

Review

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Synthalin, Buformin, Phenformin, Metformin: A Century Of Intestinal,, - Clearance“ as Oral Antidiabetic Strategy in Overweight/Obese Patients

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Review

Synthalin, Buformin, Phenformin, Metformin: A Century of Intestinal „Glucose-Excretion*“ as Oral Antidiabetic Strategy in Overweight/Obese Patients

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Abstract: After the first release of synthalin B (dodecamethylenbiguanide) in 1928 and its later retraction in the 1940s in Germany, the retraction of phenformin (N-Phenethylbiguanide) and of Buformin in the USA (but not outside), because of the lethal complication of acidosis seemed to have put an end to the era of the biguanides as oral antidiabetics. The strongly hygroscopic metformin (1,1-dimethylbiguanide), first synthesized 1922, resuscitated as an oral antidiabetic (type 2 of the elderly) compound first released in 1959 in France and in other European countries, was used in the first large multicenter prospective long-term trial in England in the UKPDS (1977-1997), released in the USA after a short-term prospective trial in healthy overweight “young” type 2 diabetics (mean age 53 years) in 1995 for oral treatment of diabetes type 2, however, mostly to multimorbid older patients (above 65 years of age) and is now the most used drug worldwide. While intravenous administration of biguanides does not have any glucose-lowering effect, their oral administration leads to enormous increase of their (metformin) intestinal concentration (up to 300-fold compared to that measured in the blood), to reduced absorption of glucose from the diet and to decrease of insulin serum level through increased hepatic uptake and decreased production. Furthermore, these compounds have also a diuretic effect (loss of sodium and water in the urine) Acute gastrointestinal side effects accompanied by fluid loss often led to dose-reduction of the drugs and strongly limit adherence to therapy. Main long-term consequences are, „chronic “dehydration, deficiency of vitamin B12 and of iron and, as observed for all the biguanides, to “chronic “increase of fasting and postprandial lactate plasma level and also to a as a laboratory marker of a clinical condition characterized by hypotension, oliguria, adynamia, and evident lactic acidosis. Intravenously injected F18-labelled glucose in metformin-treated type 2 diabetics accumulates in the small and even more in the large intestine. The densitometry picture observed in metformin-treated overweight diabetics is similar to that observed in patients after bowel-cleansing or chronically taking different types of laxatives where the accumulated radioactivity can even reach values observed in colon cancer. The glucose-lowering mechanism of action of metformin is therefore not only due to inhibition of glucose uptake in the small intestine but also to „attraction “of glucose from the hepatocyte into the intestine, possibly through the insulin-mediated uptake in the hepatocyte and its secretion into the bile. Metformin is not different from the other biguanides, synthalin B, buformin and phenformin. The mechanism of action of the biguanides as antihyperglycemic substances, and their side effects are comparable if not even stronger (abdominal pain and fluid loss) to those of laxatives.

Keywords: guanidine; biguanides; metformin; diabetes mellitus type 2; vomitus; diarrhea; lactic acidosis; loss of body weight; laxatives; malabsorption; anemia; dehydration; kidney injury; shock; death

Introduction: Development of Industrial Food Production, Hypercaloric Nutrition, Overweight/Obesity, Type 2 Diabetes and Increasing Popularity of Biguanides

Beginning with the middle of the 20th century the so-called developed countries, Germany, Italy, Japan, United States of America but also the United Kingdom, France, Finland, Sweden, Norway experienced a steady growth (3-5-fold) of the population size [1]. Those countries mainly contributed to the worldwide pandemic increase of Obesity and Overweight. Obesity is increasing also in the formerly called developing world (emerging countries, low income countries), inducing a change of the definition of malnutrition as it now comprises not only undernourishment but also overfeeding especially in children [2–5]. Japan represents an exception among the western world countries as the overweight and obesity numbers are relatively lower [6] as agriculture and livestock production is limited by the scarcity of land which can be used for agriculture and by the state control of import of food products from abroad [7,8].

Nevertheless we are still talking about several hundred million people worldwide suffering of hunger [9]. The global overfeeding can be explained with the epochal change and enormous increase of food production, which began in the middle of the 19th century when the soil fertilization, the industrialization of agriculture [10–12] and the livestock sector started to grow [13,14] first in the western world, but then expanded into the rest of the world along with the globalization, especially after the second world war [15,16]. In the middle of the 20th century it became obvious that cigarette smoke was the first cause of coronary heart disease and of lung cancer in the United Kingdom and in the United States of America [17–23]. It became apparent that consumption of alcoholic beverages would increase mortality [24].

Immediately after these publications an alternative explanation for development of arteriosclerosis also inducible by starvation [25] was offered by the biologist Ancel Kyes [26], namely hypercholesterolemia attributed to the different composition of animal fat in the food of men in north Karelia, south-east of Finland on the Russian border although with a much lower BMI than that found in American men [27,28]. Finnish women from the same area with the same hypercholesterolemia were not as much affected as men [29,30].

It has to be mentioned that the true risk factors, namely cigarette smoke and consumption of alcoholic beverages were not discussed as it was not emphasized that these men had a normal body mass index [31–36].

The meat industry favored more the hypothesis of exaggerated consumption of carbohydrates and sugar being the cause of overweight and of the co-morbidities induced by hypercaloric nutrition, including alcoholic beverages [37–40]. This hypothesis was denied in scientific contributions published in the world literature while in the meantime, however, the explosion of the overweight pandemic [41–44] can be attributed to the development of a „food addiction“ [45]. It has to be pointed out that being overweight changes the „normal“ physiology. In fact, fat accumulates in the liver in the abdominal cavity but also around of the intrathoracic organs, heart and lungs [46–48]. Overweight also is accompanied by increase of blood volume, which is however not proportional to the increase of body weight [49–51]. This may have serious consequences for the kidney [49] if one considers that blood volume physiologically decreases with increasing age [52–55] mostly due to dehydration [53] and hypoalbuminemia [56] accompanied by increased lactate production [57,58] leading to subclinical „chronic“ acidosis especially in obese type 2 diabetics [59–62].

Increase of blood volume without a corresponding increase of the vascular bed may explain the development of hypertension in obese patients [63]. In addition, increased body weight is accompanied by changes in the largest organ of the body, the skin. Increased dryness of the skin is a physiological change in obese persons (inadequate hydration) which is accompanied by increased blood flow in the capillaries, increased transdermal fluid loss and increased sweat gland activity [64]. The latent peripheral tissue hypoxia [65] could explain the increased plasma D-Dimer found in patients with hypertension and overweight [66].

As increase of endocrine pancreas function is timely limited, many obese patients develop hyperglycemia mostly in the presence of increased insulin level (compared to lean persons) which becomes however not sufficient to keep glucose serum level under the prediabetic limit of 125 mg/dl [67]. While the overweight-pandemic expands, the use of different drugs to contrast the „comorbidities“ has also expanded, as it is, the number of persons suffering from different side effects from the single drugs. As those drugs considered adequate for appetite reduction are considered life-style drugs [68] and diffusion of statin-use [69,70] only distract the attention from the causes of the obesity pandemic, a surgical subspecialty, bariatric surgery [71], appears to be the only measure to reduce food uptake, as the therapeutic escape solution for diabetes type 2. Preventive nutritional suggestions (reduction of calorie-uptake especially by reducing uptake of sweets, fat [72,73] and of alcoholic beverages remain mainly unsuccessful [73]. The treatment of „comorbidities“ caused by the diffusion of this lifestyle continues to be dealt with the increasing number of prescriptions of statins („statinisation“, oral antidiabetics and diuretics [74] aggravating maybe the most serious obesity-associated „comorbidity“, namely the „chronic kidney disease“ [75,76].

Metformin (1,1-dimethylbiguanide), a water-soluble, strong basic compound [77] belonging to the group of the biguanides, the compound, which is mostly used as antidiabetic drug for obese patients. It has a molecular weight of 129,16, while metformin hydrochloride (Glucophage) has a m.w. of 165,62. Since 1957, when the first successful use of this biguanide in overweight diabetics was published in France [77] to reach nowadays about 200 million doses/day worldwide for treatment of multimorbid, older, overweight persons with diabetes type II alone or in combination with other orally administered antidiabetic compounds or with insulin [78,79]. The review of the literature of the last 100 years strongly suggests that biguanides can be hardly considered as classical drugs as they do not bind to proteins and do not undergo any biotransformation. They not only induce loss of water and of nutrients (especially glucose) into the stool but also increase water loss through the urine creating a constant danger of kidney failure because of dehydration in elderly overweight/obese diabetics.

1. History of Biguanides: From the First Animal Experiments to the Production of the First Synthetic Compounds to Their First Oral Use as Substitute of Insulin or in Addition to Insulin in Overweight Type 2 Diabetics

Although some characteristic symptoms and the sweet urine have been reported long before Christ was born it was around the 5th century B.C that the Indian doctor Samhita mentioned that those symptoms were more frequent in rich castes and it was related to excessive food consumption. 1776 Dobson (found that glucose concentration in urine and in the blood of a diabetic was increased [80]. In the middle of the 19th century Claude Bernard measured glucose in the portal vein immediately after feeding a carbohydrate-rich diet to dogs. He also found glucose in the portal and in the hepatic vein of dogs fed with meat only. Moving to tissue analysis of glucose he found large amounts of sugar in the liver but not in other organs. He named the starchy, water-insoluble form of hepatic glucose-storing, glycogen, which is then converted into glucose and secreted into the circulation [81]. From this observation he drew the conclusion that an excess of glucose production by the liver of diabetics would be responsible for hyperglycemia. Claude Bernard could not imagine that the storing capacity of the liver for glucose and fat could be overpowered after a meal and that insulin and glucose from the portal blood could bypass the liver and reach the systemic circulation [81–83].

After the histological observations of Langerhans [84] and after Minkowski in 1889 [85] presented his observation that the pancreas is the source of a substance which regulates the glucose serum level, 1910 Sharpey-Schäfer called insulin the possibly deficient factor produced in the cells described by Langerhans [84]. At the time when Banting and coworkers [86] first successfully treated patients with deficient insulin production with pancreatic extract, most of those diabetics were young persons who died within months because of hyperglycemia, while the second increasing group of hyperglycemic patients were the overweight elderly, clinically less apparent diabetics. While the first group was

affected by an irreversible change of normal physiology, the acquired end of a physiological function, namely insulin production, the second group was affected by a reversible change induced by a protracted overstimulation of insulin production [82] due to overweight. The increase of the number in this group of diabetics was the first sign of industrialization of agricultural and livestock production initiated by the German chemist Justus Liebig [10]

Falkenberg in 1891 reported his observation on thyroidectomized dogs. Of 16 dogs, which died because of the operation, 11 presented glycosuria and two of the long-term survivors (several months) were diagnosed by Falkenberg [87] to suffer from what he called diabetes (in both cases parathyroid glands were found in place at autopsy). Glycosuria under such conditions was confirmed by Hirsch 1906 [88].

While looking for the substance responsible for tetanic symptoms observed in parathyreodectomized animals, Fühner H [89] found that the substance guanidine, a product of protein metabolism, eliminated in the urine [90,91], causes muscle contraction by directly acting on the neural plate. On the other hand, it was also found that a thyreoparathyreoidectomy in dogs also causes hypoglycemia and depletion of glycogen deposits in the liver and no glycosuria [92]. Extirpation of three parathyroid glands caused a reduction of absorption of dextrose given by mouth or subcutaneously. Normalizations of calcium serum level by administration of calcium lactate resulted in normalization of blood sugar content[93], while it had been shown that ammonia elimination into the urine was increased[94]. At the same time Burns and Sharpe demonstrated a marked increase of guanidine in the urine and blood of parathyreidectomized dogs and in the urine of children with idiopathic tetany [95]. On the basis of the assumption that parathyroids are involved in glucose metabolism, Watanabe first studied the effect of subcutaneous guanidine administration on rabbit blood sugar levels and on urinary excretion of glucose and of ammonia [96–99]. He administered guanidine hydrochloride subcutaneously into rabbits which were kept without food and water after administration, and several blood glucose tests were performed after the start of the experiments. A decrease of glucose serum level was observed, which was paralleled by a deterioration of general conditions until death ensued. He also observed the development of marked acidosis which took place after subcutaneous injection of guanidine hydrochloride into rabbits which then refused food uptake [100].

Frank, Northemann and Wagner in 1926 [101] first studied the hypoglycemic effect of guanidine on rabbits and realized that it worked like a poison after subcutaneous administration and that, before their death, the animals developed hyperglycemia. Authors then tested herring-sperm extracted- and then synthetically produced- guanidine. They called it Agmatin (aminobuthylguanidine, derived from the amino acid arginine). In the attempt to dissociate the toxic from the hypoglycemic effect, they found that a non-toxic dose of this compound was able to induce a decrease in glycemia. A further modification to aminopenthylguanidine was able to induce a decrease in blood glucose like that observed after insulin administration. To increase the glucose-lowering effect, structural modifications were performed and synthalin A and B were generated. Subcutaneous injection of the substances in pancreatectomized dogs was able to reduce blood sugar concentration in a dose-dependent manner before toxicity symptoms became evident. The speed of the hypoglycemic effect was slower than that observed after insulin injection. A small increase in the dose given orally was sufficient to achieve an effect like that observed after subcutaneous administration

~~After the maximal toxic dose in rabbits and dogs was found, the first clinical attempts in diabetic persons were ready to be tried.~~ The effect of orally administered synthalin was compared to that of subcutaneously administered insulin, namely on the effect of peripheral glucose consumption and on glucose storage as glycogen [101].

To demonstrate the first effect, insulin was injected into the arteria femoralis of a pancreatectomized dog and a decrease of venous blood glucose level compared to the arterial level was determined. ~~A similar effect was reported after intraarterial injection of synthalin. While a higher venous glucose level could be observed three hours and 50 minutes after the intra-arterial injection,~~

a continuous increase of the difference between the venous and the arterial glycemia up to 22 mg%, two hours and 45 minutes after the injection, was observed. It took two hours before the beginning of the decrease of glucose blood levels could be observed in this dog. The authors hypothesized an extrahepatic consumption of glucose as observed after injection of insulin. Contrary to what could be observed after administration of insulin, however, only trace amounts of hepatic glycogen were found in the pancreatectomized fasting dog when non-toxic amounts of synthalin were injected [101].

The animal experiments performed with the intra-arterially injected guanidine were considered successful and concluded. First diabetes patients were then treated with orally administered synthalin. Therapy started with different dosages in insulin-dependent patients. Insulin dosages could be reduced, and glycosuria disappeared. After two or three days however, therapy had to be temporarily interrupted (24-36 hours) because of gastrointestinal side effects: nausea, vomiting, diarrhea, gastric pain. Therapy was more successful if accompanied by a diet [101].

Under these conditions, authors calculated that 1 mg synthalin administered orally generated the same effect as one unit insulin [101,102] in the reduction of the amount of glucose eliminated in the urine. Furthermore, authors found that synthalin was able to reduce the serum glucose level below the glycosuria concentration, to abolish acidotic episodes and to reduce the symptoms of the disease, namely polyuria and polydipsia. Comparable results were obtained by oral and subcutaneous application of the substance.

In a comment to the presentation of his coworkers, professor Minkowski [103] ~~certified that the data generated in his own department were valid. Based on previous experiences from the use of similar compounds, he however also expressed some caveats as to the insulin like effects attributed to previously used substances, they could be due either to the calorie restricted diet alone or to the impairment of the proper intestinal glucose handling. He meant that it was justified to define synthalin as "insulin like substance". It however should not be considered as an insulin „substitute “as the effect was not as strong and rapid as that of insulin [103].~~

~~He added to his judgment stated that it was too early to decide about the grade of efficacy of the drug for the treatment of mild diabetes and for the possibility of developing substances with less side effects. He also prophetically supposed that in a short time there would be many experiences from several sites. He expressed the wish that such trials and their reporting would be performed with necessary, strong scientific rigor. He prophetically wished that previously reported similar insulin-like effects of natural anti-diabetic substances should be studied again under the actual conditions as reported by his coworkers [103].~~

After the first supporting experiences were published [104–108], Frank, Northman and Wagner published their next experience with the „new “biguanide, synthalin B (dodecamethylendiguanidin) [108]. This was a further molecular development of the drug in the attempt to reduce the gastrointestinal side effects observed with synthalin [109]. Initially, the substance induced diarrhea, which was more frequent than under synthalin but less strong and more temporary. Indication for this therapy was diabetes of the older patient normally reluctant to take the prescribed diet, while treatment of choice for the young diabetic patient remained insulin administration [108]. Synthalin B was approved in Germany and made available for further studies in England [109] and in the United States of America [110]. These experiences, especially those performed with synthalin A (diguanidinododecamethylene) delivered some support but also confirmed the high frequency of gastrointestinal side effects [109,110].

Bischoff and coworkers [110] and Batherwick and coworkers [111] reported about a special observation concerning the “poison” synthalin administered parenterally to rabbits. The fasting animals developed hypoglycemia accompanied by an increase of serum urea concentration just before dying. Authors called the damage of the tubular epithelium (while the glomeruli were intact) “nephritis”

Short-term results obtained with oral administration were unsuccessful.

The data were summarized and also critically commented in an article in the scientific journal Nature in January 1928 [112]. ~~It was underlined that synthalin, in spite of the advantages of the oral~~

~~administration and of its more prolonged action is not comparable to insulin because its action is slower in onset and because of the unpleasant side effects „suggesting intestinal irritation“ [112].~~ The author repeated the judgment of the late Minkowski 1926 that synthalin was not „a substitute for insulin“ but the first results obtained with that substance would encourage the continuation of the search for better compounds with insulin-like effects [112]. While the recently synthesized [113,114] strong ~~hygroscopic~~ hygroscopic 1-1-dimethylguanidine hydrochloride was also synthesized and tested in the same Institute in Breslau by the chemists who also first synthesized synthalin and tested for comparison by the pharmacologists Hesse and Taubmann [115] confirmed that biguanide derivatives, like synthalin were able to reduce the glucose serum level up to cramp level and that glucose administration was able to reduce the cramps but not to ameliorate the general conditions of the animals (rabbit). Compared to the insulin effect, biguanides were not able to induce glycogen synthesis in the liver. On the contrary, compound-induced hepatic glycogen depletion was observable. All the authors underlined the finding of Watanabe [99] that the substances, synthalin and the biguanides caused lactic acidosis [100]. ~~Hyperalaninemia induced (poisoned) by administration to dogs of biguanide (guanidine hydrochloride).~~ Minot and coworkers thoroughly studied the lactic acidosis production induced by guanidine intoxication [116].

Authors found that vomiting and diarrhea in addition to the tendency to hypoglycemia were even aggravated by administration of bicarbonate. They also mentioned that in some cases retention of nitrogen could be due to “nephritis” (see Blatherwick et al.). Authors stated that “fluid administration to improve kidney function is an important feature whenever the specific means employed to combat the acidosis are unsuccessful. “The removal of guanidine through increased kidney function maybe an important feature in the adjustment of acid-base equilibrium by continuous venoclysis with glucose or Ringer solution” [116]. Because of the unsatisfactory safety the search for new guanidine-compounds continued [115] and synthalin remained as an oral antidiabetic drug on the German market until 1940.

Then retraction from the market was effectuated because of the seldom- occurring but fatal acidosis, attributed to anaerobic glycolysis, [117] and because of the possible also fatal hepatotoxicity [118]. At the beginning of the fifties, however, Synthalin received interest again because of reports about its specific toxic effect on pancreatic alpha-cells [119,120]. At the same time several other („new“) biguanides were synthesized and tested in comparison also with synthalin but the side effects especially the tendency to lactate production due to anaerobic glycolysis were quite similar to those of the other biguanides [121].

Buformin and phenformin [122–129] were developed and used for treatment of obese type 2 diabetics (until today).

It was shown that phenformin administered orally for three days to hyperinsulin responders, obese non-diabetics before oral but not intravenous administration of glucose were able to reduce both glucose and insulin serum levels to the level of normal subjects. A similar effect was also observed in obese diabetics [128]. These results strongly suggested a reduction of glucose absorption and also a lack of induction of intestinal hormones by phenformin, which would have on the contrary stimulated the beta cells to synthesize more insulin (according to a still current hypothesis).

Walker and Linton during the two years of use of phenformin in at least 100 patients observed „a worrying and indeed dangerous complication“ ketonuria and acidosis in the presence of normoglycemia or mild hyperglycemia [127].

Ketonuria with or without acidosis was observed in about one third of all cases, always without a significant rise of blood sugar levels. The suggested measures to combat ketosis (“metabolic starvation”) were increased dietary carbohydrate, reduction of the dose of phenformin and supplementary use of insulin. These measures with additional alkali administration were not successful in a patient who died a few hours after admission and autopsy failed to find lesions possibly responsible for his death [127]. Walker RS, however, mentioned its efforts to clarify the mechanisms of acidosis development under phenformin therapy and reported that the patients controlled under phenformin had increased basal blood lactate levels exaggerated by the

administered compound and by exercise accompanied by a marked fall of the alkali reserve of a magnitude many times greater than that observed in normal people.

Dehydration, as the cause of hypotension and kidney injury, was not discussed neither at the clinical nor at autopsy level.

At that time Beaser in his report [129] about the short-term- treatment of 10 overweight diabetic patients with a combination of phenformin, sulfonylurea and insulin did not find toxic side-effects but mentioned that „the potential usefulness of phenethylguanidine is circumscribed by gastrointestinal intolerance, which supervenes in many patients before the most effective dosage levels are reached“. As it will happen in the following decades the focus was put on the glucose serum level [130] and less on the systemic consequences of the gastrointestinal fluid loss due, on the one end to the reduced fluid uptake as a consequence of nausea and loss of appetite and the increased loss through the intestine and, on the other hand to an increased loss of water through the kidney [131,132].

In a study performed in Japan [133] on 13 overweight diabetics in Osaka randomly divided into two groups, buformin was given in a daily dose of 150 mg (50 mg after breakfast, lunch and dinner) for two weeks, while the other group was administered a hypocaloric diet (20 kcal/kg b.w.) along with physiotherapy for two weeks. Buformin treatment not only significantly lowered fasting plasma glucose and plasma insulin-levels, but it also increased hepatic but not peripheral glucose uptake when compared to the diet alone group [133]. While phenformin had definitively been withdrawn from the market in the USA because of lactic acidosis development [135], buformin was partially taken from the market. Both are still used in many countries outside of the United States of America [129,130,134].

The use of metformin(dimethylbiguanide of Wagner and Bell),as a „new“ biguanide was first described by Mehnert H and Seitz W [136] and by Sterne 1957[137] and first approved in France 1959.It was used in the UK where the lowering effect on body weight was described when compared to the opposite result obtained with chlorpropamide after twelve months (but not after further six months) of treatment in obese diabetics [138].By that time „some non-tissue-forming catabolic process“ should be hypothesized if reduction in food intake was not the explanation for the tendency of weight loss in obese diabetics [138] as also observed with phenformin .

Bratusch-Marrain and coworkers [130] studied the effect of buformin on carbohydrate metabolism in healthy men. Authors found important results as they found that buformin altered the lactate-balance and net lactate output occurred under buformin treatment. In fact, splanchnic lactate output was increased after glucose ingestion, while hepatic glucose output was not increased. The arterial blood glucose concentration after ingestion of glucose was significantly reduced by buformin pretreatment. Buformin treatment induced a marked rise of splanchnic lactate production and of an increase of c-peptide level as measured by the hepatic venous catheter technique [130].

Despite of some critical case reports about the occurrence of fatal acidosis [139] and of the long-term low efficacy (mostly justified by improvement of microvascular changes) on a subgroup of overweight type II diabetics [140] and mainly based on the short- term (29 weeks) effect on glucose serum level, as surrogate marker [141] the trial results published [Table 1] were considered to favor introduction of metformin as an oral antihyperglycemic drug.

The findings were considered to be sufficient for approval of metformin as one more oral antidiabetic drug [142] in the United States in august 1995 while the first cases of lactic acidosis which were reported were mostly attributed to „comorbidities [143] which were then listed as contraindications [144] for metformin therapy. A confirmatory long-term prospective trial under similar conditions of the short-term was not requested by the FDA [143,144].

Metformin was defined as the „cousin “of phenformin by Campbell and colleagues [142] and not much different from buformin [128,145] and from the other biguanides as described in the previous decades (see above).

Table 1. Metformin-therapy in diabetes type 2, prospective trials:inclusion criteria.UKPDS 1996* DeFRONZO et al.1995**.

N	342	352***
Age (mean years)	53	53
BMI	27-31	29
Fasting		
Hyperglycemia:		
HbA1c	7	8
Fasting lactate --		1.41-/1.46 (p=0,08)
(<1.30 mmol/L)		1.47/1.54
Max doses		
(mg/day)	2.550	2.550
Duration	15y	29 weeks
UKPDS	De Fronzo et al.	
-symptomatic diabetes (e.g. polyuria)	-symptomatic hyperglycemia (e.g polyuria,	
dehydration, ketonuria)	dehydration,ketonuria)	
-history of myocardial infarction, angina	-symptomatic cardiovascular disease	
or sign of heart failure	-concurrent medical illness	
-more than one major vascular episode	-3 or more alcoholic drinks (>3 oz/day)	
-serum creatinine higher than 1.98 mg/ml	-use of chlortalidone or thiazide allowed	
-malignant hypertension, severe retinopathy	-smoking not mentioned	
-severe concurrent illness		
-smoking allowed		

Table 1 Comparison of the essential characteristics of the two prospective trials, which lead to the approval of metformin by the FDA as an oral compound to treat hyperglycemia in adult obese patients with type 2 diabetes. *most of the exclusion criteria were then listed as contraindication for metformin therapy.

2. Pharmacokinetic and Pharmacodynamic of Biguanides

„To Produce Its Characteristic Effects a Drug Must Be Present in Appropriate Concentration at Its Site of Action“ [146 See Also Table 1-2 of This Article]

After oral administration of metformin[147,148] like the other biguanide hydrochloride [149,150], about 30- 60% of the compound [149] is absorbed,as while the largest part accumulates in the small intestine to reach a 300-fold larger concentration than it has been measured in the blood [151] in the first part of the small intestine (into the venous blood of the portal vein, released from the fenestrated[152] hepatic sinusoidal endothelium of the liver. It was first supposed to be transported through the OCT1-hepatocyte membrane transporter [153,154] and genetic variations of this transporter were supposed to be the basis for the different efficacy of the drug .However this could not be confirmed in fasting healthy individuals with reduced-function OCT1-diplotypes [155].Treatment of those individuals with 2000 mg metformin daily for a week did not modify plasma

glucose-, insulin and C-peptide-concentration. While plasma glucose appeared to be lower during the first 2-3 hours the mean plasma lactate value increased significantly during metformin treatment, remaining within the normal range.

The absorbed part of the orally administered metformin is released from the liver into the systemic circulation and eliminated unchanged (up to 5 times faster than creatinine) through the kidney by glomerular filtration (but also by tubular secretion?) and can be found to almost 100% in the urine. After intravenous administration, Pentikäinen found some C14-labelled metformin in the saliva but not in the feces [151].

After oral administration of C14-metformin the bioavailability in three healthy volunteers was 61% and 51% of the administered dose 48 hours later. The recovery in the feces of one volunteer from whom the stool was collected for one week was 29.4% and 90.5 %, together with the activity recovered in the urine. Metformin was not bound to proteins neither in vivo nor in vitro [151] (Table 2).

Table 2. Main “metabolic” effects of metformin in the gut after oral introduction.

Metformin in the small and large bowel after oral administration

-
- reaches a local -concentration up to 300-fold higher than that in the serum
 - induces local(intestine) lactate production, abdominal discomfort and pain
 - induces water loss from the systemic circulation into the intestine
 - induces nausea, vomit and diarrhea
 - induces glucose loss from the blood into the intestinal lumen and
 - increases elimination of glucose from both the blood and the ingested food
 - induces reduction of protein digestion
 - Trace amounts remain attached to the mucosa of the large bowel for longer than 72 hours after oral ingestion of a single dose
-

C11-metformin (9.5 microSv/MBq=ca.1.1 micrograms), first injected intravenously, allowed to study its biodistribution in humans, by the PET-scan technique [153]. Most of the activity was found in the liver, the kidney and the urinary bladder. Activity peaked immediately after injection and most of the compound was cleared from the blood 20 minutes after injection. In the kidney there was quick intense activity (80% of the injected activity) with an equally rapid decrease with a reversibility velocity like that of the liver but faster. In the liver the peak of the activity was of course lower (15% of that in the kidney) because of the much larger volume of the cellular distribution (Figure 1 upper panel).

After rapid ingestion of the strong basic compound C11-metformin (18.1micro Sv/MBq, half-life= 20.4 minutes) dissolved in water containing 100mM (NH4)2-HPO4 [pH=5], consecutive whole-body scans were performed. Dosimetry calculations were performed for the stomach content, small intestine, liver, kidney and bladder content. It was demonstrated that hepatic metformin uptake is very rapid and fully reversible, but the accumulation of the activity is higher than after intravenous administration, as, although slower, the tracer delivery comes from the portal blood through the liver first. Two hours after the oral ingestion of the tracer the bulk of the radioactivity is still to be found in the intestine (Figure 1 lower panel) and no further observation of the fate of the radioactive metformin was possible.

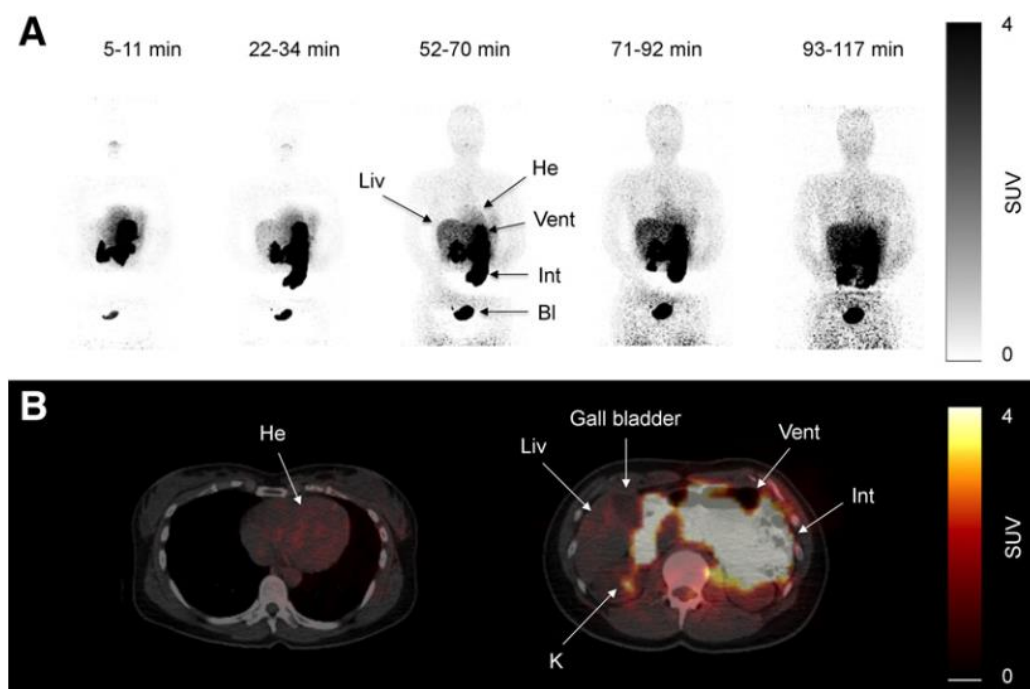


Figure 1. Scans of C11-metformin administered to humans intravenously and orally (lower and lower panel respectively) taken at different time after administration. Gormensen LC et al. (153, with permission).

It is understandable that the study performed by Pentikäinen and colleagues [150] with ^{14}C -labelled metformin could only be repeated by the addition of „cold “metformin to the ^{14}C -labelled compound. This would most probably accelerate the intestinal passage as most of the conventional drug- containing tablets also contain polyethylenglycol (3-6.000) as an additive, which is also a laxative.

Although an active transport of metformin through the membrane of the hepatocyte has been extensively discussed by Gromensen et al [153], it has to be mentioned that hepatic densitometry pictures similar to those observed after intravenous and oral administration of C11-metformin can be observed also after the injection of C11-nicotine [156] or even of C11-Donepezil, a high-affinity antagonist of acetylcholinesterase normally used as drug in the treatment of patients with Parkinson's disease [157,158]. It cannot be ruled out that there is a diffusion into the space of Disse through the fenestrae of the sinusoidal endothelial cells [152] and that the compound is then washed out from the interstitium back into the hepatic vein. It may be therefore more appropriate to speak about hepatic metformin „extraction “ [159] than about uptake.

The kidney to blood activity ratio was identical independently of the administration route of the radioactive metformin. Some discrete uptake of the tracer was found in the salivary glands and discrete uptake was found also in the intestine. No activity was found in the gallbladder. Significant amounts of the tracer passed to the small intestine 10 minutes after ingestion.

Despite the increasing popularity, the mechanism of action of metformin (gluco-phage of Sterne) has been elusive until nowadays.

Especially after the report of Bonora et al. [160] and of Sum et al [161] on the lack of metabolic changes after intravenous administration of metformin hydrochloride, attention concentrated mostly on pharmacokinetic studies performed after oral administration of immediate- or retarded release-metformin formulations and of different dosages at different time of the day.

Bonora and colleagues [160] found no significant change in fasting plasma level of glucose, insulin, C-peptide, glucagon and growth hormone after intravenous injection of 1g of metformin as a bolus in 15 non-diabetic subjects (4 males and 11 females) after a 12-hours overnight fast. Blood was drawn 15 minutes and up to 30 minutes after injection of metformin hydrochloride dissolved in 10 ml of distilled water. The finding ended with the discussion about a possible direct effect of

metformin on insulin production in the pancreas. Sum et al. [161] performed a double-blind randomized crossover study in nine type 2 diabetic patients (6 female and 3 male, HbA1c 8,8 vs 8,6 on oral metformin therapy (Glucophage, Lypha).

After a 10-hour fasting period and having omitted the morning metformin dose, D-glucose, [3-H3]-glucose and metformin/NaCl were injected intravenously. Metformin was continuously infused to achieve a serum concentration at a lower therapeutic level and then (after the first 120 minutes) increased to reach a high therapeutic range of 5-7 mg/L. Glucose was infused to reach and then maintain a concentration of 5 mmol/Blood samples were collected at 10-minute intervals. Urine samples were also collected. After the first increase of metformin serum level from 1.64 to 6.57 at the end of the second step a continuous decrease of metformin level was measured under NaCl infusion.

The study was repeated between 10 and 20 days after the first study and no difference was found, between the two study days, in fasting glucose-, insulin-plasma-levels and in metformin concentration. No difference was found between control and metformin studies in hepatic glucose disposal, peripheral glucose disposal, glucose oxidation. Although the authors did not underline this important finding they observed no change in blood lactate concentrations. These results demonstrated that there was no acute effect of increasing metformin serum concentration in type 2 diabetics on hepatic glucose production or peripheral glucose disposal and, contrary to the previous observation, after oral administration, no influence of intravenously administered metformin on lactate production was found [161].

Sombol and coworkers found that the pharmacokinetic parameters after a single dose of 850 mg of metformin hydrochloride in healthy NIDDM-patients (6 males and 3 females of the original 12) with a creatinine clearance of >85 mL/min, were not statistically significant different compared to those measured in healthy subjects (5 males and 4 females) [147].

Three diabetics, however, withdrew during the single-dose phase. One had a hypotensive episode with nausea and vomiting and one other had several headache episodes, a third patient withdrew for personal reasons. In the healthy people's group, only one withdrew for personal reasons.

A single dose (850-2.550 mg) of metformin did not significantly decrease the postprandial glucose serum concentration. When the single dose was augmented the clearance/bioavailability ratio increased in both participant groups, mainly due to a decrease of bioavailability than to an increased clearance which was lower when 1.700 or 2550 mg than 850 mg were administered in NIDDM and was significantly lower in healthy persons. It was confirmed that bioavailability decreased with an increase of the administered dose. In fact, it was previously reported that bioavailability decreased from 86% to 42% when the dose was increased from 250 mg to 2.5 gm. As an explanation for this phenomenon it was hypothesized that metformin **permeability** is limited as a non-lipophilic compound, this is confirmed by the 30-60% bioavailability in absence of hepatic extraction and by the fact that bioavailability of metformin **of intermediate release formulations is lower than** that of immediate release formulations.

In addition, it was found that intake of food decreases the absorption of metformin by about 25%. While, in diabetic participants, multiple doses of metformin decreased fasting glucose serum concentration and also postprandial glucose levels, no effect on glucose serum level was detected in healthy subjects. This is not due to different pharmacokinetics of metformin in each group and can only be observed also in non-diabetics if the serum glucose level has been artificially raised.

Furthermore, the glucose-lowering effect of metformin in NIDDM was correlated to the level of severity of fasting hyperglycemia and significant reduction of pre-prandial insulin plasma level was observable after multiple doses of metformin hydrochloride. Interestingly a statistically significant decrease of postprandial (0-2 hours post meals) insulin concentrations was observable in healthy subjects after metformin administration (4-6 hours) both of different single doses (850-2550 mg) and multiple doses.

It was justified to conclude that the pre-prandial glucose-lowering effect of metformin in NIDDM-subjects and the early postprandial effect in healthy subjects is not dependent on stimulation of insulin production.

This pivotal study, supported by Lipha Pharmaceuticals Inc., the same company, which performed the short-term trial (AM Goodman), which led to the FDA-release 1995, [141] was performed in young diabetics (47 years mean age otherwise healthy) and in young (46 years mean age) healthy controls. It had the potential to analyze the effect of metformin on creatinine clearance, as authors mentioned that urine was collected to measure metformin concentration and urine volumes were measured to calculate total metformin clearance. In the results section there is no data about creatinine clearance possible changes and eventually also lactate concentration. Both are not mentioned in the discussion as there is no mention of the possible changes in body weight.

This is surprising as it was stressed that the diabetics, participating in the study, had normal creatinine clearance.

In fact, one of the most interesting findings of this complicated study was that the serum concentration of metformin measured on day 4 and 5 was significantly higher than that measured at day 1 of study begin. The explanation for this surprising finding could have been that there was an accumulation of the compound in the blood due to reduced renal clearance. Authors introduced the "new" definition of "steady state" blood concentration as their explanation, possibly distracting from fluid loss because of adverse events (not reported).

On the other hand, some sentences in the introduction: 1) "Although the chemical structure of metformin is similar to that of phenformin, it has much less propensity for causing lactic acidosis. 2) "The pharmacologic action of metformin primarily involves an increase in cellular glucose uptake, gluconeogenesis, glucose oxidation and lactate production;" suggest a certain influence of the supporting pharmacological company as that company was working together with the FDA in preparing the release of the compound into the North American market. In fact, the conclusion of the discussion section of the publication was "This finding, together with the fact that the magnitude of the glucose-lowering effect of metformin appears to depend on the level of hyperglycemia, makes metformin an attractive alternative for treating NIDDM".

Unfortunately, authors did not discuss the results of this study (also supported by Lipha Pharmaceuticals Inc.) taking into consideration their own previously published results obtained in persons of different ages, e.g. healthy young (27 years) healthy elderly (71 years), healthy middle age (47 years) with creatinine clearance varying between 111 and 89 ml/min where metformin clearance was investigated. In that study, patients with a different degree of chronic renal insufficiency with creatinine clearance between 73 and 22 ml/min

were also included. The study clearly showed that creatinine clearance physiologically decreases with increasing age and that clearance of the absorbed metformin, administered orally (850 mg) decreases as a function of creatinine clearance. Both, creatinine and metformin-clearance could have been negatively influenced also by the consequence of "the most frequently observed adverse events" including diarrhea, abdominal cramps, nausea, headache and dizziness on loss of fluids [148]. The urine volumes were not given in the result section.

Besides the previously hypothesized fact that a clear effect of metformin on lowering glucose can only be found when administered orally by reducing glucose absorption [147] in the intestine, additional effects like inhibition of hepatic glucose production, improved glucose uptake and utilization were mentioned together with weight reduction, reduction of plasma lipid levels and prevention of some vascular complications.

McCright and coworkers [158] performed a pharmacokinetics study in obese patients with type 2 diabetes who were intolerant to metformin and compared the numbers obtained in 19 obese diabetics who tolerated oral metformin. Under fasting conditions 500 mg metformin were orally administered. Peak serum concentration of metformin was reached at 2.5 hours (2.1/2.0 mg/L respectively) in both groups and the continuous decrease for 24 hours follow-up was also comparable [162]. Lactate serum concentration increased to 2.4 mmol/L one hour after the peak of metformin

serum concentration in both groups. The curve of the decrease of the serum lactate concentration during the following hours was even more flat than that of metformin. The peak of lactate measured early after metformin ingestion is of course higher than that measured 24 hours after administration of higher doses of metformin to their patients with a lower BMI as reported by De Fronzo 1995 [141].

Somoyi and coworkers had already published a significant increase of serum lactate at four and twelve hours after oral administration of 250 mg of metformin (131). Authors supposed a correlation between plasma concentration and pharmacodynamic response, which would become more evident in patients increasing metformin doses as previously observed by Phillips and coworkers in patients with declining kidney function (132).

3. Mechanisms of Action (Lowering Effect of Serum Glucose) of Metformin- and the Other Biguanides- Therapy

An attempt to explain the mechanism of action of biguanides in lowering glucose serum level has been again discussed by Sterne [163]. After eight years of personal research, it was clear for him that: 1) in the diabetic patient the biguanides do not act by metabolic effect, 2) There is an absence of hypoglycemic effect in the non-diabetic man under biguanides treatment (dimethyl in France n-butyl in Germany and phenethyl in USA, 78) the biguanides do not stimulate insulin production, 4) the development of lactic acidosis so far observed may be due to many other reasons, especially to the coexisting comorbidities, 5) there was not enough support for the hypothesis of biguanide-induced deviation of glycolysis toward the anaerobic pathway.

Despite the many additional publications, however, the mechanism of action of metformin (and of the biguanides) has been a matter of discussion for now one hundred years since its synthesis and administration to animals and since its introduction as a therapeutic option in overweight/obese elderly type 2 diabetics [78,164–169]. It continues to be under discussion as reported in the most recent publications [200,201]. It is however important to underline the following established certainties:

1) about 40-60% of the orally administered compounds are excreted with the stool, 2) the absorbed compounds are completely excreted unchanged through the kidney, 3) no damage is observed in tissues like liver, kidney, muscle after administration of the compounds and only a trascurable amount of the biguanide is detectable in those tissues, 4) increase of serum lactate can be detected especially when administered orally after a meal and in obese persons but not after intravenous administration, 5) although an increase of lactate level can be measured in the portal blood after oral administration, lactate concentration in arterial blood is higher than that in the hepatic vein after oral administration explained, at least in part, by the use of lactate in the hepatocellular gluconeogenesis, 6) in most of the cases of hospitalization reported under biguanide therapy because of symptoms of lactate acidosis dehydration and hypotension combined with acute kidney failure (or acute on chronic) dominate the clinical picture, lactate acidosis being the marker of anaerobic glycolysis in the hypoxic tissues and reduced renal clearance as it maybe an increased serum biguanide concentration. Taken together this data strongly suggest that lactate production through tissue anaerobic glycolysis is not due to the biguanide present in blood circulation 7) Injection of lactate in healthy animals does not have any pathological consequence (Table 6 3).

In experiments performed in metformin treated fa/fa rats, Penicaud et al. [164] demonstrated that there was no glucose utilization in insulin-dependent organs and it was even decreased in skeletal muscle. Authors found an increased glucose utilization in the stomach and small intestine of the metformin-treated rats as confirmed by Ikeda et al. (172), by Abdul-Ghani (173) and recently also by experiments in the mouse (174). The experiments demonstrated that metformin does not exert its hypoglycemic action through an increased insulin secretion, but it rather causes reduced intestinal absorption of glucose and therefore an increased glucose elimination [164,167] and most probably also of glucose reaching the intestine through the bile [169].

After having observed a clinical case of lactic acidosis arisen in a 65 year-old patient who presented with a massive metformin serum concentration but with only a mild increase in serum creatinine in the presence of an acute intestinal occlusion, the clinical picture resolved soon after the abdominal operation (175). This experience stimulated Lalau and coworkers to reproduce this

clinical situation in an animal experiment, where authors could show that obstruction of the terminal ileum may induce a short-term increase of metformin serum concentration without a concomitant increase of serum creatinine and serum lactate. Among other possibilities authors propose hypovolemia as possible cause of metformin serum retention (176). Authors called the increase of serum metformin “retention” intended as reduced renal elimination.

The elevated metformin serum concentration without an increase of serum creatinine concentration could be the consequence of a reduced urine production persisting not long enough (duration of the experiment 4 days) confirming the 5-fold faster metformin renal clearance.

Additionally, the intestinal retention due to the jejunal obstruction could have allowed a further absorption of the administered compound into the systemic circulation. The retained compound could also have caused water loss into the intestine.

Table 3. ~~metformin~~ biguanides and glucose metabolism 100 years after ~~its~~ their synthesis.

-after intravenous administration elimination through the kidney unchanged and with no metabolic effect
-after oral administration concentration in the small bowel 300-fold higher than in systemic circulation
-after oral administration 40-60% eliminated with the stool
-no damage induced by the drug in the studied organs (intestine not studied)
-lactate serum level in portal blood and in systemic circulation increase only after oral administration, when
-lactate in arterial blood is higher than in the liver veins
-metformin biguanides do not increase insulin-synthesis nor insulin-activity,
-possible increase of glucose elimination through the bile into the intestine
-biguanides increase intestinal glucose-elimination through its strong hygroscopic effect and water loss into the lumen
-metformin decreases protein metabolism in the intestine, albuminemia, blood volume and blood pressure
-biguanides increase urinary sodium (and water) elimination in the urine similar to diuretics

Bailey also underlined glucose utilization in the intestine even in the fasting state using glucose from the vascular compartment [177].

Besides the inhibitory effect of glucose absorption in the small intestine, a reduction of the glucose production in the liver is still under discussion. This is however difficult to understand as intravenously injected metformin does not have any effect on glycemia [160,161]. Furthermore, there is no established therapeutic-, nor “toxic” concentration of metformin reported in the literature, which could be used for therapy monitoring or diagnostic purposes in diabetics under biguanide therapy, as recently assessed by Lalau et al. [178] given also the enormous variability of the serum level especially in obese persons [151], possibly due to a different compliance and/or hydration status, which can change suddenly through symptoms like vomiting, diarrhea, fever and leading to emergency clinical conditions. As stated by Lalau and coworkers, caution should be used when attributing a causative role for lactate acidosis to metformin therapy (178).

A further indirect mechanism of the glucose-lowering effect of orally administered metformin has been postulated, namely through the increase of the incretin GLP-1- production in the small intestine [173] eventually mediated by bile salts [179]. On the other hand it has been demonstrated ex vivo in the perfused rat ileum that only carbohydrate luminal perfusion increases the release of glucagon-like peptide-1 into the mesenteric vein [180] through the uptake of glucose into the epithelial cells. In fact, an increase of GLP-1 production could be detected in the portal blood of diabetic and non-diabetic cirrhotic patients after the introduction of a study meal (69 grams of carbohydrates) into the small intestine, but no significant difference in the peripheral insulin serum level was found [181] between diabetic and non-diabetic patients. As GLP-1 should reduce glycemia by stimulating insulin

production in the pancreas only in the presence of hyperglycemia, an increased insulin serum level under metformin therapy should be the precondition for the indirect hypoglycemic effect. Insulin serum concentration under metformin (as is the case for buformin and for phenformin) therapy, however, does not increase, either. Instead, it remains unchanged, or it is decreased as it has been repeatedly demonstrated. The administration of GLP-1 RA exenatide in type 2 diabetics seems, on the contrary, to function by a mechanism like that of metformin by inducing a significant reduction of body weight in a short-term treatment [182].

Recently a further attempt has been made to explain the early loss of body weight after starting oral metformin therapy. It has been found that the blood of diabetic overweight patients treated with metformin contained increased amounts of GDF15 [183,184] a hormone considered to act as an appetite suppressor by directly interacting with specific receptors in the brain. On the other hand, however, a decrease in the release of ghrelin (the classical appetite stimulus) into the blood but also the increase of an appetite-suppressing metabolite (185) has been reported.

It was hypothesized that increased production of the hormone would take place possibly due to a metformin-induced change of the intestinal microbiota [184]. It cannot be excluded, however, that metformin acts as stressor for intestinal cells and that the local GDF15-production together with increased lactate and also serotonin (186) production may represent a sign of local hypoxia. A similar explanation could be the basis for the recently published induction of other possible appetite inhibitors [185]

In a retrospective analysis, Kim and coworkers [187] described an accumulation of injected F18-FDG in the lumen of patients suffering of non-inflammatory diarrhea or constipation, and, for the first time intravenously injected, radioactive glucose in the stool. Gontier and colleagues [188] retrospectively studied the localization of F18-FDG injected in diabetics type 2 and found a significant increase of the radioactively-labelled glucose in the wall, but also in the lumen of the small bowel and of the colon in patients treated with metformin compared to that of non-metformin treated diabetics, which was not different from that of non-diabetics controls [188]. The authors stated that „the digestive tract is the only tissue responsible for a large glucose utilization enhancement“.

Morita and coworkers [189] by using positron emission tomography (PET)-MRI, recently found that the maximum standardized uptake value (SUVmax) of F18-FDG in the intestine (jejunum, ileum and right or left hemicolon) of metformin- treated diabetics was higher than that of the control group. More importantly, the study permitted to differentiate the SUVmax of the intestinal wall from that of the intestinal lumen. The SUVmax of the intraluminal space in metformin-treated diabetics was greater than that of controls (Figure 2). On the contrary the SUVmax of the intestinal wall was similar in both groups [189]. A temporarily increased accumulation of the injected tracer seems to be observed (Figure 3) also in the liver of metformin-treated diabetics up to 48 hours after interruption of the oral uptake of the drug [189–191] suggesting a persisting uptake of the radioactive glucose mediated by circulating insulin as consequence of the “metabolic starvation”(?) induced by the biguanide.

In a pivotal animal study Massollo and coworkers found a significantly increased intestinal uptake of intravenously injected radioactive glucose in mice that have been fed metformin for 4 weeks. At the same time serum glucose concentration decreased as was the whole-body radioactivity. The latter finding does not support the assumption of an insulin-mediated glucose uptake in the muscle but could indicate a reduction of peripheral blood volume. Authors also stated that „the data indicates that the metabolic action of metformin cannot be directly attributed to the presence of therapeutic drug concentrations; they reflect rather phenotypic modification in the gut cells that occur after a relatively long time and persist at least 2 days after drug disappearance“ [192 page 263]

Accumulation of F18-FDG in the large intestine (Figure 4) has been found also in persons who regularly use laxatives [193,194]. SUVmax can even reach levels which simulate those of colorectal neoplasms (Figure 5) in patients with chronic constipation [195].

It seems justified to conclude that biguanides such as phenformin [196] and metformin [197] not only induce glucose „malabsorption“ after oral administration, they also may „attract“ glucose from the intestinal vasculature into the lumen of the small bowel which is then transported and

concentrated as stool of the large intestine together with the remaining compound (198). Under the influence of intestinal metformin, the reduced glucose uptake from the intestine into the portal blood could also be compensated by an increase of an insulin-mediated uptake [199,200] of glucose from the systemic circulation into the liver, from there into the bile [174,201] and into the small bowel. Through the effect of metformin, radioactive glucose then reaches the large intestine, there achieving the concentration necessary to be captured by the dosimetry scan (whole-body PET-scan).

The reduction of intestinal absorption of glucose [170] of other nutrients [202] and of some life-saving drugs [203] by metformin resembles that of „irritant laxatives“ [204,205] which produce peristalsis and not only reduce absorption of glucose and other food components [206,207], an increase of serum level of homocysteine [208] but it also induces a reduction of protein digestion [209] and also and even more importantly loss of water [210]. The latter, together with increased loss of sodium and water with the biguanide may explain dehydration and increase of creatinine serum level as described in metformin-treated overweight type 2 diabetics. It may even worsen the latent hypoperfusion of peripheral tissues, eventually explaining excess of production of lactate, hyperferritinemia, increase of hepcidin serum level and hyposideremic anemia as an acute-phase-response to tissue damage [211,212].

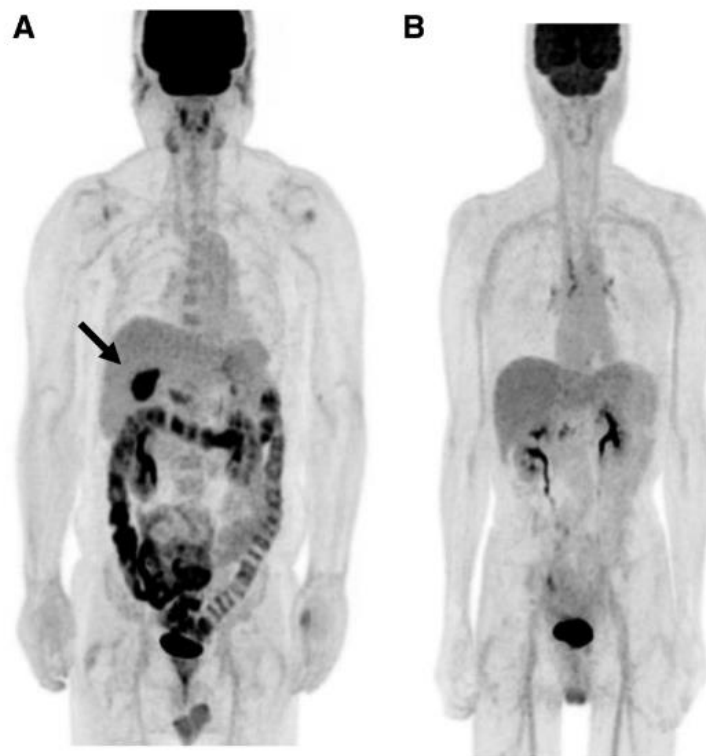


Figure 2. PET-images taken 60 minutes after intravenous administration of F18-FDG in a diabetic patient treated with metformin (A) and in a control patient (B). In A radioactivity has accumulated in the last portion of the ileum and in the colon (right hemicolon stronger than left hemicolon). The indication for the study was gall bladder cancer as confirmed by the accumulation of the tracer in the gallbladder (arrow). From Morita Y et al. (189).

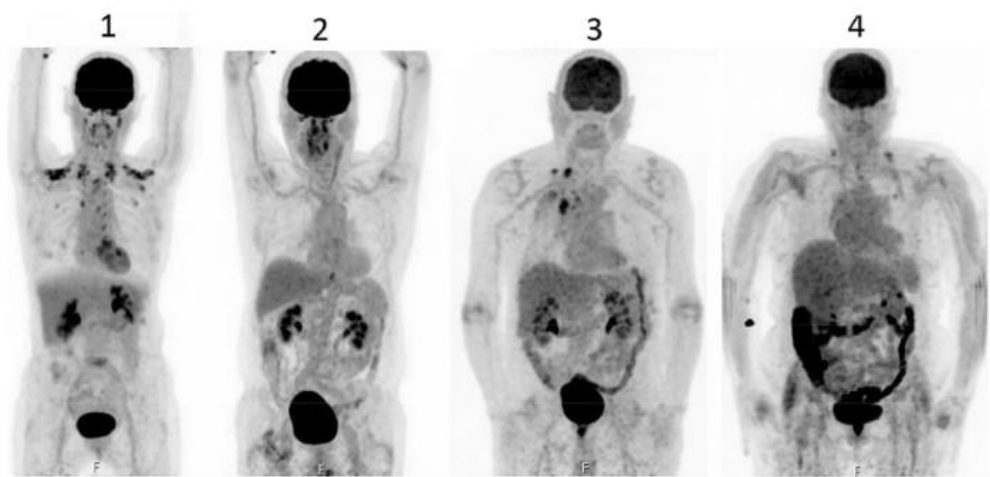


Figure 3. PET-scan was performed in diabetics at different times after interruption of metformin therapy. In patient 4 interruption time was shorter than 48 hours and showed strong accumulation of the tracer in the colon. From Schreuder N et al. (191).

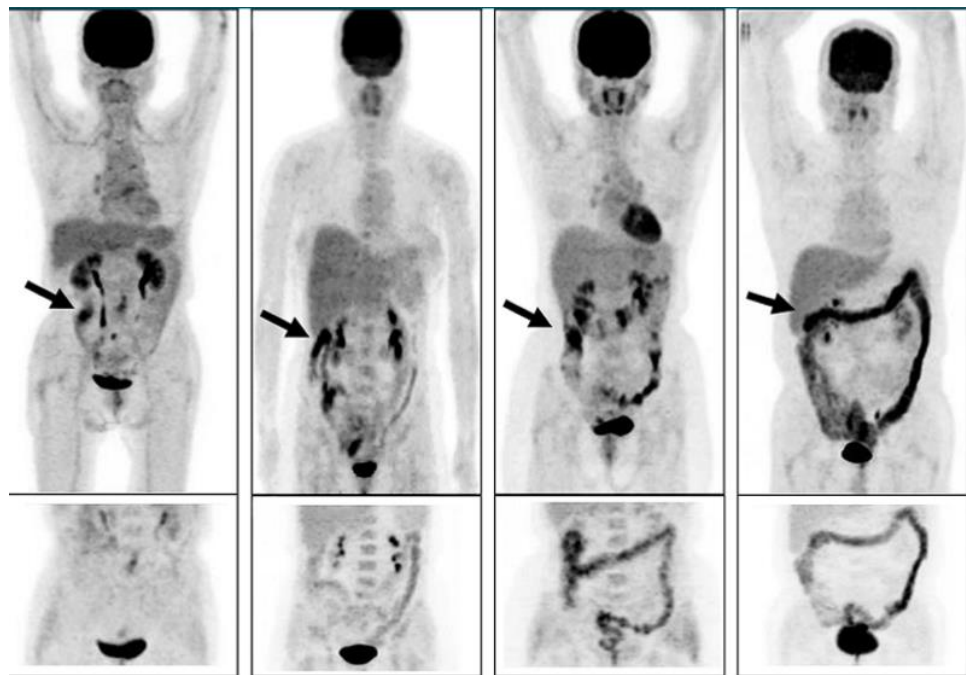


Figure 4. PET-pictures of early scans (upper row) and of late FDG-scans(lower raw) after oral administration of laxatives. The arrows in the upper row of PET-scans show the different patterns of accumulation of the tracers in the large intestine. From Chen Y-K et al. (194, with permission).

from a mild diabetes (HbA1c=6.8%) and from chronic constipation treated with

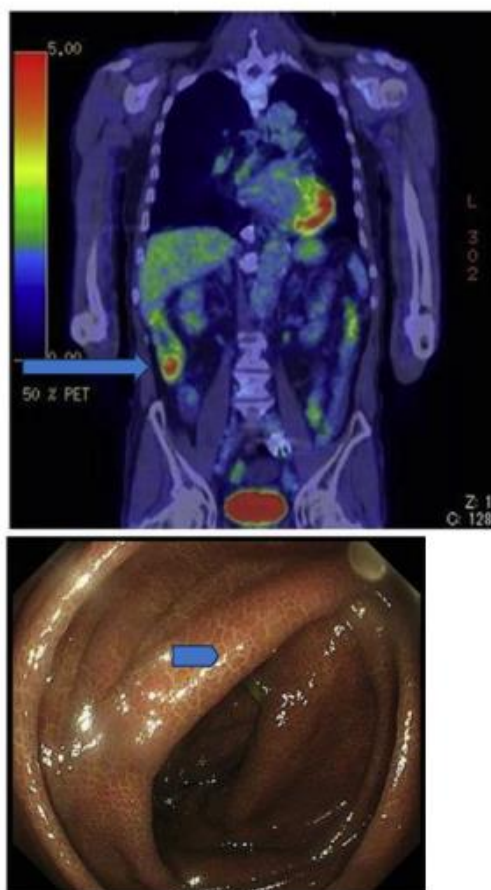


Figure 5. Accumulation olaxatives after intravenous administration) in the coecum and ascending colon (upper panel, long arrow) of a patient suffering anthraquinone laxatives as demonstrated by the presence of melanosis coli at colonoscopy performed to exclude colon cancer (lower panel, short arrow). From Katsumata R (195).

4. Short- and Long-Term Side Effects of Metformin and of the Other Biguanides ~~Contraindications and Adherence to Metformin Therapy~~

a) Short-Term Side Effects of ~~Metformin~~ Therapy with Biguanides

As described by Frank and colleagues [101] at the first administration, synthalin causes „nausea, loss of appetite, increased intestinal movements, gastric pain, vomiting and also diarrhea “. For this reason, they developed a therapeutic procedure consisting of increasing the dose for the first 3-4 days followed by an interruption of ~~drug~~ administration of the compound of 1-2 days. It is therefore understandable that many diabetics, especially older ones, cannot tolerate the medication, as those side effects may be accompanied by dizziness, tiredness abdominal cramps, asthenia, myalgia and an altered metallic taste [213,214] as confirmed to be more frequent in patients treated with metformin than in the placebo treated control diabetics. Although the side effects are like those caused by cytotoxic anticancer drugs, (215) in the case of the biguanides they have been attributed in part to the intestinal disturbance of the bile salt metabolism by ~~metformin~~ [216–219]. The increase of bile salt elimination in the stool has been observed also in patients with chronic constipation treated with elobixibat, a laxative approved in Japan for treatment of chronic constipation [220], probably like what has been discussed recently for other antihyperglycemic laxatives [221,222].

Biguanides can cause, besides the above-mentioned side effects, flatulence, abdominal bloating, heartburn, headache, agitation, chills. It is therefore not surprising that aggravation of dehydration may be the consequence especially in elderly overweight patients. Reduced blood volume could even be the mechanism behind the observed effect of metformin on blood pressure when administered to hypertensive non obese men [223,224] (Table 4) as the strong renal clearance of the compound and sodium with water (225) resembles the action of diuretics.

Table 4. Short-term side effects of biguanide treatment in elderly, obese type 2 diabetics.

-abdominal and gastric discomfort, flatulence, abdominal bloating, heartburn
-abdominal pain sometimes simulates intestinal ischemia
--vomiting, diarrhea, nausea, loss of appetite, metallic taste
-feeling cold(chills)
-agitation, headache
-dehydration, hypotension up to shock level, acute kidney failure („metabolic “acidosis)

As ~~lactic acidosis~~ increase of lactate serum level under orally administered biguanides is a continuum in the reporting from emergency and ICU-hospitalized overweight/obese diabetics (Table 5) and as biguanides are also used ~~alone or~~ in combination with other drugs for the purpose of weight loss in non-diabetics, it is worth to mention here the study published 1978 by Czyzyk and coworkers [226] who studied the effect of the three main biguanides, buformin, phenformin and metformin on blood lactate levels in 56 healthy subjects(30 men and 26 women).Of special interest is the effect of alcohol- and fructose-, a very popular sweetener, and of exercise during administration of the biguanides. Authors found only minimal differences in the effect of the individual biguanides. The highest increase in blood lactate was observed after intravenous fructose loading leading to an increase of glucose serum level. The lactate increase under biguanide was also further increased after oral ethanol, metformin showing the highest lactate increase 4-to 6 hours after ethanol administration compared to phenformin and buformin. The effect of exercise (15 minutes duration) was studied twice in 18 volunteers (7 men and 11 women, mean age 29 years). Before the second experiments biguanides were administered orally. While biguanides did not cause a significant reduction of glucose serum level, all the drugs caused a significant rise of blood lactate and a significant fall of bicarbonate levels but no change of blood ph. This has to be taken into consideration when talking about metformin-associated lactate acidosis (MALA,178)

In 1978 Luft and coworkers published [227] a review on cases of lactate acidosis after treatment with biguanides (most of the patients reported were treated with buformin or phenformin but also with metformin).

~~Lactate acidosis under oral biguanides was considered underreported. This is not only because of metformin therapy the clinical picture may not be properly recognized as many elderly patients are treated with several drugs like diuretics on hospitalization in the emergency room, but also because elderly patients may die at home or in a hospice and the cause of cardiac arrest may not be recognized to be dehydration as the consequence of metformin treatment.~~

More than 50% of the patients died soon after hospitalization showing advanced renal insufficiency and higher lactate acidosis [227]. Most common clinical signs reported were dehydration and pallor. Patients were frequently treated with vasopressors and volume expanders, but the cornerstone of the treatments reported were alkalinization with bicarbonate administration, hemodialysis, insulin and glucose.

Table 5. Symptoms attributable to lactic acidosis under long-term biguanide-treatment.

-abdominal, stomach discomfort
-nausea, vomiting, diarrhea,
-weakness
-fast breathing
-fast and weak pulse

- dry mouth and thirst
 - muscle pain or cramping
 - feeling of discomfort
 - sleeplessness
 - hypotension
 - tachycardia
-

The suggestions based on the clinical examination of the published data failed to establish the proper sequence of events after treatment with biguanides, considering that the hypoglycemic effect of the drugs is due to their Intestinal accumulation, local fluid loss and elimination of water with the nutrients and with the absorbed part of the compound cleared through the urine, leading to prerenal insufficiency.

The eventual accumulation of different amounts of the biguanides in the blood, and the increase of serum lactate concentration, can both be seen as signs of reduced kidney function due to dehydration (Table.6).

Both symptoms could be improved by mere fluid administration.

It can therefore be concluded that measurement of metformin serum level is dispensable for the determination of the therapeutic procedure and risk of death.

Development of increased serum lactate under biguanide treatment has been known since the first administration of the compounds as oral therapeutics in elderly obese type II diabetics. For this reason it was mentioned that “no clinically evident case of lactate acidosis was reported to the authors” of the UKPDS-trial from the patients treated with metformin as stated by the authors in their first long-term report [140]. No statistically significant increase of lactate serum level was reported in the short-term study of De Fronzo and colleagues [141], where determination of fasting lactate was part of the monitoring during the 29 weeks of the trial. It has however to be pointed out that only overweight, non-obese (mean BMI 29) and relatively young (not yet elderly, mean age 53 years) healthy type II diabetics were originally admitted to the trials (Table1).

After the publication of the American study testifying safety of the metformin use [141] and the approval of the compound for oral therapy in elderly overweight/obese diabetics type2, the first 10 cases of lactate acidosis with three cases of death were reported to the FDA [143] and the presence of comorbidities, which were an exclusion-criterion for the participation to the trials (see Table 1) was found as possible contributory factors for the development of acidosis (congestive heart failure, hypoxia, chronic lung disease, sepsis and impaired renal function).

Seven further deaths under metformin therapy were reported six months later by Innerfield [228]. The report was sharply criticized by the authors of the American study and a further study was announced by the FDA [229]. Additional cases reported to the FDA in the period from May 1995 to June 1996 were described by the FDA 1998 [144]. Twenty of the 47 hospitalized patients died; the mean age of these patients was 77 years (compared to 53 years of the patients in the study of de Fronzo) and 16 had a serum creatinine above 1.5 mg/dl while 8 had also furosemide, the most effective aggressive diuretic drug. The body mass index was not reported. In that report it was stated that the upper limit of lactate considered to be „normal “(2.7 mmol/L) in those patients treated with metformin in the first short-term study ([141] while seven had a lactate serum level between 2.7 and 4.9mmol/L therefore not meeting the criteria for lactate acidosis previously published by one of the reporting authors. Blood pressure values and hydration status were not mentioned.

In that report it was suggested to restrict metformin therapy to healthy diabetics who are under 80 years old although, again, the age of the patients included in the trial (141) was 53 years.

Similar to the previously used guanidins [230–233], at time of release by the Food and Drug Administration (FDA) such a life-threatening complication has been published only sporadically and has been considered to happen significantly more seldom compared to tolbutamide. It has been

described especially in diabetics with reduced kidney function [234–237] until recently [238] and in emergency conditions such as severe COVID-19-infection [177,239–241] in hospitalized diabetics.

The above-mentioned intestinal effects and adverse events can not only be the cause of the temporary dose-reduction or withdrawal of metformin intake but also the long-term complete lack of adherence to the therapy [242–247].

On the contrary, the loss of body weight observed especially at the beginning (but not at later time points) of metformin therapy [248,249], has been seen (not always, [141]) as an achievement as it is known that overweight, obese elderly diabetics do not comply with diet restrictions.

It should however be seen as a possibly dangerous acute consequence of the gastrointestinal side effects of the compound. In fact, there could not only be a negative effect of the metformin on food- but also on fluid-intake with consequent dehydration, tissue hypoperfusion, hypoxia-induced tissue damage and consequent increased lactate production, especially in type 2 diabetics performing exercise [250].

The consequent kidney injury could account for lactate accumulation and lead to acute lactic acidosis (just as marker of dehydration), the symptoms of which may be similar to the side effects of metformin with the main distinctive characteristic of dehydration, namely hypotension, tissue hypoxia with increase of anaerobic glycolysis, peripheral tissue injury and inflammation and fibrinolysis [251].

It has to be kept in mind that oral metformin administration alone can induce, besides, decrease of plasma insulin, serum cholesterol and triglycerides, a significant decrease of blood pressure as it has been found when metformin 850 mg twice a day was given for 6 weeks to nine non obese non diabetic no smoking men suffering of mild non treated hypertension [225]. A significant marked decrease of both systolic and diastolic blood pressure was observed within the first three weeks of therapy. The blood pressure increased again within two months after interruption of the metformin administration and mildly decreased again during the next three years of follow-up under dietary restrictions.

This phenomenon must be taken into consideration when interpreting the complication observed in 5 patients with type 2 diabetes and hypertension treated with metformin and angiotensin receptor blocker. The patients were admitted to the emergency room because of severe metabolic acidosis (pH 6.60–6.94) and high lactate 14–23 mmol/L) and elevated serum creatinine as signs of acute kidney failure in patients previously having normal kidney function. The clinical conditions were precipitated by severe dehydration with circulatory and respiratory collapse. Also, in these cases, however, the importance of replenishment of the blood volume (sufficient fluid and eventually albumin infusions) has not been emphasized enough [251] as it should be.

b) Long-Term Side Effects of Metformin Therapy

In their 10-year follow-up report, after the end of the UKPDS [252,253], Holman and coworkers [254] describe that the metformin group-patients were followed for a period of 17.7 years with 8.8 years of post-trial follow-up. Overall mortality was 44% (51.5% cardiovascular and 24.2% cancer as the cause of death). Baseline differences in surrogate marker glycated hemoglobin disappeared after the first year of intensive therapy. Although there was no difference in blood pressure levels and plasma creatinine was not significantly different within the two groups, the plasma creatinine levels in the metformin group were 15% higher than those in the conventional therapy group. As about 10% of the patients were treated with diuretics because of hypertension, both metformin and diuretics can eventually increase dehydration, which also naturally develops with increasing age in elderly persons.

Metformin treatment in type 2 diabetics is associated with early development of anemia, which is most probably due, but not solely, to the inhibition of Vitamin B12 absorption [255–258]. Vitamin B12 absorption inhibition could also explain the development of peripheral neuropathy in elderly diabetics treated with metformin [259].

Anemia and iron deficiency under long-term metformin treatment (> 5 years) has been described by Ahmed and colleagues [260] (Table 6).

Table 6. Long-term side effects of metformin-treatment in elderly type 2 obese diabetics.

-dehydration
-reduction of glomerular filtration rate (GFR)
-increase of fasting and postprandial plasma lactate
-increase of lactate elimination in the urine
-Vitamin B-12-deficiency
-Iron-deficiency
-hypoalbuminemia

5. Adherence to the Prescribed Dose of Metformin and Other Biguanides

After several reports claiming positive effects of orally administered drugs including metformin alone or in combination with other oral substances but also with insulin were published, „new medications for attempting to nearly normalize blood glucose levels could not have arrived at a better time“ [261] the enthusiasm about metformin, the first drug that did not induce increase of body weight, did not induce increase of insulin serum level, built up continuously although the true mechanism of action of this compound, which was poorly absorbed (>30% eliminated with the stool) and quickly eliminated unchanged via urine was only speculative. In addition, dose-dependent gastrointestinal side effects caused the drug to be withdrawn in up to 50 % of the study patients [142,262].

The results of the large UKPDS-trial, which started 1977 in England, contributed to the enormous „popularity “of the substance [248,249]. The UKPDS-reported positive results in the reduction of microvascular consequences of type 2 diabetes in the subgroup of overweight diabetics treated with metformin. They represented, despite the several critical comments [252,253] especially about the statistical significance of the obtained results, the definitive establishment of long-term oral therapy alone and/or in combination with insulin and other oral antidiabetic drugs .In one of the first reports of the UKPDS-trial, first data about adherence to protocol after 3 year follow-up was given [263] for the 2741 (of the first 3000) were still attending the clinics (91%).While 83% of those patients allocated to insulin-and 90% of those allocated to sulphonylurea were on the allocated therapy 30% of those allocated to diet policy had to be reallocated as having secondary diet failure. The adherence to protocol of the metformin (sub)group was not mentioned. Adherence to protocol of this group of patients has not been mentioned in the 10-year follow-up report [254]. In this report, however, it was found that the change in body weight was similar in the metformin and in the conventional control (diet only) groups. Of the metformin group 82% of person-years (3035 of 3682) were still treated with metformin alone (mean dose 2550 mg/day) or in combination. The most striking support to a good compliance was however the number of deaths under those patients allocated to the combination therapy metformin and sulphonylurea (median follow-up 6,6 years) with an adherence of 62% person-years was increased with a diabetes-related mortality-risk -increase of 96% (p=0.039).It was stated that after 3 years of monotherapy more than 50 of the patients needed an additional drug and after 9 years most of the patients under combination therapy will need insulin administration[263,264].In the short-term prospective trial (29 weeks) of DeFronzo and coworkers[141] 78% of the patients allocated to metformin were taking 2550 mg/day of the drug after the 5 week titration phase. Before end of the 29 weeks of the study duration 22% of the patients of the metformin group withdrew from the study and 28% in the placebo group (12 % because of treatment failure vs 1% in the metformin group, protocol 1). In protocol 2 25% of the patients in the

metformin group withdrew before the end of the 29 weeks mainly because of gastrointestinal side effects.

This was the first randomized, prospective trial showing that metformin can induce a change of the lactate serum level even with the level remaining within a „normal“ range. Interestingly the study showed no significant change of body weight in the metformin-compared with the placebo-group.

This means that adherence to the first and the only curative therapeutic intervention for diabetes type 2 of the „elderly“ (young old?) with overweight/obesity, namely diet, (restriction of the caloric intake) was negligible.

Adherence to dietetic prescription and to anti-diabetic, antihypertensive drug uptake was judged by means of the information given by the patients and contained in the questionnaire filled at the yearly visit when fasting glucose-, creatinine- and glycated hemoglobin- level together with blood pressure were measured. In the year 6 to 10 of follow-up after the UKPDS-trial was closed 1997, visits were continued every 3 years. Patients unable to attend the visits were sent the questionnaires as it was done for their general doctors at the end of the study in September 2007 [254]. Of the 2729 patients assigned to receive intensive therapy 489 (17,5%) had died at the end of the trial 1997 and of the 2118 available for the post-trial follow-up, 674 (31,8%) died within the 10 years and 1010 (37%) completed the 10 year-monitoring. Of the 1118 patients allocated to the conventional therapy (diet only) 213 (19%) at the end of the trial 1997 and further 324 (36,8%) of the 880 available, 324 died during the post-trial follow-up study 379 completed the post-trial monitoring (33,3%). While 48% of the patients allocated to the conventional therapy had died at the end of the post-trial monitoring the data available from the group of those patients allocated to the diet and intensive therapy allows the conclusion that 49,3% died within the 30 years of the two trials [254]. Of the 342 patients allocated to the intensive therapy with metformin, 50 died during the UKPDS-trial and 279 were available for the 10 year-follow-up trial. Of those 102 died (29,8%) and 136 (39,8%) completed the post-trial monitoring when 44,4% of the originally allocated patients had died.

While the mean age did not differ between the two groups (conventional vs intensive therapy) body weight and the fasting glucose and the glycated hemoglobin value were similar, more patients allocated to the intensive therapy were receiving a combination of oral and insulin therapy (64%) than those who had originally allocated to the conventional therapy (46%). In addition creatinine serum level in those allocated to intensive therapy with metformin was 15% higher than that in the conventional therapy group. Unfortunately, failure to adhere to the main curative intervention in overweight patients, namely dietetic restriction, has not been discussed as the attention was concentrated on drug administration.

The question of adherence to therapy in type 2 overweight/obese elderly patients has concentrated more on the drug prescription and less on the curative dietetic therapy.

The aggressive approach for improvement of adherence has been justified by a calculation of a possible reduction of expenses for additional drug costs and for management of comorbidities attributed mainly to the glycemic control considered a chronic disease on itself [265,266].

Although the introduction of extended release (ER)-metformin may have improved tolerability and adherence to the compound uptake, its superiority in glycemic control is not demonstrated [267].

It is however still the question of the costs of the long-term consequences of administration of a compound which can be considered to act as a laxative (and a diuretic) by withdrawing water from the intestine and from the systemic circulation in elderly obese dehydrated patients with an increased basal lactate serum level (still within the normal range).

Furthermore, the question is justified if it is rational to try to increase adherence to metformin therapy in the attempt to eliminate the „barriers“ especially when the cause of non-adherence is the large number of side effects especially when prescribed without paying attention to the numerous contraindications [265]

6. Contraindications

Every clinical decision nowadays is based on literature justification [268] called evidence-based decision. This attempt to reduce healthcare costs was thought to be objective and led to the development of guidelines, which could be followed worldwide. The basis for the development of evidence has been (multiple) multicenter (possibly international) prospective randomized, double-blind trials mostly financed by the pharmaceutical industry, whose results were published in international journals. The results should have been acceptable for national and international state release authorities, at first the American Food and Drug Administration (FDA) which regularly discusses the structure of the trials in advance.

In the last decades it has become clear that clinical trial conditions are often different from the „real world “application of the results obtained under study conditions [268] as it immediately happened after the release of metformin 1995/1996 [228,229] when only healthy overweight relatively young diabetics were included in the two prospective trials. Those results should be a guide for the clinician who is asked to judge the advantages compared to the possible disadvantages of the drug before prescribing it to his patient. To achieve this goal the clinician, however, must have knowledge about literature and about the criteria which have led to choosing the recruitment of the study patients at the beginning of the randomization process. It is, on the other hand, clear that it is almost impossible to have the results of at least one prospective trial available for each clinical situation in each single patient.

In fact, often results from retrospective analysis (especially those published after the release of a drug), are used even for guideline purposes. Such studies are used to perform and publish meta-analyses in the attempt to answer so far unaddressed questions, often extrapolating the answer to the „special question “from subgroup analysis with doubtful statistical significance.

Despite the long history of the use of synthetic biguanides as oral antidiabetic drugs in overweight/obese diabetics and of the common side effects, the basis for the interpretation of these side effects and for what is considered the real danger arising from administration, namely lactate acidosis (as sign of anaerobic glycolysis and hypoxia caused by dehydration) is still considered to be unclear [173].

It is still not yet clear how orally administered metformin (and other biguanides) cause the main side effects, namely, abdominal discomfort, nausea, vomiting, loss of appetite, bad taste (paralleled by intestinal lactate production!).

Although metformin is the most prescribed oral drug for diabetes type 2 in obese patients worldwide, the other two biguanides buformin and phenformin are still available in many countries outside of the USA such as Europe, South America, Asia [269], the controversy about the fundamental question and the real reasons of avoiding metformin (and the other biguanides) therapy is still ongoing.

L-lactate is a product of anaerobic glycolysis taking place mainly in the muscle even under normal conditions [270], its clearance takes place mainly in the liver where it is converted into glucose (under fasting conditions) with a potential increase of the capacity of the normal liver up to six fold [178,235,271]. Lactate however increases also after a meal [272] and the increase seems to be due to increased release from the liver [273].

Serum lactate concentration is increased after exercise especially after meal in overweight type 2 diabetics [250].

A recent report underlined the importance of the hydration status when judging the effect of exercise on the lactate serum concentration even in young non-obese people [275].

The clearance capacity of the liver can be temporarily impaired only by a strong reduction of the blood flow into the liver (over 75%) by an acute-phase situation induced by endotoxemia [251] or massive liver failure. On the other hand, about 30% of lactate is cleared by the kidney and every acute condition reducing this capacity will induce an increase of serum concentration of lactate, which however is not the cause of the clinical picture which is characterized by the massive reduction of the blood volume resulting in hypotonia and diffuse peripheral tissue hypoxia.

Chronically reduced kidney function will contribute to an increase of lactate blood concentration only in the contemporary presence of a reduced clearance capacity of the liver. Normal liver function could lead to an underestimation of the danger of incipient kidney failure.

Elderly (above 60 years of age) overweight/obese type 2 diabetics represent the largest group of diabetics type 2 [275]. They suffer from potential dehydration [276] and subclinical hypoxia.

Doar and coworkers [277] after having shown that fasting blood lactate level is higher in obese than in non-obese subjects independently of their glucose tolerance test-results, they demonstrated that the level of lactate remains elevated in the diabetics (maturity onset with a mean age of 50 years) after glucose administration up to the end of the test. Removal of infused lactate was impaired in obese type 2 diabetics but not significantly impaired in obese nondiabetics (mean age 25.4 years) although the fasting lactate levels were similar. Increasing dehydration has been found with increasing age, weight and metabolic syndrome conditions which may serve as explanation for fasting and postprandial increased lactate levels [277]. It is therefore understandable that conditions leading to a worsening of peripheral oxygen delivery due to further reduction of blood volume may precipitate the clinical picture of hypovolemia and shock outside of septicemia and sepsis, such as therapy with diuretics like furosemide and/or with laxatives frequently used in depressed constipated elderly diabetics [278–280]. These conditions may all have in common the last consequence of fluid withdrawal from the systemic circulation through the skin (e.g. heat) [281,282], kidney (diuretics) or intestine (chronic diarrhea induced by laxatives and other drugs e.g. metformin) [283–285] even be responsible for clinical pictures like mesenteric ischemia [286–289].

Metabolic acidosis induced by increased production of lactate in the hypoxemic peripheral tissues (subclinical dehydration of the obese elderly diabetic type 2) and/or reduced clearance mainly by decreased kidney function (mainly prerenal due to dehydration) may become a marker of acute clinical worsening due to sudden fluid loss in case of an acute illness, like viral infections of the respiratory tract as it may be the case for influenza- or Coronavirus-infections [290] or of the gastrointestinal viruses like noroviruses. Hypovolemic shock may be the common clinical emergency complicated by the so-called multiorgan failure such as acute kidney failure (AKI) and pulmonary insufficiency (ARDS) [289,290].

It is important to clearly identify the cause of the emergency in order to start with the proper therapeutic approach intended first to gradually reestablish the normal hydration and blood volume by administration of fluids and of albumin. It is not necessary to administer bicarbonate in the assumption that acidosis is the cause of the clinical emergency condition [291]. Under these preconditions the use of substances like biguanides represents an unjustified danger for the diabetics when considering the long list of contraindications beginning with the age and the body mass index higher than that of the two main prospective studies [140,142] which should be added to the long list published by Sulkin et al 1997 (292) besides dehydration, obstipation, depression and polypharmacy.

7. Conclusions

Metformin, the latest and most often prescribed biguanide to overweight/obese, mostly elderly type 2 diabetics does not seem to differ from the previous biguanides used as oral antidiabetics. The mechanisms of serum glucose-lowering effect and the acute-and long-term consequences are like those of conventional laxatives apart from abdominal symptoms and the taste and appetite disturbance frequently caused by biguanides. Dehydration, prerenal kidney injury and worsening of lactate acidosis (as marker of hypoxia) are the most serious and, for older multimorbid patients, life-threatening consequences of protracted metformin therapy (Figure 6). Rehydration is the first mandatory therapeutic intervention.

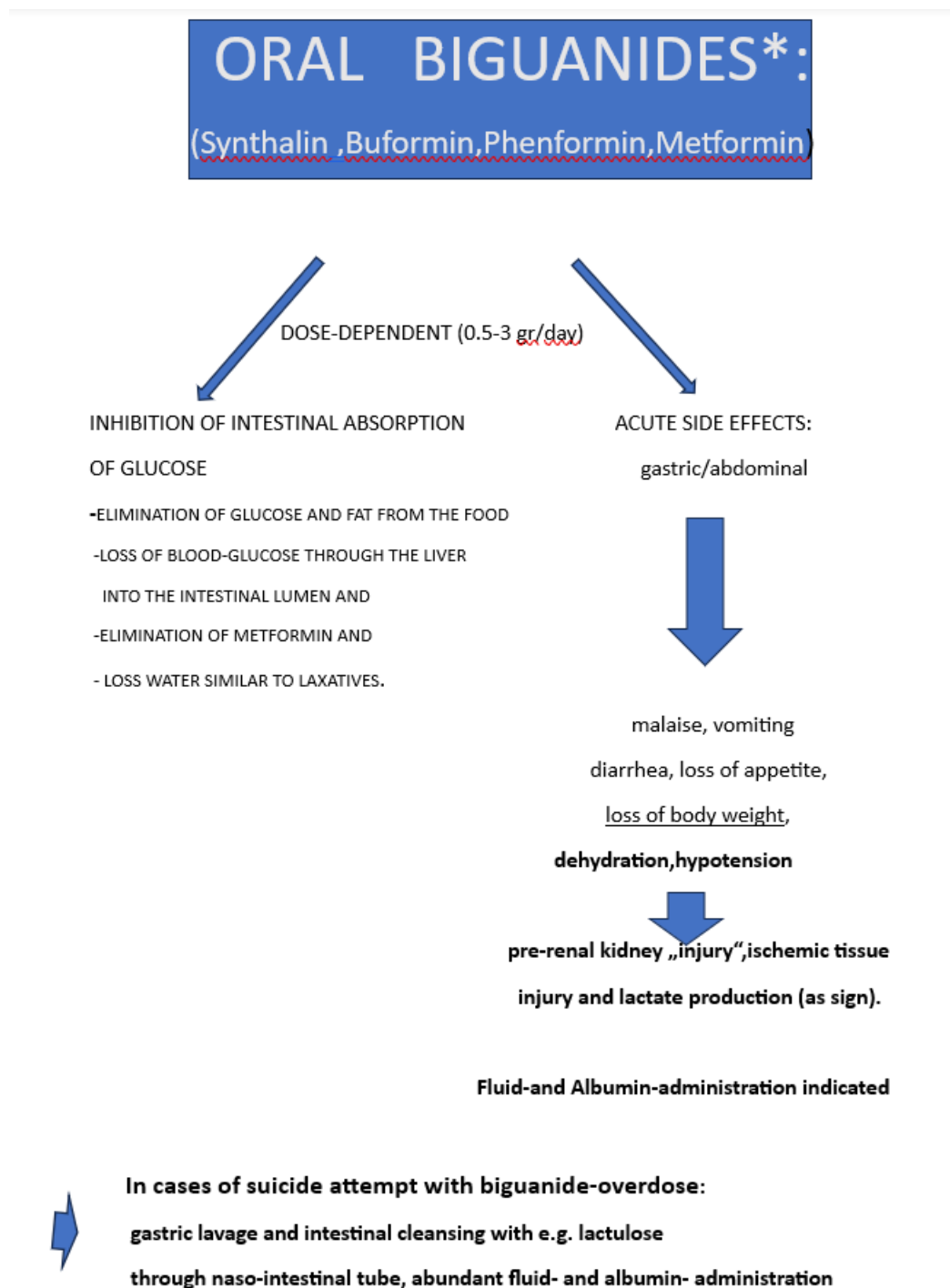


Figure 6. Graphical Abstract of the content of the review.

*Physicochemical characteristics of biguanides

- small molecules
- strongly basic and water-soluble
- strongly hygroscopic
- not protein-binding
- no metabolic change induced by the plasmatic biguanides
- complete elimination from the blood (through the kidney, faster (5x) than creatinine) by inducing sodium and water loss into the urine

and from the intestine (40-70%) accompanied by water loss
-compound in the urine and in the stool unchanged (no biotransformation-process)

References

1. Estimated global population from 10,000BCE to 2100 (in millions) Statista 2024 <https://www.statista.comstatistics>
2. The GBO 2015 Obesity Claborators.Health effects of Overweight and Obesity in 195 Countries over 25 Years.New Engl J Md.2017;372(113-27.
3. New WHO report:Europe can reverse ist obesity „epidemic“.https://www.who.int/europe/news/03-05-2022-new-who-report.
4. WHO Obesity and Overweight. 2024.https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
5. Kerac M,McGrath M,Connell N,Kompala Ch,Moore WH,Bailey J,Bansma R,Berkley JA,Briend A,Collins S,GirmaT,Wells JC.“Severe Malnutrition”:thinking deeply,communicating simply.BMJGlobal Health 2020;5:e003023.doi:10.1136/bmjgh-2020-003023.
6. Kuriyama S,Ohmori K,Miura C,Suzuki Y,Nakaya N,Fujita K,Sato Y,Tsubono Y,Tsuji I,Fukao A,Hisamichi S..Body Mass Index and Mortality in Japan:The Miyagi Cohort Study.J Epidemiol 2004;14:S33-S38
7. Nippon.com.Japan Sets New Record Low for Food Self-Sufficiency on a Production Value Basis.Oct 28 2024.https://www.nippon.com/en/japan-data/h01758/
8. Klonowska-Siwak E.The japanese Food Market and the japanese business culture 2022.https://www.nippoland.com/
9. WHO hunger numbers stubbornly high for three consecutive years as global crises deepning report.1 in 11 people worldwide faced hunger in 2023,1 in 5 in Africa.24.07. 2024.https://www.who.int/news/intern/24-07-2024-hunger-numbers-stubb.
10. Rosenfeld L.Justus Liebig and Animal Chemistry.Clinical Chemistry 2003;49:1696-1707.
11. 11 Ritchie H,Roser M.Half of the world's habitable land is used for agriculture.OurWorldInData.https://ourworldindata.org/global-land-for-agriculture2019
12. FAO2023.WORLD FOOD AND AGRICULTURE-Statistical Yearbook 2023.Rome. https://doi.org/10.4060/cc8166en
13. Cordain L,Brand J,Eaton SB,Man N,Holt SMA,Steta JD.Plant-Animal subsistence ratios and macronutrient energy estimations in worldwide hunter-gather diets.Am J Clin Nutr 2000;71:682-692
14. Stafford N.History:The Changing notion of foodNature 2010;468:16-17.
15. Wright I Livestock,engine for economic growth and sustainabilityCFSHLPE.https://www.fao.org/c/chshlpe/insights/news-insight/news-detail..
16. The World BankUnderstanding Poverty.Moving Towards Sustainability:The Livestock Sector and the World Bank.October 21 2021.https://www.worldbank.org/en/topic/agriculture/brief/moving-tow...
17. Doll R,Hill AB.:Lung cancer and other causes of death in relation to smoking.A second report on mortality of british doctors.BMJ 1956; 2(5001) :1070-1081. DOI: 10.1136/bmj.2.5001.1071
18. Hopkinson NS.Smoking and Lung Cancer-70 long years on.BMJ2024.384.q443.doi:10.1136/bmj.q443.
19. Dawber TR,Moore E,Mann GV:II.Coronary heart Disease in the Framingham study.Am J Publ Health.1957;47:4-24.
20. Hammond C,Horn D.Smoking AND DEATH RATES-REPORT ON FORTY-FOUR MONTHS OF FOLLOW-UP OF 187,783 MEN.II DEATH RATES BY CAUSE.JAMA 1958;166(11):1294-1308.nnnn
21. Sheps MC.: SHALL WE COUNT THE LIVING OR THE DEAD?N Engl J Med 1958;259 (25):1210-1214.
22. Doyle JT,Dawber TR,Kannel WB,Heslin SA,Kahn HA:Cigarette smoking and coronary heart disease.Combined experience of the Albany and Framingham Studies.N Engl J Med 1962;266(16):796-801.
23. Doll R,Peto R.:Mortality in relation to smoking:20 years observations on male british doctors BMJ 1976;2:1525-1536.

24. Doll RD,Peto R,Hall E,Wheatley K,Gray R::Mortality in relation to consumption of alcohol:13 years observation of male british doctors.BMJ 1994;309:911-918.
25. Freund K,Belanger AJ,D'Agostino RB,Kanner WB.:The Health Risk of Smoking.The framingham Study:34 years of follow-up.AEP 1992;3(4):417-424.
26. Magowska AM.Historical Perspectives.The changing face of hunger:from fasting tot he concept of aatherogenesis.Adv Physiol Edu 2020;44:734-740.
27. Keys A,Anderson JT,Grande F.Prediction of serum-cholesterol responses of man to changes in fats in the diet.Lancet 1957;Nov 16(7003):959-966.
28. Roine P,Pekkarinen M,Karvonen MJ,Kihlberg J.Diet and cardiovascular disease in Finland.Lancet1958July 26:173-175.
29. Huijbregets P,Feskens E,Raesanen L,Fidanza F,Nissinen A, Menotti A, Kromhout D.:Dietary pattern and 20 year Mortality in elderly men in Finland,Italy,and the Netherlands:longitudinal cohort study.BMJ 1997;315:13-17.
30. Karvonen MOrma E,Keys A,Fidanza F,Brozek J.Cigarette Smoking,Serum-Cholesterol,Blood-Pressure and Body Fatness.Observations In Finland.Lancet1959 March 7:492-494*aided by the tobacco industry Research Committee (New York).
31. Keys A,Karvonen MJ,Fidanza F.Serum-Cholesterol Studies in Finland.Lancet 1958;July 26:175-178.
32. Menotti A,Puddu PE,Kafatos AG,Tolonen H,Adachi H,Jacobs Jr DR.Cardivascular Mortality in 10 Cohorts of Middle-Aged Men Followed-up 60 Years until Extinction:The seven Countries Study.J Cardiovasc Dev Dis 2023;10:201.DOI:10.3390/icdd10050201.
33. Puddu PE,Piras P,Kafatos A Adachi H,Tolonen H,Menotti A.Competing Risks of Coronary Heart Disease Mortality versus Other Causes of Death in 10 Cohorts of Middle-Aged Men oft he Seven Countries Study Followed for 60 Years to Extinction.J CCardiovasc Dev Dis 2023;10:482.DOI:10.3390/jcdd10120482.
34. Bucheley RW,Drake RM,Breslow L.Relationship of Amount of Cigarette Smoking to Coronary Heart Disease Mortality Rates in Men.Circulation1958;XVIII:1085-1090.
35. Matilainen TKM,Puska P,Berg MAK,Pokusajeva S,Moisejeva N,Uhanov M,ArtemjevA.Health- related
36. Behaviours in the Republic of Karelia,Russia,and North Karelia, Finland.International Journal of behavioural Medicine 1994;1(4):285-304
37. YLE.Health.Report:Alcohol abuse a „big problem“ in Finland,main cause of preventable deaths.30/12/22.<https://yle.fi/a/74-20010916>.
38. Manthey J,Probst C,Kilian C,Moskalewicz,Steroslowski,Karlsson T,Rehm J.Unrecorded Alcoh Consumption in Seven European Union Countries.European Addiction Research 2020;26:316-325.
39. EUROSTAT.Alcohol consumption statistics.Statistics Explained.<https://ec.europa.eu/eurostat/statisticsexplained/-/09/08/2023>.
40. Garcia MJ, McNamara PM, Gordon T, Kannell WB. Morbidity and mortality in diabetics in the Framingham population. Sixteen-year follow-up study. Diabetes. 1974; 23:105–111.
41. Mayes CR,Thompson DB.What Should We Eat? Biopolitics,Ethics,and Nutritional Scientism.Bioethical Inquiry 2015;12:587-599.
42. Mayes C Healthy Eating Policy:Racial Liberalism,Global Connections and Contested Science.Food Ethics 2023;8:1.DOI:10.1007/s41055-022-00111-5.
43. The GBD 2015 Obesity Collaborators.Health Effects of Overweight and Obesity in 195 Countries over 25 Years.New Engl J Med 2017;377:13-27.
44. Antoniotti E Com'è cambiata la salute degli Italiani 1861-2011:centocinquant'anni.quotidianosanita`.it2011.https://www.quotidianosanita`.it/stampa_articolo.php?articolo_id=3269.
45. Liu B,Hu Y,Rai SK,Wang M,Hu FB,Sun Q.Low-Carbohydrate Diet Macronutrient Quality and Weight Change.JAMA Network Open 2023;6(12):e2349552.doi:10.1001/jamanetworkopen.2023.49552.
46. Adams RC,Sedgmond J Mizey L,Chambers CD,Lawrence N.Food addiction:implications fort he diagnosis and treatment of overeating.Nutrients 2019;11(9):2086.

47. Lee JJ, Pedley A, Hoffman U, Massaro JM, Levy D, Long MT. Visceral and Intrahepatic fat are associated with cardiometabolic risk factors above other ectopic fat depots: The Framingham Heart Study. *Am J Med* 2018;131(6):684-692.
48. Manka P. Liver, Heart, Death.... Can This Sequence Be Broken? *Dig Dis Sci* 2023;68:3490-3491.
49. Mavrogeni S, Bacopoulou F, Markousis-Mavrogenis G, Chrousos G, Charmandari E. Cardiovascular Imaging in Obesity. *Nutrients* 2021;13,744. DOI:10.3390/nu13030744.
50. Lemmens HJ, Bernstein DP, Brodsky JB. Estimating Blood Volume in Obese and Morbidity Obese Patients. *Obesity Surgery* 2006;16:773-776.
51. Csige I, Ujvarosy D, Szabo Z, Lörincz I, Paragh G, Harangi M, Somodi S. The impact of Obesity on the cardiovascular System. *Journal of Diabetes Research* 2018. DOI:10.1155/2018/3407306.
52. Stapleton PA, James ME, Goodwill AG, Fresbee JC. Obesity and Vascular Dysfunction. *Pathophysiology* 2008;15(2):79-89.
53. Faizan U, Rouster AS. Nutrition and Hydration Requirements in Children and Adults. *StatPearls* Jan 2023. <https://www.ncbi.nlm.nih.gov/books/NBK562207/#:~:text=Anoth.>
54. Volkert D, Beck AM, Cederholm T, Cruz-Jentoft A, Goisser S, Hooper L, Kiesswetter E, Maggio M, Raynaud-Simon A, Sieber CC, Sobotka L, van Asselt D, Wirth R, Bischoff SC. ESPEN guideline on clinical nutrition and hydration in geriatrics. *Clinical Nutrition* 2019;38:10-47.
55. Flatharta TO, Flynn A, Mulkerrin EC. Heat-related chronic Kidney disease mortality in the young and old: differing mechanisms, potentially similar solutions? *BMJ Evidence Based Medicine* 2019;24:45-47.
56. Gunanithi K, Sakthidasan S. Serum Albumin levels in uncontrolled type II diabetes mellitus- An observational study. *MedPulse International Journal of Biochemistry* 2021;20(2):13-17.
57. Di Nicolantonio JJ, O'Keefe J. Low-grade metabolic acidosis as a driver of chronic disease: a 21st century public health crisis. *Open Heart* 2021;8:e001730. DOI:10.1136/openhrt-2021-001730.
58. Alpern RJ, Sakhaee K. The clinical Spectrum of Chronic Metabolic Acidosis: Homeostatic Mechanisms Produce Significant Morbidity. *Am J Kid Dis* 1997;29(2):291-302.
59. Doar JWH, Cramp DG. The effects of Obesity and Maturity-Onset Diabetes Mellitus on L(+) Lactic Acid Metabolism. *Clinical Science* 1970;39:271-279.
60. Crawford SO, Hoogeveen RC, Brancati FL, Astor BC, Ballantyne CM, Schmidt MI, Young JH. Association of Blood Lactate with type 2 diabetes: the Atherosclerosis Risk in Communities Carotid MRI Study. *International Journal of Epidemiology* 2010;39:1647-1655. DOI:10.1093/ije/dyq126.
61. Chirumbolo S, Bertossi D. Insights on the role of L-lactate as a signaling molecule in skin aging. *Biogerontology* 2023;24:709-726.
62. De-Cleua R, CARDIA L, VEIRA-CARDUCCI A, GREVE JM, SANTO MA. LACTATE CAN BE A MARKER OF METABOLIC SYNDROME IN SEVERE OBESITY? *ABCD Arq Bras Cir Dig* 2021;34(1):e1579. DOI:10.1590/0102-67202021000e1579.
63. Re RN. Obesity-Related Hypertension. *The Ochsener Journal* 2009;9:133-136.
64. Trayurn P. HYPOXIA AND ADIPOSE TISSUE FUNCTION AND DYSFUNCTION IN OBESITY. *Physiol Rev* 2013;93:1-21.
65. Rodriguez AJ, Boonya-Ananta MT, Gonzalez M, Du Le VN, Fine J, Palacios C, McShane MJ, Cote' GL, Ramella-Roman JC. Skin optical properties in the obese and their relation to body mass index: a review. *Journal of Biomedical Optics* 2022;27(3). DOI:10.1117/I.JBO.27.3030902.
66. Long Y, Li Y, Zhang L, Tao L, Xiao H, Li Y. Plasma D-dimer levels are correlated with disease severity among hypertensive patients. A comparative cross-sectional study. *Medicine* 2022;101:36(e30281).
67. Nolan JJ, Faerch K. Estimating insulin sensitivity and beta cell function: perspectives from the modern pandemics of obesity and type 2 diabetes. *Diabetologia* 2012;55:2863-2867.
68. Saunders KH, Shukla AP, Igel LI, Kumar RB, Aronne LJ. Pharmacotherapy for Obesity. *Endocrinol Metab Clin N Am* 2016;45:521-538.
69. Guadamuz JS, Shooshtari A, Qato DM. Global, regional and national trends in statin utilisation in high-income and low/middle-income countries, 2015-2020. *BMJ Open* 2022;12:e061350. DOI:10.1136/bmjopen-2022-061350.

70. Wondmkun YT. Obesity, insulin Resistance, and Type 2 Diabetes: Associations and Therapeutic Implications. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy* 2020;13:3611-3616. DOI.
71. Coucouls AP, Daigle CR, Arterburn DE. Long-term outcomes of metabolic/bariatric surgery in adults. *BMJ* 2023;303. DOI:10.111133/bmj-2022-071927.
72. Lardinio CK. Time for new APPROACH TO Reducing Cardiovascular Disease: Is Limitation on Saturated Fat Meat Consumption Still Justified? *Am J Med* 2020;133(9):1009-1010.
73. Lee CD, Hardin C, Longo DL, Ingelfinger JR. Nutrition in Medicine-A New Review Article Series. *New Engl J Med* 2024;390:1324-1325.
74. Blais JE, Wie Y, Yap KKW, Alwafi H, Ma TT, Brauer R, Lau WCY, Man KKC, Siu CW, Tan KCB, Wong ICK, Wie L, Chan EW. Trends in Lipid-modifying agent use in 83 countries. *Atherosclerosis* 2021;328:44-51.
75. Kang H, Hong SH. Risk of Kidney Dysfunction from Polypharmacy among Older Patients: A Nested Case-Control Study of the South Korean Senior Cohort. *Scientific Reports* 2019;9:10440. DOI:10.1038/s41598-019-46849-7.
76. Oosting IJ, Colombijn JMT, Kaasenbrood L, Liabeuf S, Laville SM, Hooft L, Bots M, Verhaar MC, Vernooij RWM. Polypharmacy in Patients with CKD: A Systematic Review and Meta-Analysis. *Kidney* 2024;5:841-850.
77. Foretz M, Viollet B. Les nouvelles promesses de la metformine. Vers une meilleure compréhension de ses mécanismes d'action. *Medicine/sciences* 2014;30 (1):82-92. DOI:10.1051/medsci/20143001010.
78. Staub H. Übersichten. 40 Jahre Insulin und intermediärer Stoffwechsel. *Klin Wschr* 1965;42(2): 61-69.
79. Bailey CJ. The origin of type 2 diabetes medications. *Br J Diabetes*. 2022;22:112-120
80. Dobson M (1776). Experiments and observations on the urine in diabetes. *Medical Observations and Inquiries*. Volume 5. London: T Cadell, p 298-316.
81. Robin ED, Claude Bernard. Pioneer of Regulatory Biology. *JAMA* 1979;242(12):1283-1284.
82. Polonsky KS. The past 200 Years in Diabetes. *New Engl J Med*. 2012;367:1333-1340.
83. Langerhans P. Beiträge zur mikroskopischen Anatomie der Bauchspeicheldrüse. Inauguraldissertation, Medicine and Surgery. Berlin 1869. p.32.
84. Raddatz D, Ramadori G. Carbohydrate Metabolism and The Liver: Actual Aspects From Physiology and Disease. *Z. Gastroenterol* 2007;45(1):51-62.
85. Minkowski O. Verhandlungen des I. internationalen Physiologenkongresses. Basel 1889. *Zentbl. Physiol.* 1889;312-313.
86. Banting FG, Best CH, Collip JBC, Campbell WR, Fletcher AA. Encore: pancreatic extracts in the treatment of diabetic mellitus: preliminary report. *Can Med Assoc J* 1922 Mar;12(3):141-6.
87. Falkenberg M. Verhandlungen des X. Congresses für Innere Medizin. Wiesbaden 1891 S.502
88. Hirsch R. Glykosurie nach Schilddrüsenextirpation bei Hunden. *Z. exp. Path. u. Therap.* 1906;3:393-400.
89. Fühner H. Über den Angriffsort der peripheren Guanidinwirkung. Zugleich eine Erwiderung. *Archiv f. experiment. Pathol. u. Pharmacol.* 1909;65:402-427.
90. Koch WF. On the occurrence of methyl Guanidine in the urine of parathyroidectomized animals. *J Biol Chem* 1912;12(3):313-315.
91. Koch WF. Toxic bases in the urine of parathyroidectomized dogs. *J Biol Chem* 1913; 15:43-64.
92. Underhill FP, Blatherwick NR. Studies in carbohydrate metabolism. VI. The influence of Thyreoparathyroidectomy upon the sugar content of the blood and the glycogen content of the liver. *J Biol Chem* 1914;118 (1):87-90.
93. Underhill FP, Blatherwick NR. Studies in Carbohydrate Metabolism. VII. The influence of subcutaneous injections of dextrose and of calcium lactate upon the blood sugar content and upon tetany after thyreoparathyroidectomy. *J Biol Chem* 1914;119(1):119-126.
94. Underhill FP, Saiki T. The influence of complete thyroidectomy and thyroid feeding upon certain phases of intermediary metabolism. *J Biol Chem*. 1908;5:225—241.
95. Burns D, Sharpe JS. THE PARATHYROID-TETANIA PARATHYREOPRIVA, ITS NATURE, CAUSE, AND RELATIONS TO IDIOPATHIC TETANY. PART V: GUANIDIN AND METHYL-GUANIDINE IN THE BLOOD AND URINE IN TETANIA PARATHYREOPRIVA AND IN THE URINE IN IDIOPATHIC TETANY. *Quart. J Exp Physiol* 1916;10:345-354.

96. Watanabe CK. Studies in the metabolic changes induced by administration of guanidine bases. I. Influence of injected guanidine hydrochloride upon blood sugar content. *J Biol Chem* 1918;33:253-265.
97. Watanabe CK. Studies in the metabolic changes induced by administration of guanidine bases. II. The influence of guanidine upon urinary ammonia and acid excretion. *J Biol Chem* 1918;34(1):51-63.
98. Watanabe CK. Studies in the metabolic changes induced by administration of guanidine bases. IV. The influence of the administration of calcium upon blood sugar content in rabbits with guanidine hypoglycemia. *J Biol Chem* 1918;34(19):73-76.
99. Watanabe CK. Studies in the metabolic changes induced by the administration of guanidine bases. V. The change of phosphate and calcium content in serum in guanidine tetany and the relation between the calcium content and sugar in the blood. *J Biol Chem* 1918; 34(1):531-546.
100. Watanabe CK. Studies in the metabolic changes induced by the administration of guanidine bases. *J Biol Chem* 1922;1(2):195-200.
101. Frank E, Northmann M, Wagner A.: Über Synthetisch Dargestellte Körper mit Insulin artiger Wirkung auf den Normalen und diabetischen Organismus. *Klin Wochenschr.* 1926; 45:2100-2107.
102. Banting FC, Best CH. The internal secretion of the pancreas. *J Lab clin. Med.* 1922;7(5):251-266.
103. Minkowski O.: Synthetische Insulinähnlich Wirkende Substanzen. Bemerkungen zu den vorstehenden Mitteilungen von Frank, Northmann und Wagner. *Klin Wochenschr.* 1926;45:2107.
104. Calvert EGB.: Observations on the treatment of Diabetes By Synthalin. *Lancet* 1927;5430(2):649-651. DOI:10.1016/S0140-6736(01)31246-1.
105. Anonimus.: Synthalin in the treatment of Diabetes. Preliminary reports to the medical research council. *Lancet* 1927;5427 (2):517-521. DOI:10.1016/S0140-6736(01)31155-8.
106. Bodo R Marks A.: The relation of SYNTHALIN TO CARBOHYDRATE METABOLISM. *J Physiol* 1928;65:83-99.
107. Ringer AI, Bilon S, Harris MM, Landy A.: SYNTHALIN. its use in the treatment of Diabetes. *Arch Int Med.* 1928;41(4):453-471.
108. Staub H, Küng O.: Zum SANTHALINMECHANISMUS. *Klin Woch.* 1928;7(29):1365-1366.
109. Frank E, Northmann M, Wagner A.: Über die experimentelle und klinische Wirkung des Dodekamethylendiguanids (Synthalin B). *Klin Woch.* 1928;42:1996-2000.
110. Bischoff F, Sahyun M, Long L.: GUANIDINE STRUCTURE AND HYPOGLYCEMIA. *J Biol Chem* 1929;81:325-349.
111. Blatherwick NR, Sahyun M, Hill E. Some effects of Synthalin on Metabolism. *J Biol Chem* 1927;75:671-683
112. Anonymus. Insulin and Synthalin. *Nature* 1928;3039(121):151-153.
113. Werner EA, Bell J. The preparation of Guanidine by the interaction of Dicyanodiamide and Ammonium Thiocyanate. *Journa of the Chemical Society, Transactions.* CXXVII 1920;117:1133-1136.
114. Werner EA, Bell J. The Preparation of methylguanidine, and of BB-dimethylguanidine by the interaction of dicyanodiamid, and methylammonium and dimethylammonium chlorides respectively. *Journal of the chemical society, Transactions.* 1922;121:1790-1794.
115. Hesse E, Taubmann G. Die Wirkung des Biguanids und seiner Derivate auf den Zuckerstoffwechsel. *Archiv f. experiment. Pathol. u. Pharmacol* 1929;142:290-308.
116. Minot AS, Dodd K, Saunders JM: The acidosis of guanidine intoxication. *J Clin Invest* 1934;13:917-932
117. Mortensen J. Effect of guanidine and synthalin on the citric Acid Metabolism. *Acta Medica Scandinavica* 1946;CXXV(1):82-94
118. Goldner MG. Oral hypoglycemic agents Past and Present. *JAMA* 1958;102:830-840.
119. Davis JC. Hydropic Degeneration of the aCells of The Pancreatic Islets Produced by Synthalin A. *J Path Bact* 1952; 56:575584
120. Ferner H, Runge W. Synthalin A as elective Mitotic Poison Acting on aCells of the Islet of Langerhans. *Science* 1955;122:420.
121. Goldner M.: Oral Hypoglycemic Agents Past and Present. *Archiv Int Med.* 1958;102:830-840.
122. Bloom A, Richards JG.: PHENFORMIN AS ADJUVANT ORAL THERAPY IN DIABETES. *Br Med J* 1961; 1796-1799.

123. Krall LP, Camerini-Davalos R. Clinical Trials with DBI, a New Nonsulfonylurea Oral Hypoglycemic Agent. *AMA Archiv Int Med* 1958;102:25-31.
124. Williams RH, Tanner DC, Odell WD. Hypoglycemic Actions of Phenethyl-Amyl-, and Isoamyl-Diguanide. *Diabetes* 1958;7(2):87-92.
125. Unger G, Freedman L, Shapiro SL. Pharmacological Studies of a New Oral Hypoglycemic Drug. *Proc Soc Exp Biol Med* 1957;95:190-192.
126. Tranquada RE, Kleeman C, Brown J. Some effects of Phenylmethylbiguanide on Human Hepatic Metabolism as Measured by Hepatic Vein Catheterization. *Diabetes* 1960;9(3):207-214.
127. Walker RS, Linton A, Thomson WST. Mode of action and Side effects of Phenformin Hydrochloride. *BMJ* 1960 Nov 26;1567-1569.
128. Grodsky GM, Karam JH, Pavlatos FCh, Forsham PH. Reduction by Phenformin of excessive Insulin Levels after glucose loading in Obese Diabetic Subjects. *Metabolism* 1963;12(4):278-286.
129. Beaser S.: THERAPY OF DIABETES MELLITUS WITH COMBINATIONS OF DRUGS GIVEN ORALLY. *N Engl J Med* 1958;259 (25):1207-1210.
124. Walker RS, Preliminary Observations on Phenethyldiguanide. *BMJ* 1959; 2:405-406.
130. Bratusch-Marrain PR, Korn A, Waldhäusl WK, Gasic S, Nowotny P. Effect of Buformin on Splanchnic Carbohydrate and Substrate Metabolism in Healthy Man. *Metabolism* 1981;30(10):946-952.
131. Somogyi A, Stockley C, Keal JR, Rolan P, Bochner F. Reduction of Metformin renal tubular secretion by cimetidine in man. *J clin Pharmacol* 1987;231:545-551.
132. Phillips PJ, Scicchitano R, Clarkson AR, Gilmore AR. Metformin-associated lactic acidosis. *Aus NZ J Med* 1978;8:281-284.
133. Ohara S, Komatsu R, Matsuyama T. Short-term effect of buformin, a biguanide, on insulin sensitivity, soluble fraction of tumor necrosis factor receptor and serum lipids in overweight patients with type 2 diabetes mellitus. *Diabetes Reserch and Clinical Practice* 2004;66:133-1126.38.
134. Knatterud G, Meinert CL, Klimt CR, Osborne RK, Martin DB. Effects of Hypoglycemic Agents on Vascular Complications in Patients With Adult-Onset Diabetes. *JAMA* 1971;217(6):777-784.
135. Misbin RI. Phenformin-Associated Lactic Acidosis: Pathogenesis and Treatment. *Ann Int Med* 1977;87:591-595.
136. Mehnert H, Seitz W. Weitere Ergebnisse der Diabetesbehandlung mit blutzuckersenkenden Biguaniden. *Münch Med Wschr* 1958;100:1849-1851.
137. Sterne J. Du nouveau dans les antidiabetiques. La NNdimethylamino guanyl guanidine (NNDG). *Maroc Med* 1957;36:1295-1296.
138. Clarke BF, Duncan UP. Comparison of chlorpropamide and metformin treatment on weight and blood glucose response and uncontrolled obese diabetics. *Lancet* 1968;1:123-126.
139. Lebacqz EG, Tirmalis A. Metformin and Lactic Acidosis. *The Lancet* 1972;12:314-315.
140. UK. prospective diabetes study group 16. Overview of 6 years therapy of type 2 diabetes: a progressive disease. *Diabetes* 1995;44(11):1249-1258.
141. DeFronzo R, Goodman A. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. *N Engl J Med* 1995;333(9):541-549.
142. Campbell RK, White JR, Saulie BA. Metformin: A new Oral Biguanide. *CLINICAL THERAPEUTICS* 1996;18(3):360-370.
143. Guriguian J, Green L, Misbin RJ, Stadel B, Fleming GA. Efficacy of Metformin in non-insulin-dependent diabetes mellitus. *N Engl J Med* 1996;334(4):269.
144. Misbin RI, Green L, Stadel BV, Gueriguian JL, Gubbi A, Fleming GA. *N Engl J Med* 1998;338:265-266.
145. Krishnamurthy M, Sahouria JJ, Desai RC, Aguiat J. Buformin induced lactic acidosis-A symptom of Modern Health Care Malady. *JAGS* 2004;52(10):1785. DOI:10.1111/J.1532-5415.2004.52479_7x.
146. Fingl E, Woodbury DM. General Principles. 1. Pharmacokinetics 2-26 pp. In: *The Pharmacological Basis of Therapeutics*. Goodman LS, Gilman A eds 11975 5th edition. Macmillan Publishing Inc. New York.
147. Sambol NC, Chiang J, O'Conner M, Liu CY, Lin ET, Goodman AM, Benet LZ, Karam JH. Pharmacokinetics and Pharmacodynamics of Metformin in Healthy Subjects and Patients with Non-Insulin-Dependent Diabetes Mellitus. *J clin Pharmacol*. 1996; 36:1012-1021.

148. Sombol NC,Chiang J,Lin ET,Goodman AM,Liu ChL,Benet LZCogan MG.Kidney Function and Age Are Both Predictors of Pharmacokinetics of Metformin.J Clin Pharmacol 1995;35:1094-1102.
149. Lintz W,Berger W,Aenishaenslin W,Kutova V,Baerlocher Ch,Kapp JP,Beckmann R.Buthylbiguanide Concentration in Plasma,Liver,and intestine after intravenous and Oral Administration to man.Eur J Clin Pharmacol 1974;7:433-448.
150. 150Alkalay D,Khermani L,Wagner WHBartlett MF.:Pharmacokinetics of Phenformin in Man.J Clin Pharmacol 1975;15(5-6):446-448.
151. Pentikäinen PJ,Neuvonen PJ,Penttilä A.Pharmacokinetics of Metformin After intravenous and Oral Administration to Man.Eur J Clin Pharmacol 1979;16:185-202.
152. Wisse E,De Zanger RB, Van Der Smissen CP,McCuskey RS.The Liver Sieve:Considerations Concerning the Structure and Function of Endothelial Fenestrae,the Sinusoidal Wall and the Space of Disse.Hepatology 1985;5(4):683-692.
153. Le Couteur DG,Fraser R,Hilmer S,Rivory LP,McLean AJ.The Hepatic Sinusoid in Aging and Cirrhosis.Effects on Hepatic Substrate Disposition and Drug Clearance.Clin Pharmacokinet.2005;44(2):187:200
154. Gormensen LC,Sundelin EI,Jensen JB,Vendelbo MH,Jakobsen S,Munk OL,Christensen MMH,Broesen K,Froekiaer J, Jessen N.In vivo Imaging of Human C11-Metformin in Peripheral Organs:Dosimetry,Biodistribution,and Kinetic Analyses.J Nucl Med 2016;57(12):1920-1926.
155. Shu Y,Sheardown SA,Brown C,Owen RP,Zhang S,Castro RA,Ianculescu AG,Yue L,Lo JC,Burchard EG,Brett CM,Giacomini KM.Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action.J Clin Invest 2007;117(3):1422-1431.
156. Christensen MMH,Hoejnd K,Hother-Nielsen O,Stage TB,Damkler P,Beck-Nielsen H,Brosen K.Steady-state pharmacokinetics of metformin is independent of OCT1 genotype in healthy volunteers.Eur J Clin Pharmacol 2015;71:691-697.
157. Garg, PK,Lokitz SJ,Nazih R,Garg S.Biodistribution and Radiation Dosimetry of C11-Nicotine from Whole-Body PET Imaging in Humans.J Nucl Med 2017;58:473-478.
158. Gierloeff T,Jakobsen S,Nahimi A,Munk OL, Bender D,Alstrup AKO,Vase KH,Hansen SB,Brooks DJ,Borghammer P.In Vivo Imaging of Human Acetylcholinesterase Density in Peripheral Organs using C11-Donpezil:Dosimetry,Biodistribution,and Kinetic Analyses.J Nucl Med 2014;55:1818-1824.
159. Gierloeff T,Fedorova T,Knudsen K,Munk OL,Nahimi A,Jacobsen S,Danielsen EH,Terkelsen AJ,Hansen J,Pavese N,Brooks DJ,Borghammer P.Imaging acetylcholinesterase density in peripheral organs in Parkinson's disease with C11-donpezil PET.BRAIN 2015;138:653-663.
160. Chou C-H.Uptake and Dispersion of Metformin in the Isolated Perfused Rat Liver.J Pharm Pharmacol 2000;52:1011-1016.
161. Bonora E,Cigolini M,Bosello O,Zancanaro C,Capretti L,Zavaroni I,Coscelli CButturini U.:Lack of effect of intravenous metformin on plasma concentrations of glucose,insulin,C-peptide,glucagon and growth hormone in non-diabetic subjects.Curr.Med.Res.Opin.1984;9:47-51.
162. Sum CF,Webster JM,Johnson AB,Catalano C,Cooper BG,Taylor R.:The effect of intravenous Metformin on Glucose Metabolism During Hyperglycemia in Type 2 Diabetes.DIABETIC MEDICINE 1992;9:61-65.
163. McCreight LJ,Stage TB,Connelly P,Lonergaum M,NielsenF,Prelin C,Adamsky J,Broesen K,Pearson ER,Pharmacokinetics of metformin in patients with gastrointestinal intolerance.Diabetes Obesity Metabolism 2018;20:1593-1601.
164. Sterne J.:The Present State of Knowledge on the Mode of Action of the Antidiabetic Diguanydes.Metabolism 1964;13(9):781-798. CJ
165. Penicaud L,Hitier Y,Ferre P,Girard J.Hypoglycemic effect of metformin in genetically obese(fa/fa) rats results from an increased utilization of blood glucose by intestine.Biochem J.1989;262:881-885.
166. Sahi J,Grepper S,Smith C.:Hepatocytes as a Tool in drug metabolism,Transport and Safety Elimination in Drug Discovery.Current Drug iscovery Technologies.2010;7:188-198.
167. Bailey JC **Metformin and intestinal glucose handling.** Diabetes Metab Rev 1995; Suppl 1:S23-32. doi: 10.1002/dmr.5610110505.

168. Wilcock C, Bailey CJ.: Reconsideration of inhibitory effect of metformin on intestinal glucose absorption. *J. Pharm. Pharmacol.* 1991;43:120-121.
169. Christensen MMH, Hojhund K, Hother-Nielsen O, Stage TB, Damkier P, Beck-Nielsen H, Brosen K.: Endogenous glucose production increase in response to metformin treatment in the glycogen-depleted state in humans: a randomized trial. *Diabetologia* 2015;58:2494-2502.
170. Wu T, Xie C, Wu H, Jones KI, Horowitz M, Rayner CK.: Metformin reduces the rate of the small intestinal glucose absorption in type 2 diabetes. *Diabetes, Obesity and Metab* 2017;119(2):290-293. DOI:10.1111/dom.12812.
171. Horakova O, Kroupova P, Bardova K, Buresova J, Janowska P, Kopecky J, Rossmeisl M.: Metformin acutely lowers blood glucose levels by inhibition of intestinal glucose transport. *Scientific Reports* 2019;9:6156. DOI:10.1038/s41598-019-42531-0.
172. Campbell IW.: Metformin: 60-year odyssey with the journey still continuing—a personal commentary from Professor Ian Campbell. *Current medical research and opinion*. 2022;38(1):55-58.
173. Ikeda T, Iwata K, Murakami H. Inhibition Effect of Metformin on Intestinal Glucose Absorption in the perfused Rat Intestine. *Biochem Pharmacology* 2000;59:887-890.
174. Abdul-Ghani M, DeFronzo RA.: Is The time To change the Type 2 Diabetes Treatment Paradigm? Yes! GLP-1 Ras Should Replace Metformin in the Type 2 Diabetes Algorithm. *Diabetes Care* 2017;40:1121-1127.
175. Tobar N, Rocha GZ, Santos A, Guadagnini D, Assalin HB, Camargo JA, Goncalves AESS, Pallis FR, Oliveira AG, Rocco SA, Neto RN, Layane de Sousa I, Alborghetti MR, Storca ML, Rodrigues PB, Ludwig RG, Vanzela EC, Brunetto SQ, Boer PA, Gontijo JAR, Geloneze B, Carvalho CRO, Prada PO, Folli F, Curi R, Mori MMA, Vinolo MAR, Ramos CD, Franchini KG, Tonnena CF, Saad MJA. Metformin acts in the gut and induces gut-liver cross-talk. *PNAS* 2023;120(4):e2211933120.
176. Lalau JD, Race J-M, Brinquin L, Lactic Acidosis in Metformin Therapy. *Diabetes Care* 1998;21(8):1360-1362
177. Lalau JD, Race J-M, Andreelli P, Lacroix C, Canarelli JP. Metformin retention Independent of Renal Failure in Intestinal Occlusion. *Diabetes Metab (Paris)*. 2000;27:24-28
178. Bailey CJ, Gwilt M. Diabetes, Metformin and Clinical Course of COVID-19: Outcomes, Mechanisms and Suggestions on the Therapeutic Use of Metformin. *Front Pharmacol* 2022;13:78459. DOI:10.3369/iphar.2022.784459.
179. Lalau JD, Kajbal F, Protti A, Christensen MM, De Bore M-E, Wirsperger N. Metformin-associated lactic acidosis (MALA): moving towards a new paradigm. *Diabetes Obes Metab* 2017;19(11):1502-1512
180. Bronden A, Alber A, Rohde U, Rehfeld JF, Holst JJ, Vilsboell T, Knop FK.: Single-dose Metformin enhances Bile-Acid-induced Glucagon-like Peptide-1 Secretion in Patients with type-2 Diabetes. *J Clin Endocrinol Metab* 2017;102(11):4153-4162.
181. Ritzel U, Fromme A, Otteleben M, Leonhardt U, Ramadori G. Release of glucagon-like peptide-1 (GLP-1) by carbohydrates in the perfused rat ileum. *Acta Diabetol* 1997;34:18-21.
182. Raddatz D, Nolte W, Roßbach C, Leonhardt U, Buchwald A, Scholz KH, Ramadori G. Measuring the effect of a Study Meal on Portal Concentrations of Glucagon-Like Peptide-1 (GLP-1) in Non Diabetic and Diabetic Patients with Liver Cirrhosis: Transjugular Intrahepatic Portosystemic Stent Shunt (TIPSS) as a New Method for Metabolic Measurement. *Exp Clin Endocrinol Diabetes* 2008;116:461-467.
183. Li B, Hu Y, Wang G, Liu L. The effect of exenatide on fasting bile acids in newly diagnosed type 2 diabetes mellitus patients, a pilot study. *BMC Pharmacology and toxicology* 2020;21:44. DOI:10.1186/s40360-020-00422-5.
184. Coll AP, Chen M, Taskar P, Rimmington D, Patel S, Tadross JA, Cimino I, Yang M, Welsh P, Virtue S, Goldspink D, Medzybrodzka EL, Konopka AR, Ruiz Esponda R, Huang JT, Tung YCL, Rodriguez-Cuenca S, et al. GDF15 mediates the effects of metformin on body weight and energy balance. *Nature* 2019. DOI:10.1038/s41586-019-1911-y
185. Kincald JWR, Rimmington D, Tadross JA, Cimino I, Zwetkova I, Kaser A, Richards P, Patel S, O'Rahilly S, Coll AP. The gastrointestinal tract is a major source of the acute metformin-stimulated rise in GDF15. *Scientific Reports* 2024;14:1899. DOI:10.1038/s41598-024-51866-2.

186. Scott B, Day EA, Lynch L, O'Brien KL, Scanlan J, Cromwell G, Scannal AL, Mc Donnell E, Finlay DK. Metformin and Feeding increase levels of appetite-suppressing metabolite Lac-Phe in humans. *NATURE METABOLISM* 2024;6:651658
187. Cubeddu LX, Boenisch H, Goethert M, Moldering SG, Racke K, Ramadori G, Miller KJ, Schworer H. Effects of Metformin on Intestinal 5-Hydroxytryptamine (5-HT) Release on 5-HT₃-receptors. *Naunyn Schmiedbergers Archiv Pharmacol* 2000;361:85-91
188. Kim S, Chung JK, Kim BT. Relationship between gastrointestinal F-18-fluorodeoxyglucose accumulation and gastrointestinal symptoms in whole-body PET. *Clin Positron Imaging* 1999;2:273-279.
189. Gontier E, Fourme E, Wartski M, Blondet C, Bonardel G, Le Stanc E, Mantzarides M, Foehrenbach H, Pecking A-P, Alberini J-L. High and typical F18-FDG bowel uptake in patients treated with metformin. *Eur J Med Mol Imaging* 2008;35:95-99.
190. Morita Y, Nogami M, Sakaguchi K, Okada Y, Hirota Y, Sugawara K, Tamori Y, Zeng F, Murokami T, Ogawa W. Enhanced Release of Glucose Into Intraluminal Space of the Intestine Associated With Metformin Treatment as Revealed by [18F]Fluorodeoxyglucose PET-MRI. *Diabetes Care* 2020;43:1796-1802. DOI:10.2337/dc20-0093.
191. Özülkur T, Özülkur F, Mert M, Özpacaci T. Clearance of high intestinal F18-FDG uptake associated with metformin after stopping the drug. *Eur J Nucl Med Mol Imaging* 2010;37:1011-1017.
192. Schreuder N, Klarenbeek H, Vendel BN, Jager PL, Kosterink JGW, van Puijenbroek EP. Discontinuation of metformin to prevent metformin-induced high colonic FDG uptake: is 48 h sufficient? *Annals of Nuclear Medicine* 2020;34:833-839.
193. Massollo M, Marini C, Brignone M, Emionite B, Salani BR, Iondato M, Capitano S, Fitz F, Democrito A, Amaro A, Morbelli S, Piana MM, Maggi D, Cilli MP, Pfeffer U, Sambucetti G. Metformin Temporal and Localized Effects on Gut Glucose Metabolism Assessed Using F18-DG PET in Mice. *J Nucl Med* 2013;54:259-266.
194. Tu DG, Chen C-R, Wang Y-W, Tu C-W, Huang YC. Bowel-cleansing methods affecting PET-CT Image interpretation. *Nucl Med Commun* 2011;32:570-574.
195. Chen Y-K, Chen J-H, Tsui C-C, Chou H-H, Cheng R-H, Chiu J-S. Use of Laxative-augmented Contrast Medium in the Evaluation of Colorectal Foci at FDG PET. *Radiology* 2011;259 (2):525-533.
196. Katsumata R, Manabe N, Ayaki M, Tanikawa T, Fujita M, Ono Y, Fujiwara H, Suehiro M, Monobe Y, Kato K, Kawamoto H, Haruma K. Increased Colonic Fluorodeoxyglucose Uptake in Melanosis Coli-A Case Series of Three Patients. *Gastro Hep Advances* 2022;1:125-128.
197. Czyzyk A, Tawcki J, Sadowsky J, Ponnikowska I, Szezepanik Z. Effect of biguanides on intestinal absorption of glucose. *Diabetes* 1968;17:192-198.
198. Berchtold P, Bolli P, Arbenz U, Keiser G. Intestinale Absorptionsstörung infolge Metforminbehandlung (Zur Frage der Wirkungsweise der Biguanide). *Diabetologia* 1969;5:405-412.
199. Paleari L, Burhenne J, Weiss J, Poersch S, Roth W, Parodi A, Gnant MB, Achleitner-Hofmann T, Scherer D, Ulrich CM, Stabuc B, Puntoni M, Coccia G, Petrera M, Haefeli WE, Decensi A. High Accumulation of Metformin in Colonic Tissue of Subjects With Diabetes or the Metabolic Syndrome. *Gastroenterology* 2018;154:1543-1545.
200. Madison LL. Role of insulin in the hepatic handling of glucose. *Arch Int Med* 1969;123:284-292.
201. Balks H-J, Jungermann K. Regulation of peripheral insulin/glucagon levels by rat liver. *Eur J Biochem* 1984;141:645-650.
202. Guzelian PH, Boyer JL. Glucose reabsorption from bile. *J Clin Invest* 1974;53:526-535.
203. Infante M, Leoni M, Caprio M, Fabbri A. Long-term Metformin therapy and Vitamin B12 deficiency: An association to bear in mind. *World Journal Diabetes* 2021;12(7):916-931.
204. Wijnen JCF, Van De Riet IR, Lijfering WM, Van Der Meer FJM. Metformin use decreases the anticoagulant effect of phenprocoumon. *J Thromb Haemost* 2014;12:887-890.
205. Baker EH, Sandle GI. Complications of Laxative Abuse. *Ann Rev Med* 1996;47:127-134.
206. Hart SL, McColl I. The effect of the laxative oxyphenisatin on the intestinal absorption of glucose in rat and man. *Br J Pharmacol* 1968;32:683-686.
207. Sturtzel B, DIETRICH A, Wagner KH, Gisinger C, Elmafa I. The status of vitamins B6, B12, Folate, and of homocysteine in Geriatric home residents receiving laxatives or dietary fiber. *The Journal of Nutrition Health*. 2010;14(3):219-223.

208. Sugimoto H,Yamada U.Iron deficiency anemia induced by magnesium overuse:a case report.BioPsychoSocial Medicine 2019;13:18.DOI:10.1186/s13030-019-0159-y.
209. Nilsson SE,Takkinen S,Johansson B,Dotevall G,Melander a,Berg S,McClearn G.Laxatie treatment elevates plasma homocysteine:a study on a population-based Swedish sample of old people.Eur J Clin Pharmacol 2004;60:45-49.
210. Kelley CJ,Verdegaal AA,Anderson BW,Show WL,Ben CVenga-Barry N,Fogga-Stognew NG,Goodman AL.Metformin inhibits Digestive Proteases andImpairs Protein Digestion in Mice.J Bil Chem 2023;299(12):105363.DOI:10.1016/jbc.2023.105363.
211. Han Y,Yun C.Metformin Inhibits Na⁺/H⁺Exchanger NHE3 Resulting in Intestinal Water Loss.Front Physiol 2022;13867244.DOI:103389/fphysiol2022.867244.
212. Naz N,Malik IA,Sheikh N,Ahmad S,Kahn S,Blaschke M,Schultze F,Ramadori G.Ferroportin-1 is a nuclear negative acute-phase protein in rat liver:a comparison with other iro-transport proteins.Lab Invest 2012;92:842-856.
213. Ahmad S,Moriconi F,Naz N,Sultan S,Sheikh N,Ramadori G,Malik IA.Ferritin L and Ferritin H are differentially located within hepatic and extra hepatic organs under physiological and acute-phase conditions.Int J Clin Exp Pathol 2013;6(4):622-629.
214. Vidon N,Chaussade S,Noel M,Franchisseur C,Huchet B,Bernier JJ..Metformin in the digestive tract.Diabetes Research and Clinical Practice.1988;4:223-229.
215. Ghosal S,Ghosal S.:The Side Effects of Metformin-A Review.J Diabetes Metab.Disorders 2019.DOI:10.24966/DMD-201X/100030.
216. Wickham RJ.Revisiting the physiology of Nausea and vomiting-challenging the paradigm.Supportive Care in Cancer 2019.DOI:10.1007/s00520-019-05012-8.
217. Caspary WF,Creutzfeldt W.:Inhibition of Bile salt absorption by blood sugar lowering biguanides.Diabetologia 1975;11:113-117.
218. Scarpello JHB,Hodgson E,Howlett HCS..Effect of Metformin on Bile salt Circulation and Intestinal Motility in Type 2 Diabetes Mellitus.Diabetic Medicine 1998;15:651-656.
219. Sansome DJ,Xie C,Veedfald S, Horowitz M, Rayner CK,Wu T.:Mechanisms of glucose-lowering by metformin in type 2 diabetes:Role of bile acids.Diabetes Obes Metab.2019;DOI:10.1111/dom.13869.
220. Metry M,Krug SA,Karra VK,Kane MA,Fink JC,Shu Y,Wang H,Polii JE.:Differential effects of metformin-mediated BSEP repression on pravastatin and bile acid pharmacokinetics in humans: A randomized controlled trial.Clin Transl Sci.2022;15:2468-2478.
221. Nakajima A,Ishizaki S, Matsuda K,Kurosu S,Taniguchi S,Gilberg PG,Mattsson JP,Hasumuma T,Camillen M.:Impact of elobixibat on serum and fecal bile acid levels and constipation symptoms in patients with chronic constipation.Journal of Gastroenterology and Hepatology 2022;37:883-890.
222. Spiller R.Inhibiting glucose absorption to treat constipation.Lancet Gastroenterol Hepatol 2018.DOI:10.1016/S2468-1253 (18)30214-0.
223. Chu N,Ling J,Jie H,Leung K,Poon E.The potential role of lactulose pharmacotherapy in the Treatment andprevention of diabetes.FrontEndocrinol 2022;13.956203.DOI:10.3589/fendo2022.956303.
224. Landin-Wilhelmsen K,Metformin and blood pressure.Journal of Clinical Pharmacy and Therapeutics 1992;17:75-79.
225. Gudmundsdottir H,Aksnes H,Heldal K,Krogh A,Froyshov S,Rudberg N,Os I.Metformin and antihypertensive therapywith drugs blocking the renin angiotensin system,a cause of concern?.Clin Nephrol 2006;66(5):380-385.
226. Hashimoto H,Nomura N,Shoda W,Isobe K,Kikuchi H,Yamamoto K,Fujimaru T,Ando F,Mori T,Okado T,Rai T,Uchida S Sohara E.Metformin Increases urinary sodium excretion by reducing phosphorylation of the sodium-chloride cotransporter.Metabolism2018;85:23-31
227. Czyzyk KA,Lao B,Bartosiewicz W,Szczepanik Z,Orlowska K.The Effect of Short-Term Administration of Antidiabetic Biguanide Derivatives On The Blood Lactate Levels in Healthy Subjects.Diabetologia 1978;2:89-94.DOI:10.1007/BF01263445.
228. Luft, D., Schmülling, R.M., Eggstein, M.: Lactic acidosis in biguanide-treated diabetics. A review of 330 cases. *Diabetologia* 14, 75–87 (1978).

229. Innerfield JR. Metformin-associated mortality in US Studies. *New Engl J Med* 1996;334(24):1611-1612.(correspondence)
230. Stadel BV, Guerigulian J, Fleming GA. Metformin-associated lactic acidosis. *New Engl J Med* 1996;334(24):1613.
231. Unger RH, Madison LL, Carter NW.: Tolbutamide-Phenformin in Ketoacidosis-Resistant Patients. *JAMA* 1960;174(17):2132-2136.
232. Bloom A, Richards JG. Phenformin as adjuvant oral therapy in Diabetes. *Br Med J* 1961;1(5242): 1796-1799
233. Cornfield J. The university Group Diabetes Program. A Further Statistical Analysis of the Mortality Findings. *JAMA* 1971;217(12):1676-1687.
234. Palmer JP. Farewell to Phenformin for treating diabetes Mellitus. *Ann Int Med* 1975;83(4):567-568.
235. Fitzgerald E, Mathieu S, Ball A.: Metformin associated lactic acidosis. *BMJ* 2009;339:1254-1257.
236. William DA, Marples J.: Biguanides and Lactic acidosis. *Lancet* 1977;309(8004):191-192.
237. Blough B, Moreland A, Mora A.: Metformin-induced lactic acidosis with emphasis on the anion gap. *Proc(Bayl Univ Med Cent)* 2015;28(1):31-33.
238. Pena Porta CM, Villafuerte Ledesma HM, DE Vera Floristan CV, Ferrer Dufol A, Salvador Gomez T, Alvarez Lipe R. Incidence, factors related to presentation, course and mortality of metformin-associated lactic acidosis in the healthcare area of a tertiary hospital. *Nefrologia* 2019;39(1):35-43.
239. Faggian G, Cesaro A, Faggian R, Del Piano C, Vitagliano A, Del Piano D, Salzano M, Diglio A, Faggian A. Insufficienza renale acuta, acidosi lattica e metformina: due casi clinici e review della letteratura. *Giornale Italiano di Nefrologia*. 2023;3(3): ISSN 1724-5990
240. Cheng X, Liu MY-M, Li H, Zhang X, Lei F, Quin JJ, Chen Z, Deng KQ, Lin L, Chen M-M, Song X, Xia M et al. Metformin is associated with higher incidence of acidosis, but not mortality, in individuals with COVID-19 and pre-existing type 2 diabetes. *Cell Metabolism* 2020;32:537-547.
241. Vordoni A, Theofilis P, Vlachopoulos G, Koukoulaki M, Kalaitzidis RG. Metformin-associated lactic acidosis and acute kidney injury in the era of COVID-19. *Frontiers in Bioscience Scholar* 2021;13 (2):202-207. DOI:10.52586/S563
242. Takayama K, Obata Y, Maruo Y, Yamaguchi H, Kosugi M, Irie Y, Hazama Y, Yasuda T. Metformin-associated Lactic Acidosis with Hypoglycemia during Covid-19 Pandemic. *Intern Med* 2022;61:2333-2337. DOI:10.2169/internal medicine.9179-21.
243. Donnelly LA, Morris AD, Pearson ER.: Adherence in patients transferred from immediate release metformin to a sustained release formulation: a population based study. *Diabetes, Obesity and Metabolism*. 2009;11:338-342.
244. Rashid M, Warraich NY, Laique T, Shujaat K, Zawar S, Munir A.: Reasons of NON-Compliance to metformin among type 2 diabetics attending diabetic clinic in Lahore. *Journal of Akhtar Saeed Medical and Dental College* 2019;01(3):111-116
245. Christofides EA.: Practical Insight Into Improving Adherence to Metformin Therapy in Patients With Type 2 Diabetes. *CLINICAL DIABETES JOURNALS.ORG*. 2019;37 (3):234-241. DOI:10.2337/cd18-0063.
246. Walker EA, Gonzalez JS, Tripputi MT, Dagogo-Jack S, Matulik MJ, Montez MG, Tadros S, Edelstein SL.: Long-term metformin adherence in the Diabetes Prevention Program Outcomes Study. *BMJ Open Diab Res Care* 2020;8:e001537. DOI:10.1136/bmjdr-2020-001537.
247. Syafhan NF, Donnelly R, Harper R, Harding J, Mulligan C, Hogg A, Scott M, Fleming G, Scullin C, Hawwa AF, Chen G, Pearson C, McEnlay JC.: Adherence to Metformin in adults with type 2 diabetes: a combined method approach. *Journal of Pharmaceutical Policy and Practice* 2022;15:61. DOI:10.1186/s40545-022-00457-5.
248. McGovern A, Tippu Z, Hinton W, Munro N, Whyte M, de Lusignan S.: A comparison of adherence and persistence by medication class in type 2 diabetes: A systematic review and meta-analysis. *Diabetes Obes Metab*. 2018;20(4):1040-1043.
249. UKPDS (33). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 1998;352:837-853.
250. UKPDS(34). Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes(UKPDS 34) *Lancet* 1998; 352:854-865.

251. Heden TA, Liu Y, Kanaley JA. Exercise Timing and Blood Lactate Concentration In Individuals With Type 2 Diabetes. *Appl Physiol Nutr Metabolism* 2017;42:732-737.
252. Tapia P, Soto D, Bruhn A, Alegria L, Jarufe N, Luengo C, Kattan E, Regueria T, Meissner A, Menchaca R, Viver MI, Echeverria N, Ospina-Tascon G, Bakker J, Hernandez G. Impairment of exogenous lactate clearance in experimental hyperdynamic septic shock is not related to total liver hypoperfusion. *Critical Care* 2015;19:88. DOI:10.1186/s13054-015-0928-3
253. Nathan DM.: Some answers, more controversy, from UKPDS. COMMENTARY. *The Lancet* 1998;352:832-833
254. Ewart RM.: The UKPDS: what was the question? *The Lancet* 1999;353:1882.
255. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil AW.: 10-Year Follow-up of intensive Glucose Control in Type 2 Diabetes. *N Engl J Med* 2008;359:1577-1589.
256. Tomkin GH, Hadden DR, Weaver JA, Montgomery DAD.: Vitamin-B12 Status of Patients on long-term Metformin Therapy. *BMJ* 1971;2:685-687.
257. Jager J, Kooy A, Lehret Ph, Wulfele`MG, Kolk J, Bets D, Verbung J, Donker AJM, Stehouver CDA.: Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomized placebo controlled trial. *BMJ* 2010;340:c2181. DOI:10.1136/bmj.c2181.
258. Kim J, Ahn CW, Fang S, Lee HS, Park JS. Association between metformin dose and vitamin B12 deficiency in patients with type 2 diabetes. *Medicine* 2019;98:46(e17918).
259. Donnelly L, Dennis JM, Coleman RI, Sattar N, Hattersley AT, Holman RR, Pearson ER.: Risk of anemia with metformin Use in Type 2 diabetes: A MASTERMIND Study. *Diabetes Care* 2020;43:2493-2499.
260. Serra MC, Kancherla V, Khakharia A, Allen LL, Phillips LS, Rhee MK, Wilson PWF, Vaughan CP.: Long-term metformin treatment and risk of peripheral neuropathy in older Veterans. *Diabetes Research and Clinical Practice* 2020;170:108486.. DOI:10.1016/j.diabres.2020.108486.
261. Ahmed HH, Fadl NN, Kotob SE. Impact of long term Metformin therapy on hepcidin and Iron status in type II Diabetic Patients. *IJPCR* 2015;7 (3):185-193.
262. Hermann LS, Schersten B, Bitzen P-O, Kjellström T, Lindgärde F, Melander A.: Therapeutic Comparison of Metformin and Sulfonylurea, Alone and in Various Combinations. A double-blind controlled study. *Diabetes Care* 1994;17(10):1100-1109.
263. Dandona P, Fonseca V, Mier A, Beckett A.: Diarrhea and Metformin in Diabetic Clinic. *Diabetes Care* 1983;6(5):472-474.
264. UK Prospective Diabetes Group Study (UKPDS). VIII. Study Design, Prog RRress and Performance. *Diabetologia* 1991;34:877-890.
265. Turner RC, Cull CA, Fright V, Holman RR. Glycemic Control with Diet, Sulphonylurea, Metformin, or Insulin in Patients with Type 2 Diabetes Mellitus. Progressive Requirement of multiple Therapies (UKPDS 49). *JAMA* 1998;281(21):2005-2012.
266. Flory JH, Mushlin AI. Effect of Cost and Formulation in Persistence and Adherence to Initial Metformin in Therapy For Type 2 Diabetes. *Diabetes Care* 2020;43:e62-e-67. DOI:10.2337/dc19-2426.
267. Piragine E, Petri D, Martelli A, Calderone V, Lucentoforte E. Adherence to Oral Antidiabetic Drugs in Patients with Type 2 Diabetes: Systematic Review and Meta-Analysis. *J Clin Med* 2023;12(5):1981. DOI:10.3390/jcm12051981.
268. Mammutovic A, Lekic L, Zamovic I, Mamovic N, Kikanovic A, Pavlovic A. Investigations Affecting Adherence to Metformin Therapy In Type 2 Diabetes. *Medis. Medical Sciences and Research* 2024;3(2):43-51.
269. Greenes, Robert A. A Brief History of Clinical Decision Support. Technical, Social, Cultural, Economic, and Governmental Perspectives. *Clinical Decision Support* 2014;2:49-109. Academic Press doi:10.1016/B978-0-12-398476-0.00002-6
270. Sam Wang G, Hoyte Ch. Review of biguanide (Metformin) Toxicity. *J Int Care Med* 2018;34(11-12). DOI:10.1177/0385066618793385.
271. Dietze O, Wicklmayr M, Hepp KD, Bogener W, Mehnert H, Czempiel H, Henftling HG. On Gluconeogenesis of Human Liver. Accelerated Hepatic Glucose Formation Induced by Increased Precursor Supply. *Diabetologia* 1976;12:555-561

272. Agyekum AK, Klarie E, Walsh MC, Nyachoti CM Postprandial portal glucose and lactate fluxes, insulin production, and portal vein-drained viscera oxygen consumption in growing pigs fed a high-fiber diet supplemented with a multi-enzyme cocktail. *Anim Sci* 2016;94(9):3760-3770. DOI:10.2527/jas.2015-0076
273. Davis MA, Williams PE, Cherrington AD. Effect of a mixed meal on hepatic lactate and gluconeogenic precursor metabolism in dogs. *Am J Physiol* 1984;247(3Pt 1):E362-E369.
274. Green JM, Hornsby JH, Pritchett RC, Pritchett K. Lactate Threshold Comparison in Anaerobic vs Aerobic Athletes and Untrained Participants. *Int J of Exercise Sc* 2014;7(4):329-358.
275. Crawford B, Steckse, Sander DP, Merchant AT, Woo JM, Prk JMM. Dietary Patterns, Socioeconomic Disparities, and Risk of Type 2 Diabetes in Sister Study. *Diabetes Research Clin Pract* 2023;204:11096. DOI:10.1016/j.diabetes.2023.110906.
276. Chang T, Ravi N, Plegue MA, Sonnevile KR, Davis MM. Inadequate Hydration, BMI, and Obesity Among US Adults: NHANES 2009-2012. *Ann Fam Med* 2016; 14:320-324.
277. Volkert D, Beck AM, Cederholm T, Cruz-Jentoft A, Hooper L, Kiesswetter E, Maggio M, Raynod-Simon A, Sobotka L, Vanasselt D, Wirth R, Bischoff SC. ESPEN Practical Guidelines Clinical Nutrition And Hydration In Geriatrics. *Clin Nutr* 2022;2022;41(4):958-989.
278. Doar JW, Cramp DG, Maw DS, Seed M, Wynn V. Blood Pyruvate and Lactate Levels During Oral and Intravenous Glucose Tolerance Tests in Diabetes Mellitus. *Clin Sci* 1970;39 (2):253-269.
279. Badescu SV, Tataru C, Kobilinea L, Georgescu EL, Zahiv DM, Zagrean L. The association between Diabetes mellitus and Depression. *J Med Life* 2016;9(2):120-125.
280. Wei L, Ji L, Miao Y, Han X, Li Y, Wang Z, Fu J, Guo L, Su Y, Zhang Y. Constipation in DM are associated with both poor glycemic control and Diabetic Complications: Current Status and Future Directions. *Biomedicine and Pharmacotherapy* 2023;165:115202.
281. Haines ST. Treating Constipation in the Patient With Diabetes. *The Diabetes Educator* 1995;21(3):223-232.
282. Darienski R, Mihaylova V, Handjieva-Darienska T. The link between Obesity and the skin. *Front Nutr* 2022;9:855573. DOI:10.3389/fnut.2022.855573.
283. Svensson R, Hahn RG, Zdolsek JH, Bahlmann H. Plasma volume expansion reveals hidden metabolic acidosis in patients with diabetic ketoacidosis. *Intensive Care Medicine Experimental* 2022;10:36. DOI:10.1186/s40635-022-00464-5.
284. Délia JA, Segal AR, Pharm D, Weinrauch LA. Metformin-SGLT2, Dehydration, and Acidosis Potential. *JAGS* 2017. DOI:10.1111/jgs.14724.
285. Dobrica EC, Gaman MA, Cozma MA, Bratu OG, Stoian AP, Diaconu CC. Polypharmacy in Type 2 Diabetes Mellitus: Insights from an Internal Medicine Department. *Medicina* 2019;55:436. DOI:10.3390/medicina55080436.
286. Ghiraldi EM, Nourian A, Chen M, Friedlander JI. Investigating Fluid Intake in an Underserved Community: What Factors Are Associated with Low Urine Volume on 24-Hour Urine Collection? *J Endocrinol* 2021;35(1):17231728.
287. Andraska EA, Tran LA, Haga LM, Mark AK, Madigan MC, Makaroun MS, Eslami MH, Chaer RA. Contemporary management of acute and chronic mesenteric ischemia: 10-year experience from a multihospital healthcare system. *J Vascular Surgery* 2022;75(5):1624-1635.
288. Zhang QC, Hastings C, Johnson K, Slaven E. METFORMIN-ASSOCIATED LACTIC ACIDOSIS PRESENTING LIKE ACUTE MESENTERIC ISCHEMIA. *The Journal of Emergency Medicine* 2019;. DOI:10.1016/j.uermed.2019.04.024.
289. Correia CS, Bronander KA. Metformin-associated lactic acidosis Masquerading as Ischemic Bowel. *Am J Med* 2012. DOI:10.1016/j.amjmed.2011.012.
290. Canfora A, Ferronetti A, Marte G, Di Maio V, Mauriello C, Maida P, Bottino V, Aprea G, Amato B. Predictive Factors of Intestinal necrosis in acute mesenteric Ischemia. *DE GRUYTER Open. Med.* 2019;14:883-889.
291. Ramadori G. Albumin Infusion and Critically Ill COVID-19 Patients: Hemodilution and Anticoagulation. *Int J Mol Med* 2021;22(13):7126. DOI:10.3390/ijms22137126.
292. Adeva-Anday MM, Fernandez-Fernandez C, Moorigna-Bayad D, Castro-Quintela E, Dominguez-Montero A. Sodium Bicarbonate Therapy in Patients with Metabolic Acidosis. *Scientific World Journal* 2014;21:627673. DOI:10.1155/2014/627673.

293. Sulkin TV, Bosman D, Krentz AJ. Contraindications to Metformin Therapy in Patients with NIDDM. *Diabetes Care* 1997;20(6):925-928. DOI:10.2337/diacare.20.6.925.

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