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Chapter 3

METFORMIN: WHO ARE SUITABLE PATIENTS?

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ABSTRACT

Metformin is currently the most widely-used drug for the treatment of diabetes mellitus. Perhaps the main therapeutic effect of metformin (not only an unwanted one) is restriction of aerobic metabolism resulting in lower energy income and adenosine triphosphate (ATP) production.

The ultimate effect is increased income of glucose to the cell enabling constant ATP production. This mechanism explains why metformin decreases glucose production by liver cells and restores its uptake by skeletal muscle cells from the blood, even if they do not respond sufficiently to insulin-stimulated impulses. Metformin increases intestinal absorption of glucose, but its turnover into lactate is significantly increased, with intestinal metabolism subsequently becoming ineffective (more anaerobic).

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The rate of glucose absorption is reduced, and the process occurs more distally along the tract. In the end, metformin decreases net intestinal glucose uptake and aerobic glucose utilization.

However, the side effects of this drug can be dangerous. Metformin-induced lactic acidosis (MILA) is a lethal disorder. The first significant deviation – and probably the cause of MILA development in monitored patients – is gastrointestinal tract involvement. Common infection or the effect of metformin itself cause vomiting and diarrhea, with subsequent hypovolemia causing pre-renal acute kidney injury, which in turn raises metformin levels up to toxic values. Development of kidney failure (followed by heart failure with dyspnea and brain failure with a delirious state) is often unexpected and fast.

Metformin has become the most widely-used drug for the treatment of Type-2 diabetes mellitus. Strictly speaking – we use this drug in cases of treatment of "syndrome of intracellular overfeeding with secondary syndrome of chronic hyperglycemia", today known as "DMT2 syndrome", or "Type-2 diabetes mellitus with obesity". It is the drug of choice for the treatment of T2DM in obese people today. Its use is not recommended in cases of DM with intracellular starvation, i.e., true "Type-1 diabetes mellitus, T1DM (a disease characterized by lack of insulin), but can be useful in patients with either type of diabetes mellitus, i.e., both T1DM and T2DM.

Many patients with intracellular starvation due to a lack of insulin (T1DM) tend to overfeed – then treated with high, supra-normal, doses of insulin subsequently develop intracellular overfeeding and, hence, T2DM. On the other hand, many obese patients with T2DM lose their ability of insulin overproduction and they develop ***either*** type of diabetes mellitus.

Today, metformin belongs to the safest medications in its class. Nevertheless – if we are forced to use drugs that limit the surplus of energy and decrease the effectiveness of metabolism – we can still expect an adverse effect characterized by aerobic metabolism collapse, i.e., MILA. Today we have some other modern drugs: incretins acting by the anorectic effect; gliflozins modulating direct glucose elimination via the urine. While adequate food intake and physical training have the same beneficial effect, they are definitely not associated with any potential harm.

Keywords: lactic acidosis, metformin, Type-2 diabetes mellitus, T2DM, AMP-activated protein kinase, respiratory complex I

1. INTRODUCTION

Metformin is currently a widely-used drug in the treatment of Type-2 diabetes mellitus (T2DM).

The drug reduces the efficiency of metabolism of energy-rich substances. This is the basic effect of the drug.

Metformin allows patients to receive more energy-rich substances than their cells actually need. It increases the need for glucose by tissue (cells), reduces glucose production thereby lowering blood glucose levels.

The effect of metformin is similar to that of other biguanides (phenphormin and buformin) and is quite different from – and in many aspects opposed to – the effect of insulin.

Unlike insulin, metformin provides protection to the body from the accumulation of energy-rich substances. Besides, it protects the body from obesity as well as steatosis of the liver and muscles including the myocardium.

The main therapeutic effect of the drug is reduction of aerobic metabolism.

In our center specializing, among other things, in the management of acid-base disturbances, we have encountered, over the last 30 years, a relatively large number of cases of biguanide intoxication. These included 31 cases of lactic acidosis caused by the use of buformin in the 1989–1998 period [1] with another 23 cases of metformin-induced lactic acidosis (MILA) between 1999 and 2017 [2], followed by 4 cases (2017–2019) (unpublished data).

The symptoms of intoxication with metformin and buformin were similar. In contrast, they were quite different from those of an insulin overdose. It was for this reason that we decided to revise some of the traditional views on the effect of metformin.

2. VARIOUS EXPERIMENTAL RESULTS

Experiments with metformin bring very ambiguous results. One may ask why?

Metformin exerts its effect at sites where the energy is acquired (transformed). It acts in the mitochondria, where it modulates production of the basic cell energy carrier – adenosine triphosphate (ATP). To reach the mitochondria, it is necessary to cross three membranes, the cell membrane and the outer and inner membranes of the mitochondria.

For metformin to exert its effect, a high concentration at the site of action is required; this is accomplished by either a high dose of metformin or its long-term administration. This is the only way to allow sufficient transfer of the drug across the membranes and to achieve the necessary high concentration in the mitochondrial matrix.

The other two biguanides, buformin and phenformin, are (unlike metformin) effective at significantly lower concentrations. Phenformin is lipophilic, readily crossing cell membranes and penetrating significantly faster to its site of action. This, on the one hand, makes it easy to determine the effect of this substance but, on the other, it is responsible for its high toxicity index.

In the body, the metformin molecule is in ionized form, carrying an electric charge. It is hydrophilic and does not pass rapidly through cell membranes.

For this reason, unfortunately, studies using inadequately low metformin concentrations as well as those using inadequately short observation period reported confusing and, occasionally, contradictory results.

How high is then the necessary effective concentration of the drug and how long does it take to evaluate the effect of metformin?

Compare:

The plasma concentrations of metformin found in patients are about 20.9 μM (= μmol/l), e.g., 2.7 mg/l [3].

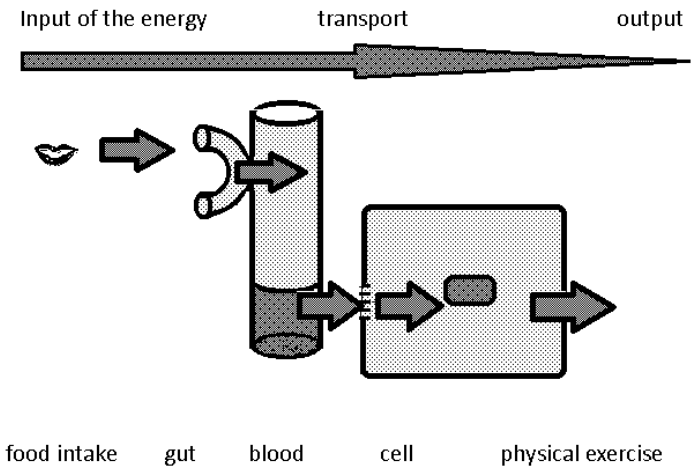

Figure 1. Transport of the energy. Eating is the first principal step of energy transport. Food enters the mouth, passes through intestine to blood. Intracellular metabolism, determine the need of glucose. Physical exercise is the last step of energy transport in the muscle [135].

In experiments the concentrations needed for metabolic changes to occur, in tissue homogenates containing disrupted mitochondria, were 30 000 μM [4]. The concentrations required for 50% inhibition of nicotinamide adenine dinucleotide, NADH oxidation by metformin is 79 000 μM in the case of submitochondrial particles [5].

Thus, the necessary concentration at the site of action is a thousand times higher than the plasma concentrations.

3. INTRACELLULAR METABOLISM

The most important metabolic processes take place inside the cells. These processes are controlled by external (extracellular) hormones and, (mainly), by internal regulatory mechanisms, through "intracellular hormones". The basic metabolic processes, in which energy is obtained, take place even in the inner organelles inside the cells, in the mitochondria. Similarly, the metabolic processes occurring in the mitochondria are

controlled primarily by intramitochondrial regulatory mechanisms through what is referred to as "intramitochondrial hormones".

While the hormones determine the amount of energy-rich substances (glucose, triacylglycerols, or amino acids) entering the cells, intracellular regulatory mechanisms determine how many energy-rich substances enter the mitochondria. Metabolism is linked to the presence of substrates that actually reach the mitochondria and of the effect of respiratory complexes that produce the bulk of the major energy carrier, adenosine triphosphate (ATP). It is necessary to produce a certain constant amount of ATP. If we reduce the efficiency of the (aerobic) metabolism, then more glucose – that the cell will consume – will be needed. Thus, it takes more glucose from the blood allowing to reduce hyperglycemia.

Why is it not possible to meet the increased need for ATP and, consequently, the need for glucose by increasing physical activity, muscle exercise? The answer is that people today are generally reluctant to exercise physically. On the very contrary, they are keen on eating more food than their body actually needs. And the imbalance between low energy expenditure and high energy intake is solved through medication.

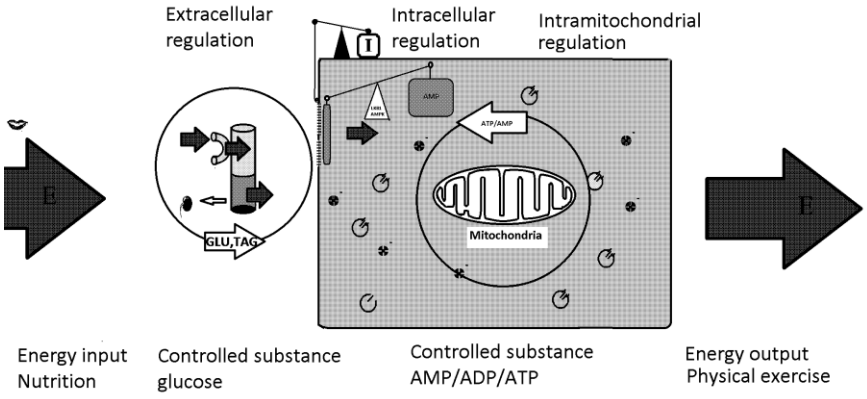

Figure 2. Regulation. 1/ Extracellular regulatory mechanisms - hormonal system. (insulin, glucagon, growth hormone, adrenaline, leptin, adiponectin and others). 2/ Intracellular regulation mechanism (AMPK, LKB1, CaMKK and others). 3/ Intra-mitochondrial regulatory mechanism.

4. Inhibition of Respiratory Complex I in Submitochondrial Particles

As mentioned above, the basic effect of metformin consists in the inhibition of mitochondrial respiratory complex I [4-7]. This complex is located on the inner side of the mitochondria. To assess the effect of metformin, it is necessary to obtain sub-mitochondrial particles (SMPs). To do so, tissues were homogenized and sonicated to disrupt all cells and mitochondria.

In this process, the pH gradient disappears (the pH within the mitochondria is 7.6, while the intracellular pH is 7.0), a fact limiting our ability to accurately determine the effect of other substances or pH to some extent.

Electron transfer by respiratory complex I was partially blocked even at extremely high concentrations of metformin. Metformin inhibited complex I in sonicated tissue homogenates contained disrupted mitochondria at concentrations of 30 000 μmol/l. [3] whereas the concentrations needed for 50% inhibition of NADH oxidation by metformin was 79 000 μmol/l in case of submitochondrial particles [5].

Restricted energy income, restricted ATP production and increasing AMP concentrations all influence the basic mechanism which controls the acquisition and conversion of energy by the cell.

(By the way, the concentrations required to achieve 50% inhibition of NADH oxidation by phenformin is significantly lower, 2230 umol/l, in the case of submitochondrial particles [11].)

5. Inhibition of Respiratory Complex I in Intact Mitochondria

To investigate the effect of metformin on intact mitochondria, not extremely high concentrations but extremely long periods of time are necessary. Metformin is slowly transmitted through membranes by specific

carriers. This process is very time consuming. While metformin at concentrations of 10 000 μmol over a short period of time (30 minutes) did not inhibit oxygen consumption of isolated liver mitochondria [8], this agent at relatively low concentrations (50 μM) caused significant inhibition after a long time period (24 and 60 hours) [5] (in permeabilized hepatoma cells).

To be more specific, metformin (50 μM) caused 12.6% inhibition after 24 hours, whereas, for 100 μM, the inhibition rate was 25.8%. At 60 hours, metformin (50 μM) caused 29.4% inhibition, whereas for 100 μM metformin, the inhibition rate was 37.1% [4]. Similar results were obtained in experiments using the rat soleus muscle [4]. Experiments designed to assess the effect of short periods (e.g., 20 minutes) of metformin incubation permeabilized cells showed no effect of metformin on respiratory function [9].

The time and concentration dependence of complex-1 inhibition by metformin in the mitochondria and SMPs suggests a slow rate of permeation of the drug across the inner mitochondrial membrane to directly inhibit complex I [5]. The positive charge on metformin may account for its accumulation within the matrix of energized mitochondria driven by the membrane potential ($\Delta\psi$). Thermodynamic considerations predict that, with a $\Delta\psi$ of -180 mV, metformin should accumulate 1000-fold within the mitochondrial matrix [5]. Metformin probably helps to avoid life-threatening complications because of its (partially) self-limiting inhibition. As the matrix concentrations of the drug rise, progressive inhibition of the respiratory chain will lead to a drop in the membrane potential, thereby preventing further accumulation of the drug [5].

6. Inhibition of Respiratory Complex I in Intact Cells

Preparation of the cells (we do to be able to evaluate intracellular or intramitochondrial processes) is associated with significant cell damage.

Digitonin permeabilization, which leads to cell membrane disruption, permits the penetration of ions from cells and vice versa. However, the huge concentration differences between sodium, potassium, calcium and pH levels are essential to maintain the vital processes within the cells. Also, breakage of the mitochondrial membrane e.g., by sonification, results in a loss of concentration gradients, pH among other things, which are responsible for gaining energy by these organelles. Reachable data come from experiments obtained under completely non-physiological conditions incompatible with life. Therefore, any processes that require a longer duration of experiment are very difficult to evaluate. The results we get with living intact cells are therefore thus even more important.

In experiments with live undamaged cells, the researcher is faced with another problem. Cells respond to any significant deviation in vital functions, hence processes whereby energy is acquired are controlled by a number of parallel control and performance mechanisms.

Imagine this situation: Metformin will reduce ATP production (and increase the concentrations of energy-poor AMP). The regulatory mechanism detects this dangerous deviation and immediately changes energy metabolism. Regulatory mechanisms limit the formation of energy-rich substances such as cholesterol. Furthermore, regulatory mechanisms increase the ATP production from fatty acids, glucose or amino acids. Regulatory processes very effectively restore ATP (and thus also ADP and AMP) levels to previous values. As a result, an observer, while noticing changes in metabolism but will remain unaware of any significant changes in the AMP/ATP ratio.

First, let us mention the results of experiments where metformin was used at a relatively high concentration. The effect on energy metabolism of intact cell is relatively more intensive and faster than that in permeabilized cells. In intact hepatocytes, metformin 10 000 μM (i.e., 10 mM) decreases the mitochondrial membrane potential ($\Delta\Psi$) by approximately 30 mV after 30 minutes of incubation (DC -175… -141 mV). It likewise reduces the cytosolic ATP/ADP ratio (from 7.3 to 2.5) and mitochondrial ATP/ADP ratio (from 1.7 to 0.5) while increasing the lactate/pyruvate ratio by 23% (from 19.8 to 24.4) and 3-hydroxybutyrate/acetoacetate ratio by 111%

(from 0.18 to 0.38) and decreasing the cellular respiratory rate (JO2) (from 100% to 54%) [8].

Incubation with a lower concentration (2000 μM) of metformin for a longer period of time (3 hours) increases the ratio of β-hydroxybutyrate/acetoacetate by 249% and that of L-lactate/pyruvate by 166% [5]. Similar results were obtained with the isolated rat soleus muscle: its 24-hour exposure to metformin (at a lower concentration) reduced cell respiratory and increased anaerobic glycolysis rates, with 270 μmol metformin increasing lactate production by 84% and decreasing oxidation of glucose [4].

Is it possible to register deviations caused by low metformin concentrations? Assessment of the effect of metformin on the amounts of AMP or ATP in intact cells is relatively difficult. The majority of ATP/ ADP/AMP is processed intramitochondrially. Regulatory mechanisms are set to respond to minor deviations rather than those that cause noticeable changes in the total ATP/AMP concentration in the cell.

The total AMP content in a well perfused rat heart is approx. 350 μM, with cytosolic AMP content being about 0.4 μM [6]. Cytosolic [ADP] and [AMP] represent the metabolically active fractions of the total content of these two nucleotides. In metformin-perfused hearts, cytosolic [AMP] in 10 mM metformin-perfused hearts increased significantly, this, however, after a long period of time (42–50 minutes) [10].

7. INTRACELLULAR REGULATION

Each cell has its own regulatory system which maintains its energy homeostasis. We are looking for a single molecule that controls the cell's energy status. One molecule cannot control such a complex regulatory mechanism. There are a number of molecules that monitor and affect a cell's energy status. The number is likely to be as extensive as the number of regulatory molecules in the extracellular space. The same as tens or hundreds of extracellular hormones are currently known, tens or hundreds

of intracellular signaling and executive molecules are most likely to be gradually identified.

Which molecules are considered the most important today?

An important energy sensor and regulator of energy homeostasis is AMP-activated protein kinase, AMPK. In the presence of AMP, the activity of AMPK is increased approximately 4–5-fold. AMP or ADP can directly bind to the γ regulatory subunits of AMPK, leading to a conformational change that protects activating phosphorylation of AMPK [11, 12]. AMPK phosphorylation results in its activation by at least 100-fold. AMPK is phosphorylated by upstream AMPK kinases, i.e., AMP-activated protein kinase kinase, AMPKK [13]. The following two molecules are considered to be significant: LKB1 and CaMKKB. However, LKB1 has several superior kinases: protein kinase C ZETA, PKC-ζ [14], which is important for the metformin effect, and another two protein kinases A, PKA and p90 ribosomal S6 kinase, RSK [14]. Another potential mechanism for AMPK activation is through TGFβ-activated protein kinase (TAK-1). [15]. Thus, the central energy sensor is a whole network of upstream kinases. Are these superior kinases controlled by AMP or ADP or ATP? The most likely answer is yes.

AMPK can be phosphorylated in response to calcium flux via the calmodulin-dependent protein kinase kinase-beta, CaMKKβ [16-19].

Liver kinase B1, LKB1, otherwise called serine-threonine kinase 11 (STK11) is an important upstream kinase of adenosine monophosphate-activated protein kinase [20]. Active LKB1 kinase is actually a complex of three proteins: LKB1, Ste20-related kinase adaptor alpha (STRADA) and calcium-binding protein 39 (CAB39; also known as mouse protein 25, MO25). Thus, the enzyme complex is sometimes referred to as LKB1-STRAD-MO25.

LKB1 can phosphorylate AMPK (and some other members of this AMPK subfamily) increasing their activity >50-fold [21].

Later, other AMPK-like enzymes have been identified: BRSK1, BRSK2, NUAK1, NUAK2, QIK, QSK, SIK, MARK1, MARK2, MARK3, MARK4, and MELK. They are called AMPK-related kinase RK family enzymes [20, 21]:

- BRSK1 and BRSK2 are brain-specific serine/threonine kinases 1 and 2, respectively.
- NUAK1 refers to nuclear AMPK-related kinase – AMPK-related kinase 5 (ARK5).
- NUAK2 is also called SNF1/AMPK-related kinase (SNARK).
- SNF1 is sucrose non-fermenting.
- SIK stands for salt-induced kinase. SIK2 was identified as a SIK1-related gene expressed in adipose tissue. SIK3 is also known as QSK.
- MARK1–4 are microtubule affinity-regulating kinases 1 through 4.
- MELK is maternal embryonic leucine zipper kinase.

Which of these molecules are significant or even more significant than AMPK? When will we define all their possible interactions? And are they important for the development of diabetes? Certainly they are important. Long-term research is warranted to assess all their possible effects.

8. AMPK PATHWAY

5´AMP- activated protein kinase or AMPK is a multi-subunit enzyme which plays the essential role in cellular energy homeostasis. AMPK induces catabolic processes and enables the production of ATP.

It regulates the cellular uptake of glucose, beta-oxidation of fatty acids and biogenesis of the GLUT4 glucose transporter. AMPK increases the glucose input in cases of lack of energy, intracellular starvation, as well as high concentrations of AMP and low ATP concentrations [22-24]. It is a regulator of the lipid biosynthetic pathways due to its role in the inactivation of key enzymes such as acetyl-CoA carboxylase (ACC) [25]. AMPK inhibits anabolic processes that consume ATP, such as protein synthesis [26]. AMPK is a major signaling protein that represses the rate of protein synthesis [27].

AMPK acts as an ultrasensitive cellular energy sensor system responding to increases in AMP concentrations [AMP] or the AMP/ATP ratio [25].

The original idea was simple: AMPK recognizes a lack of energy-rich ATP and an excess of energy-poor AMP molecule to change cell metabolism and restore the equilibrium with sufficient ATP. Later studies showed that the response to the AMP/ATP ratio directed by AMPK alone is relatively small. Superior kinases (LKB1, CaMKKβ and others) have a significant effect. For example: a deficiency of LKB1 in the skeletal muscle attenuates AMPK activation and glucose uptake during muscle contraction [28].

All substances that influence the effect of superior kinases are not yet known. Extracellular signals transmitted from extracellular fluid through hormones and their receptors (e.g., through insulin, leptin, and adiponectin) reflect the needs of the whole body whereas intracellular signal (AMP, ATP or calcium and other substances) express the requirements of a single cell.

An explanation for the effect of calcium and CaMKKβ seems to be relatively easy. The superior kinase CaMKKβ responds to elevated calcium concentrations as a manifestation of pump failure that removes calcium from the cell. The intracellular calcium concentrations are 100 times lower than those in extracellular fluid. High calcium concentrations may indicate targeted opening of calcium channels, necessary for actin and myosin interaction allowing for muscle contraction. Energy in the form of ATP will be needed to relax the muscle. Therefore, calcium concentrations in a cell's cytoplasm is a significant signal of the need for energy supply that is transmitted via calmodulin-dependent protein kinase kinase-beta, CaMKKβ, to AMPK.

The cells respond to very small variations in the plasma AMP/ATP ratio, which are not yet laboratory detectable. Conversely, it is these regulatory mechanisms that we observe and misinterpret as a putative effect of the drug.

Under conditions of severe stress, when the levels of AMP might approach those of ADP and free ATP, the 10-fold allosteric activation

caused by AMP would multiply with the >200-fold activation caused by Thr 172 phosphorylation to yield a >1000-fold activation overall. Thus, the mammalian kinase can respond over a wide range of the cellular ADP/ATP and AMP/ATP ratios and over a very wide dynamic range [29].

AMPK has three subunits. Different isoforms exist for each of the AMPK subunits. Two α (α1 and α2), two β (β1 and β2), and three γ (γ1, γ2, and γ3) isoforms enable the creation of up to 12 distinct AMPK configurations. However, in human skeletal muscle, these configurations are likely limited to α2/β2/γ1 (most abundant), α2/β2/γ3, and α1/β2/γ1 [30]. Of these three, α2/β2/γ3 accounts for the majority of AMPK activation due to high-intensity exercise [31].

Metformin activates AMP-activated protein kinase. Directly or indirectly? Via AMP concentrations or via AMPK phosphorylation? Either way?

Some drugs – such as metformin or pioglitazone – affect ATP production. In a system that would not have regulatory mechanisms, a variation in ATP concentration would arise.

Regulatory events in the living cell (maintaining cell homeostasis) alleviate immediately deviation of the ATP concentration and thus deviation of the AMP/ATP ratio. The deviations that occur in a living organism are so small that they are very difficult to detect. Besides, the total amount of ATP and AMP is largely stored in the mitochondria, with only a small portion of these compounds, which are present extra-mitochondrially in the cytoplasmic space, affecting cell metabolism. For this reason, intracellular cytosolic concentrations (not total concentrations) of ATP and ADP should be evaluated. Nevertheless, it was possible to prove the effect of the drug on the energy of living cells: AMPK activity responds to cytosolic concentration ([AMP] hereinafter), e.g., in the isolated rat heart [32, 33].

In these experiments, metformin activated AMPK in the heart by increasing *cytosolic* AMP concentrations [10]. In hearts treated with 10 mM metformin, [AMP] was increased (after a relatively short period, 50 min) and AMPK activity, phosphorylated AMPK, and phosphorylated acetyl-CoA carboxylase were elevated [10]. Whereby *total* ATP content

and *total* AMP/ATP ratio remained unchanged or the difference was too small to detect.

Metformin is able to increase the net phosphorylation of the AMPK catalytic α subunit at Thr-172, a crucial phosphorylation site in the activation of AMPK, and activate AMPK activity in primary hepatocytes [34]. However, upstream kinase LKB1 is necessary for this process to set in: hepatic knock-out of LKB1 (liver kinase B1), the upstream kinase for AMPKα phosphorylation at Thr-172, abolishes metformin suppression of hepatic glucose production [35]. Furthermore, activation of AMPK results in the phosphorylation of CRTC2 (CREB-regulated transcription coactivator 2) and CBP (CREB-binding protein), which then inhibit gluconeogenic gene expression [36, 37].

Metformin increases the translocation of LKB1S from the nucleus to cytosol. When inactive, LKB1 is localized to the nucleus and is able to translocate to the cytosol and become active when it associates with STRAD and MO25 [38].

Nucleocytoplasmic translocation of LKB1L, which requires phosphorylation of Ser-428/431 and Ser-307 in the LKB1L C-terminal domain, is needed for metformin-mediated activation of AMPK [39, 40].

9. TRANSPORT

Metformin is transported by organic cation transporters (OCTs) (solute carrier 22, SLC22) OCT1, OCT2 [41-43] and other polyspecific organic cation transporters.

The following transporters are involved in the drug's absorption, OCT3 (SLC22A3), PMAT (SLC29A4) [44], SERT (SLC6A4), distribution, OCT1 (SLC22A1) OCT3, and elimination OCT2 (SLC22A2), MATE1 (SLC47A1), and MATE2 (SLC47A2) [45].

OCT1 has been shown to play an important role in hepatic metformin uptake, which is an important mechanism for its therapeutic effect as well as side effects (lactic acidosis) [42].

OCT2, which is expressed on the basolateral membrane of kidney tubular cells, was implicated in the renal excretion of metformin [43, 46].

OCT3 is distributed in brain tissue, in the liver, kidney, intestine, and other organs.

PMAT, proton-activated organic cation transporter, or plasma membrane monoamine transporter, is expressed in the human small intestine and concentrated on the tips of the mucosal epithelial layer. PMAT transports many organic cations and also plays a role in the intestinal uptake of metformin [44].

Metformin is also a substrate for and an inhibitor of the human thiamine transporter, THTR-2 (SLC19A3), which is highly expressed in the small intestine [47].

Genetic disorders, drug interactions or pH changes influence the function of transporters. The transport activity of the OCTs is generally significantly decreased at lower pH [48-50], and the acidic environment in the gut lumen (pH 4.0–7.0) may limit the ability of OCT3 in drug absorption. PMAT-mediated metformin transport is greatly stimulated by an acidic pH, with the uptake rate being ~4-fold higher at pH 6.6 than at pH 7.4 [51].

10. THIAMINE

Selective thiamine deficiency is just another possible mechanism for metformin therapeutic (as well as adverse) effects.

Vitamin B1 or thiamine, is essential for normal glucose metabolism. Thiamine pyrophosphate is a cofactor for pyruvate dehydrogenase (PDH), α-ketoglutarate dehydrogenase, and transketolase, all of which have fundamental roles in regulating cellular metabolism [52].

Pyruvate dehydrogenase is a multienzyme complex in the inner mitochondrial membrane that catalyzes the oxidative decarboxylation of pyruvate to acetyl coenzyme A (CoA), which can then enter the citric acid cycle. In thiamine deficiency, when pyruvate cannot undergo this

conversion, it is converted to lactate by the action of lactate dehydrogenase [53].

OCT1, the major hepatic transporter for metformin, is also the primary hepatic uptake transporter for thiamine [54].

The human thiamine transporter 2 THTR-2 mediates thiamine uptake in the intestine, CNS, and various peripheral tissues. Metformin inhibits the THTR-2-mediated uptake of thiamine [47].

The gastrointestinal syndrome of thiamine deficiency consisting of nausea, vomiting, abdominal pain and lactic acidosis – gastrointestinal beri-beri – resembles metformin intoxication [55-57].

11. MITOCHONDRIAL GLYCERO-3-PHOSPHATE DEHYDROGENASE INHIBITION

The glycero-3-phosphate shuttle has an important role in the regulation of hepatic gluconeogenesis [58, 59].

It is a mechanism that regenerates NAD^+ from NADH. Cytoplasmic glycero-3-phosphate dehydrogenase 1 (GPDH-C) converts dihydroxyacetone phosphate to glycerol-3-phosphate, by oxidizing one molecule of NADH to NAD^+. Glycero-3-phosphate gets converted back to dihydroxyacetone phosphate by inner membrane-bound mitochondrial glycero-3-phosphate dehydrogenase 2 (GPDH-M or mGDP), reducing one molecule of enzyme-bound flavin adenine dinucleotide (FAD) to $FADH_2$ [60]. $FADH_2$ subsequently reduces coenzyme Q (ubiquinone to ubiquinol) which enters the oxidative phosphorylation pathway. [60]. The net gain is 2 ATP molecules [60].

Metformin non-competitively inhibits the redox shuttle enzyme mGPD, resulting in an altered hepatocellular redox state, reduced conversion of lactate and glycerol to glucose, and decreased hepatic gluconeogenesis [61].

12. METFORMIN REDUCES LIVER GLUCOSE PRODUCTION BY INHIBITION OF FRUCTOSE-1-6-BISPHOSPHATASE

Hepatocytes are equipped with a mechanism to control the rate of hepatic gluconeogenesis in response to energy status, and fructose bisphosphatase 1 (FBP1) has long been recognized as a key component [62]. FBP1 catalyzes the irreversible hydrolysis of fructose-1,6-bisphosphate (F-1,6-P2) to fructose-6-phosphate (F6P) and inorganic phosphate (Pi) in the presence of divalent cations. FBP1 activity is regulated synergistically by the allosteric inhibitors AMP and F-2,6-P2. Metformin induces a mild energy stress in the liver, leading to an increase in the concentrations of AMP that allosterically inhibits FBP1 to lower hepatic glucose production (HGP). The AMP-inhibited fructose-1,6-bisphosphatase-1 is also considered to be the major contributor to metformin's therapeutic action (glucose-lowering effect) [63].

12.1. Other Theories

Recently, another study reported that metformin inhibits HGP through hepatic AMPK-independent mechanisms, by attenuating the ability of glucagon to increase 3',5'-cyclic adenosine monophosphate (cAMP) levels and promote HGP [64].

13. AEROBIC METABOLISM INHIBITION CAUSED BY METFORMIN

What do all the theories that explain the effect of metformin in the body have in common? All theories show that metformin reduces the efficiency of metabolism and increases glucose consumption for basic metabolic processes. Metformin reduces the processes that lead to aerobic

metabolism of substances that are rich in energy and contain carbon (especially carbohydrates, fats and amino acids).

The end product of glucose metabolism is not CO_2 +H_2O (with 38 (32 more exactly) ATP molecules being obtained) but lactate (with a 2 ATP molecule gain). The need for glucose in the intestine or muscle increases.

Lactate is produced under physiological conditions, too. At rest, it is formed mainly by the skin, muscle, brain, red blood cells or the intestine. During heavy exercise, the most important source of lactate is white skeletal muscle. Placenta during pregnancy or lung in sepsis produce excessive amounts of lactate. Approximately two thirds of lactate are metabolized in the liver and one third in the kidney.

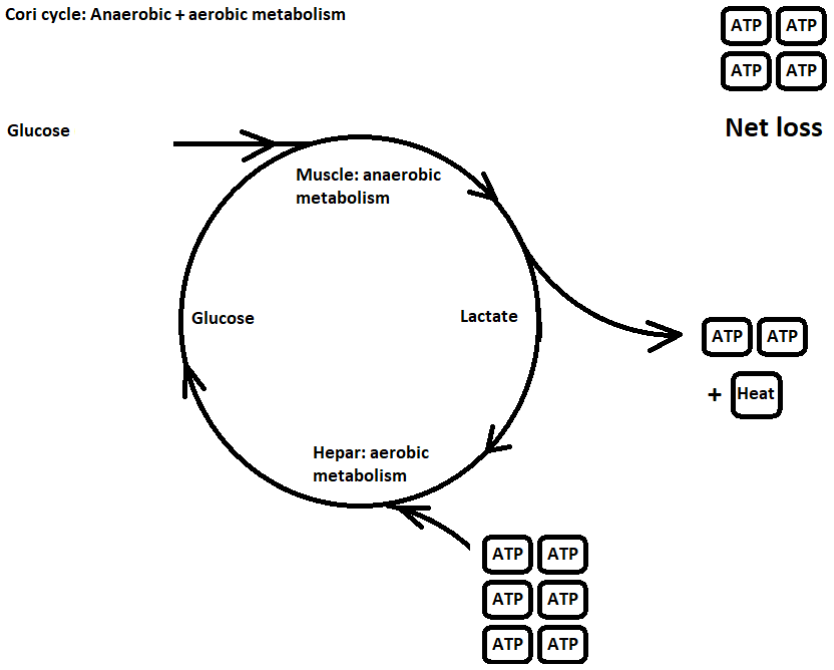

Figure 3. Cori cycle. The Cori cycle (Lactic acid cycle). Lactate produced by anaerobic glycolysis in the muscles moves to the liver and is converted to glucose. Glucose returns to the muscles and is cyclically metabolized to lactate and back.

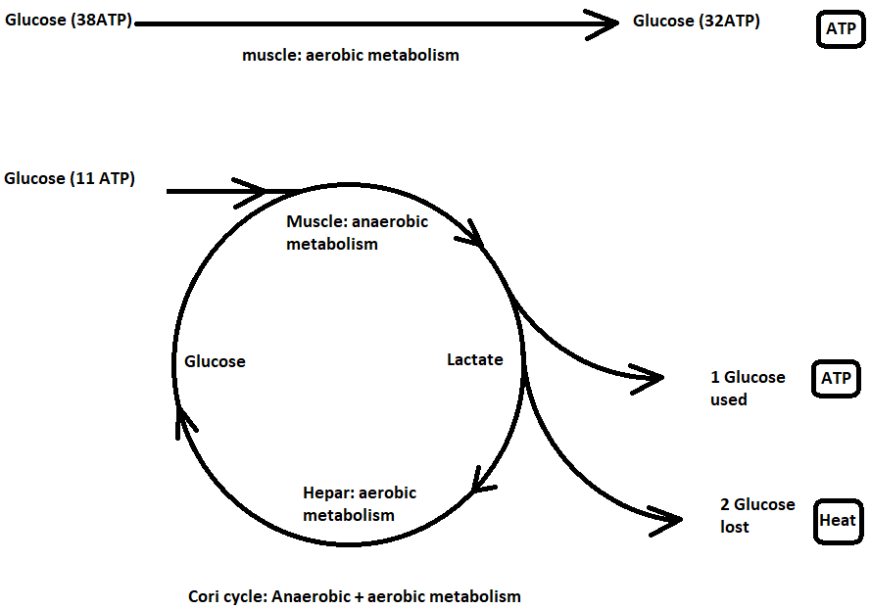

Figure 4. Effectiveness of the Cori cycle. Theoretical metabolic efficiency of the aerobic metabolism: 38 ATP molecules per 1 glucose. Real efficiency of aerobic metabolism: about 32 ATP per 1 glucose, 84% effectiveness (due to leaky membranes or cost of transport into the mitochondrial matrix). Efficiency of anaerobic metabolism (lactate lost by kidney): 2 ATP per 1 glucose, 5.3%. Efficiency of combined metabolism, anaerobic + aerobic in the Cori cycle (lactate converted back to glucose in the liver) 11 ATP per 1 glucose, 33%. 4 molecules ATP are lost every cycle.

It can get converted into glucose (or into CO_2 and H_2O if ATP is needed and aerobic metabolism possible). Metformin has been shown to drive lactate production. It is worth noting that the lactate-glucose-lactate cycle (or glucose-lactate-glucose cycle) is a highly energy-consuming process. Lactate enters the Cori cycle: it is transmitted from the site of origin (muscle, intestine) to where glucose is produced from it (liver), but at the cost of losing 4 ATP molecules (!) to recover each glucose molecule. Metformin causes a futile intestinal-hepatic cycle [65] (and other futile, muscle-hepatic and muscle-kidney cycles) thus increasing energy expenditure [65].

Metformin reduces ATP production with a larger amount of energy-rich compounds consumed by cells to meet the body's metabolic requirements. Glucose, triglycerides and fatty acids are consumed and do not accumulate in the blood.

14. THE INTESTINE

1) Metformin increases intestinal glucose absorption. 2) Metformin lowers intestinal glucose gain. Which of these statements is correct? Both.

Metformin would stimulate glucose uptake from the intestinal lumen to its tissue, primarily in the proximal jejunum – if it were possible – but, in fact reduces transport of glucose to the blood. Enterocytes, which are responsible for glucose entry into the body, highly accumulate metformin. Metformin significantly reduces the metabolism of these cells. Enterocytes themselves consume increased amounts of glucose. This mechanism explains why less glucose is transferred from the intestine to the liver in metformin-treated patients. Blood glucose levels are significantly reduced depending on the dose of metformin.

14.1. Re 1) Metformin Increases the Rate of Glucose Absorption in the Human Intestine

Metformin really increases glucose uptake and utilization [66, 67]. Metformin has been shown to cause an increase in labeled (intravenously administered) glucose uptake (18-fluoro-deoxy-glucose, 18F-FDG) in the colon and small intestine on the PET-CT scan [68-71]. It is critical to withdraw metformin for 2 days before an 18F-FDG PET/CT scan is performed for intra-abdominal neoplasic lesion assessment [70].

Physiological focal uptake – sometimes intense – has been reported within the right lower quadrant, corresponding to the cecum and right

colon; it was suggested to be related to the high concentrations of glucose-metabolizing lymphatic cells in this particular region [72].

14.2. Re 2) Metformin Reduces the Amount of Glucose Absorbed by the Intestine

While this statement may seem to contradict the first one, both are correct. Metformin reduces glucose transport across the intestinal epithelia in the proximal jejunum as well as in the proximal ileum in a dose-dependent manner [73]. As all dietary glucose is almost completely absorbed in the upper small intestine, it is not possible to further increase the amount of glucose received by the enterocytes. Enterocytes need more glucose from the intestinal lumen or from the blood. If no food is taken, enterocytes themselves consume glucose from the blood.

In the blood that comes from the intestine to the liver there is a reduced amount of glucose and an increased amount of lactate. The concentration of this less energy-rich substance is increased up to 50% [66, 67]. The lactate concentration is significantly higher in the blood that is transmitted from the intestines to the liver than in the peripheral blood that we usually examine. A significant – 2.5-fold – increase in lactate concentration in the hepatic portal vein was recorded [74]. Under usual conditions, lactate is almost completely metabolized in the liver. In one study, metformin-treated patients had only slightly higher plasma lactate concentrations (in peripheral blood) than non-metformin-treated subjects, 1.86 vs 1.58 mmol/l respectively. Metformin-treated patients had a mean plasma lactate 0.16 mmol/l higher than subjects not taking the drug [75].

It should be remembered that the human body only gains from the nutrients in the colon what remains after the energy-rich substances have been drained by bacteria. Metformin also reduces the effectiveness of metabolism in bacteria residing in the colon. Its influence changes the intestinal microbiome. A common finding was a reduction in butyrate-producing bacteria and an increase in opportunistic pathogens [76-78]. Metformin cause an overall decrease in bacterial diversity [79, 80]. It can

be expected that studies conducted in the future will demonstrate that the total amount of nutrients recovered in the colon is reduced by metformin.

14.3. .Metformin Absorption

Metformin concentrations in the jejunum peak at 500 μg/g, a value 30–300 times higher compared with its plasma concentrations [67]. After oral administration, metformin is slowly absorbed from the proximal small intestine (e.g., duodenum) [81, 82]. Metformin absorption is mediated by an active, saturable absorption process [81]. OCT1 and OCT3 play an important role in the transfer of metformin from the intestinal lumen to the interstitium. OCT3 is associated with metformin uptake and efflux in the salivary glands, which may account for the dysgeusia associated with metformin therapy [83].

Furthermore: Human thiamine transporter 2THTR-2 (hTHTR-2), SLC19A3, which is highly expressed in the small intestine, transports metformin too. The uptake mechanism for hTHTR-2 is pH- and electrochemical gradient-sensitive [50].

Metformin is efficiently taken up across the apical (luminal-facing) surface of enterocytes via bi-directional transporters, but that efflux across the basolateral surface of enterocytes is limited, resulting in metformin accumulation in the epithelium [84].

The human serotonin transporter (SERT, SLC6A4) plays a role in metformin intestinal absorption [47]. Serotonin (5-HT) release from the intestine is associated with nausea, vomiting and diarrhea, i.e., symptoms similar to those associated with metformin intolerance. Therefore, one possible mechanism for the GI intolerance associated with metformin use may relate to altered serotonin transport or to a direct serotonergic-like effect of metformin. Metformin uptake via SERT or OCT1 results in reduced serotonin transport and resultant GI side effects [85]

Metformin concentrations in the gastrointestinal (GI) lumen of patients taking 1-g oral doses are estimated to be 24 mM [50]. Thirty to 50% of

patients on metformin report GI side effects such as diarrhea and bloating [86, 87].

Modified-release formulations of metformin have been developed to spread the absorption of metformin along the intestine thus reducing the drug's local concentrations, in an effort to raise its tolerability. A new metformin formulation has recently been developed, metformin XR (extended release) and metformin DR (delayed release), which is designed to target the ileum via pH-dependent dissolution of the tablet. The bioavailability of metformin DR is lower, yet its glucose-lowering efficacy is similar despite lower systemic metformin exposure [88].

What is the main effect of metformin in the intestine? This drug reduces the efficiency of (aerobic) metabolism and reduces the amount of glucose obtained from food.

15. THE LIVER

Patients with T2DM show increased rates of endogenous glucose production, which can be attributed to increased rates of gluconeogenesis. Metformin lowers the rate of glucose production in these patients through a reduction in gluconeogenesis.

The rate of gluconeogenesis is three times higher [94] in diabetic subjects compared with controls (0.59 vs. 0.18 mmol $\cdot$ $m^{-2} \cdot min^{-1}$). Metformin reduces that rate by 36% (to 0.38 mmol $\cdot$ $m^{-2} \cdot min^{-1}$) [88].

The above findings highlight the importance of correct interpretation of the phenomena observed in experiments. The liver of a patient with diabetes is not a perpetual motion machine. It is unable to create matter. Increased glucose production reflects increased entry of energy-rich substrates into the liver such as glucose, lactate, amino acids, free fatty acid and other energy-rich substances (e.g., so-called hepatic gluconeogenic substrate, i.e., alanine, glutamine, glutamate, glycerol, glycine, lactate, threonine or serine).

Even metformin is unable to annihilate the matter. The liver of metformin-treated patients receives smaller amounts of glucose precursors; moreover, its metabolism is less effective due to metformin use.

Nonetheless, experiments have produced quite different data. Hence the question arises which experiments provide accurate information about the effect of metformin on the liver? Experiments with low or high metformin concentrations? The answer is: both. After oral administration, the plasma concentrations of metformin peak within 1 hour in man as well as in animals [70, 88, 89] to achieve values of 10–40 μM in humans following a therapeutic dose [70, 89, 90]. As metformin is absorbed from the GI tract and delivered directly to the liver through the portal vein, the plasma concentrations of metformin in the portal vein are relatively high (40–80 μM) [70]. The most accurate results will be obtained by experimenting on cell cultures with this (80 μM) concentration for several days. However, in order to define the influence of all confounders and regulatory mechanisms, the duration of trial should be months or years.

There was in other studies considerable glycogen cycling in individuals with poor control of diabetes, which accounted for about 25% of endogenous glucose production. Hyperglucagonemia and hyper-insulinemia, conditions typically present in T2DM patients, have both been shown to promote glycogen cycling [91-93]. However, glycogen stores in diabetic patients, whether treated or not with metformin, are not extremely elevated or reduced in the long term. Thus, this mechanism is of little significance and only temporary.

Metformin treatment also lowered the plasma free fatty acid concentrations by 30% in the diabetic subjects [94-96]. Reduction in plasma free fatty acid concentrations after metformin treatment also contributed to reduced rates of gluconeogenesis [88].

Metformin decreases the rate of hepatic lactate uptake while increasing the amount of lactate taken up by the liver. Which of the two claims is correct? Both.

Metformin was observed to decrease gluconeogenesis in perfused liver, primarily through inhibition of hepatic lactate uptake [105]. The drug would have reduced lactate entry to liver cells if the lactate levels were the

same as in those not treated with metformin. However, since lactate production (in the intestine or in the muscles) in metformin-treated patients is increased and the lactate levels in the portal blood are increased, the liver's lactate entry is not reduced but elevated.

Metformin decreases hepatic glucose production [67, 88, 98, 99]. Restricted energy income, restricted ATP production and increasing AMP concentrations influence the basic mechanism defining energy gain of the cells. Reduced amounts of some energy-rich substrates and lack of energy (ATP) in hepatocytes curb the ability of the liver to produce glucose and thus reduce total glucose output from the liver to the blood. The total effect is given by the sum of the simple ATP deficiency and the influence of the regulatory mechanisms (LKB1, AMPK).

Metformin inhibits gluconeogenesis from L-lactate in isolated hepatocytes in a time- and concentration-dependent fashion. This is consistent with the time-dependent accumulation of the drug within the mitochondria and subsequent inhibition of the respiratory chain. After 2.5 hours of incubation with 2 mM metformin, the percentage of inhibition reached 75% [5].

The K0.5 (50% efficacy) value with respect to extra-mitochondrial metformin is about 80 μM at equilibrium and is consistent with the 29% inhibition of ADP-stimulated glutamate-malate oxidation observed in the mitochondria from cultured rat hepatoma cells exposed to 50 μM metformin for 60 hours. Fifty percent inhibition of respiration led to 20% inhibition of gluconeogenesis [100].

Metformin, via an AMP-activated protein kinase (AMPK)-dependent mechanism, suppresses glucose production and gluconeogenic gene expression in primary hepatocytes at concentrations found in the portal vein of (60–80 μm). Oral metformin inhibits gluconeogenic gene expression in the liver. Furthermore, the cAMP-PKA pathway negatively regulates AMPK activity through phosphorylation at Ser-485/497 on the α subunit, which in turn reduces net phosphorylation at Thr-172. Because diabetic patients often have hyperglucagonemia, AMPKα phosphorylation at Ser-485/497 is a potential target of metformin efficacy [101].

What is the main effect of metformin in the liver? The drug reduces the efficiency of metabolism and reduces the ability to produce glucose.

16. MUSCLE

Muscles make up a significant part of human body weight. Optimally, 35% (female) to 40% (male) of a healthy person's body mass is made up by skeletal muscle. Any change in muscle metabolism will entail a major change in the need for glucose and fatty acids, and significantly change blood glucose levels. Metabolism is predominantly aerobic. Especially the slow twitch (red) fibers focused on long-term or repetitive contraction are powered by aerobic processes.

Muscles that are capable of rapid physical performance work on the principle of anaerobic glycolysis. Fast twitch (white) fibers contain lesser amounts of mitochondria. Anaerobic metabolism allows faster development of muscle strength but its by-product is lactic acid converted back to glucose in the liver.

Most of the processes that continually consume ATP energy (e.g., pumps that transfer ions against concentration gradients) are dependent on the aerobic (more energy-efficient) process (in both red and white muscle).

Insulin increases glucose input into the muscle. However, when a certain upper limit is exceeded, further glucose input into the cell becomes unsuitable, and the internal regulatory mechanisms begin to limit glucose supply into the muscle. A secondary feedback event arises, which is referred to as "resistance". Maintenance of high insulin and glucose blood concentrations will trigger feedback the regulatory mechanism fast, within seven days [102].

Insulin increases glycogen production in the muscle. While the amount of glycogen is slightly increased, it does not reach the levels found that patients with an inherited glycogen-type metabolic disorder. A regulatory mechanism that detects excessive amounts of glycogen produces counter-regulatory events that curbing further glycogen production.

(Compare: glycogen storage diseases: glycogen synthase, acid alpha glucosidase, glycogen debranching enzyme, glycogen branching enzyme, muscle glycogen phosphorylase, muscle phospho-fructokinase, phosphoglycerate mutase, aldolase A, beta-enolase, glycogenin 1 and other deficiencies: Pompe's, Cori's, Forbes', Andersen, Mc Ardle diseases.)

Insulin increases the production of lipids in the muscle. The patient suffering from diabetes (T2DM) has intramyocellular lipid stores comparable to those of endurance athletes [103], but will not use them.

Metformin inhibits aerobic metabolism even in red and resting white muscle. The drug reduces the rate of ATP production and, conversely, causes increased concentrations of ADP or AMP. The end product of glucose metabolism is not CO_2 (with 32 ATP molecules obtained) but, to some extent, lactate (with a 2 ATP molecule gain). The need for glucose in the muscle increases. Lactate enters the Cori cycle: it is transmitted from the site of origin (the muscle) to where glucose is produced in (the liver), but at the cost of losing 4 ATP molecules to recover each glucose molecule. (The efficiency of this process is about 33%, 11 ATP/glucose.)

Likewise, metformin increases skeletal or heart myocyte glucose uptake [104-106], directly via AMP, AMP/ATP, via AMPK (AMPKα2) [107], via pyruvate dehydrogenase inhibition [52], via mitochondrial glycerol-phosphate dehydrogenase inhibition [61], or via fructose-1-6-bisphosphatase inhibition [63]. What is the main effect of metformin in muscle? This drug reduces the efficiency of (aerobic) metabolism and increases the amount of glucose consumed by the muscle.

17. ADIPOSE TISSUE

In many people, the mass of adipose tissue significantly exceeds that of muscle tissue. Adipose tissue is highly metabolically and hormonally active. Adipose tissue secretes hormones that contribute to energy homeostasis (adipokines such as leptin, adiponectin, adipsin, interleukin-6, tumor necrosis factor-α and others). Leptin acts through the brain to

regulate food intake and energy expenditure [109]. Another type of adipose tissue serves as an energy storage depot to maintain lipid homeostasis.

Insulin increases fat synthesis and fatty acid esterification while decreasing lipolysis. Disorders caused by severe insulin resistance (Rabson-Mendenhall syndrome, Donohue syndrome) are characterized by a deficiency or absence of adipose tissue.

Metformin treatment brought in a study significant reduction in visceral fat mass compared to controls accompanied by up-regulation of a fat oxidation-related enzyme in the liver, UCP-1, in brown adipose tissue [110]. Besides, the drug was shown to reduce the amount of body fat and improve body composition in studies performed in patients with T2DM [96, 111]. It has also been shown to enhance the expression of uncoupling proteins (UCPs), the key enzymes of adaptive thermogenesis [112]. Thus, it has been hypothesized that metformin administration promotes the reduction of body fat amount via acceleration of fat oxidation and adaptive thermogenesis *in vivo*. Has increased production of lactic acid been proven in adipose tissue due to the metformin therapy? Yes. The rate of net postabsorptive subcutaneous lactate release was increased (from 149 to 475 nmol · 100 g^{-1} · min^{-1}) [113]. What is the main effect of metformin in adipose tissue? The drug reduces the ability to store fat deposits.

18. BRAIN

Metformin may also exert an anorectic effect as metformin-treated patients tend to receive smaller amounts of food.

Besides, metformin has a direct anorexigenic effect [114-117]. It increases the plasma levels of the anorectic gut hormone peptide YY (PYY) via cell internalization through PMAT and SERT and intracellular activation of AMPK [188]. Other effects are also most likely – transporter interactions in the gastrointestinal tract, signs of local thiamine deficiency [55-57] and others. It has been hypothesized that metformin activates GLP-1 receptors to increase protein kinase A (PKA) activity on intestinal vagal afferents (gut-brain axes) [119].

The effect of metformin on the brain (through what mechanism this drug reduces the craving for food) is also believed to be extremely important, but evaluation of any possible effects warrants further long-term research.

19. SIDE EFFECTS

Generally, metformin is safe, with the most frequent adverse effects being gastrointestinal disorders, nausea, vomiting, flatulence and diarrhea. The most serious side effect is lactic acidosis, i.e., metformin-induced lactic acidosis (MILA). Metformin is contraindicated in patients developing hypoxic states and severe renal impairment (effective glomerular filtration, eGFR below 30 ml/min/1.73 m^2)!

A systematic review concluded no data exists to definitively link metformin to lactic acidosis [120]. Lactic acidosis never occurs with metformin exposure during routine medical care [121]. The incidence of MILA is about 9 per 100,000 person-years [122].

Unfortunately, we cannot confirm these optimistic data. The incidence of MILA in our catchment area of about 90,000 inhabitants is relatively high. Overall, 27 cases of MILA were identified over a period of 21 years (1998–2019), i.e., 21.3 cases a year per 100 000 patient-years. Mixed disturbances of acid-base balance (with other acidifying but, also, alkalizing disorders) were usually present. Blood pH was on average low (pH 7.1), with the lowest and highest values being 6.65 and 7.47, respectively. The patients usually presented with significant hypocapnia (average pCO_2, 2.73 kPa). Hypochloremia caused alkalizing disorder. Lactate levels were 11.9 mmol/l (max. 19.8 mmol/l). Levels of Base Excess, BE were on average lowered (by 20.2 mmol/l) as were bicarbonate levels (9.06 mmol/l). Levels of Anion Gap, AG were high: 37.97 mmol/l. Patients' partial pressure of oxygen was relatively high (pO_2, 13.2 kPa). Patients were usually diagnosed to have pre-renal acute failure (creatinine level 575 umol/l). Renal function gradually became fully normalized in most survivors. Levels of glycemia were on average high (14.6 mmol/l),

with hypoglycemia detected in only five cases. The highest plasma metformin concentration was 61.1 mg/l (i.e., 473 μmol/l).

Hyperventilation and hypocapnia seem to be the underlying mechanisms preventing the development of critical intracellular acidosis in MILA.

The basic sign of metformin intoxication was organ failure, especially of those organs that require a lot of energy. Disorientation or unconsciousness was indicative of impaired cognitive function. Patients who were able to speak reported severe vomiting and diarrhea.

The first significant disturbance and, probably, also the cause of MILA development in our patients was gastrointestinal tract involvement. While common infection – or the effect of metformin itself – caused vomiting and diarrhea, subsequent hypovolemia resulted in pre-renal failure, which in turn led to an increase in metformin levels up to toxic values. Development of kidney failure was often unexpected and fast. The most effective therapeutic option in our group of patients was elimination of metformin from the body (using hemodialysis or continuous veno-venous hemodialysis, CVVHD).

Metformin is generally a safe drug, significantly safer than phenformin, buformin (and probably relatively safer than insulin). However, we believe that MILA is a serious condition with an incidence significantly higher than reported in the relevant literature.

20. INSULIN

Insulin is one of the most important anabolic hormones.

Insulin stimulates the translocation of the GLUT4 from intracellular pools to the surface cell membrane [123]. Besides, the hormone stimulates glycogen synthase, the key regulating enzyme for glycogen synthesis, and many other enzymes.

Let us mention its main “clinical” effects:

Lipid metabolism:

1. Insulin stimulates triacylglycerol synthesis in adipose tissue, muscles and the liver
2. Insulin increases the rate of cholesterol synthesis in the liver
3. Insulin decreases the rate of lipolysis in adipose tissue
4. Insulin increases the uptake of triglycerides from the blood into adipose tissue and muscles
5. Insulin decreases plasma fatty acid levels

Protein metabolism:

6. Insulin increases the rate of protein synthesis in muscles, the liver and other tissues
7. Insulin decreases the rate of protein degradation in muscles
8. Insulin increases the rate of transport of amino acids into tissues

Minerals:

9. Insulin decreases renal sodium excretion
10. Insulin promotes the transfer of potassium to cells

Carbohydrate metabolism:

11. Insulin inhibits the rate of gluconeogenesis in the liver
12. Insulin increases the rate of glycogen synthesis in muscles, adipose tissue and the liver
13. Insulin decreases the rate of glycogen breakdown in muscles and the liver
14. Insulin enables glucose to enter into insulin-sensitive tissues (muscles, adipose tissue, and other cells containing the GLUT4 transporter)
15. Insulin decreases the blood glucose concentration

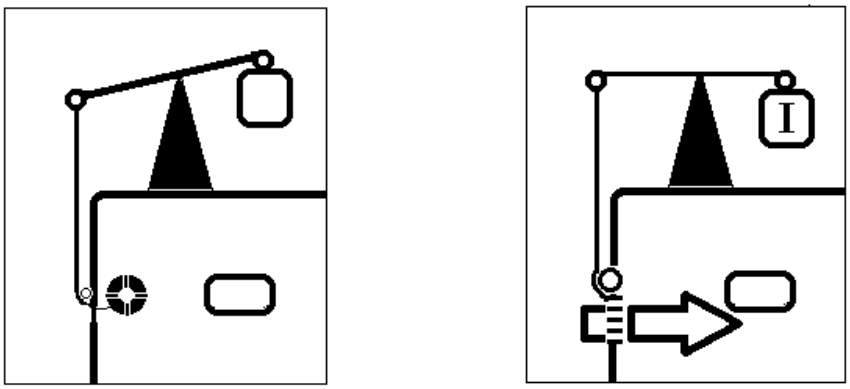

Figure 5. GLUT 4. Insulin "opens" the "cellular gate" via GLUT4 in the muscle. This reaction enables glucose transport from blood into the cell. [129, 135].

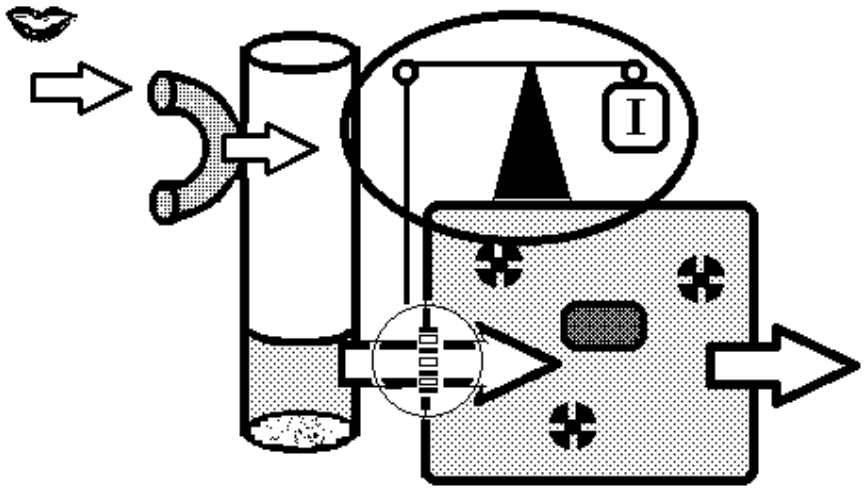

Figure 6. Insulin gate. Insulin "opens" the "cellular gate" via GLUT4 in the muscle. This reaction enables glucose transport from blood into the cell [129, 135].

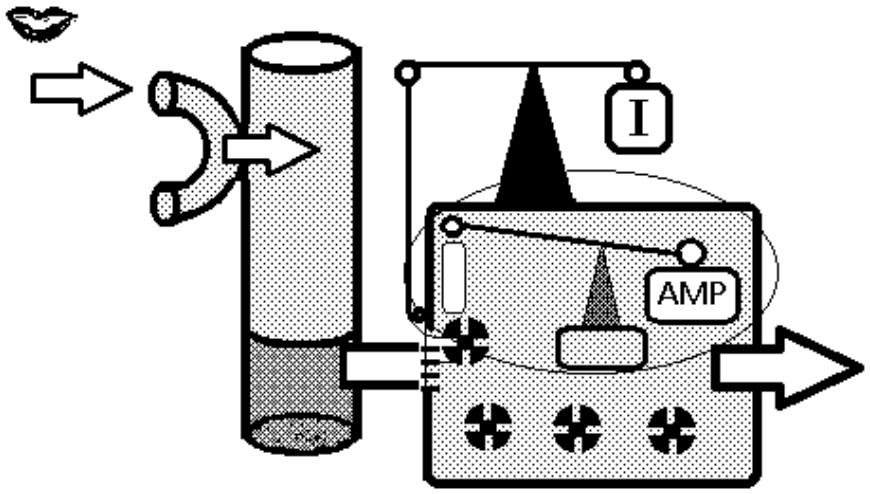

Figure 7. Intracellular gate. AMPK and other members of the AMPK family determines the amount of glucose entering the cell [129, 135].

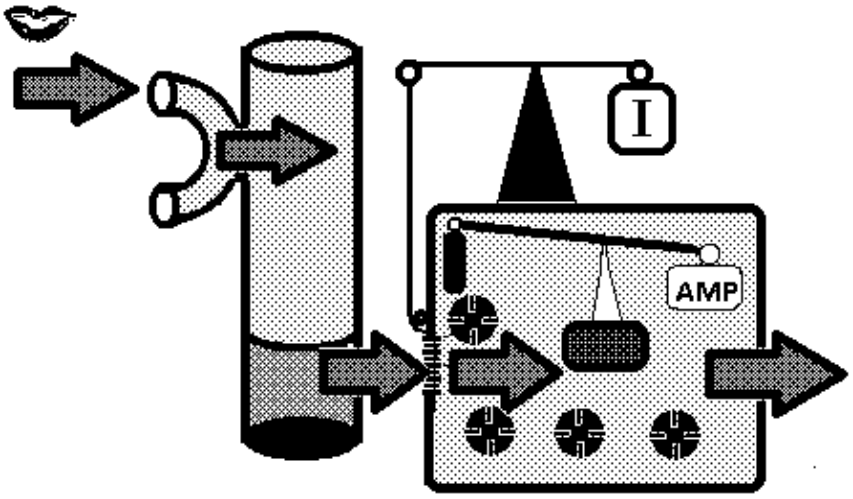

Figure 8. Physiological situation. Food intake and energy expenditure are in the optimal correlation [129, 135].

A sudden increase in insulin concentration – and, hence, a rapid surge in its effect – causes a rapid fall in blood glucose levels (point 15).

A slow yet steady increase in insulin concentrations and, hence, chronically enhanced effect of insulin primarily promotes the anabolic processes (items 1 to 14). The blood glucose levels depend on the rate of delivery of energy-rich substances to the body, which is usually normal or elevated. The patients du not suffer from *lack of insulin* but they suffer from *surplus of insulin* (and from transformation of its effect). While the term "*hyperinsulinism*" is rarely used today, it most befittingly reflects the real-life situation. Hyperinsulinism and *insulin uncoupling*:

21. INSULIN RESISTANCE AND INSULIN-UNCOUPLING (PSEUDO-RESISTANCE)

Insulin resistance (IR) is a condition whereby cells fail to respond to the actions of insulin with the result being that insulin-resistant cells cannot take in glucose.

Primary *congenital* insulin resistance is a rare disorder caused by the lack of a fully functional insulin receptor, a condition also referred to as Donohue syndrome [124, 125] or Rabson–Mendenhall syndrome [126]. None of the clinical effects of insulin (items 1–15) is expressed.

By contrast, *acquired* insulin resistance is a more frequent phenomenon occurrence. Severe infection, sepsis, burns and other stressful conditions impair glucose metabolism and involve insulin resistance. In these cases, the muscle is rapidly degraded. The released amino acids are converted to glucose. Catabolic processes are harmful for the whole body of the patient.

Quite a different situation occurs in the case of "insulin pseudo-resistance", "resistance" present in obese diabetics.

This phenomenon arose from misinterpretation of experiments with the induction of "comparable conditions". In clamp studies, we ensure completely identical conditions for diabetics and healthy control persons. Insulin and glucose levels are identical [127, 128]. Under these conditions, paradoxically, there is less glucose input into the obese diabetic tissues than in controls. What is the explanation for this phenomenon?

In fact, we have mixed up the manifestations of the *disorder* and the *feedback* mechanism that protects the diabetic cells from overfilling with energy-rich substrates.

As the disease advances, the excessive glucose uptake by adipose tissue cells and muscle is ensured by the high concentration gradient, hyperglycemia and hyperinsulinemia. Induction of "comparable conditions" in clamp studies leads to paradoxical results. Due to relative hypoglycemia and hypoinsulinemia (as compared to normal conditions) – during the clamp experiment – the tissues of diabetic patients take up a smaller amount of glucose than those of non-diabetic subjects. This phenomenon is called the "paradox of insulin resistance" [129]. Reduced glucose input to cells takes only a short time while we perform the experiment and is a manifestation of intracellular feedback.

On the contrary, glucose uptake by the muscle and adipose tissue are *higher* in obese diabetics during their previous as well as subsequent life.

Insulin pseudo-resistance can be induced by mere maintenance of hyperinsulinemia [102]. It can be minimized by reducing nutrient intake and by increasing physical exertion [130-132]. The clinical effects of insulin (items 1–14) are fully expressed. Only the last item – item 15 – is not accomplished, with hyperglycemia persisting in patients. This is, however, not due to an impaired insulin effect, but to an excessive energy intake.

In the text below, we use the term "insulin uncoupling", a term expressing disconnection (split) of insulin functions.

22. UNUSUAL COMPARISON

The following lines present a seemingly absolutely unscientific, but well-remembered comparison.

Let's compare the human body with a well-functioning car.

This car would be able to drive at 200 kilometers per hour for 14 hours a day. The car would travel 2800 kilometers a day and its fuel consumption would be 1 liter of gasoline per hundred kilometers, thus 28 liters per day.

Imagine that we would like to use 50 liters of gasoline (much more than needed) every day and drive just 500 kilometers with this car. And every other day we would have to draw another 50 liters into the car.

Nobody can imagine that we would want a worse car that needs more fuel. Such a wish is absolutely pointless. Yet the contemporary man wants their body to consume a large amount of food without moving. The question is: *How do we have to damage the car to meet the above owner's requirements?*

One option is to make a small hole in the tank to permanently lose fuel. This corresponds to the effect of gliflozin. This drug allows urine glucose losses.

Another option is to pour gasoline inside the car. Unfortunately, there is no room left for passengers, but more gasoline can be poured. This is how (high doses of) insulin and sulfonylureas work. High doses of insulin (high insulin concentrations) makes it possible to store energy-rich substances in cells. The most preferred form of energy storage is fat. Excess of insulin causes creates fat deposits in the organs. The side effects include morbid obesity, liver steatosis or myocardial steatosis.

Another possibility is to convince the gas station attendant not to pump 50 liters but less. Anorectics work on this principle. Above all, incretins reduce our desire for food. Also metformin reduces our craving for food.

The last option is to pull the handbrake. The car is able to drive but consumes more gasoline. This will allow you to draw more fuel as we wish. Metformin ensures exerts this effect in a safe and predictable way.

Preparations that acted too strongly (phenformin and buformin) caused complete loss of metabolic processes and are currently banned. Glitazones also reduce the effectiveness of metabolism too intensely so their use is limited today.

In fact, the drugs we are now more or less successfully treating T2DM are actually causing the loss of energy-rich substances.

23. DIAGNOSTIC ENTITIES WITHIN THE CHRONIC HYPERGLYCEMIC SYNDROME

Regarding cell energy intake, there are two main groups of diagnostic entities:

23.1. Diabetic Syndrome with Intracellular Starvation

This type includes the following diagnoses:

- True type-1 diabetes mellitus (T1DM), i.e., diabetes mellitus that occurs in childhood. This disease is characterized by real lack of insulin. More precisely: Untreated T1DM is characterized by lack of insulin and intracellular starvation.
  Diabetes mellitus results from autoimmune destruction of the insulin-producing beta cells in the pancreas and subsequent lack of insulin.
- Latent autoimmune diabetes in adults (LADA) is the identical form of T1DM, a form that occurs in adulthood. It is characterized by autoimmune destruction of the insulin- producing cells and, also, by real lack of insulin.
- Diabetes mellitus with true insulin resistance. Insulin resistance (IR) is a condition whereby cells fail to respond to the actions of insulin. Insulin-resistant cells cannot take in glucose. Though extremely rare, this disorder does occur and is termed Donohue syndrome or leprechaunism [134, 135] caused by a mutation of the insulin receptor [143, 144]. The mutant allele is not as effective as it should be, the usual doses of insulin are insufficient and the insulin-dependent cells begin to starve.
- Others diseases with real lack of insulin. For example: diseases caused by the destruction or removal of pancreatic beta-cells (i.e., after surgical pancreatectomy due to injury or carcinoma), intoxications et al.

- A number of other diseases that are associated with genetic disorders at the level of individual enzymes within intracellular glucose processing, intra-mitochondrial ATP production, or many diseases associated with other hormone and regulatory disorders.

23.2. Diabetic Syndrome with Intracellular Overfeeding

- Diabetes mellitus type 2 (T2DM) with obesity. This metabolic disorder is not caused by a relative (or absolute) lack of insulin, nor is it caused by inborn insulin resistance. [135]. T2DM is caused by insulin uncoupling.

Type-2 diabetes mellitus is a disease caused by chronic excessive energy intake that has damaged the basic regulatory systems [135]. Other contributing factors include excessive intake of energy-rich substances [102], excess of food, and lack of physical exercise. Type-2 diabetes mellitus with obesity is characterized by excessive filling of cells by energy-rich substances. The patients create excessive intra-myocellular fat deposits comparable to those of endurance athletes [138]. In fact, T2DM develops due to a collision of basic (extracellular) hormonal systems (insulin and other hormones) and the intracellular regulatory system (AMPK and other "intracellular hormones") [135]. T2DM is caused by inadequate, unhealthy lifestyle - incompatible with the genetic predisposition of the overwhelming proportion of the general population [136, 139, 140], a population with a thrifty genotype [137]. Diabetes with obesity is the result of maladaptive habits, amenable to behavioral therapy.

23.3. Combinations

The disorders that afflict the aforementioned patient populations can combine. As the number of patients suffering from diabetes is extremely

high, it is well possible that the patient develops one or more of the congenital as well as acquired types of the hyperglycemic syndrome.

The following two disorders are common: T1DM causing T2DM and T2DM causing T1DM!

Many patients treated for T1DM (syndrome of T1DM with intracellular starvation due to lack of insulin) tend to overfeed and, consequently, become hyperglycemic. As a result, they require high, supra-normal, doses of insulin. These patients, of necessity, suffer from intracellular overfeeding – and obesity – and, hence, inevitably develop T2DM.

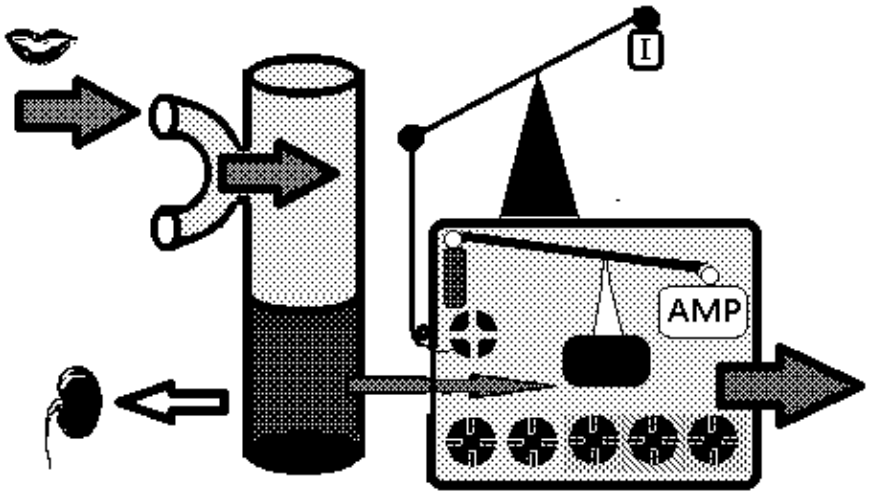

Figure 9. T1DM. T1DM results from the destruction of the insulin-producing beta cells and the subsequent lack of insulin. Insulin "gate" is closed. The most important symptoms are intracellular starvation, shortage of intracellular ATP [135].

On the other hand, many obese patients with T2DM receiving treatment with sulfonylureas gradually lose their ability of insulin production (or they suffer from LADA) to eventually develop real, absolute lack of insulin (typical of T1DM). They suffer from both type of diabetes mellitus T2DM and T1DM. In these cases, the combination of metformin and insulin will be beneficial.

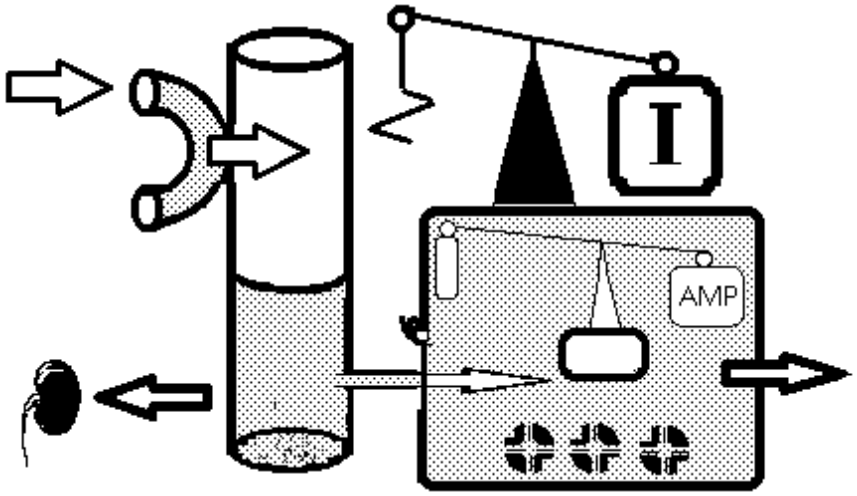

Figure 10. Donohue syndrome. Insulin resistance (IR) is a condition in which cells fail to respond to the actions of the insulin. Insulin resistant (GLUT4-dependent) cells cannot take in glucose. Subcutaneous adipose tissue and muscle are diminished [135].

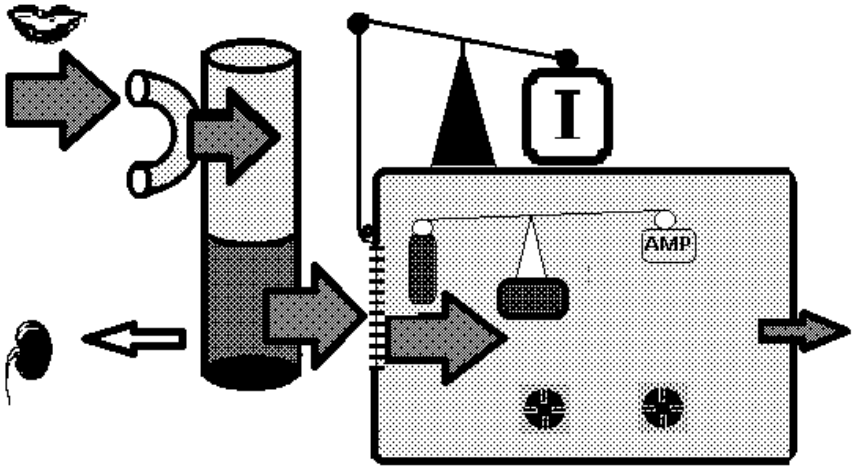

Figure 11. Diabetes mellitus type 2, T2DM**.** The Insulin production is high, (but it gradually decreases). The input of the glucose into cells is increased. Energy expenditure is low. The blood glucose level is high and rising. There are no signs of intracellular starvation. The insulin uncoupling ("pseudo-resistance") is present [135].

24. OTHER DISEASES

24.1. Cardiovascular Effect of Metformin

The prognosis of patients with T2DM used to be grim. Despite being treated with sulfonylureas or insulin, they would die significantly earlier from cardiovascular and other diseases. The UK Prospective Diabetes Study (UKPDS) and a number of other projects (which were subsequently performed) showed that the effect of metformin is significantly more beneficial than that of sulfonylureas. Why? Probably because metformin reduces hyperglycemia but has no other (insulin-like) anabolic effects. The UKPDS investigators reported that metformin treatment lowered the risk of myocardial infarction (by 39%) when compared with traditional treatments over a period of 10 years [141] as well as during subsequent 10-year follow-up [142].

24.2. Non-Alcoholic Fatty Liver Disease

With a prevalence of approximately 25% worldwide, non-alcoholic fatty liver disease (NAFLD) is thought to be currently the most common liver disease in the world [143]. Metformin was considered to be very usefull in treatement of the NAFLD - and the first information from a meta-analysis indicated that metformin improves liver function, ALT, AST (but not histological response) in NAFLD patients [144]. Regrettably, no studies have shown whether metformin improves long-term outcomes. It is to be hoped that experiments carried out in the future will offer more favorable and robust data.

24.3. Obesity

Metformin has been shown to have beneficial effects in the treatment of obesity [145-148].

Let us present the results of several studies.

24.3.1. Effectiveness of Metformin on Weight Loss in Non-Diabetic Individuals with Obesity

In one study, treatment with metformin for 6 months was successful, with mean weight loss in the metformin-treated group being 5.8 kg (5.6%) whereas untreated controls gained 0.8 kg (0.8%) on average. Patients with severe insulin resistance lost significantly more weight as compared to insulin-sensitive patients. The percentage of weight loss was independent of age, sex or BMI [145]. In another report, metformin reduced, compared with placebo, BMI by 1.42 kg/m^2 in obese children and adolescents [146]. Yet other authors showed that six months of metformin therapy improved weight loss and reduced abdominal adiposity in obese children with normal glucose tolerance. Weight loss was modest but more pronounced in the metformin group (-4.9 kg) than in the diet/exercise group (-1.7 kg) [147]. Similarly, metformin treatment significantly reduced body weight after 1-month hypocaloric dieting and 6-month combined treatment with a hypocaloric diet plus metformin. Another study in women with preexistent abdominal or visceral obesity and polycystic ovary syndrome reported a weight loss of 3 kg versus 7 kg in abdominally obese women not developing polycystic ovary syndrome. Visceral adipose tissue area values significantly decreased during metformin treatment [148]. These results are most encouraging.

24.4. Cancer

Today, metformin is generally viewed as a promising candidate drug for cancer treatment in combination with other cytotoxic agents. Many clinical trials designed to investigate metformin anticancer activity

including breast cancer, lung, esophagus, liver, pancreatic cancer or leukemia are currently underway (see overview) [149 et al.].

25. METFORMIN – FOR WHOM

Metformin was discovered in 1922 to be subsequently forgotten for many years although its blood glucose-lowering effect was known. Jean Sterne reported the use of metformin to treat diabetes in 1957. The long-term cardiovascular benefits of metformin were identified by the UK Prospective Diabetes Study (UKPDS) in 1998. Metformin has become the preferred first-line oral blood glucose-lowering agent to manage type 2 diabetes mellitus [150].

Strictly speaking – we use this drug for the treatment of "syndrome of intracellular overfeeding - with secondary syndrome of chronic hyperglycemia", today known as "syndrome T2DM", or "Type-2 diabetes mellitus with obesity".

At present, metformin is the drug of choice for the treatment of T2DM in obese individuals. While not recommended in cases of DM syndrome with intracellular starvation, i.e., real T1DM (a disease which is characterized by lack of insulin), the agent can be useful in patients with *either* type of diabetes mellitus, i.e., both T1DM and T2DM.

Metformin has also been shown to have beneficial effects in the treatment of obesity. When it becomes one of the basic drugs used in the management of this serious disease is a difficult question to answer. After thirty years (as in the case of diabetes treatment)?

Today, metformin belongs to the safest medications in its class. Nevertheless, if forced to use drugs that limit the influence of energy surplus and decrease the effect of metabolism, we can still expect an adverse effect characterized by aerobic metabolism lapse, MILA. Therefore, we do not use high doses of metformin alone but a combination of metformin with other drugs such as incretins, gliflozins or even insulin. While adequate food intake and physical training have the same beneficial effect, their advantage no one can deny is that they do not cause any harm.

25.1. Summary

The main effect of metformin is apparently *a decrease in metabolism efficiency*. It is a medicine that eliminates the problem of *damage caused by energy excess*. So what is the likely number of patients for whom metformin would be beneficial? One or two billion today?

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