study period, expressed as a percentage of the value recorded on admission.



This figure shows the reduction over time in metformin concentrations as a percentage of the value on admission. Although the shape of the curve is suggestive of an asymptotic relationship, there was great variation in the degree of the reduction over the first few days post-admission (especially for plasma). Moreover, the reduction did not reach a value of 100% at the time the last sample was collected.

Finally, the mean terminal half-life of metformin in plasma and in erythrocytes was estimated to be 51.9 and 43.4 hours, respectively.

Discussion

Impact of the time frame on metformin levels

The present study is the first to document patients with marked metformin accumulation as a function of the time frame for blood sampling. Lactic acidosis was observed in 10 out of the 17 reports in our series.

In view of the issues highlighted in the Introduction, the following observations may be made: (i) in most cases (13 out of 17), the pH, lactate and metformin values were not determined simultaneously on admission; (ii) the time interval between the initial sampling (for pH and lactate) and the subsequent sampling for obtaining other study parameters (including the metformin level) was generally rather short (about 12 hours) but varied markedly when considered the SD (16 hours) and especially the range (up to 52 hours); most importantly, (iii) the lactic acidosis status (i.e. resolution or occurrence) had changed in half the patients for whom blood metformin levels had been determined with some delay.

It is also noteworthy that the three patients with the longest time interval between admission and subsequent sampling (about two days) still had very high plasma and erythrocyte metformin levels. At the time of the metformin measurement, the pH and lactate values no longer met the criteria for lactic acidosis. Although a high erythrocyte metformin concentration was expected (because of the drug's long half-life in these cells [Lalau 2003]), the persistent, high plasma concentration is surprising – especially since dialysis had been initiated in two of the three patients.

This study shares the limitations of all descriptive studies. However, it is fair to say that a trial limitation may be strength when it reflects what happens actually in clinical practice. More precisely, the present study's main objective was not to rigorously study the effect of metformin accumulation on lactate metabolism but rather to examine possible pitfalls when considering the link between metformin and so-called “metformin-associated lactic acidosis” under emergency conditions. In other words, we wanted to know which factors have an effect on this link, such as the time frame for blood sampling for pH, lactate, and metformin measurements.

There are two levels of complexity in this context: factors that modify the metformin level (such as the time to sampling after admission, in particular) and the physiopathological mechanism complexity of so-called “metformin-associated lactic acidosis” in itself [Lalau 2015]. Indeed, metformin accumulation may be related to either acute, primary renal failure or isolated metformin intoxication [Vecchio 2011] (which occurred in less than one-third of the patients studied here) or secondary to renal failure as a complication of a severe, systemic disease (such as sepsis, heart failure, etc.) [Mizock 1992, Kajbaf 2013B]. For this reason, the existence of the term “metformin-associated lactic acidosis” does not imply that all the associated factors (e.g. the degree of metformin accumulation and the severity of any associated diseases) are present to the same degree at any given time.

Given the above results and considerations, our results suggests that when the time interval between the first lactate measurement and that of blood metformin is very long, caution must be taken when considering the putative relationship between metformin accumulation and lactic acidosis. Indeed, the patient's lactic acidosis status will have changed in some cases by the time the metformin assay is performed - even though metformin accumulation may still be present.

Metformin elimination

To the best of our knowledge, the present study is the first to measure long-term plasma and erythrocyte metformin elimination in the real-life context of so-called “metformin-associated lactic acidosis”. The lack of knowledge about elimination of accumulated metformin is surprising, since (i) this drug has been available for more than 50 years now, and (ii) this information is needed to establish a link between metformin and any case of lactic acidosis occurring in metformin-treated patients [Lalau 2010]. In an emergency setting, lactate and pH levels in critically ill patients are measured almost immediately upon admission. In contrast, blood metformin concentrations are not always determined (or at least not immediately, since assaying for metformin is not necessarily the prime concern in an emergency setting). Moreover, metformin levels are not readily available.

The present findings provide valuable insight into the pharmacokinetics of metformin in the particular context of accumulation. The metformin concentration-time curves suggest that the initial elimination of metformin is rapid -even when the drug has accumulated massively (i.e. with a plasma concentration of almost two hundred times the normal value in our centre (0.5 ± 0.6 mg/L [Lalau 1995])). This finding agrees with the short half-life in plasma estimated in healthy subjects (i.e. 2.7 hours) [Robert 2003]. Quoting a $t_{1/2}$ for elimination is difficult, however, since the plasma metformin concentration-time curves are bi- or even tri-exponential, indicating the existence of a deep compartment. Indeed, the estimated $t_{1/2}$ for elimination in healthy subjects is far longer in

erythrocytes (reflecting the deep-compartment) than in plasma (23.4 vs. 2.7 hours, respectively). Furthermore, in the present setting of metformin accumulation, the terminal $t_{1/2}$ in erythrocytes (43.4 hours) is twice as high as that previously measured in healthy subjects. This difference is not surprising, given the context of dramatic metformin accumulation caused by severe renal failure. In contrast, we did not expect to detect metformin in plasma for up to 13 days, given (i) the rapid elimination in healthy subjects (as specified above), (ii) the use of dialysis and (iii) the drug's rather high dialyzability [Lalau 1989]. Indeed, the mean terminal $t_{1/2}$ in plasma (51.9 hours) was greater than that in erythrocytes.

Metformin is a small, non-metabolized molecule that is not bound or linked to proteins and is normally cleared rapidly from the plasma by the kidneys or by dialysis. The long observed terminal $t_{1/2}$ for metformin elimination might therefore be due a long-lasting redistribution of the drug into deep compartments; indeed, metformin has been found in significant amounts in some tissues [Wilcock 1994]. This redistribution cannot be explained by conventional passive diffusion rules in which transfers are merely dependent on the respective concentrations on either side of the cell's plasma membrane. It remains to be established whether the activity of metformin transporters (OCT1, OCT2, MATE1, MATE2 and PMAT) may influence this type of active “shuttle phenomenon”. Indeed, variations in the genes coding for these transporters may modulate metformin's pharmacodynamics and pharmacokinetics (in addition to metabolic effects; for a review, see [Graham 2011, Todd 2014, Pawlyk 2014, Chen 2013, Christensen 2011]). The striking clinical impact of this type of polymorphism was confirmed in a study of a cohort of type 2 diabetes patients taking 1 g of metformin twice a day over a 24-month period; the researchers observed an 80-fold inter-individual variation in trough steady-state plasma metformin concentrations [Christensen 2011].

The present study had however some limitations. Firstly, it was a descriptive study that lacked true daily monitoring of all patients throughout the post-admission period. Secondly, the terminal $t_{1/2}$ of elimination of metformin was calculated from the last two available, quantifiable concentrations prior to the patient's discharge; the time interval between these determinations varied markedly.

Conclusions

When considering metformin accumulation, metformin levels are generally measured many hours after patients' admission. Because of this delay the patient's metabolic status (i.e. the presence or the absence of lactic acidosis) had changed in half the cases studied here -even while metformin accumulation persisted. This delay should be taken into consideration when classifying patient with so-called “metformin-associated lactic acidosis”.

The rapid initial elimination of metformin (especially from the plasma) following accumulation of the drug does not rule out a long terminal $t_{1/2}$. Indeed, the drug could be detected in plasma for at least 2

weeks -even after dialysis. This observation suggests the presence of a “shuttle phenomenon” between the deep compartments and plasma for this drug. In the setting of lactic acidosis and massive metformin accumulation, dialysis should be rather performed with a view to correcting metabolic disturbances in these generally oligo-anuric patients, rather than eliminating the drug rapidly.

The measurement of metformin is ultimately of importance to improve assessment of prognosis during the course of various situation of metformin accumulation.

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PART IV. Metformin therapy in CKD

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Background

In view of metformin's first-line position in the treatment of patients with T2DM, the drug's potential effects (both beneficial and harmful) have prompted much debate. According to most official guidelines, use of metformin is contraindicated when the renal creatinine clearance is <60 ml/min. In fact, the proposals for continuing or stopping metformin therapy in CKD involve a threshold (whether based on serum creatinine or eGFR) rather than the dose adjustment as a function of renal status (in patients with stable renal function) performed for other drugs excreted by the kidney. In the most-populated countries, the criteria for metformin withdrawal are mainly qualitative; hence, the current contraindications for metformin rule out its use in a large proportion of patients with CKD [Kajbaf 2013]. Until very recently, this was also the case for the Glucophage® license holder itself [EMA guidelines]. This means that although lactic acidosis is rare, the fear of this condition in metformin-treated patients still influences today's treatment strategies. Indeed, a high proportion of patients present an established or suspected contraindication - particularly in the context of renal failure.

However, the debate over metformin's beneficial and harmful effects should include a critical analysis of the current guidelines most frequently applied to patients with renal impairment [Holstein 1999]. Some researchers consider the latter guidelines to be exaggerated [McCormack 2005, Jones 2003], whereas others even suggest that metformin is safe in patients with stable, severe CKD [Nye 2011]. This position was supported by the results of a 4-year study of a large cohort of metformin-treated patients at varying CKD stages [Ekström 2012]. Compared with other oral antidiabetic agents, metformin was associated with lower all-cause mortality and a lower risk of cardiovascular disease, acidosis and serious infection (one of the study's endpoints). Compared with insulin, metformin was associated with lower all-cause mortality. It is noteworthy that these beneficial effects were consistently found in patients with eGFR values ranging from 45 to 60 ml/min/1.73 m², who did not display an elevated incidence of acidosis/infection. Ekström et al.'s observational study extended the results of the United Kingdom Prospective Diabetes Study to patients with impaired kidney function. The study's results are also in line with those of an observational study of almost 20,000 patients with diabetes and established cardiovascular disease or three atherothrombotic risk factors (i.e. patients who could be considered as particularly vulnerable to metformin) [Roussel 2010]. Relative to other antidiabetic treatments, metformin was associated with a lower risk of all-cause mortality after nearly 2 years. The recent observation that long-term metformin treatment augments myocardial resistance in an animal model of ischemia-reperfusion constitutes further evidence of a protective cardiovascular effect [Whittington 2013]. In this regard, it is important to bear in mind that cardiovascular disease is the main cause of morbidity and mortality in CKD patients [Shanahan 2011].

Given the critical importance of antidiabetic therapies and the fact that metformin has been available for over 50 years, it is very surprising that there are so few literature data from clinical studies of metformin therapy in CKD patients. Indeed, only four such studies have been published:

1. A retrospective study featured data on metformin dose, erythrocyte metformin level and eGFR in 240 metformin-treated CKD stage 1-5 patients [Briet 2012] (68.5% of whom had an eGFR <60 mL/min/1.73 m²). The percentage of CKD stages 2, 3 and 4 patients with a metformin level above the 95th percentile (for the total study population as a whole) were low (1.7%, 3.3% and 10.5%, respectively), when compared with stage 1 patients. Unfortunately, the small number of CKD stage 5 patients (n=6) prevented this calculation in this particular group. Importantly, the metformin dose had been pragmatically reduced (by up to two-thirds) in CKD patients. This indicates that many physicians did not comply with the conventional contraindication for metformin and had preferred to adjust the metformin dose to match the patient's renal status.
2. A proposal for dose adjustment was based on a single prospective study (published in 1990) of elderly CKD patients on a two-month course of metformin [Lalau 1990]. Mean plasma metformin concentrations remained within the therapeutic range (i.e. <1.65 mg/L) when subjects were given either 1700 mg per day of metformin (for creatinine clearances above 60 ml/min, i.e. CKD 2) or 850 mg per day (for clearances between 30 and 60 ml/min, i.e. CKD 3). The difference in plasma metformin concentration between the two dosage groups was not statistically significant.
3. An open-label observational study conducted in 22 patients with severe renal impairment (creatinine clearances rates: 15–40 ml/min) and who were receiving a range of metformin daily doses (250-2000 mg). The lactate concentration was >2.7 mmol/L in only 3 patients and the plasma metformin concentrations were 3-5 mg/L in two other patients; there was no correlation between metformin and lactate concentrations. The authors concluded that in patients with stable renal function, a metformin dose reduction and metformin monitoring might enable the safe use of metformin in CKD patients [Duong 2012].
4. A 4-week prospective study conducted in 35 diabetic patients undergoing automated, peritoneal dialysis and taking a metformin daily dose of 500-1000 mg. There were no significant differences in the anion gap or pH when comparing the pre- and post-administration data. After 4 weeks, the mean overall lactate level was 1.39 ± 0.61 mmol/l. Hyperlactatemia (i.e. a lactate value of between 2 and 5 mmol/L) was reported in only 4 of 525 determinations. The researchers concluded that metformin can be used in patients with insulin-dependent T2DM on automated peritoneal dialysis [Al-Hwiesh 2014].

Data from animal studies have emphasized the potential value of metformin therapy in CKD. Metformin displays several renoprotective effects (independently of its well-known beneficial effects on cardiovascular risk factors), i.e. protection of podocytes (through the modulation of oxidative stress), reduction of endothelial cell apoptosis, inhibition of the unfolded protein response in tubular epithelial cells, and prevention and even reversal of renal injury [Kim 2012, Rafieian-Kopaie 2013, Thériault 2011, Alhaider 2011].

Given the above considerations, it is now time to examine metformin's effects in a more convincing clinical study of CKD patients.

Hypothesis

Given that metformin is normally eliminated solely by the kidneys (at a rate 4 to 5 times higher than that of creatinine), it may be possible to continue metformin therapy under safe conditions in patients with CKD (and even severe CKD), provided that the metformin dose is appropriately adjusted.

Method and research design

Selection of study subjects

Diabetic patients not treated with metformin at any stage of chronic kidney disease (stages 1-5) consulting department of endocrinology of University Hospital of Amiens-France.

Inclusion criteria

- Diabetic patients non-treated with metformin, or patients previously treated with other antidiabetic drugs but have poorly diabetes control (HbA1c > 6.5%) at any stage of stable kidney failure (stage 1-5 according to MDRD classification), as follows:

Table 4.1. Staging of CKD.

CKD stage	GFR (ml/min/1,73m ²)	Description
1	≥ 90	Normal or high
2	60 - 89	Mildly decreased
3A	45 - 59	Mildly to moderately decreased
3B	30 - 44	Moderately to severely decreased
4	15 - 29	Severely decreased
5	< 15	Kidney failure

- Patients having a renal function control at least 3 months before start of study to evaluate stability of renal function;
- Patients aged 18-80 years old whose renal function is stable (less than 30% of fluctuation in two previous consecutive eGFR at least in the last three months before start of study). In the other terms, a reference kidney function of at least 3 months is compared to the inclusion values.
- Patients without severe hepatic failure (Child > A).

Exclusion criteria

- Patients without the former background of renal function control (i.e. absence of renal creatinine clearance);
- Fluctuation of more than 30% of renal creatinine clearance at least in the last three months before start of study;
- Severe hepatic impairment (Child stage > A);
- Absence for liver functional test;
- Patients having an X-ray with contrast medium injection;
- Pregnancy, lactation;
- Any acute systemic illness with lactate concentration > 2.5 mmol/l.

Withdrawal criteria

- The occurrence of any factor that could interfere kidney and/or liver function;
- The observation of a significant hyperlactatemia (≥ 2.5 mmol / l) after a processing phase;
- Initiation of use of the drugs that alter the creatinine clearance (diuretics, ACE inhibitors, ARBs, NSAIDs) or interfering with the renal tubular secretion of metformin (cimetidine) after start of study.

Metformin dose and blood sampling

Metformin (Glucophage)[®] is administered orally as conventionally recommended, i.e. at the end of the meal. All patients undergo 3 one week-blocks of metformin treatment at an increasing dosage, each of which is followed by a one week-wash-out period: 500 mg/day in the evening (E) in phase 1; 1,000 mg/day (500 mg morning (M) and E) in phase 2; 2,000 mg/day (1,000 mg M and E) in phase 3. CKD 1 patients have a complementary phase using 3,000 mg/day (1,000 mg M and 2,000 mg E).

At the end of each phase GFR, blood glucose, metformin level of the patients are evaluated and blood lactate concentrations are assayed in CKD 3-5.

The design is summarized as follows:

Table 4.2. Schematic table of the protocol.

CKD stage	1						
	2-5						
Phase	1	Wash-out	2	Wash-out	3	Wash-out	4
Duration (week)	1	1	1	1	1	1	1
Metformin daily dose (mg)	500 qpm		500 b.i.d		1000 b.i.d		1000 qam + 2000 qpm

Analytical methods

Metformin was isolated from plasma and erythrocytes according to a previously described method [Lacroix 1991]. Solutes were separated on a 10 µm, 4.6 x 250 mm Spherisorb® SCX column (Waters, Saint-Quentin en Yvelines, France) using a mobile phase consisting of 50% acetonitrile and 50% 2 mM NaH₂PO₄ pH 3.8 buffer pumped at a flow rate of 1 ml/min. The eluate was monitored at 230 nm using a diode array detector (Waters). The retention time for metformin was 8 min. The method showed good linearity ($r > 0.997$) over the concentration range 0.10-15.0 mg/L. The lower limits of detection and quantification were 0.05 mg/L and 0.1 mg/L, respectively. The upper limit of quantification was confirmed as 100 mg/L. The precision and accuracy determination were carried out with three different concentrations (0.5, 2 and 8 mg/L) of quality control samples. The intra- and inter-assay precisions (measured as the percent coefficient of variation over the concentrations range of the quality controls) were all <15%. The intra- and inter-assay accuracy (defined as the absolute value of the ratio of the calculated mean values of quality controls to their respective nominal values, expressed as a percentage) ranged from 91% to 109.8%. This method is valuable for the invasive monitoring of diabetic patients treated with this hypoglycaemic agent. All concentration data are quoted with regard to basic metformin. An ion-exchange chromatography method (Variant II Turbo, Bio-Rad, Hercules, CA) was used to assay HbA_{1c}, according to the manufacturer's instructions.

Estimation of the glomerular filtration rate

The eGFR was estimated according to the Modification of Diet in Renal Disease equation, which includes four variables: $\text{eGFR (mL/min per 1.73 m}^2\text{)} = 175 \times (\text{serum creatinine})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African-American})$ (conventional units).

Reclassification of CKD stages

Because eGFR may have varied throughout the study, we decided to reclassify patients when the CKD stage determined at the end of each study phase has changed at least 3 times compared to the CDK stage at inclusion.

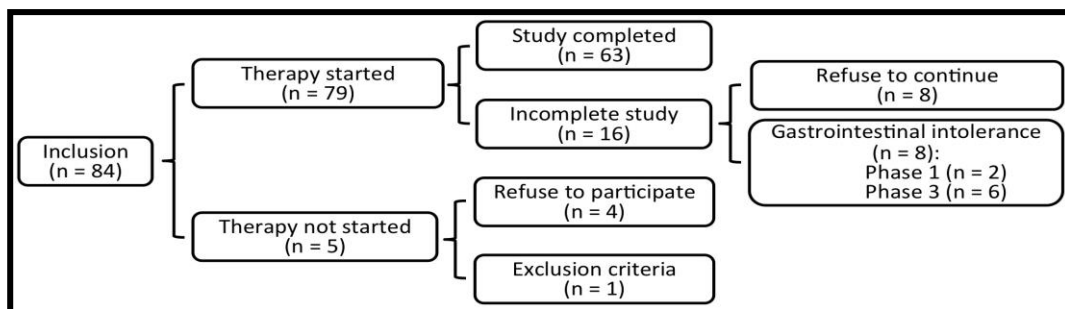
Statistical analysis

For each stage of renal failure, quantitative variables are expressed as mean \pm standard deviation or median (minimum - maximum) according to their distribution. Kruskal-Wallis and Mann-Whitney U tests were used for comparison studies. The results obtained with the Mann-Whitney test have been reexamined using the Bonfroni correction according to the number of study groups.

Results

Eighty-four patients were included into the study from December 2012 to July 2015. However 4 patients refused secondarily to take metformin (for personal reasons) and 1 patient had a change in his antihypertensive treatment (exclusion criterion). Among the 79 participants to the study, 63 completed the study. The 16 remaining patients stopped metformin therapy either because of gastrointestinal intolerance (n = 8, of whom 6 at a metformin dose of 2,000 mg/day) or for personal reason (n = 8). No patient discontinued the study because of withdrawal criteria.

Figure 4.1. Patients' flow chart.



A reclassification into CKD stages was necessary in 11 patients, as follows:

- CKD1 → CKD2: n = 2
- CKD2 → CKD1: n = 4
- CKD3B → CKD3A: n = 1
- CKD4 → CKD3B: n = 2
- CKD4 → CKD5: n = 2.

However this change was generally marginal and concerned 6 patients in CKD 1-2.

Table 4.3. Baseline demographic data of the patients (data are those retained after CKD stage reclassification, mean±SD).

CKD Stage	No.	GFR (ml/min)	Age (year)	M/F No.	BMI	HbA1c (%)	Blood glucose (mmol/l)	Lactate (mmol/l)	ASALT (U/l)	ALAT (U/l)	GGT (U/l)
1	17	112.6±30.2	52.4±12.8	11/6	30.3±5.5	10.6±2.9	11.6±3.8	1.32±0.52	27.8±40.3	34.6±21.9	71±46.6
2	11	78.3±15.6	63.8±4.5	5/6	30.8±4.2	8.8±2.2	9.1±3.5	1.21±0.30	23.5±7.2	36.2±22.3	66.4±53.3
3A	14	51.6±5.5	65.9±5.9	10/4	31.3±5.1	8.2±1.5	10.6±3.5	1.20±0.41	28.4±12.1	34.3±19.5	60.8±45.8
3B	16	36.2±5.2	66.4±9.4	10/6	30.8±4.1	8.8±1.7	8.9±2.9	0.99±0.49	20.1±10.9	23.6±8.2	90.9±80.3
4	12	23.2±3.8	63.6±7.2	4/8	33.4±4.9	8.1±1.8	8.2±6.1	1.22±0.34	22.5±11.3	29.3±15.2	61.6±57.3
5	6	15.5±4	63±7.2	3/3	34.7±5.3	7.73±1.0	9.2±3.1	0.73±0.3	17±4.8	18.8±6	24.5±7.9

Table 4.4. Study data (mean±SD (range)).

CKD Stage		1	2	3A	3B	4	5
Phase 1 (after 500 mg/day for a week)	No.	17	11	14	16	12	6
	eGFR (ml/min)	121.2±20.1 (90-150)	77.7±9.7 (59-96)	55.9±6 (41-65)	37.8±5.4 (30-47)	22.6±4.7 (16-29)	12.7±1 (11-14)
	Blood glucose (mmol/l)	9.56±3.35 (3-16.3)	8.84±2.86 (5.6-16)	9.86±3.28 (5.2-16)	8.75±2.89 (4.8-16.7)	8.55±3.82 (3.9-16)	8.96±2.11 (6.2-12)
	Lactate (mmol/l)	—	—	1.34±0.55 (0.62-2.66)	1.12±0.37 (0.5-1.6)	1.18±0.43 (0.7-1.97)	0.73±0.52 (0.16-1.7)
	Plasma metformin (mg/l)	0.23±0.11 (0.10-0.44)	0.46±0.26 (0.10-1.03)	0.86±0.54 [‡] (0.10-2.11)	0.97±0.52 (0.33-2.0)	1.44±1.13 [‡] (0.29-4.17)	1.38±0.57 (0.83-2.44)
	Erythrocyte metformin (mg/l)	0.23±0.11 (0.10-0.57)	0.54±0.31 (0.12-1.16)	0.65±0.23 (0.40-1.09)	0.96±0.76 (0.10-3.12)	1.02±0.64 [◊] (0.46-2.82)	1.43±0.24 (0.80-1.38)
Phase 2 (after 1000 mg/day for a week)	No.	17	11	14	15	12	5
	GFR (ml/min)	123.3±18 (91-150)	78.5±11.9 (62-96)	53.5±6.2 (39-61)	35.7±6.3 (30-44)	22.3±5.5 (11-32)	11.8±2.6 (8-15)
	Glycemia (mmol/l)	9.17±2.96 (4.7-14.4)	8.32±3.73 (5.6-18.7)	8.42±1.97 (5.5-12.2)	9.03±2.64 (5-14.6)	6.75±3.89 (2-15.9)	7.74±1 (6.3-8.7)
	Lactate (mmol/l)	—	—	1.34±0.68 (0.53-3)	1.31±0.56 (0.54-2.4)	1.16±0.38 (0.5-1.67)	0.44±0.17 (0.2-0.61)
	Plasma metformin (mg/l)	0.50±0.51 (0.10-2.18)	0.66±0.36 (0.32-1.45)	1.09±0.51 (0.31-1.82)	1.24±0.50 (0.5-2.07)	2.28±1.16 (0.89-4.9)	1.92±0.95 (1.03-3.53)
	Erythrocyte metformin (mg/l)	0.55±0.40 (0.10-1.86)	0.76±0.43 (0.24-1.84)	0.96±0.30 (0.59-1.49)	1.47±0.68 (0.73-3.12)	1.63±0.58 (0.62-2.47)	1.20±0.90 (0.10-2.44)
Phase 3 (after 2000 mg/day for a week)	No.	15	11	13	13	11	5
	GFR (ml/min)	121.7±22.3 (85-150)	80.3±7.5 (69-96)	55.3±8.1 (42-76)	37.3±5.5 (30-45)	22.5±3.3 (17-27)	12±1.2 (10-13)
	Glycemia (mmol/l)	8.59±2.95 (4.4-14.9)	7.92±1.59 (5.1-10)	8.72±2.73 (4.2-13.2)	7.85±1.67 (5.7-11.3)	7.11±3.40 (2.7-14.5)	9.62±4.16 (6.7-16.6)
	Lactate (mmol/l)	—	—	1.4±0.56 (0.64-2.45)	1.21±0.64 (0.1-2.7)	1.47±0.67 (0.74-2.7)	0.63±0.14 (0.43-0.79)
	Plasma metformin (mg/l)	0.49±0.30 (0.10-1.1)	0.84±0.62 (0.11-2.0)	1.31±0.90 (0.48-3.04)	2.07±1.03 (0.76-4.06)	3.09±1.58 (1.55-7.2)	4.37±1.73 (3.07-7.19)
	Erythrocyte metformin (mg/l)	0.73±0.27 (0.10-1.15)	1.19±0.42 (0.49-1.88)	1.48±0.70 (0.83-3.34)	2.32±0.90 (0.82-4.18)	2.68±0.64 (1.61-3.57)	4.54±2.03 (1.56-6.74)
Phase 4 (after 3000 mg/day for a week)	No.	10	—	—	—	—	—
	GFR (ml/min)	123.8±19 (94-150)	—	—	—	—	—
	Glycemia (mmol/l)	8.77±2.88 (5.6-14.7)	—	—	—	—	—
	Lactate (mmol/l)	—	—	—	—	—	—
	Plasma metformin (mg/l)	0.82±1.09 (0.17-3.88)	—	—	—	—	—
	Erythrocyte metformin (mg/l)	0.90±0.50 (0.25-1.83)	—	—	—	—	—
*: No.=13 ‡: No.=10 ◊: No.=11							

Figure 4.2A. and Figure 4.2B. present plasma and erythrocytes metformin levels according to the study phase and the CKD stage (mean±SD).

Figure 4.2A.

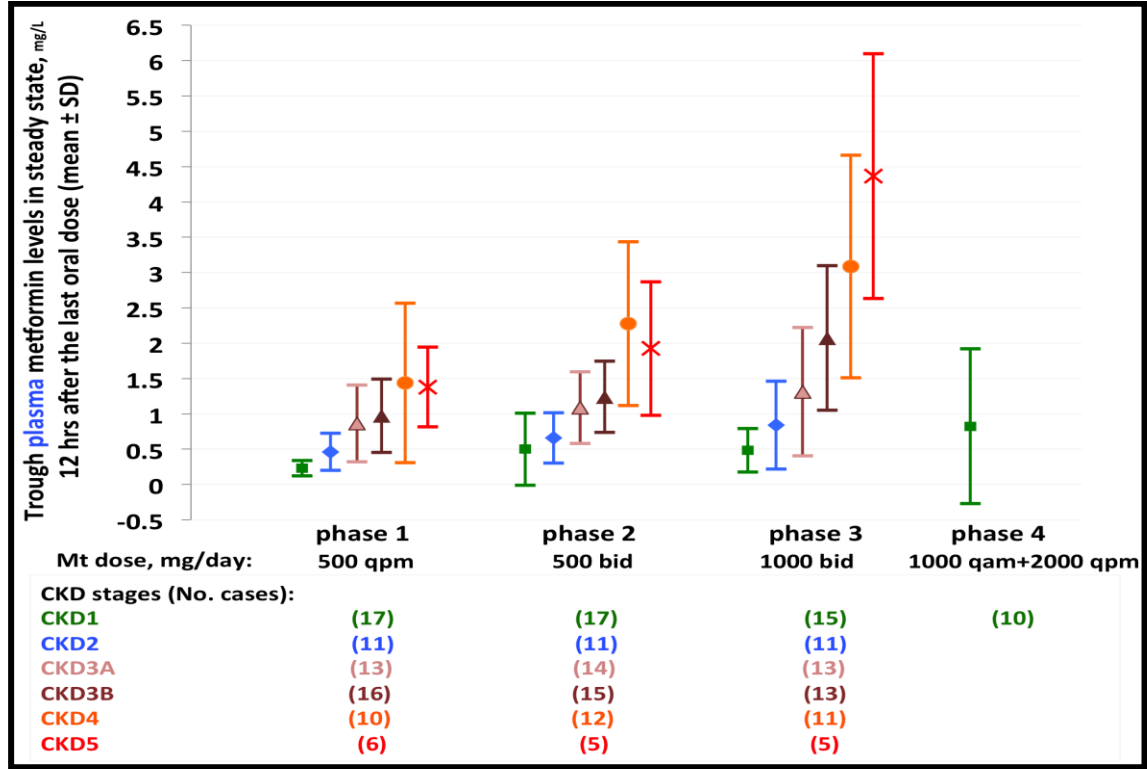


Figure 4.2B.

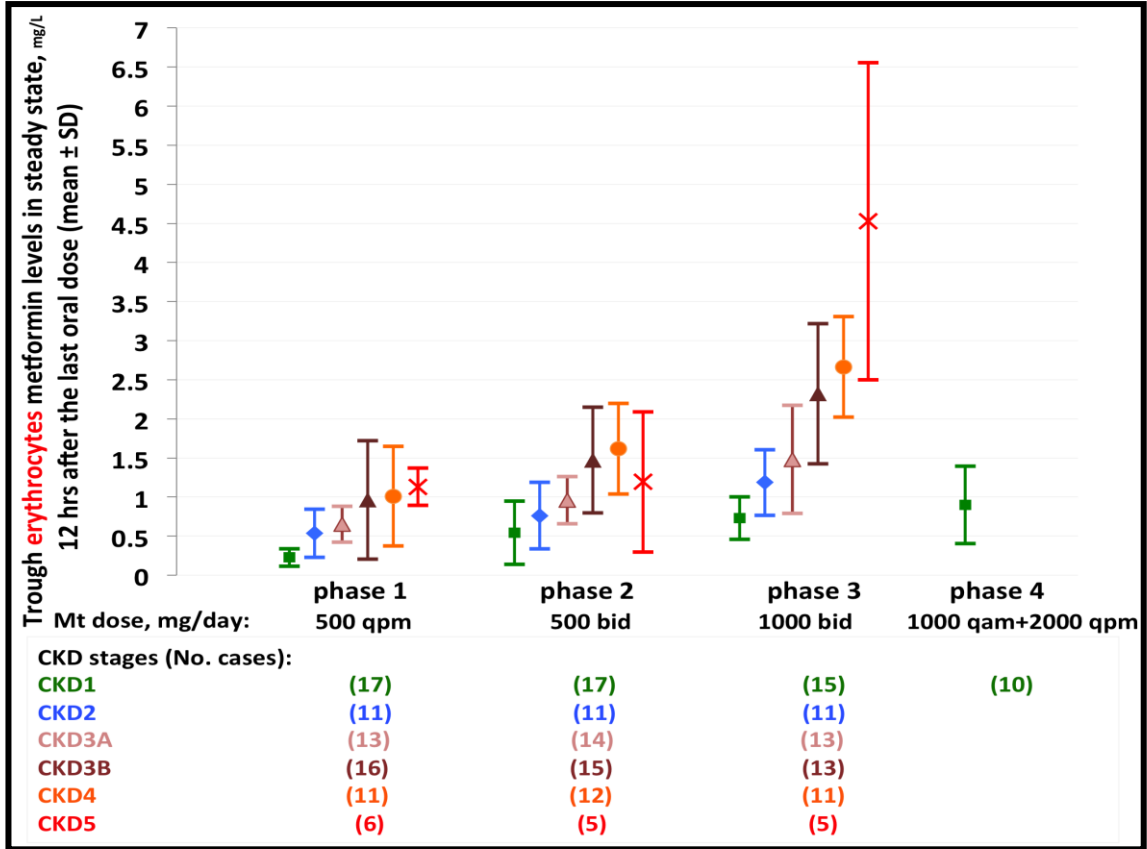


Figure 4.3A. and Figure 4.3B. present the individual levels of metformin per study phase and CKD stage.

Figure 4.3A.

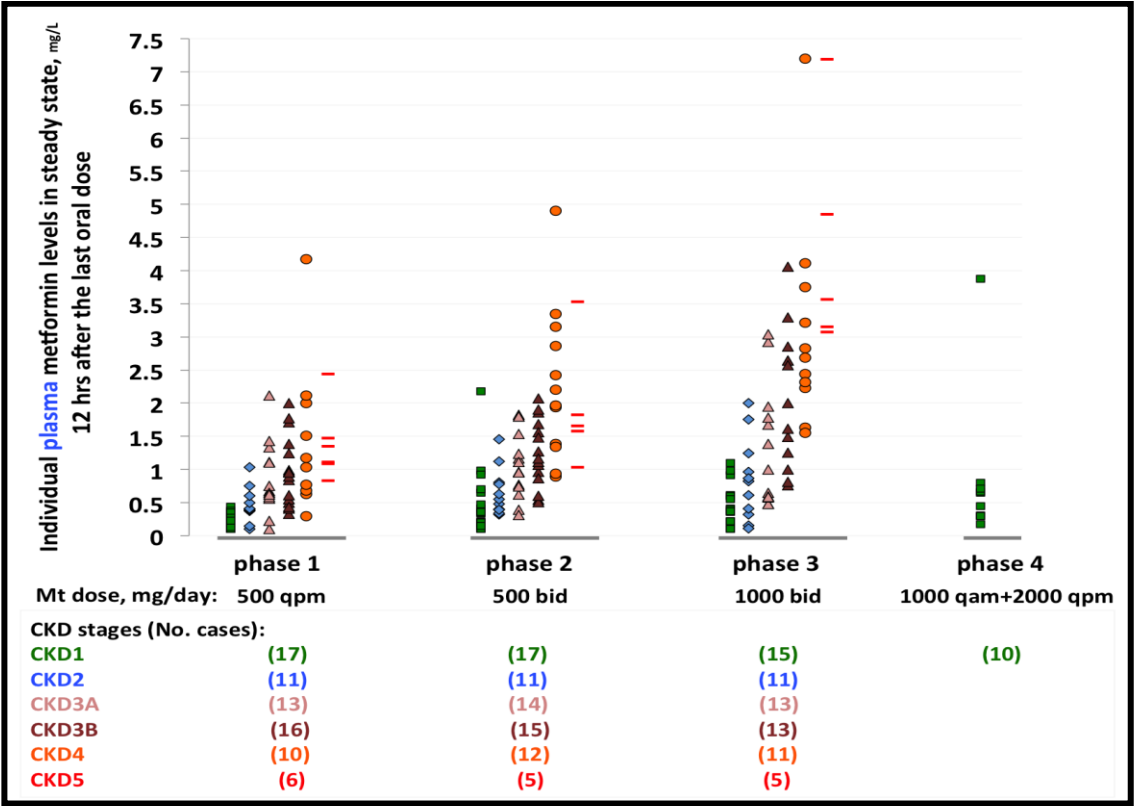
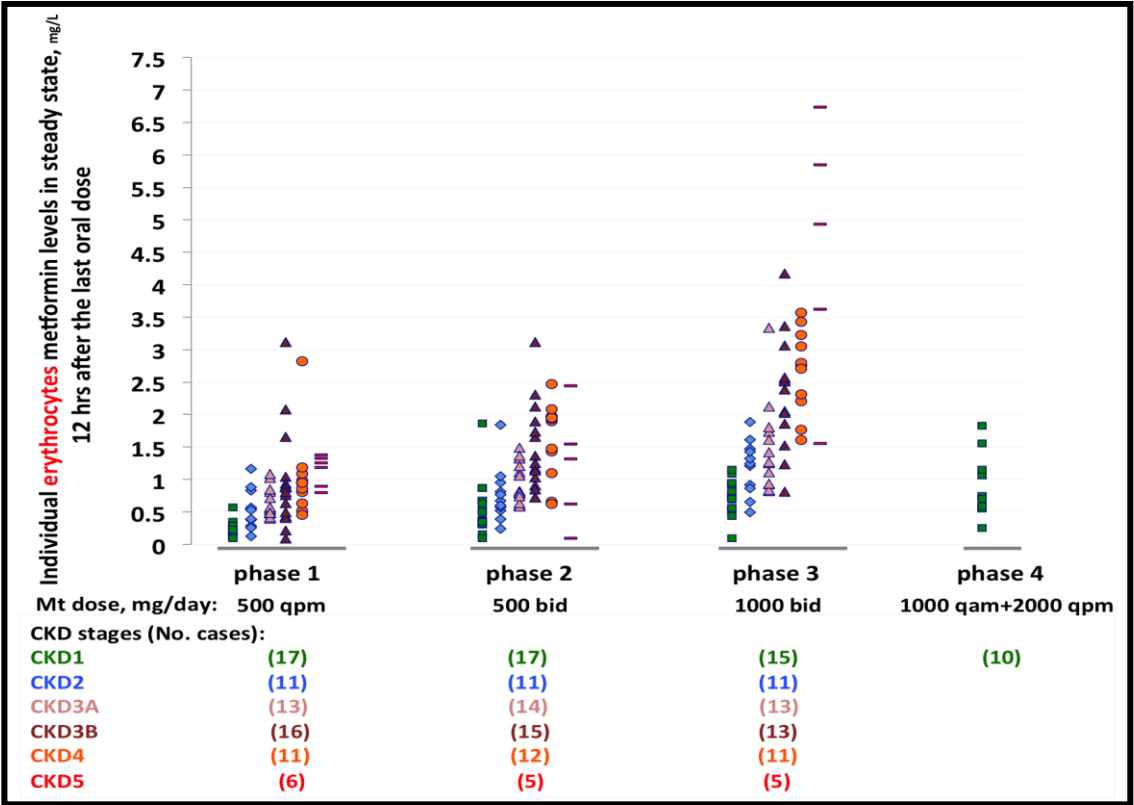
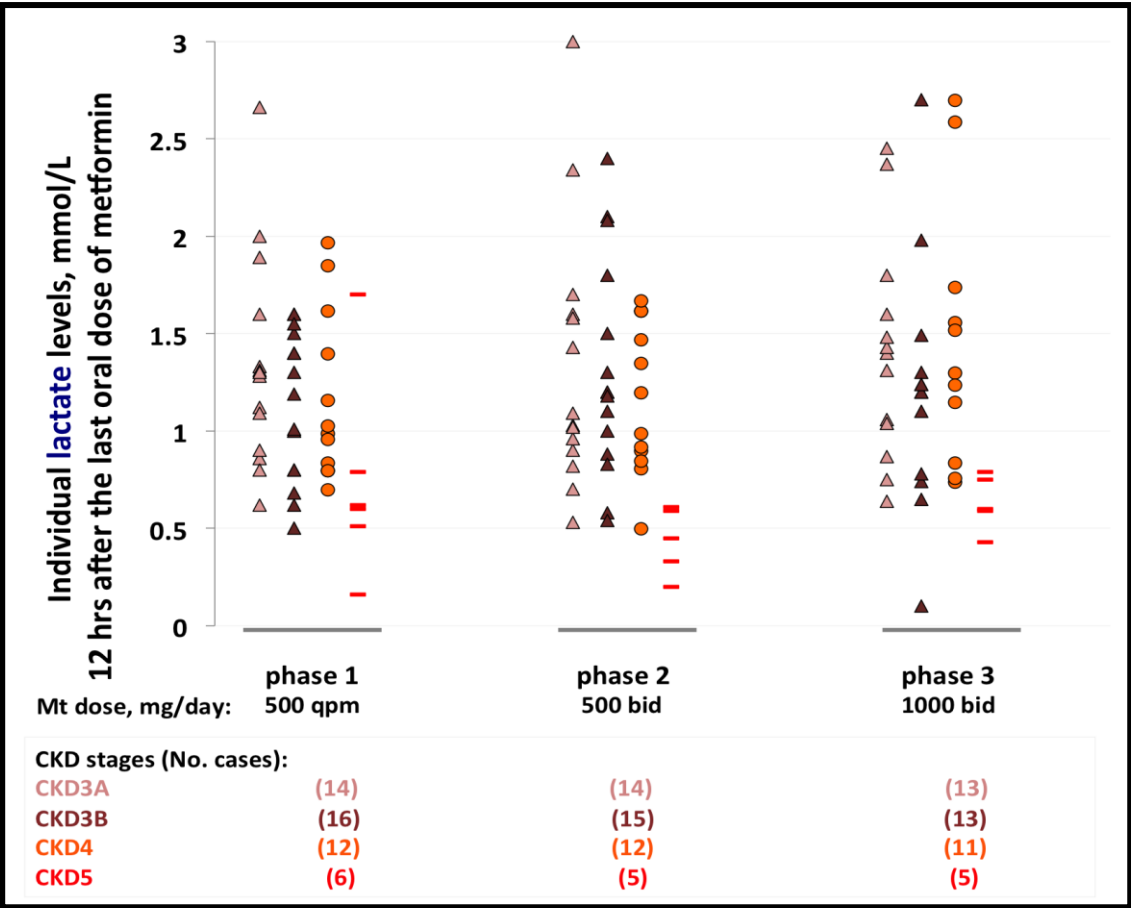


Figure 4.3B.



The maximal metformin levels were 7.2 mg/l in plasma and 6.7 mg/l in erythrocytes (respectively in a CKD4 patient and in a CKD5 patient, both at 2,000 mg/day).

Figure 4.4. Individual lactate levels per study phase in CKD 3A, 3B, 4 and 5.



A total of four patients experienced a lactate level above the criterion of metformin withdrawal. However in those patients the increase in lactate level was very modest. The maximal value was 3 mmol/l in a CKD 3A patient at the end of the phase 2. We have nevertheless decided to continue metformin therapy in the latter because of intolerance to other antidiabetic agents and of the absence of metformin accumulation (plasma metformin level of 1.8 mg/l), and despite this the following lactate value (phase 3) was not above 2.5 mmol/l. Moreover lactate values were not increased in the patients having the highest metformin levels (> 5 mg/l).

Table 4.5. Interphase comparisons of plasma metformin concentration (at increasing metformin dose) per CKD stage (see **Figure 4.2A.**)

CKD stage	Metformin doses	<i>p</i> value
1	500-1000-2000-3000	0.010
2	500-1000-2000	0.251
3A	500-1000-2000	0.470
3B	500-1000-2000	0.005
4	500-1000-2000	0.009
5	500-1000-2000	0.009

Table 4.6. Interphase comparisons of erythrocytes metformin levels (at increasing metformin dose) per CKD stage (see **Figure 4.2B.**)

CKD stage	Metformin doses	<i>p</i> value
1	500-1000-2000-3000	<0.0001
2	500-1000-2000	0.0034
3A	500-1000-2000	<0.0001
3B	500-1000-2000	0.0003
4	500-1000-2000	0.0001
5	500-1000-2000	0.0108

At least for erythrocytes values, in each CKD stage metformin levels were statistically increased in parallel with increasing in metformin dose.

Table 4.7. Intra-phase comparisons of plasma metformin concentration per study phase

PLASMA						
Study phase, Metformin dose (mg /day)	<i>p</i> -values (Mann-Whitney Test)					
	CKD stage	2	3A	3B	4	5
1 (500)	1	0.025	0.002	<.0001	0.001	0.002
	2	—	NS	0.027	0.024	0.008
	3A	—	—	NS	NS	NS
	3B	—	—	—	NS	NS
	4	—	—	—	—	NS
2 (1000)	CKD stage	2	3A	3B	4	5
	1	NS	0.008	0.002	<.0001	0.015
	2		NS	0.022	0.002	0.024
	3A			NS	0.016	NS
	3B				0.046	NS
	4					NS
3 (2000)	CKD stage	2	3A	3B	4	5
	1	NS	0.012	<.0001	<.0001	0.006
	2		NS	0.021	0.001	0.011
	3A			NS	0.016	0.008
	3B				NS	0.039
	4					NS

Table 4.7. Intra-phase comparisons of erythrocytes metformin levels per study phase

ERYTHROCYTES						
Study phase, Metformin dose (mg /day)	p-values (Mann-Whitney Test)					
1 (500)	CKD stage	2	3A	3B	4	5
	1	0.016	<.0001	0.001	<.0001	0.002
	2		NS	NS	NS	0.021
	3A			NS	NS	0.022
	3B				NS	NS
	4					NS
2 (1000)	CKD stage	2	3A	3B	4	5
	1	NS	0.007	<.0001	0.001	NS
	2		NS	0.013	0.010	NS
	3A			NS	0.03	NS
	3B				NS	NS
	4					NS
3 (2000)	CKD stage	2	3A	3B	4	5
	1	0.056	0.002	<.0001	<.0001	0.006
	2		NS	0.010	0.001	0.024
	3A			NS	0.006	0.039
	3B				NS	NS
	4					NS

These tables contains many statistical data. If one focuses on CKD stages 3-5 and on erythrocyte metformin levels, it appears that there was generally no difference between the different groups (3A, 3B, 4, and 5) for each metformin dose.

Discussion

Hitherto, the safety of metformin in patients at different CKD stages had not been evaluated in a prospective study. Hence, we decided to perform the first non-comparative, open-label pilot study (Phase II) of increasing dose levels of metformin in patients at different CKD stages (1-5). Plasma and erythrocyte metformin levels were determined during the treatment period, in order to know whether or not metformin may accumulate. In CKD stage 3-5 patients, metabolic tolerance was checked by assaying the blood lactate concentration.

Each treatment phase lasted for 7 days. This duration was chosen according to the pharmacokinetic characteristics of metformin, with (i) the rapid elimination of metformin in the absence of renal failure [Graham 2011], and (ii) elimination of the drug within 24 hours in severe CKD after administration of a single dose of metformin [Sambol 1995]. We therefore hypothesized that the metformin level obtained after 7 days of treatment corresponds to a steady-state trough concentration.

The CKD stage had to be reclassified in several patients (n = 11). However, this change concerned 6 patients with normal renal function (initially classified as CKD 1-2). Moreover, the change in eGFR

during the study was generally small; in one patient, for example, the initial eGFR value was 28 ml/min (corresponding to CKD4), whereas the values obtained at the end of the study phases were 31, 33, and 32 ml/min (thus corresponding to CKD3B). Sixty-three of the 79 study participants completed the study. The remaining 16 patients stopped taking metformin because of poor gastrointestinal tolerance (n=8, including 6 at a metformin dose of 2,000 mg/day) or for personal reasons (n = 8). None of the discontinuations were related to the criteria for emergency withdrawal of metformin.

We made the following observations: (i) the mean ages were similar in CKD2-5 groups (around 65 years of age) and only CKD1 patients were markedly younger (mean: 52.4) than the others; (ii) mean plasma and erythrocyte metformin levels exhibited the same gradual increase as the metformin dose increased; (iii) the increase in metformin levels was more pronounced in CKD 3B-5 patients; (iv) despite the increase in metformin levels in severe CKD, erythrocytes levels (reflecting a deep-compartment) were not higher to plasma levels; (v) a descriptive analysis of individual metformin levels revealed marked overlap in the range of values in the various patient groups (from CKD1 to 5), although the ranges were particularly broad in CKD 3-5 patients, as early as the first treatment phase, with some peak values of 5 mg/l or more (i.e. accumulated); (vi) the dispersion of metformin levels explains why intergroup differences appear to be less pronounced in moderate to severe CKD; and (vii) in contrast to metformin levels, there was complete overlap of the lactate values when considering the CKD groups (for example, one of the highest individual lactate values was observed at the lowest metformin dose in a patient with normal renal function). *In other words (and even though correlations were not measured), it is clear that there was no correspondence between the highest metformin values and the highest lactate values.*

Defining an upper limit for “therapeutic levels” of metformin and a threshold for “supratherapeutic levels” or even “accumulation” is problematic. As noted in the Introduction, the scientific literature is of no help in this regard because the only valid way of defining the therapeutic concentration window for metformin would be to relate dose-efficacy (in terms of blood glucose control) to the corresponding plasma metformin concentrations in patients on long-term metformin therapy, and this has never been attempted. A pertinent basis for defining the therapeutic concentration of metformin should at least be the range of trough plasma concentrations associated with well-tolerated doses (while taking account of the time interval after metformin administration). To further complicate the debate, sequence polymorphisms in the genes coding for solute carriers significantly modulate metformin's pharmacodynamics and pharmacokinetics [Maruthur 2014, Tzvetkov 2009, Goswami 2014]. A clinical impact of this type of polymorphism (an 80-fold inter-individual variation in plasma metformin level) was observed in a cohort of type 2 diabetes patients taking 1 g of metformin twice a day over a 24-month period [Christensen 2011]. This implies that in a particular

patient, a given metformin concentration might be therapeutic, subtherapeutic or supratherapeutic or might even correspond to metformin accumulation.

The FDA states that “during controlled clinical trials, which served as the basis of approval for metformin, maximum metformin levels did not exceed 5 mg/l, even at maximum doses” [FDA 2013]. However, the studies performed as part of a marketing authorization application necessarily exclude patients with severe CKD. In the present study, two patients showed metformin values > 5mg/l in plasma and in erythrocytes, with a peak value of 7.2 mg/l in plasma and 6.7 mg/l in erythrocytes. However, these values were observed only in 3 patients with severe CKD (CKD4 and CKD5) and at the highest dose of metformin (2000 mg/day), i.e. clearly outside the guidelines. Moreover, despite the high metformin levels these patients did not experience hyperlactatemia; their lactate values were even in the lower half of the normal range (< 1.2 mmol/l).

The short study duration constitutes a major limitation. It is therefore possible that metformin at supratherapeutic levels in CKD patients on short-term therapy may accumulate on long-term. This is why we are performing an additional, 4-month study based on a fixed and adjusted dose of metformin (CKD 3A: 500 mg in the morning and 1000 mg in the evening; CKD 3B: 500 mg in the morning and 500 mg in the evening; CKD 4: 500 mg in the morning). At the end of each month, the GFR and blood metformin and lactate levels are measured. HbA1C is measured at time 0 and at the end of month 4. The study will conclude with a 12-hour pharmacokinetic study in subgroups of 3 patients.

Conclusion

In short-term metformin therapy conducted in all stages of CKD patients, the rather linear metformin dosage/level relationship (observed without significant hyperlactatemia) forms a solid base to validate on a longer term the adjustment of the metformin dosage for continuing metformin in moderate to severe CKD in safe conditions.

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General discussion

The set of studies described here had an important objective: to develop a practical guide for the use of metformin therapy in CKD. We sought to resolve the paradox whereby on one hand, metformin should be prescribed more widely because of its beneficial, pleiotropic effects; on the other, it should be prescribed less widely because an increasing proportion of diabetic patients have poor renal function.

To achieve this objective, several different domains have been addressed (epidemiology, diagnosis, pathophysiology, prognosis, and therapies) in order to obtain an overall view of this issue. Nevertheless, since it is quite clear that the scientific literature suffers from major shortcomings; the present work corresponds to “deconstruction” rather than brick-by-brick construction.

The critical analysis of the so-called “metformin-induced lactic acidosis” (MILA) and of “metformin-associated lactic acidosis” (MALA) was a prerequisite to the study of metformin therapy in CKD. It should be remembered that metformin may accumulate because of defective elimination or excessive intake. In fact, overdose should be considered as the only cause of “pure” MILA. Indeed when metformin accumulates because of kidney disease, impairment in renal function has *per se* a negative impact on lactate clearance and the acid-base equilibrium. Hence, “metformin-induced lactic acidosis” is exceptional, since isolated metformin overdoses are rare.

With regard to MALA, literature analyses of published reports are purely quantitative, whereas the issue is qualitative. Although it is already difficult to specify the actual prevalence/incidence of MALA in clinical practice, it is even more difficult to avoid falling into the trap represented by the following syllogism: MALA is rare but is nevertheless potentially fatal; metformin can accumulate; metformin is therefore potentially dangerous. In straightforward terms, the key question is whether metformin accumulation is life-threatening or not (regardless of the frequency of MALA).

In summary, we have shown that:

- The great majority of cases denoted as “metformin-associated lactic acidosis” in the largest pharmacovigilance database available were not comprehensively described [Kajbaf 2013A];
- The overall mortality rate associated with MALA fell from around 50% in the 1960s to around 25% at present [Kajbaf 2014];
- When compared with common forms of lactic acidosis, severe MALA is particular in that the majority of patients survive – despite a mean pH that is usually thought to be fatal. For this type of MALA patient, the outcome is related to the nature and number of triggering factors, rather than the severity of lactic acidosis or the extent of metformin accumulation *per se* [Kajbaf 2013B];

- By applying what was probably a pragmatic metformin dose reduction in our center's patients with decreased eGFR, we found that elevated metformin values were rare and never encountered true metformin accumulation [Briet 2012];
- When considering metformin accumulation, metformin levels are generally measured many hours after patients' admission. Given this delay, the patient's metabolic status (i.e. the presence or the absence of lactic acidosis) had changed by the time the metformin assay is performed in half the cases studied -even though metformin accumulation may still be persisted [Lalau 2014];
- The rapid initial elimination of metformin (especially from the plasma) following accumulation of the drug does not rule out a long terminal $t_{1/2}$. Indeed, the drug could be detected in plasma for at least 2 weeks, and even after dialysis [Kajbaf 2015].

In general, it is hard to believe that more than half a century after the most common antidiabetic drug became available (i.e. a drug prescribed in one of the most common debilitating diseases), the concept of MALA is still common misunderstood.

The literature about MALA is indeed weakened by the following major errors: blood metformin levels (and especially erythrocyte levels) are rarely measured; there is significant confusion about the information provided by the assessment of metformin levels, since subnormal levels are generally not clearly distinguished from major metformin accumulation capable to induce hyperlactatemia; the time frame for metformin measurement is never taken into consideration; the prognostic significance of the metabolic disorders is poorly understood; the role of associated pathologies (such as cardiovascular failure, sepsis, hemorrhage, hepatic failure, pulmonary failure, etc.) is insufficiently explored; little attention is given to liver failure (i.e. the liver's critical ability to remove excess lactate); lastly, failure to consider an unexpected, favorable outcome of some MALA patients under very severe conditions - leading to the challenging hypothesis whereby metformin might even protect against lactic acidosis!

All these shortcomings can be summarized as follows: MALA is necessary misclassified since it is generally presented as a single-factor event. Likewise, the mortality in MALA is still expressed as a mean value.

Although the above discussion concerns metformin, the debate should be broadened to the entire pharmacopoeia. Indeed, metformin therapy should never be judged in isolation but should be compared with the different treatment options available to the diabetologist. We need to assess an all-inclusive, objective, global benefit/risk ratio. In this regard, the number of serious or even dramatic events associated with the "direct competitor" of metformin (sulfonylureas) is impressive: the increase in mortality due to hypoglycemia (in particular in the elderly), and the increase in

cardiovascular events due to alteration of the ischemic preconditioning effect (for non-selective sulfonylureas). Moreover, the reported increase in the risks of weight gain, cardiac events, and bone loss with glitazones [Nissen 2007, Habib 2010] is of concern, and the debate about the risk of cancer associated with the administration of sulphonylureas or insulin is not completely over [Monami 2007, Draznin 2010]. In contrast, a growing body of evidence shows that metformin provides pleiotropic benefits in diabetic patients who are free of the above-mentioned complicating conditions.

The above reasoning is particularly important in fragile CKD patients. It should be borne in mind that MALA occurs more frequently in acute renal failure patients (up to three times more frequently, according to Lalau et al.: Drug Safety 1999 [Lalau 1999]) and that metformin can be considered to be a vasoprotective drug with antidiabetic properties and a long list of possible clinical benefits [Wiernsperger 2007, Lalau 2010]. The list of the proposed mechanisms of vascular protection conferring clinical benefits is indeed impressive [Lalau 2010]. This is certainly why some researchers have suggested that metformin can be used safely in CKD [Adam 2014, Nye 2011, McCormack 2005]. This viewpoint has been reinforced by the observed efficacy and safety of metformin in a four-year study of a large cohort of patients at varying CKD stages [Ekström 2012]. Compared with other oral antidiabetic agents, metformin was associated with lower all-cause mortality and a lower risk of cardiovascular disease, acidosis and serious infections (one of the study's endpoints). It is noteworthy that these beneficial effects were consistently found in patients with eGFR values ranging from 45 to 60 ml/min/1.73 m², who did not display an increase in the risk of acidosis/infection. These results are also in line with those of an observational study of almost 20,000 diabetic patients and either established cardiovascular disease or the presence of several cardiovascular risk factors. Relative to other antidiabetic treatments, metformin significantly lowers the risk of all-cause mortality after nearly 2 years [Roussel 2010].

The results of our prospective study (the only one of its kind, at present) support the use of metformin in CKD provided the metformin dose is adjusted to the renal function since elevated or even accumulated values were observed in severe CKD. Given that this was a short-term study, the findings should be considered with a degree of caution; however, the ongoing 4-month study should reinforce our conclusions. This is a follow-up study, performed with a fixed dose of metformin according to the CKD stage: CKD stage 3A (GFR: 59-45): 500 mg in the morning, 1000 mg in the evening; CKD 3B (GFR: 44-30): 500 mg in the morning, 500 mg in the evening; and CKD 4 (GFR: 29-15): 500 mg in the morning. At the end of each month, GFR, blood metformin levels, and lactate levels are measured. HbA1C is measured at the start and end of the 4-months trial period. The preliminary results (obtained with around 20 patients) are encouraging: metformin levels remain stable during the study period in the usual range in therapeutic conditions (i.e. below 5 mg/l), and

lactate levels were not significantly elevated. Hence, our pragmatic dose-adjustment approach appears to be relevant when assessed *a posteriori*. This means that ultimately metformin could be used in moderate-to-severe CKD – as long as renal function remains stable.

Once again, it is surprising that (i) official guidelines still reason in terms of thresholds for the withdrawal of metformin, (ii) a guide to dose adjustment has never been proposed (as is usually the case for a drug excreted by the kidney), (iii) emphasis has been placed on the severity of CKD rather than the stability of renal function, and (iv) a prospective study in CKD has never been performed.

Our reasoning is as follows: metformin dose must be reduced as renal function declines - not primarily because metformin would otherwise be a toxic drug but merely because it is possible to maintain the therapeutic efficacy with a lower dose. This has the advantage of facilitating compliance, given that CKD patients generally receive many different treatments.

Lastly, we would not have fully achieved our goal if we had not tried to understand why dose adjustment guidelines for metformin have never been developed (as has always been done for other drugs eliminated by the kidneys). For example, the dose of an aminoglycoside is always adjusted to match the patient's renal function, on the basis of a practical guide.

There are several possible reasons (which are not mutually exclusive) for the lack of guidelines. Firstly, there is a strong class effect. Metformin has inherited the poor image of phenformin. This early biguanide was indeed associated with frequent, severe and even fatal lactic acidosis, with a clear temporal relationship between the initiation of treatment and the occurrence of lactic acidosis. However, although phenformin and metformin belong to the same family, the differences in structure mean that the two drugs do not have the same effects on metabolism and the cardiovascular system. Secondly, it is more difficult to publish conceptual work than publications that have a “hard” methodology. Thirdly, and perhaps more importantly, since metformin is an old drug, no appropriate studies were performed at the time of coming on the market. Moreover, once a drug becomes generic pharmaceutical companies are no more interested in doing such (expensive!) studies.

In conclusion, we should clearly avoid metformin accumulation in CKD. It is however difficult to maintain the view according to which metformin is a true *pharmakon* (i.e. a substance acting both as a remedy and a poison).

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Conclusion

“Lactic acidosis associated with metformin therapy” and “metformin use or contraindications in chronic kidney disease (CKD)” are two inseparable issues. Indeed these two facts that “metformin is normally well eliminated by the kidney” and “lactic acidosis can be induced by severe metformin accumulation” strongly influence treatment strategies in type 2 diabetes patients with CKD and in those at risk of kidney failure consequently in the elderly.

At this stage, we would rephrase the debate “concerns in proposing metformin in CKD while assuming an acceptable risk level” into “possibility of continuation of metformin therapy in stable CKD patients, with an adjusted dose, as a defensible therapeutic choice (compared to the risk of switching to drugs having their own side effects and contraindications)”. In fact, such fragile patients should not be deprived from the beneficial effects of metformin, after having informed of the risk factors (diarrhea, vomiting, severe infection, etc) for developing metformin accumulation.

The results of our prospective short-term study of metformin in all CKD stages show a progressive dose-related increase of the trough levels of metformin with supra-usual to even accumulated values in severe CKD (without significant hyperlactatemia however in those cases).

We are therefore waiting for the results of a complementary, follow-up study, conducted over 4 months, with this time *a fixed and adapted dose* to the renal function of metformin to provide a solid basis for the adoption of metformin therapy in moderate to severe CKD.

The next step consists in the exploration, based on experimental and clinical studies, what will be the impact of metformin once introduced in treatment of CKD patients concerning vascular protection, atherothrombosis, endothelial function, calcifications etc....

Summary

Although metformin is acknowledged as having a pivotal role in the treatment of type II diabetes mellitus, there is still a debate concerning the drug's beneficial and adverse effects. The purpose of the present work was three-fold: (i) To perform a critical, qualitative examination of reports of so-called MALA, with a focus on the criteria for the definition of MALA, the prognostic value of the latter criteria in severe MALA, and the mortality rate; (ii) To examine the advantages and limitations of measuring metformin concentrations in blood (plasma and erythrocytes) when screening for metformin accumulation and assessing its extent and consequences; and (iii) to conduct an open-label pilot study with metformin therapy in patients at all chronic kidney disease (CKD) stages, given the absence of this type of study in the literature. Patients underwent 1 week-blocks of metformin treatment at an increasing dosage (each block followed by a 1 week-wash-out period). Metformin levels were measured in plasma and in erythrocytes (the tissue compartment) according to metformin dosage and CKD severity. Lactate levels were also measured. A progressive dose-related increase of the trough metformin levels was observed, leading to some accumulated values in severe CKD. There was however no case of significant hyperlactatemia. Our conclusion is that it may be possible to continue metformin therapy under safe conditions in patients with CKD (and even severe CKD), provided that the metformin dose is appropriately adjusted. We are waiting for the results of a complementary, follow-up study, conducted over 4 months, with a fixed and adapted dose to the renal function of metformin to provide a solid basis for the adoption of metformin therapy in CKD.

Resume in French

Metformine dans l'insuffisance rénale : réévaluation du rapport bénéfices/risques

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Introduction

La metformine est reconnue comme le traitement de première intention dans le diabète de type 2. Pour autant, le débat relatif à la balance bénéfice/risque demeure très vif, concernant en particulier le sujet insuffisant rénal.

Le but du présent travail était quadruple :

- 1) Mener une étude critique des données de la littérature relatives aux « taux thérapeutiques de metformine » et des critères de contre-indication à la metformine relatifs à l'insuffisance rénale (**Parties 1 et 2**) ;
- 2) Mener une étude critique des rapports d'acidose lactique associée à la metformine, et plus précisément de ses critères, de la signification pronostique de ces critères, et du taux de mortalité (**Parties 3, 4, et 5**) ;
- 3) Examiner l'intérêt et les limites du dosage de la metformine dans le plasma et les érythrocytes dans le dépistage d'une accumulation et dans l'évaluation du degré et des conséquences d'une telle accumulation (**Parties 6 et 7**) ;
- 4) Réaliser une première étude prospective de traitement systématique par metformine à dose croissante dans tous les stades d'insuffisance rénale (**Partie 8**).

Partie 1. Connaît-on les taux thérapeutiques de metformine?

BUT

La metformine est vieille de plus d'un demi-siècle, mais connaît-on pour autant ses taux thérapeutiques ; et ce de façon consensuelle ? C'est ce que nous avons cherché à savoir en interrogeant la littérature.

METHODES

Nous avons réalisé une étude systématique à partir des bases de données (PubMed, Scopus, etc.) à l'aide des mots clés suivants: 'therapeutic concentrations' (ou 'range' ou encore 'normal level'), depuis la disponibilité de la metformine, en retenant les documents proposant une valeur ou un intervalle de valeurs. Dans les cas où une proposition renvoyait à une référence antérieure, nous avons étudié la concordance entre les données de la publication et celles de la référence.

RESULTATS

120 documents ont été identifiés (parmi plus de 900), proposant au total 65 valeurs ou intervalles de valeurs différents. Les valeurs varient de 0,129 à 90 mg/l ; les intervalles varient de 0 et 1800 mg/l, le plus étroit étant de 0,000225 à 0,003 mg/l et le plus large de 150 à 1800 mg/l. La plupart des valeurs s'inscrivent toutefois dans la fourchette de 0,1 à 4 mg/l. Les propositions émanaient d'une étude originale dans seulement 4 cas ; aucune référence n'était citée dans 53% des cas. Dans les publications renvoyant à une référence antérieure, une concordance des données est notée moins d'une fois sur deux (dans 41,6% des cas). Enfin, les publications ou les références citées étaient généralement des articles de revue, des travaux de pharmacocinétique, ou encore des études in vitro, mais non des travaux conçus pour caractériser des « taux thérapeutiques ». Aucun travail n'affichait d'ailleurs un tel objectif.

CONCLUSION

De façon surprenante, au regard de l'ancienneté de la metformine, non seulement les « taux thérapeutiques » ne sont toujours pas connus, mais la littérature s'avère même particulièrement incohérente à ce sujet.

- **Kajbaf F**, De Broe ME, Lalau JD. "Therapeutic concentrations" of metformin: a systematic review.
Clin Pharmacokin (accepted for publication)

Partie 2. Contre-indications de la metformine dans l'insuffisance rénale : état des lieux à l'échelle mondiale.

BUT

Si le degré de sévérité de l'insuffisance rénale devant faire cesser le traitement par metformine ne fait pas l'unanimité, des positions dominantes pourraient malgré tout être dégagées. C'est ce que nous avons recherché en passant en revue les contre-indications au traitement par metformine à travers le monde.

METHODES

Nous avons colligé les informations disponibles selon trois types de source : 1) les recommandations ou les directives émanant de différents organismes (la Fédération Internationale du Diabète, les Agences nationales et internationales du médicament, les associations nationales de diabétologie et de néphrologie, les ministères de la santé, les associations nationales des pharmaciens, les formulaires nationaux des médicaments), cela pour les 20 pays les plus peuplés et quelques autres aussi (Afrique du Sud, Australie, Canada, Nouvelle-Zélande, Royaume-Uni, et Suisse) ; 2) les prises de positions publiées dans des revues internationales ; et 3) les études d'intervention dans l'insuffisance rénale publiées et accessibles via PubMed.

RÉSULTATS

L'analyse des informations, colligées à partir de 46 sources au total, montre une hétérogénéité à la fois de la nature des critères de contre-indication (pas de critère chiffré : 41,5 %, créatininémie 24,4 %, clairance de la créatinine 34,1%), et des degrés pour un même critère. Pour la clairance de la créatinine, par exemple, les seuils proposés varient de 60 à 30 ml/min/1,73 m². Quatre publications adoptent un raisonnement différent en prônant une adaptation de la posologie de metformine à la fonction rénale. Un élément n'est jamais pris en compte : le caractère stable, ou pas, de la fonction

rénale. Enfin, une seule étude d'intervention est disponible dans l'insuffisance rénale (une étude réalisée chez le sujet âgé).

CONCLUSIONS

Une telle hétérogénéité des critères de contre-indication de la metformine rend un consensus international nécessaire, idéalement sur la base d'une étude prospective de traitement à posologie ajustée à la fonction rénale.

Nous parvenons à la conclusion essentielle suivante : contrairement aux autres médicaments éliminés par le rein, les recommandations ne sont pas celles d'un ajustement de la posologie de metformine selon la fonction rénale.

- **Kajbaf F**, Arnouts P, de Broe M, Lalau JD. Metformin therapy and kidney disease: a review of guidelines and proposals for metformin withdrawal around the world.

Pharmacoepidemiol Drug Saf. 2013 Oct;22(10):1027-35.

Partie 3. Critères d'acidose lactique associée à la metformine : qualité des rapports de pharmacovigilance dans une base de données mondiale.

BUT

Le but était d'étudier la qualité des rapports de pharmacovigilance d'acidose lactique associée à la metformine, et plus précisément de savoir si les critères pour un tel événement étaient réellement remplis, ou pas nécessairement.

METHODES

Nous avons recherché les critères d'acidose lactique associée à la metformine (pH artériel < 7,35, lactatémie > 5 mmol/L, et metforminémie au moins détectable sur une période de 15 ans (de 1995 à 2010) dans la base de données mondiale du fabricant de la metformine (Merck Serono).

RESULTATS

Nous avons identifié 869 rapports « d'acidose lactique associée à la metformine », issus de 32 pays différents. Parmi ces rapports, les critères d'acidose, d'hyperlactatémie, et de metforminémie ont été remplis dans 51,2%, 53,3%, et 13,9% des cas ; et dans 10,4% des cas seulement pour les 3 critères à la fois. Au long de la période d'étude, la qualité des rapports ne s'est pas améliorée.

CONCLUSIONS

Les critères d'imputabilité de la metformine dans l'acidose lactique ne sont pas pris en compte dans la grande majorité des cas déclarés, et ce sans qu'il y ait d'amélioration de la qualité des rapports au fil du temps.

- **Kajbaf F.,** Lalau JD. The criteria for metformin-associated lactic acidosis: the quality of reporting in a large pharmacovigilance database.
Diabet Med. 2013 Mar;30(3):345-8.

Partie 4. Taux de mortalité dans l'acidose lactique associée à la metformine: une revue des données depuis la disponibilité de la metformine.

BUT

Le but était d'étudier le taux de mortalité dans l'acidose lactique associée, de la disponibilité de la metformine (en 1957) à la metformine à nos jours, afin si ce taux a pu varier au long de cette longue période d'étude, et si oui dans quelle proportion.

METHODES

Nous avons étudié de types de source : les séries de la littérature scientifique comprenant au moins 20 cas, et la base de données mondiale du fabricant de la metformine (Merck Serono). Nous avons retenu les cas pour lesquels l'information sur la mortalité était disponible.

RESULTATS

Nous avons identifié 12 séries dans la littérature (jusqu'en 1993), avec un nombre moyen de cas de 51,4 et 722 cas dans la base de données de pharmacovigilance (de 1995 à 2010). De façon générale, les cas déclarés ne remplissaient pas les critères « d'acidose lactique associée à la metformine ». Dans la littérature, la mortalité est apparue variable, avec des écarts de 3 à 61% (avec un pH moyen lui-même très variable : de 6,89 à 7,20) mais globalement aux environs de 50 % pour la période des premières années à 1993, et de 25% pour les années ultérieures. Le taux de mortalité s'est réduit de la même façon d'un facteur 2, d'environ 50% à 25%, dans la période d'étude, et ce alors même que le pH est, lui, demeuré relativement stable au fil des années autour de 7.0.

CONCLUSIONS

Il est régulièrement dit que la mortalité moyenne dans l'acidose lactique associée à la metformine est d'environ 50%. Cela ne s'avère plus vrai de nos jours, la mortalité s'étant au contraire réduite d'un facteur 2.

- **Kajbaf F**, Lalau JD. Mortality rate in so-called "metformin-associated lactic acidosis": a review of the data since the 1960s.

Pharmacoepidemiol Drug Saf. 2014 Nov;23(11):1123-7.

Partie 5. Valeur pronostique du pH et de la lactatémie artérielle et de la metforminémie plasmatique dans l'acidose lactique associée à la metformine sévère.

BUT

La mortalité dans l'acidose lactique associée à la metformine est jugée avec des niveaux d'acidose, d'hyperlactatémie, ou de metforminémie extrêmement variables. Il importe donc de savoir ce qu'il en est plus spécifiquement dans l'acidose lactique sévère.

METHODES

A partir de la base de données de pharmacovigilance mondiale du fabricant de la metformine (Merck Serono), nous avons sélectionné les cas d'acidose lactique associée à la metformine sur les critères suivants : un taux de pH artériel $< 7,0$, une lactatémie > 10 mmol/L, une metforminémie plasmatique disponible, et l'information sur le pronostic vital également disponible. Le pronostic a secondairement été comparé à celui qui a été rapporté dans l'acidose lactique indépendante de la metformine (Friessecke, 2010).

RESULTATS

56 patients remplissaient les critères d'étude. Leurs valeurs moyennes de pH et de lactate étaient de $6,75 \pm 0,17$ et de $23,07 \pm 6,94$ mmol/L, respectivement. Le taux global de survie était de 53%, même avec un niveau de pH aussi bas que 6,5 et une lactatémie et une metforminémie aussi élevées que 35,3 mmol/L and 160 mg/L (normal < 1 mg/L), respectivement. Les taux moyens de pH et de lactatémie ne différaient pas entre les sous-groupes selon la survie ou le décès. La metforminémie moyenne est apparue plus élevée chez les patients décédés mais la différence tenait uniquement à une valeur très élevée (188 mg/L, de surcroît chez un patient combinant des pathologies sévères). Chez les patients décédés, les états septiques, les intoxications multiples, et les pathologies

combinées étaient plus fréquentes, comparativement aux survivants ($p = 0,007$; $0,04$; et $0,005$, respectivement). Enfin, nos données contrastent avec celles observées dans l'acidose lactique indépendante, où il n'y avait pas de survivant avec un pH moyen pourtant moins bas que dans notre série (pH: 6,86).

CONCLUSION

Dans cette série d'acidose lactique sévère associée à la metformine, les taux de pH et de lactate n'ont pas de valeur pronostique. On peut raisonnablement exclure aussi une signification pronostique du taux de metformine. Le pronostic apparaît en définitive dépendant de la sévérité et du nombre des pathologies générales. De façon frappante, de nombreux patients ont pu survivre malgré des niveaux de pH qui paraissent incompatibles avec cette survie en dehors du traitement par metformine.

- **Kajbaf F.**, Lalau JD. The prognostic value of blood pH and lactate and metformin concentrations in severe metformin-associated lactic acidosis.

BMC Pharmacol Toxicol. 2013 Apr 12; 14(1):22.

Partie 6. Accumulation de metformine: impact du retard pour prélever la metformine sur l'évaluation du statut métabolique.

BUT

Le dosage de la metformine n'étant pas la première préoccupation dans un contexte d'urgence, nous avons voulu décrire le statut métabolique en prenant en compte le facteur temps pour le dosage de la metformine.

PATIENTS ET METHODES

Nous avons sélectionné nos cas selon trois critères : une accumulation de metformine (valeur plasmatique > 5 mg/l) ; une gazométrie disponible à l'admission ; une gazométrie et une metforminémie plasmatique et érythrocytaire mesurées simultanément, que ce soit à l'admission ou au décours. Nous avons ensuite cherché à savoir si une acidose lactique était présente ou absente au moment de ces prélèvements.

RÉSULTATS

Nous avons recensé 17 cas concernant 16 patients, dont 11 ont présenté une acidose lactique. L'intervalle de temps entre le prélèvement de l'admission et celui réalisé pour doser la metformine était de $12,5 \pm 16$ heures. La gazométrie et la metforminémie ont été dosées à l'admission dans 4 cas seulement (dont 1 avec acidose lactique). Chez les 10 patients présentant une acidose lactique à l'admission et pour lesquels la metformine a été dosée avec retard, cette anomalie métabolique n'était plus notée au moment du dosage de la metformine dans 4 cas. Réciproquement, un cas acidose lactique est apparu au décours de l'admission. Dans les retards les plus importants pour doser la metformine (de 39 à 52 h), les taux de cette dernière étaient encore élevés.

CONCLUSION

Ces résultats incitent à interpréter prudemment le lien entre l'accumulation de metformine et l'acidose lactique dans les rapports de la littérature ou de la pharmacovigilance : l'acidose lactique a généralement disparu au moment où l'accumulation de metformine a été documentée.

- Lalau JD **Kajbaf F.** Interpreting the Consequences of Metformin Accumulation in an Emergency Context: Impact of the Time Frame on the Blood Metformin Levels.

Int J Endocrinol, Volume 2014. Doi: 10.1155/2014/717198

Partie 7. Etude de l'élimination de la metformine dans les cas d'accumulation.

BUT

La demi-vie de la metformine étant brève dans le plasma chez le sujet sain (inférieure à 3 h), nous avons cherché à savoir si la décroissance du taux plasmatique est rapide aussi, ou pas nécessairement, en cas d'accumulation.

PATIENTS ET METHODES

Nous avons passé en revue tous les dosages de metformine effectués dans notre établissement et retenus ceux répondant aux critères suivants : une metforminémie plasmatique ≥ 5 mg/l ; la détermination à au moins deux reprises de la metformine dans le plasma et dans les érythrocytes avec un intervalle d'au moins 5 jours entre la première détermination à l'admission et la dernière (le traitement par metformine étant nécessairement suspendu à l'admission).

RESULTATS

Nous avons retenus 12 patients répondant aux critères, tous sauf un avec une acidose lactique sévère et traités par dialyse. Le taux initial de metformine était de 6 à 88 mg/l dans le plasma (valeur normale pour notre laboratoire : $< 1,35$ mg/l). Le délai entre le premier et le dernier prélèvement sanguin a été de $8,3 \pm 0,9$ jours en moyenne et de 14 jours au maximum. Au 5ème jour, la metformine était détectable chez tous les patients, dans les érythrocytes et aussi dans le plasma ; cette détection était encore possible dans ces 2 compartiments à J13. Les demi-vies terminales moyennes ont été estimées à 52 h dans le plasma et à 43 h dans les érythrocytes, respectivement.

CONCLUSIONS

L'élimination prolongée de la metformine accumulée, même après traitement par dialyse, remet en cause l'idée commune selon laquelle la molécule est rapidement éliminée du plasma en raison de sa

demi-vie brève ; et suggère fortement un phénomène de navette entre le plasma et un ou plusieurs compartiment(s) profond(s).

- **Kajbaf F**, Lalau JD, Bennis Y, Lemaire-Hurtel AS, Andréjak M. Unexpectedly long half-life of metformin elimination in cases of metformin accumulation.

Diabetes Metab. (accepted for publication)

Partie 8. Traitement par metformine dans l'insuffisance rénale.

BUT

Nous avons mené une étude pilote dans le diabète de type 2 aux différents stades d'insuffisance rénale afin de savoir s'il est possible de poursuivre le traitement par metformine dans l'insuffisance rénale dans des conditions de sécurité.

METHODES

Il s'agit d'une étude ouverte des taux de metformine dans le plasma et les érythrocytes mesurés au terme d'une semaine de traitement à dose progressive de metformine (mg/jour) : 500 ; 1000 (500 matin et soir) ; 2000 (1000 matin et soir) ; et, pour le stade 1 d'insuffisance rénale seulement, 3 000 (1000 matin et 2000 soir). Chaque semaine de traitement est entrecoupée d'une semaine de « wash-out ».

Phase	1	wash-out	2	wash-out	3	wash-out	4 (seulement stade 1)
Durée de traitement, semaines	1	1	1	1	1	1	1
Posologie de metformine, mg/j	500	–	500 x 2	–	1 000 x 2	–	1000 + 2000

Critères de sélection

Critères d'inclusion :

- Patients non-diabétiques ou diabétiques non traités par metformine, ne recevant aucun traitement antidiabétique ou déjà traités mais mal équilibrés (HbA1c > 6.5%) et requérant un

renforcement par metformine ; à tout stade d'insuffisance rénale stable (stades 1 à 5 selon la classification MDRD).

- Patients âgés de 18 à 80 ans ;
- Patients disposant d'un bilan de la fonction rénale datant de 3 mois au maximum ;
- Patients dont la fonction rénale est stable, sur le critère de l'absence de fluctuation de plus de 30% de la fonction rénale de la clairance de la créatinine dans les trois derniers mois ; une référence de la fonction rénale d'au moins 3 mois par rapport au bilan d'inclusion est donc nécessaire.

Critères de non inclusion :

- Absence de référence antérieure de créatinine ;
- Fluctuation de plus de 30% de la fonction rénale (clairance de la créatinine) durant les trois derniers mois ;
- Insuffisance hépatique sévère (stade Child > A) ;
- Absence de référence de bilan hépatique pour les stades 3-5
- Les patients devant faire l'objet d'une radiographie avec injection de produit de contraste.

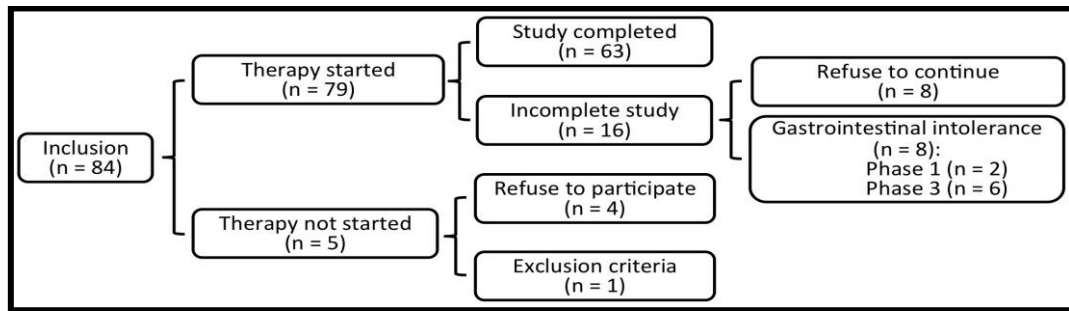
Critères d'exclusion :

- Toute modification de la clairance de la créatinine faisant changer de stade d'insuffisance rénale en cours d'étude ;
- La survenue d'une insuffisance hépatique aiguë.

Les valeurs de metformine sont comparées avec les tests de Kruskal-Wallis et de Mann-Whitney. La lactatémie est dosée dans les stades 3 à 5 d'insuffisance rénale.

RÉSULTATS

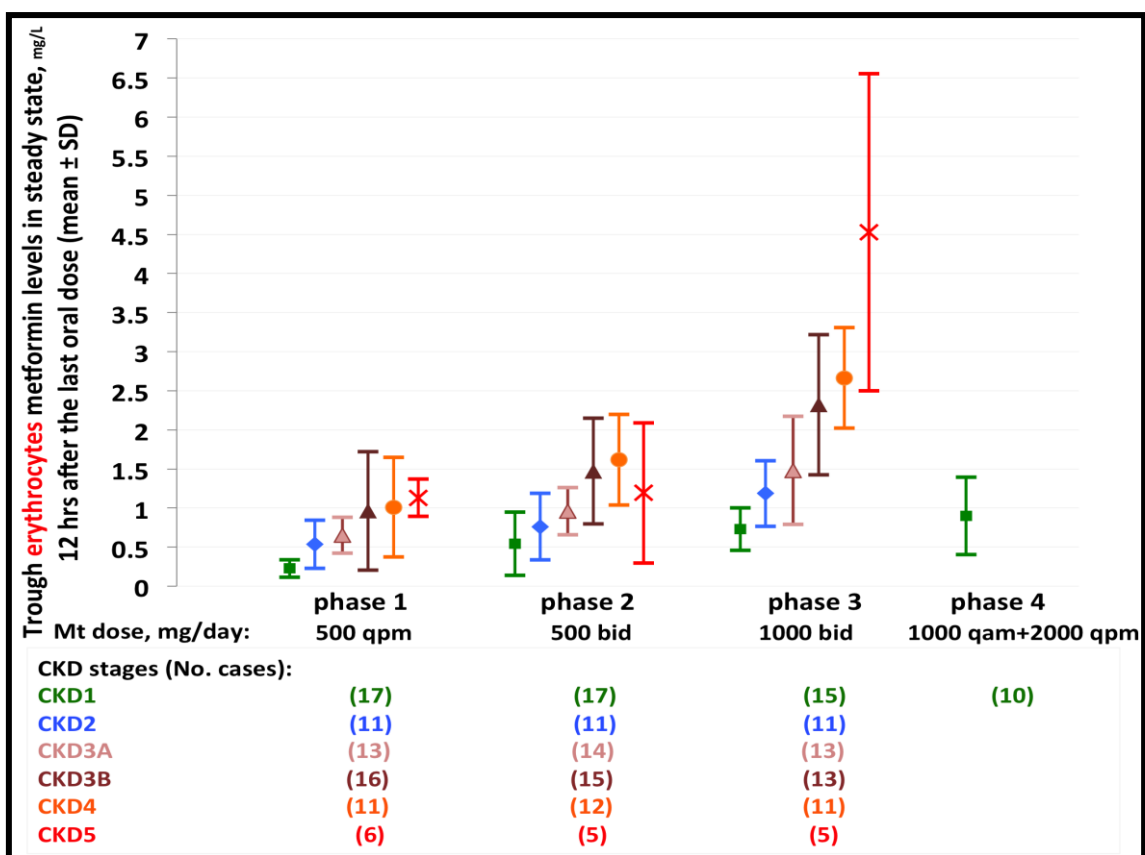
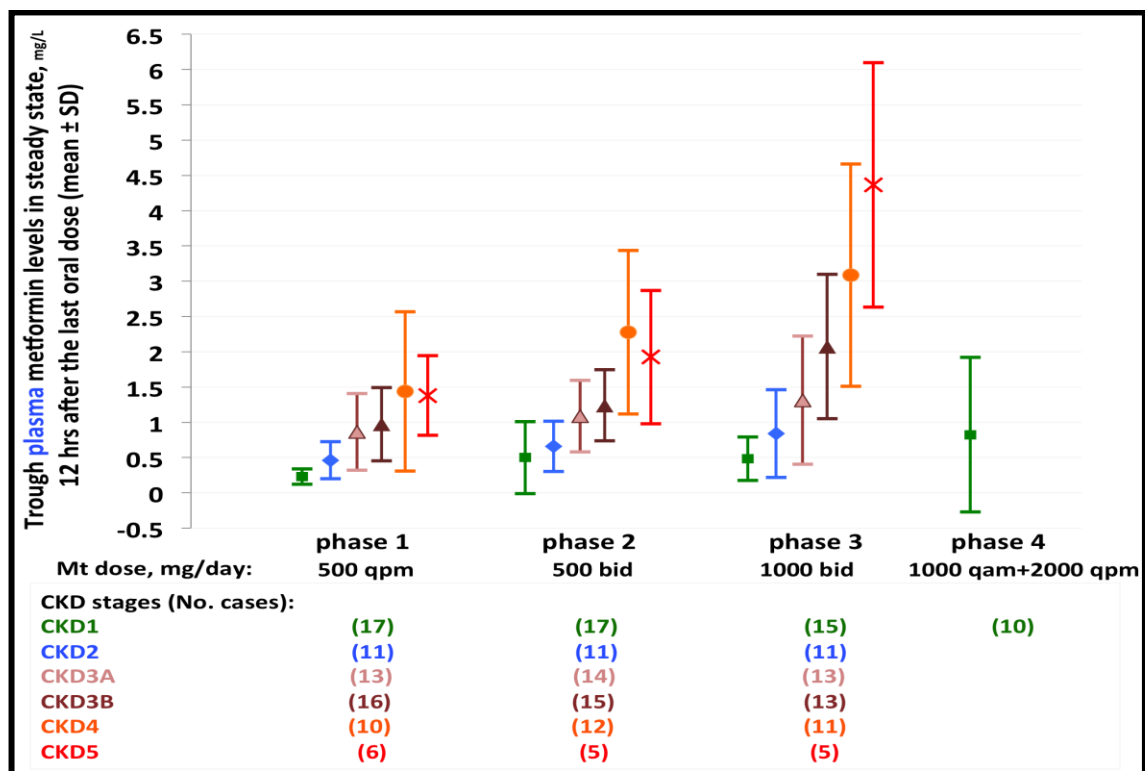
Quatre-vingt-quatre patients ont été inclus dans l'étude de décembre 2012 à juillet 2015. Leur participation au protocole est présentée à la figure qui suit :



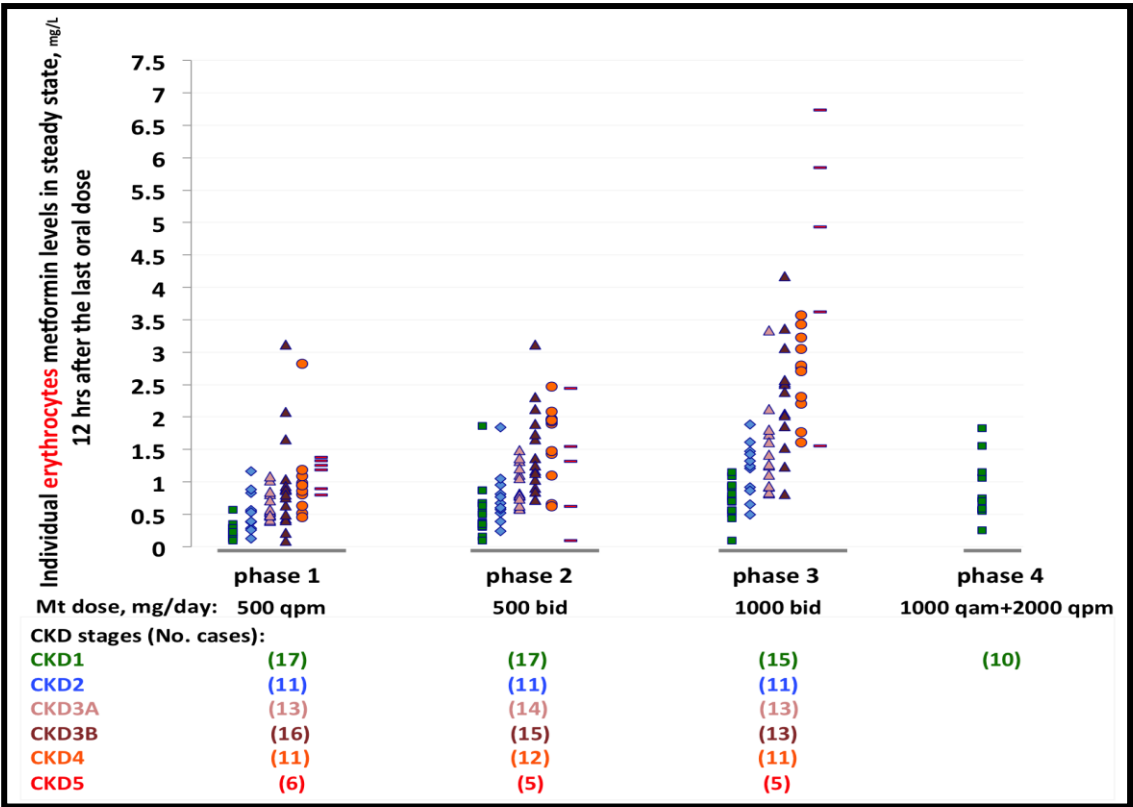
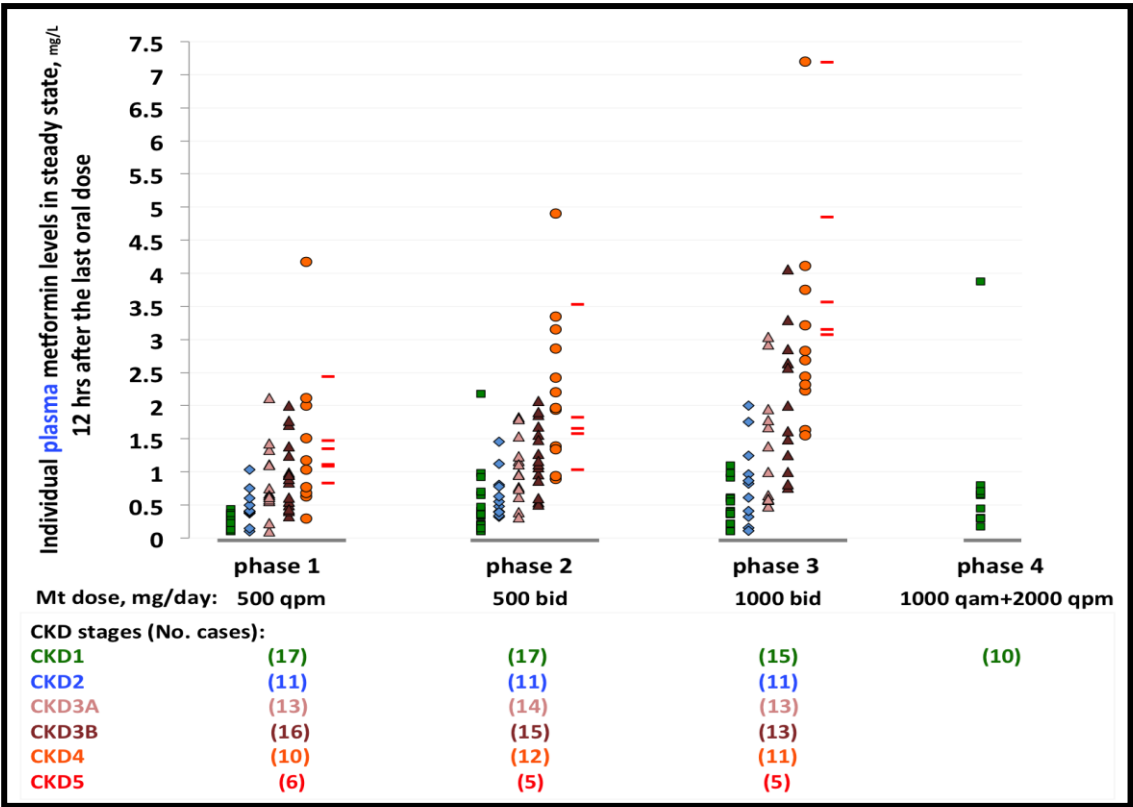
La classification en stades d'insuffisance rénale a dû être révisée selon l'évolution de la fonction rénale. C'est ainsi que 11 patients ont pu être reclassifiés (mais pour des variations de débit de filtration glomérulaire souvent minimales) :

- CKD1 → CKD2: n = 2
- CKD2 → CKD1: n = 4
- CKD3B → CKD3A: n = 1
- CKD4 → CKD3B: n = 2
- CKD4 → CKD5: n = 2

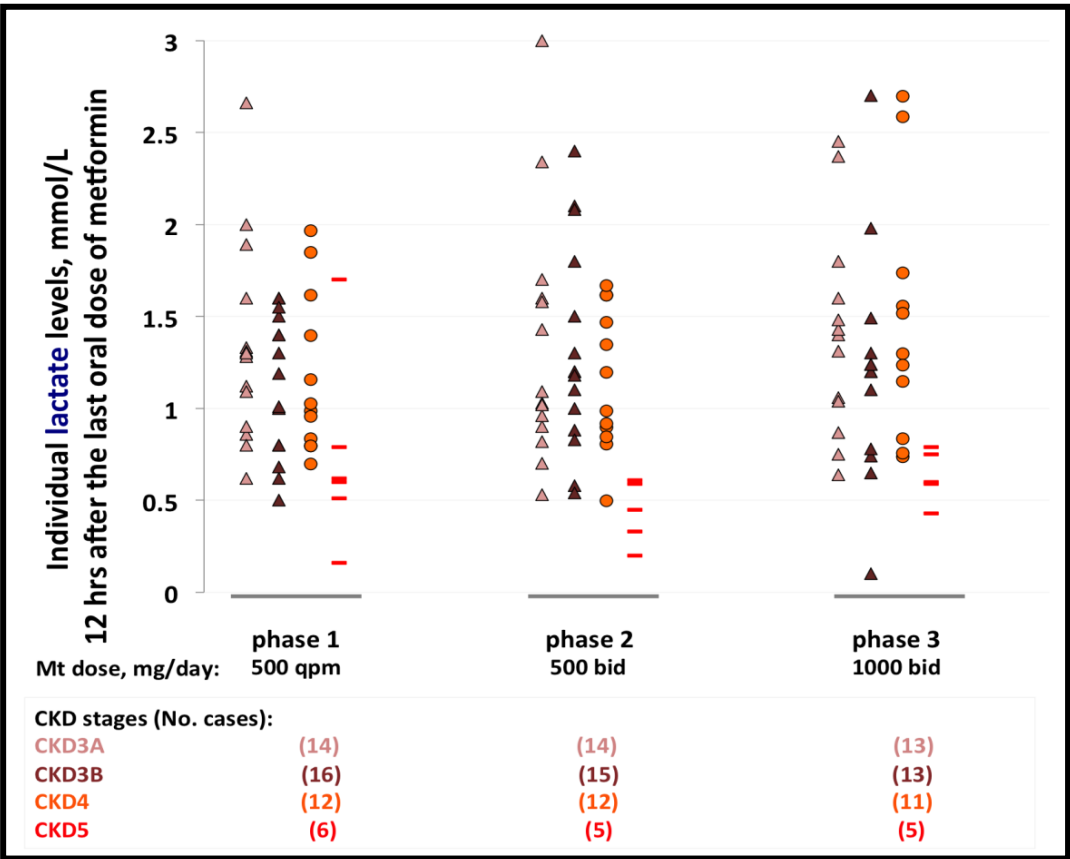
Les figures qui suivent présentent l'évolution des taux de metformine, dans le plasma et les érythrocytes (moyenne ± DS).



Les figures qui suivent présentent l'évolution des taux cette fois individuels de metformine



S’agissant de la tolérance métabolique, la figure qui suit montre l’évolution des taux de lactate:



Les metforminémies ont été comparées en interphase, pour le plasma puis pour les érythrocytes:

Plasma :

Stade CKD	Doses de metformine	<i>p</i>
1	500-1000-2000-3000	0,010
2	500-1000-2000	0,251
3A	500-1000-2000	0,470
3B	500-1000-2000	0,005
4	500-1000-2000	0,009
5	500-1000-2000	0,009

Erythrocytes:

Stade CKD	Doses de metformine	<i>p</i>
1	500-1000-2000-3000	<0,0001
2	500-1000-2000	0,0034
3A	500-1000-2000	<0,0001
3B	500-1000-2000	0,0003
4	500-1000-2000	0,0001
5	500-1000-2000	0,0108

Les comparaisons des taux de metformine ont aussi été faites en intraphase :

PLASMA						
Phase thérapeutique, Dose de metformine (mg /j)		p (test de Mann-Whitney)				
1 (500)	CKD stage	2	3A	3B	4	5
	1	0,025	0,002	<,0001	0,001	0,002
	2	—	NS	0,027	0,024	0,008
	3A	—	—	NS	NS	NS
	3B	—	—	—	NS	NS
	4	—	—	—	—	NS
2 (1000)	CKD stage	2	3A	3B	4	5
	1	NS	0,008	0,002	<,0001	0,015
	2		NS	0,022	0,002	0,024
	3A			NS	0,016	NS
	3B				0,046	NS
	4					NS
3 (2000)	CKD stage	2	3A	3B	4	5
	1	NS	0,012	<,0001	<,0001	0,006
	2		NS	0,021	0,001	0,011
	3A			NS	0,016	0,008
	3B				NS	0,039
	4					NS

ERYTHROCYTES						
Phase thérapeutique, Dose de metformine (mg /j)		p (test de Mann-Whitney)				
1 (500)	CKD stage	2	3A	3B	4	5
	1	0,016	<,0001	0,001	<,0001	0,002
	2		NS	NS	NS	0,021
	3A			NS	NS	0,022
	3B				NS	NS
	4					NS
2 (1000)	CKD stage	2	3A	3B	4	5
	1	NS	0,007	<,0001	0,001	NS
	2		NS	0,013	0,010	NS
	3A			NS	0,03	NS
	3B				NS	NS
	4					NS
3 (2000)	CKD stage	2	3A	3B	4	5
	1	0,056	0,002	<,0001	<,0001	0,006
	2		NS	0,010	0,001	0,024
	3A			NS	0,006	0,039
	3B				NS	NS
	4					NS

CONCLUSION

Nous montrons une augmentation graduelle des taux de metformine parallèlement à l'augmentation de la dose de metformine, de la même façon dans le plasma et dans les érythrocytes, et de façon plus prononcée dans l'insuffisance rénale modérée à sévère.

Discussion générale

Notre analyse de la littérature et des observations cliniques d'acidose lactique associée à la metformine montre, de façon surprenante au regard de l'ancienneté de la metformine, que :

- Les taux thérapeutiques ne sont toujours pas connus, et la littérature s'avère même particulièrement incohérente à ce sujet ;
- Les critères de contre-indication de la metformine relatifs à l'insuffisance rénale demeurent d'une grande hétérogénéité, et même souvent purement qualitatifs ;
- Les critères d'imputabilité de la metformine dans l'acidose lactique ne sont pas pris en compte dans la grande majorité des cas déclarés ;
- Le taux de mortalité dans l'acidose lactique associée à la metformine n'a pas été réévalué au fil du temps ;
- Une dangerosité a été attribuée à la metformine dans l'acidose lactique puisque le pronostic est au contraire nettement meilleur que dans l'acidose lactique indépendante de la metformine ;
- Dans l'acidose lactique associée à la metformine, les valeurs de metforminémie ne sont pas appréciées en fonction du temps, et en particulier de façon simultanée aux valeurs de pH et de lactate ;
- Les paramètres d'élimination de la metformine accumulée ne sont toujours pas connus.

En bref, si l'accumulation de metformine est authentiquement capable d'induire une acidose lactique, surtout dans l'accumulation aiguë, la dangerosité réelle d'une telle accumulation est largement sur-estimée.

Aussi nous est-il apparu licite d'étudier la possibilité de traiter par metformine le sujet insuffisant rénal. Les résultats de l'étude que nous avons menée dans tous les stades de l'insuffisance rénale et avec une dose de metformine croissante sont très en faveur de la poursuite du traitement par metformine dans l'insuffisance rénale, même sévère, *à la condition* que la dose soit adaptée à la fonction rénale.

Pour donner une base plus solide à cette proposition, nous poursuivons l'étude qui n'avait été menée qu'à court terme avec une phase de suivi de 4 mois et cette fois une dose fixe de metformine adaptée à la sévérité de l'insuffisance rénale.

RESUME

La metformine est reconnue comme le traitement de première intention dans le diabète de type 2 mais le débat relatif à la balance bénéfice/risque demeure vif, concernant en particulier le sujet insuffisant rénal. Le but du présent travail était triple : 1) mener une analyse critique des rapports d'acidose lactique associée à la metformine, et plus précisément de ses critères, de la signification pronostique de ces critères, et du taux de mortalité ; 2) examiner l'intérêt et les limites du dosage de la metformine dans le plasma et les érythrocytes dans le dépistage d'une accumulation et dans l'évaluation du degré et des conséquences d'une telle accumulation ; 3) réaliser une première étude prospective de traitement systématique par metformine dans tous les stades d'insuffisance rénale, avec monitoring des taux plasmatiques et érythrocytaires de metformine et aussi de lactate. La metformine a été administrée à dose croissante par phases d'une semaine entrecoupées d'une semaine de « wash-out ». Nous montrons une augmentation graduelle des taux de metformine parallèlement à l'augmentation de la dose de metformine, de la même façon dans le plasma et dans les érythrocytes, avec obtention de valeurs élevées dans l'insuffisance rénale modérée à sévère. Notre conclusion est qu'il devrait être possible de poursuivre le traitement par metformine dans l'insuffisance rénale, même sévère, à la condition que la dose de metformine soit adaptée à la fonction rénale. Pour donner une base plus solide à cette proposition, nous poursuivons cette étude avec une phase de suivi de 4 mois et cette fois une dose fixe de metformine adaptée à la sévérité de l'insuffisance rénale.

Appendix I

Criteria for "therapeutic concentrations" (ccs) of metformin in the literature: values, ranges, wording and sources

Author (year)	Criteria		For citations	
	Type of work	Data: clearly specified value(s) or range(s); or the wording used to describe these items [converted into mg/L]	Type of study	Concordance of data between the publication and the supporting reference; if discordance, details
	Original research (details)	Citation of a literature study: author (year)		
Frayn (1972)	No	Pignard (1962)	Pharmacokinetic study (PKS) in diabetic patients (DPs)	Discordance: the source data were not presented as "therapeutic values" ("plasma ccs were always found < 10 mg/L even after an oral dose of 2 g")
Adnitt (1972)	No	No ref.		NA
Clarke (1979)	No	Sirtori (1978)	PKS in DPs	Discordance: the source data were not presented as "therapeutic values" (they were presented as the steady state plasma ccs range and C _{max})
Isnard (1980)	No	Pignard (1962)	PKS in DPs	Discordance: cf. ref. 1
		Bruneder (1979)	PKS in DPs	Discordance: the source data were not presented as "therapeutic values" (they were presented as the fasting plasma metformin concentration)
Mountjoy (1987)	No	Clarke (1979)	Review	Discordance (0.3-1 mg/L)
Lambert (1987)	No	No ref.		NA
Tymms (1988)	No	No ref.		NA
Wollen (1988a)	No	Beckmann (1971)	Review	Discordance: the source data were not presented as "therapeutic values" (citing ref. 2 with different data)
		Noel (1979)	PKS in patients with normal renal function	Discordance: the source data were not presented as "therapeutic values" (they were presented as the PK parameters, and incorrect citation of Caporicci (1979) citing himself the study of Delorenzi (1975))
Wollen (1988b)	No	No ref.		NA
Bailey (1988)	No	Wollen (1988a)	<i>In vitro</i> study (animal)	Discordance (0.129-6.45 mg/L)
Gregorio (1989)	No	Benzi (1986)	Measurement of metformin in DPs	Discordance: the source data were not presented as "therapeutic values" ("Metformin concentration 2 h after the last ingestion of 500 mg. Metformin level ranged ~ 0.05-2.1 mg/L")
Wilcock (1990)	No	No ref.		NA
Lalau (1990)	No	No ref.		NA

Gregorio (1991)	No	No ref.	1.5 µg/mL [1.5]	NA	
		Benzi (1986)	For both refs: "Recent finding have suggested metformin even at ccs ranging from 1.2 to 4.8 µg/mL-which fall within the therapeutic plasma level" [1.5 & 1.2-4.8]	Measurement of metformin in DPs	Discordance: cf. ref.18
		Tucker (1981)		PKS in DPs	Discordance: the source data were not presented as "therapeutic values" (they were presented as the PK parameters (Cmax, etc.))
Sarabia (1992)	No	Pentikainen (1979)	12-100, µM [1.548-12.9]	PKS in non-DPs	Discordance: the source data were not presented as "therapeutic values" (they were presented as the PK parameters (Cmax, etc.))
Freislebena (1992)	No	Wilcock (1990)	0.5 -5 µM [0.0645-0.645]	<i>In vivo & vitro</i> (in the animal)	Discordance (0.129-6.45)
Bailey (1992a)	No	No ref.	"Ccs (10^{-6} - 10^{-2} mol/L) were considered appropriate to investigate therapeutic and supratherapeutic effects" [0.129]	NA	
Bailey (1992b)	No	No ref.	10^{-5} M [1.29]	NA	
Sum (1992)	No	No ref.	Lower therapeutic range (1-2 mgL ⁻¹)	NA	
Hermann (1992)	No	Marchetti (1987)	For both refs: "1-2 µg/mL (approximately 10 µmol/L) or even lower" [1-2 "1.29"]	<i>In vivo</i> (in humans)	Discordance: the source data were not presented as "therapeutic values" (they were presented as the plasma concentration)
		Marchetti (1988b)		<i>In vivo</i> (in humans)	Discordance: the source data were not presented as "therapeutic values" (they were presented as the fasting plasma concentration)
Chalmers (1992)	No	Bailey (1988)	1-5 µg/mL [1-5]	Review	Discordance: the source data were not presented as "therapeutic values" ("doses of 1500-1700 mg daily, giving plasma metformin levels up to 5 mg/L usually achieve a stable effect in about one week")
Galuska (1994)	No	Bailey (1992b)	0.01 mmol/L [1.29]	Review	Concordance
Denno (1994)	No	Bailey (1992b)	For both refs: "Mouse embryos were exposed to therapeutic ccs (metformin 500-2,550 mg per day for 24-48 h. Ccs of metformin in culture ranged from 0.15 to 1.8 mg/mL [...] blood values have been reported from 2×10^{-3} to 1.755 mg/ml" [150-1800]	Review	Discordance (1-2 (~1.29) mg/L)
		Marchetti (1989)		Review	Discordance: the source data were not presented as "therapeutic values" (they were presented as the plasma ccs after oral administration)
Fischer (1994)	No	Hermann (1992)	$\leq 10^{(-4)}$ M [≤ 12.9]	Review	No: discordant values (1-2 (~1.29) mg/L)
		Bailey (1993)		Review	No: source data not presented as "therapeutic values" ("Plasma concentration is maximally 1-2 mg/l (about 10^{-5} M) at 1-2 hr after an oral dosage of 500-1000 mg")

Lalau (1995)	Measurement of plasma ccs before drug intake in patients undergoing well-tolerated chronic metformin treatment	NA	0.6±0.5 mg/L [0.1-1.1]	NA	
Miao (1995)	No	No ref.	0-5 µg/mL [0-5]	NA	
Wiernsperger (1996)	No	Freislebena (1992)	0.5-5 µM [0.0645-0.645]	Review	Concordance
Stith (1996)	No	Wiernsperger (1996)	"Maximal stimulation of insulin action occurred at metformin concentration in the range of 1-10 µg/mL (~ 7.7-77 µM) metformin, this concentration is similar to that found to be therapeutic in diabetic human" [1-10]	Review	Discordance (0.0645-0.645 mg/L)
Sasson (1996)	No	Marchetti (1990)	For both refs: ~10 µM [~ 1.29]	PKS in DPs	Discordance: the source data were not presented as "therapeutic values" (they were presented as the PK parameters and fasting plasma levels)
		Caille (1993)		PKS in non-DPs	Discordance: the source data were not presented as "therapeutic values" (they were presented as the PK parameters)
Scheen (1996)	No	No ref.	0.5 to 1.0 mg/L (fasting state) and 1 to 2 mg/L (after a meal)	NA	
Radziuk (1997)	No	No ref.	"Metformin was added at high therapeutic levels (90 µg/mL) to the medium perfusing an isolated rat liver" [90]	NA	
Schulz (1997)	No	No ref.	0.1-1 (0.6-1.3) (µg/mL) [0.1-1 "0.6-1.3"]	NA	
Stith (1998)	No	No ref.	~1 µg /mL [~1]	NA	
Lalau (1998a)	No	Lalau (1995)	0.6±0.5 mg/L [0.1-1.1]	<i>In vivo</i> (in humans)	Concordance
Lalau (1998b)	No	Lalau (1995)	"normal overnight value of 0.6 ± 0.5 mg/L (SD) in diabetic subjects chronically taking 850 mg of metformin b.i.d. or t.i.d." [0.1-1.1]	<i>In vivo</i> (in humans)	Concordance
Al-Jebawi (1998)	No	No ref.	0.465-2.5 mg/L	NA	
Detaille (1998)	No	Wilcock (1994)	20 µmol/L [2.58]	<i>In vivo</i> (in the animal)	Discordance: the source data were not presented as "therapeutic values" (they were presented as the maximum ccs observed after oral administration, from previous studies: Frayn 1972, Gregorio 1989, Wilcock 1990)
Detaille (1999)	No	No ref.	10 µM [1.29]	NA	
Lupi (1999)	No	No ref.	2.4 µg/mL [2.4]	NA	
Wiernsperger (1999)	No	Freislebena (1992)	0.5-5 µM [0.0645-0.645]	<i>In vitro</i> (in humans)	Concordance
Lalau (1999)	No	No ref.	"plasma metformin concentrations of up to 1 mg/l are classified as normal"	NA	

Nelson (2000)	No	Sambol (1996)	For both refs: 0.5-2.0 µg/mL [0.5-2]	PKS in DPs and non-DPs	Discordance: the source data were not presented as "therapeutic values" (they were presented as the PK parameters)
		Scheen (1996)		Review	Concordance
Desel (2000)	No	Schulz (1997)	0.1-1.3 mg/L [0.1-1.3]	Review	Concordance
Reeker (2000)	No	Package insert of Glucophage (1997)	0.1-1.3 µg/mL [0.1-1.3]	Not a study – a package insert	Discordance: the source data were not presented as "therapeutic values" (they were presented as the fasting plasma metformin concentration and C _{max})
Lalau (2000)	No	Lalau (1995)	0.6±0.5 mg/L [0.1-1.1]	<i>In vivo</i> (in humans)	Concordance
Mueller (2000)	No	Scheen (1996)	0.005-0.02 mM [0.645-2.58]	Review	Discordance (0.5-2 mg/L)
Lalau (2001a)	No	No ref.	0.6±0.5 mg/L [0.1-1.1]	NA	
Kruse (2001)	No	No ref.	0.5-2.5 µg/mL [0.5-2.5]	NA	
Lalau (2001b)	No	No ref.	0.6±0.5 mg/L [0.1-1.1]	NA	
Yuan, (2002)	No	No ref.	"The concentration of metformin was selected based upon the dose-response experiment, in which 0.1 mmol/L metformin consistently produced the highest inhibitory effect at a therapeutic concentration" [12.9]	NA	
Lupi (2002)	No	Lupi (1999)	2.4 µg/mL [2.4]	<i>In vitro</i> (in humans)	Concordance
Barrueto (2002)	No	No ref.	1-2 µg/mL [1-2]	NA	
Lalau (2003)	Measurement of ccs before drug intake in patients undergoing well-tolerated chronic metformin treatment	NA	Plasma: 0.5 ± 0.4 mg/L [0.1-0.9] Erythrocytes: 0.8 ± 0.4 mg/L [0.4-1.2]	NA	
Schulz (2003)	No	Desel (2000)	0.1-1 (0.6-1.3) mg/L	<i>In vivo</i> (in humans)	Concordance
		Reeker (2000)		<i>In vivo</i> (in humans)	Concordance
Nisse (2003)	No	Kruse (2001)	0.5-2.5 µg/mL [0.5-2.5]	<i>In vitro</i> (in humans)	Concordance
Dawson (2003)	No	No ref.	< 2 mg/L	NA	
Sweeney (2003)	No	Howlett (1999)	15-20 µM [1.935-2.58]	Review	Discordance: the source data were not presented as "therapeutic values" (they were presented as the plasma metformin ccs, from Sirtori1994)
Moore (2003)	No	No ref.	"Hospital admission serum metformin concentration was 141 mg/L, or approximately two orders of magnitude above therapeutic ccs" [70.5]	NA	
Nelsson (2004)	No	Scheen (1996)	For both refs: 0.5-2.0 µg/mL [0.5-2]	Review	Concordance
		Sambol (1996)		PKS in DPs and in non-DPs	Discordance: cf. Dettaille1980

Guigas (2004)	No	No ref.	100 µM [12.9]	NA	
Feng (2004)	No	No ref.	0.1-10 µg/mL [0.1-10]	NA	
Stades (2004)	No	No ref.	≤ 5 mg L ⁻¹	NA	
Holland (2004)	No	No ref.	1-10 µg/mL [1-10]	NA	
Marchetti (2004)	No	Patane (2000)	For all of the 3 refs: "Experiments were performed either with or without a 24-h preincubation period in the presence of 2.4 µg/mL Metformin [...] This concentration of the drug is in the therapeutic range." [2.4]	<i>In vitro</i> (in the animal)	Discordance: no therapeutic value was given in the cited source
		Lupi (1999)		<i>In vitro</i> (in humans)	Concordance
		Lupi (2002)		<i>In vitro</i> (in humans)	Concordance
Lalau (2005)	No	No ref.	"Therapeutic levels of metformin are 0.5±0.4 mg/L in plasma and 0.8±0.4 mg/L in erythrocytes" Plasma: [0.1-0.9] Erythro: [0.4-1.2]	NA	
Detaille (2005)	No	No ref.	100 µmol/L [12.9]	NA	
Kimura (2005)	No	Sambol (1996)	For all of the 4 refs: "The maximum plasma concentration of metformin was reported to be 9–12 µM after a single oral administration of metformin HCl (850 mg) in patients with type 2 diabetes and up to 15 µM and 25 µM in healthy elderly patients and patients with moderate chronic renal impairment, respectively the transport of metformin by hOCT2 should not saturate at therapeutic ccs." [1.161-1.548 & 1.935-3.225]	PKS in DPs and in non-DPs	Discordance: cf. Detaille1980
		Scheen (1996)		Review	Discordance (0.5-2 mg/L)
		Davidson (1997)		Review	Discordance: the source data were not presented as "therapeutic values" (they were presented as the PK parameters, from ref. 30 and studies of Arafat, 1994 (ref. 97), Brookes, 1991 (ref. 98)
		Sambol (1995)		PKS in DPs with CKD and in non-DPs	Discordance: the source data were not presented as "therapeutic values" (they were presented as the PK parameters)
Lacher (2005)	No	Scheen (1996)	0.5-2 mg/L	Review	Concordance
Isoda (2006)	No	No ref.	20 µmol/L [2.58]	NA	
Friesecke (2006)	No	No ref.	0.3-1.2 mg/L	NA	
Vigersky (2006)	No	Al-Jebawi (1998)	0.465-2.5 µg/mL [0.465–2.5]	<i>In vivo</i> (in humans)	Concordance
Del Prato (2007)	No	Marchetti (2004)	2.4 µg/mL [2.4]	<i>In vivo</i> (in humans)	Concordance
Wessler (2007)	No	No ref.	"maximal therapeutic plasma concentration (4 µg/mL)" [< 4]	NA	
Prikis (2007)	No	No ref.	1-2 µg/mL [1-2]	NA	
Galea (2007)	No	Nisse (2003)	0.5-2.5 µg/mL [0.5–2.5]	<i>In vivo</i> (in humans)	Concordance
Bruijstens (2008)	No	No ref.	"Therapeutic levels generally do not exceed 4 mg/L" [< 4]	NA	
Seidowsky (2009)	No	No ref.	0.5-2 mg/L	NA	
Dell'Aglio (2009)	No	No ref.	1-2 µg/mL [1-2]	NA	
Stambolic (2009)	No	No ref.	0.465-2.5 mg/L	NA	

Liu, (2009)	No	www.rxlist.com/cgi/generic/fortamet_cp.htm (2009)	6-30 µM [0.774-3.87]	Review	Discordance: the source data were not presented as "therapeutic values" (they were presented as the PK parameters)
Lalau (2010)	No	No ref.	0.6±0.5 mg/L [0.1-1.1]	NA	
Friesecke (2010)	No	No ref.	0.2-1.3 mg/L	NA	
Kane (2010)	No	Scheen (1996)	For both refs: "∼1000 mg/dose, ∼0.5–1 mg/L or ∼2.5–5 µM" [0.5-1 "0.3225-0.645"]	Review	Discordance (0.5-2 mg/L)
		Bailey (1992b)		Review	Discordance (1.29 mg/L)
Frid (2010)	Metformin measurement in DPs with CKD and proposal of safe levels.	NA	"20 µmol/L may be used as preliminary upper therapeutic limit" [< 2.58]	NA	
Giuliani (2010)	No	No ref.	"Plasma metformin levels reached 4.5 µg/mL, compatible with a normal therapeutic range" [4.5]	NA	
Protti (2010)	No	No ref.	"above safe limits (metformin 61 ± 25 vs. <4 µg/mL)" [< 4]	NA	
Dell'Aglia (2010)	No	No ref.	1-2 µg/mL [1-2]	NA	
Sørensen (2011)	No	TIAFT (2004)	1-4 mg/L	Review	Concordance
Yeung (2011)	No	No ref.	"The supra-therapeutic plasma metformin level is conventionally defined as any reading above 40 µg/mL" [40]	NA	
Lalau (2011)	No	Lalau (2003)	"Plasma and erythrocyte levels (0.5±0.4 mg/L and 0.8±0.4 mg/L, respectively)". Plasma: [0.1-0.9] Erythrocyte: [0.4-1.2]	<i>In vivo</i> (in humans)	Concordance
		TIAFT (2004)	"Although TIAFT (2004) reference list of 'therapeutic substances' quotes serum metformin ccs as being 'therapeutic' (between 1 and 4 mg/L)"	Review	Concordance
Graham (2011)	Study of mean ccs in steady state in DPs not having lactic acidosis	NA	Up to 2.5 mg/L.	NA	
de Oliveira Baraldi, (2011)	No	No ref.	"Maternal metformin plasma ccs were 0.4 mg/L (range 0.1–2.4 mg/L) and umbilical cord plasma ccs were 0.3 mg/L (range 0.1–1.4 mg/L), showing that the fetus was being exposed to therapeutic plasma ccs of metformin" [0.1-2.4]	NA	
Correia (2011)	No	No ref.	0.5-2.5 µg/mL [0.5-2.5]	NA	
Vecchio (2011)	No	No ref.	< 4 µg/mL [< 4]	NA	
Takiyama (2011)	No	Frid (2010)	For both refs: 10 µmol/L [1.29]	<i>In vivo</i> (in humans)	Discordance (< 2.58 mg/L)
		Bailey (1996)		Review	Discordance: the source data were not presented as "therapeutic values" (they were presented as maximal plasma concentration)
Roche (2011)	No	No ref.	< 1.34 mg/L	NA	
Perrone (2011)	No	No ref.	1-2 µg/mL [1-2]	NA	
Pikwer	No	No ref.	< 20 µmol/L [< 2.58]	NA	

(2011)					
Cosenza (2011)	No	No ref.	1-2 µg/mL [1-2]	NA	
Berstein (2011)	No	Lalau (2003)	0.465-2.5 mg/L	<i>In vivo</i> (in humans)	Discordance (plasma: 0.1-0.9 mg/L)
Jagia (2011)	No	No ref.	0.5-2 mg/L	NA	
Briet (2012)	No	Lalau (2003)	"The upper limit for a reference group without kidney failure was 1.6 mg/L"	<i>In vivo</i> (in humans)	Discordance (erythrocyte: 0.4-1.2 mg/L)
Schulz (2012)	No	Desel (2000)	0.1-1 (0.6-1.3) mg/L	<i>In vivo</i> (in humans)	Concordance
		Reeker (2000)		<i>In vivo</i> (in humans)	Concordance
Protti (2012a)	No	Protti (2010)	< 4 mg/L	<i>In vivo</i> (in humans)	Concordance
Protti (2012b)	No	No ref.	< 4 mg/L	NA	
Lam (2012)	No	No ref.	1-2 µg/mL [1-2]	NA	
Dowling (2012)	No	Stambolic (2009)	0.465-2.5 mg/L or 2.8-15 µM [0.465-2.5 "or 0.3612-1.935"]	Review	Concordance
Duong (2013)	No	Lalau (2011)	< 5 mg/L	Review	Discordance (0.1-0.9 and 1-4 mg/L)
Kajbaf (2013)	No	Luft (1983)	"Relative to the proposed peak therapeutic concentration of 2.5 mg/L"	<i>In vivo</i> (in humans)	Discordance, no therapeutic value available in the source (error in citation, Luft 1983 instead of Graham 2011)
Kajbaf (2013)	No	Graham (2011)	"Therapeutic range (based on the upper limit of 2.5 mg/L recently proposed" [< 2.5])	Review	Concordance
Bonsignore (2013)	No	No ref.	0.5-2.5 µg/mL [0.5-2.5]	NA	
Al-abri (2013)	No	No ref.	0.465-2.5 µg/mL [0.465-2.5]	NA	
Rena (2013)	No	Bailey (1996)	For both refs: 4.5 µmol/L [4.5]	Review	Discordance (1-2 (1.29) mg/L)
		Hardie (2007)		Review	Discordance: the source data were not presented as "therapeutic values" ("The concentrations of metformin to activate AMPK in cultured cells were 1 to 2 order of than magnitude higher than those (10-40 µM) estimated to occur in peripheral plasma after therapeutic doses in humans")
Vecchio (2013)	No	TIAFT (2004)	"values between 1 and 4 µg/mL" [1-4]	Review	Concordance
		Moffat (2004)		Review	Concordance
		Repetto (2004)		Review	Concordance
Lalau (2014)	No	No ref.	< 1.65 mg/l	NA	
Geerling (2014)	No	No ref.	(in Fig. 6): 1-100 µmol ⁻¹ [0.129-12.9]	NA	
Launiainen (2014)	No	Schulz (2012)	0.1-1 mg/L	Review	Concordance
Fremin (2014)	No	No ref.	1-2 µg/mL [1-2]	NA	
Rehnan (2014)	No	Dowling (2012)	0.465-2.5 mg/L or 2.8-15 µM [0.465-2.5 "or 0.3612-1.935"]	Review	Concordance
Adam (2014)	No	No ref.	1 mg/L [1]	NA	

Acquistapace (2014)	No	No ref.	"normal value < 4 µg/mL" [< 4]	NA
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AMPK AMP-activated protein kinase ccs concentrations Cmax maximum (or peak) serum concentration DPs diabetic patients NA not applicable PKS Pharmacokinetic study refs references TIAFT The International Association of Forensic Toxicologists

^aNA: the analysis of concordance/discordance was not applicable because a reference was not cited.

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Appendix II

Thresholds for guiding metformin withdrawal: official documents from the world's 20 most populated countries

Country	Year	Ref.	Organization	National formularies, official guidelines, websites, reference textbooks, marketing authorizations or documents issued by diabetes associations	Criterion	Threshold for withdrawing metformin
China	2005	[i]	Asian-Pacific Type 2 Diabetes Policy Group	Type 2 Diabetes Practical Targets and Treatments http://www.idf.org/	Serum creatinine	> 150 µmol/L
	2010	[ii]	—	China National Formulary 2010	Qualitative	“Abnormal kidney dysfunction” or “creatinine clearance rate”
India	2005	[iii]	The Indian Council of Medical Research	ICMR Guidelines for Management of T2 Diabetes http://icmr.nic.in	Qualitative	“Renal insufficiency”
	2011	[iv]	The National Rural Health Mission, Health & F.W. Department	Standard Treatment Guideline & Essential Medicine List (For Pregnant Women) http://nrhmorissa.gov.in	Qualitative	“Presence of renal disease”
	2012	[v]	Indian diabetes association	The Management Of The Metabolic Syndrome: An Indian Scenario http://www.diabetesindia.com	Qualitative	Not stated
USA	2008	[vi]	US Food and Drug Administration	NDA 20-357/S-031 and NDA 21-202/S-016	Serum creatinine	Males: ≥ 1.5 mg/dL (>135 µmol/L) Females: ≥ 1.4 mg/dL (> 110 µmol/L)
	2009	[vii]	American Diabetes Association	A consensus statement of the American Diabetes Association	Qualitative	Renal dysfunction
	2012	[viii]	American Diabetes Association	Position Statement of the American Diabetes Association	Serum creatinine	Males: ≥1.5 mg/dL Females: ≥ 1.4 mg/dL
	2012	[ix]	US National Kidney Foundation	Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease, Guideline 2 http://www.kidney.org	Serum creatinine	Males: ≥1.5 mg/dL Females: ≥ 1.4 mg/dL
Indonesia	2000	[x]	Indonesian Diabetes Association	The epidemiology and management of diabetes mellitus in Indonesia	Qualitative	“Severe renal dysfunction”
	2005	[xi]	International Diabetes Federation	Global Guideline for Type 2 Diabetes “Version for Indonesia” http://www.idf.org	Qualitative	“Evidence or risk of renal impairment”
	2005	[i]	Asian-Pacific Type 2 Diabetes Policy Group	Type 2 Diabetes Practical Targets and Treatments http://www.idf.org/	Serum creatinine	> 150 µmol/L
Brazil	2010	[xii]	Medical associations of Latin American countries	Consensus statement	eGFR	<30 mL/min/1.73 m ²
Pakistan	2012	[xiii]	Pakistan Diabetes Institute	Guidelines for Management of Diabetes Mellitus http://www.diabetespakistan.com/	Qualitative	“Impaired renal function”
Nigeria	2012	[xiv]	Unofficial drug information website	Metformin HCl webpage http://niger-gouv.org/medicaments/	Serum creatinine	Males: ≥1.5 mg/dL Females: ≥ 1.4 mg/dL
Russia	2005	[xi]	International Diabetes	Global Guideline for Type 2 Diabetes “Version for Russia”	Qualitative	“Evidence or risk of renal

			Federation	http://www.idf.org		impairment"
	2011	[xv]	Russian Diabetes Federation	Metformin profile in "Diabetes", the journal of the Russian Diabetes Federation http://www.rda.org.ru/	Qualitative	"Renal failure"
	2012	[xvi]	Russian encyclopaedia of drugs products	http://www.rlsnet.ru/	Qualitative	"Renal impairment"
Bangladesh	2003	[xvii]	Bangladesh Institute Of Research and Rehabilitation for Diabetes Endocrine and Metabolic Disorders	Guidelines for care of type 2 diabetes mellitus in Bangladesh	Serum creatinine	≥ 2.5 mg/dL
	2005	[i]	Asian-Pacific Type 2 Diabetes Policy Group	Type 2 Diabetes Practical Targets and Treatments http://www.idf.org/	Serum creatinine	> 150 µmol/L
Japan	2012	[xviii]	Japan pharmaceutical and medical device agency	Metformin HCl properties webpage http://www.info.pmda.go.jp	Qualitative	"Renal dysfunction (including mild failure)"
Mexico	2010	[xii]	Medical associations of Latin American countries	Consensus statement	eGFR	<30 mL/min/1.73 m ²
	2005	[i]	Asian-Pacific Type 2 Diabetes Policy Group	Type 2 Diabetes Practical Targets and Treatments http://www.idf.org/	Serum creatinine	> 150 µmol/L
Philippines	2006	[xix]	—	Philippine National Formulary	Qualitative	"Renal impairment"
	2005	[i]	Asian-Pacific Type 2 Diabetes Policy Group	Type 2 Diabetes Practical Targets and Treatments http://www.idf.org/	Serum creatinine	> 150 µmol/L
Vietnam	2008	[xx]	—	Ethiopian National Drug Formulary	Qualitative	"Renal impairment"
	2010	[xxi]	Drug Administration and Control Authority of Ethiopia	Standard treatment guideline for general hospitals http://apps.who.int	Qualitative	"Renal diseases"
Egypt	2007	[xxii]	—	Egyptian National Formulary	Qualitative	"Renal failure"
	2001	[xxii]	European Medicines Agency	CPMP/4082/00	Serum creatinine	Males: ≥1.5 mg/dL (>135 µmol/L) Females: ≥ 1.4 mg/dL (> 110 µmol/L)
	2011	[xxiv]	—	Iranian Physician Desk Reference	Serum creatinine	Males: ≥1.5 mg/dL Females: ≥ 1.4 mg/dL
Iran	2012	[xxv]	Iranian Drug and Poison Information Center website	Metformin HCl properties webpage http://www.darooyab.ir	Qualitative	"Renal disease"
Turkey	2005	[xi]	International Diabetes Federation	Global Guideline for Type 2 Diabetes "Version for Turkey" http://www.idf.org	Qualitative	"Evidence or risk of renal impairment"
Congo	2012	[xxvi]	"Diabaction-Congo"	Congo Diabetes Association guideline	Serum creatinine	Males: ≥1.5 mg/dL Females: ≥ 1.4 mg/dL
Thailand	2005	[i]	Asian-Pacific Type 2 Diabetes Policy Group	Type 2 Diabetes Practical Targets and Treatments http://www.idf.org/	Serum creatinine	> 150 µmol/L

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- IV. Indian diabetes association declaration; http://www.diabetesindia.com/diabetes/mets_impaired_glycemia.htm [December 2012].
- V. http://icmr.nic.in/guidelines_diabetes/section7.pdf
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- IX. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease GUIDELINE 2; http://www.kidney.org/professionals/kdoqi/guideline_diabetes/guide2.htm [December 2012].
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- XVIII. Japan pharmaceutical and medical device agency; Metformin HCl Properties Webpage;
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- XIX. Philippine National Formulary 2006.
- XX. Ethiopian National Drug Formulary 2008.
- XXI. Drug Administrations and Control Authority of Ethiopia Contents. Standard Treatment
Guideline for General Hospitals
<http://apps.who.int/medicinedocs/documents/s17822en/s17822en.pdf> [January 2013].
- XXII. Egyptian National Formulary 2007.
- XXIII. Metformin Summary Information - European Medicines Agency CPMP/4082/00.
- XXIV. Iranian Physician Desk Reference 2011.
- XXV. <http://www.darooyab.ir> Persian Drug Information Site;
<http://www.darooyab.ir/DrugInfoD.aspx?MGID=NjQw> [January 2013].
- XXVI. Congo Diabetes Association Diabaction-Congo declaration.

Appendix III

Thresholds for guiding metformin withdrawal: official documents from the 20 top countries (according to the International Science Ranking) in the field of diabetology.

Country	Year	Ref.	Organization	National formularies, official guidelines, websites, reference textbooks, marketing authorizations or documents issued by diabetes associations	Criterion	Threshold for withdrawing metformin
USA: cf. Appendix II						
UK	2009	[i]	National Institute for Health and Clinical Excellence	The Management of Type 2 Diabetes: NICE Clinical Guideline 87 http://www.nice.org.uk	eGFR	<30 mL/min/1.73 m ²
	2011	[ii]	—	British National Formulary	eGFR	<30 mL/min/1.73 m ²
Germany: cf. Appendix II						
Italy: cf. Germany in Appendix II (European Medicines Agency)						
France: cf. Germany in Appendix II (European Medicines Agency)						
Japan: cf. Appendix II						
Spain: cf. Germany in Appendix II (European Medicines Agency)						
Canada	2008	[iii]	Canadian Diabetes Association	Clinical Practice Guidelines for the Prevention and Management of Diabetes	eGFR	<30 mL/min/1.73 m ²
	2009	[iv]	The Canadian Pharmacists Association	Practical Guideline: Pharmacologic management of type 2 diabetes	eGFR	<30 mL/min/1.73 m ²
	2010	[v]	British Columbia Advisory Committee	Guidelines & Protocols for Diabetes Care www.bcguidelines.ca	eGFR	<30 mL/min/1.73 m ²
Netherlands: cf. Germany in Appendix II (European Medicines Agency)						
Sweden: cf. Germany in Appendix II (European Medicines Agency)						
Australia	2005	[vi]	Asian-Pacific Type 2 Diabetes Policy Group	Type 2 Diabetes Practical Targets and Treatments http://www.idf.org/	Serum creatinine	> 150 µmol/L
	2009	[vii]	Australian National Health and Medical Research Council	National Evidence Based Guideline for Blood Glucose Control in Type 2 Diabetes	eGFR	<30 mL/min/1.73 m ²
	2012	[viii]	Australian Therapeutic Goods Administration	Summary of product characteristics (SPC/RCP)	Creatinine clearance	< 60 mL/min
Denmark: cf. Germany in Appendix II (European Medicines Agency)						
Brazil: cf. Appendix II						
China: cf. Appendix II						
Poland: cf. Germany in Appendix II (European Medicines Agency)						
Switzerland	2012	[ix]	Compendium Switzerland	Metformin HCl webpage http://www.kompendium.ch	Creatinine clearance	< 60 mL/min
Finland: cf. Germany in Appendix II (European Medicines Agency)						
Belgium: cf. Germany in Appendix II (European Medicines Agency)						
India: cf. Appendix II						
Turkey: cf. Appendix II						

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- iii. Can J Diabetes. 2008 32(Suppl 1): S53–S61.

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- v. British Columbia Advisory Committee for Guidelines & Protocols; www.bcguidelines.ca/pdf/diabetes.pdf [January 2013].
- vi. Asian-Pacific Type 2 Diabetes Policy Group Type 2 Diabetes Practical Targets and Treatments http://www.idf.org/webdata/docs/T2D_practical_tt.pdf [December 2012].
- vii. Colagiuri S, Dickinson S, Girgis S, Colagiuri R. National Evidence Based Guideline for Blood Glucose. Control in Type 2 Diabetes. Diabetes Australia and the NHMRC, Canberra 2009.
- viii. Metformin Approved Leaflet by TGA;
<https://www.ebs.tga.gov.au/ebs/picmi/picmirepository.nsf/pdf?OpenAgent&id=CP-2010-PI-05648-3>.
- ix. Metformin HCl properties webpage
<http://www.kompendium.ch/mpro/mnr/1943/html/fr#7200> [January 2013].

Appendix IV

Thresholds for guiding metformin withdrawal: proposals from the scientific literature

Year	Author	Basis for proposal	Blood metformin measurement	Criterion	Threshold for withdrawing metformin	Metformin dose adjustment
1990	Lalau	Prospective 2-month study in the elderly: no difference in plasma metformin concentration between patients with metformin 1700 mg/d and creatinine clearance > 60 ml/min, and those with 850 mg/d and 30-60 ml/min clearance.	Yes	Creatinine clearance	No	Clearance >60: 1700 mg/d Clearance 30-60: 850 mg/d
1995	Sambol	Administration a single 850-mg oral dose of metformin in healthy subjects and 15 subjects with CKD ('mild to severe')	Yes	Qualitative	Moderate to severe CKD and unstable mild CKD	Reduction of around a third of the dose in the elderly and in patients with stable, mild CKD
2003	Jones	Personal suggestion, not based on a study	No	Serum creatinine	> 150 μ mol/L	No
2004	Nisbet	Personal suggestion, not based on a study	No	Creatinine clearance	< 30 ml/min	No
2005	McCormack	Personal suggestion, not based on a study	No	Creatinine clearance	No	A ~50% decrease in the maximum metformin dose for a creatinine clearance < 60 mL/min
2007	Shaw	Personal suggestion to convert serum creatinine to eGFR, not based on a study	No	eGFR	eGFR < 30 ml/min/1.73 m ²	No
2007	Warren	Personal suggestion to convert serum creatinine to eGFR, not based on a study	No	eGFR	<36-40 ml/min/1.73 m ²	No
2008	Herrington	Personal suggestion, not based on a study, not based on a study	No	eGFR	< 30 ml/min/1.73 m ²	GFR 90-60 in patients aged over 70: reduce by half. GFR<60 ml/min: reduction again by half
2009	Haneda	Personal suggestion, not based on a study	No	eGFR	< 60 ml/min/1.73 m ²	No
2009	Mani	Personal suggestion, not based on a study	No	eGFR	Stage 5 CKD (<15 ml/min/1.73 m ²)	No
2010	Chen	Personal suggestion, not based on a study	No	eGFR	< 40 ml/min/1.73 m ²	No
2010	Frid	Metformin measurement in plasma: "patients above the limit of GFR proposed in NICE rarely had metformin levels above presumed upper therapeutic limit"	Yes	eGFR	< 30 ml/min/1.73 m ²	No
2010	Hartmann	Personal suggestion, not based on a study	No	eGFR	< 60 ml/min/1.73 m ²	No
2010	Vasisht	Personal suggestion to convert serum creatinine to eGFR, not based on a study	No	eGFR	< 60 ml/min/1.73 m ²	No
2011	Graham	Personal suggestion, not based on a study	No	Creatinine clearance	No	The initial maximum target dose of 1500 mg/d if creatinine clearance is ~ 60 mL/min. The started dosage should be commenced at a lower level of clearance and

						750 mg/d if creatinine clearance ~ 30 mL/min.
2011	Klachko	Personal suggestion, not based on a study	No	eGFR	< 30 ml/min/1.73 m ²	GFR≥90: 2500 mg/d GFR≥60: 2000 mg/d GFR≥45: 1000 mg/d GFR≥30: 500 mg/d
2011	Lipska	Personal suggestion, not based on a study	No	eGFR	< 30 ml/min/1.73 m ²	GFR ≥ 60: no contraindication GFR <60 ≥45: increased monitoring of renal function GFR <45 ≥30: decrease the dose by 50% or use the half-maximum dose
2012	Duong	Metformin levels in 24 stage 3-5 CKD patients (of whom 2 were on dialysis), compared with healthy subjects	Yes	Creatinine clearance	< 20 ml/min	No
2012	Martínez-Castelao	Personal suggestion, not based on a study	No	eGFR	<30 ml/min/1.73 m ² (<45 ml/min/1.73 m ² in patients at risk for lactic acidosis)	No

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