Is non-insulin dependent glucose uptake a therapeutic alternative?
Part 1: physiology, mechanisms and role of non insulin-dependent glucose uptake in type 2 diabetes

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Summary
Several decades of research for treating type 2 diabetes have yielded new drugs but the actual experience with the available oral antidiabetic compounds clearly shows that therapeutic needs are not matched. This highlights the urgent need for exploring other pathways. All cell types have the capacity to take up glucose independently of insulin, whereby basal but also hyperglycaemia-promoted glucose supply is ensured. Although poorly explored, insulin-independent glucose uptake might nevertheless represent a therapeutic target, as an alternative to the clear limits of actual drug treatments. This review not only critically examines some major pathways not requiring insulin (although they may be influenced by the hormone) but importantly, this analysis extends to the clinical applicability of these potential therapeutic principles by also considering their predictable tolerability for long-term therapy. In particular vascular safety (the ultimate problem linked with diabetes) will be envisaged because of the ubiquitous distribution of glucose transporters and some linked mechanisms.

Several mechanisms can be identified which do not require insulin for their functioning. The first part of this review deals with the description, the regulation and the limits of some mechanisms representing potential pharmacological targets capable of having a highly significant impact on glucose uptake. These selected topics are: a) unmasking and/or activation of glucose transporters in cell plasma membranes, b) insulin mimetics acting at postreceptor level, c) activation of AMPK, d) increasing nitric oxide and e) increasing glucose-6P and glycogen stores.

Key-words: Type 2 diabetes · Glucose transporter · Non-insulin dependent glucose transport · AMPK · Glucokinase · Nitric oxide.

Résumé
Le captage du glucose non insulinodépendant représente-il une nouvelle voie thérapeutique ? 1ère partie. Physiologie, mécanisme et rôle du captage du glucose non insulinodépendant dans le diabète de type 2
Malgré plusieurs décennies de recherche intensive et l’apparition de molécules nouvelles, l’expérience clinique montre clairement leur insuffisance à combattre l’évolution du diabète de type 2 sur le long terme. Le besoin de molécules plus puissantes est aujourd’hui une évidence, ce qui implique d’envisager des voies nouvelles de recherche pharmacologique. L’une d’elles, très peu abordée jusqu’ici, pourrait être l’augmentation du transport du glucose par l’activation de voies non dépendantes de l’insuline. Cet article examine de façon critique plusieurs mécanismes susceptibles de répondre à cette attente mais l’analyse intègre également des aspects essentiels comme la tolérance (souvent prévisible) à long terme de ces éventuels traitements. En particulier, il envisage les effets secondaires possibles sur le système vasculaire, l’angiopathie étant in fine la cible du traitement du patient diabétique.

La première partie de cette revue décrit la physiologie de plusieurs mécanismes pouvant, par leur place dans le métabolisme et sa régulation, représenter une réponse quantitative à la question posée. Ceux-ci sont : a) le démasquage et/ou l’activation des transporteurs du glucose dans la membrane cellulaire ; b) les insulinomimétiques qui agissent au niveau postrécepteur, c) la stimulation de l’AMPK, d) l’augmentation de l’oxide nitrique (NO) et e) l’augmentation des taux de glucose-6P et du glycogène.

Mots-clés : Diabète de type 2 · Transporteurs du glucose · Transport du glucose non dépendant de l’insuline · AMPK · Glucokinase · Glycogène · Oxide nitrique.

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Introduction

The ultimate goal of treating diabetes is to slow down and reduce the severity of large and small vessel complications, which have such disastrous consequences in social and economic terms. Recent clinical data have considerably changed our views by revealing that hyperglycaemia, while being tightly linked to microangiopathy, is poorly, if at all, linked to large vessel disease [1, 2]. Indeed several major human trials (DCCT, UKPDS) as well as others showed that the benefit afforded by glycaemic control was mainly due to the improvement in small vessel lesions [3, 4]. While this does not negate the role of hyperglycaemia as an aggravating factor in cardiac and cerebral accidents [5], it “just” means that the reduction in glucose levels actually achieved by conventional therapies does not positively translate into the progression of atherosclerotic lesions. Therefore lowering hyperglycaemia remains a priority target, although other metabolic abnormalities such as lipid disorders now also stand in front. Because the mean glycaemic reduction achieved by oral antidiabetic agents today is only around 20%, more potent therapies must become available to try to reach glycemic levels as close as possible to normoglycemia. [6, 7]. All possible approaches for finding more potent drugs must therefore be considered. The spectacular burden of information about insulin cellular molecular action over the last decade has somewhat obscured research on non-insulin dependent pathways.

However one should recall that about 75% of whole body glucose uptake (GU) is operated through pathways which are non-insulin dependent. This raises the question as to whether this quantitatively important part contains potential targets for new therapies fulfilling the requirements imposed by the “efficacy challenge” [8]. Moreover new drugs will also have to be considered for their tolerance because of increasing duration of treatment resulting from earlier appearance of this disease. The present review aims at evaluating the validity and potential of some selected mechanisms (Fig 1) and confronts them with the predictable difficulties in terms of side effect acceptability.

Abbreviations:

- AICAR: 5-aminoimidazole-4-carboxamide riboside
- AMPK: AMP (stimulated) kinase
- GE: glucose effectiveness
- GSK3: glycogen synthase kinase 3
- GT: glucose transport
- GTr: glucose transporter
- GU: glucose uptake
- IA: intrinsic activity
- IPG: inositolphosphoglycan
- PIG: phosphoinositolglycan
- IPO: inositolphosphate-oligosaccharide
- NIMGU: non-insulin dependent glucose uptake
- NO: nitric oxide
- INOS: inducible nitric oxide synthase

Figure 1
Some main pathways which are able to promote glucose uptake without requiring insulin (GTr: Glucose Transporter; GK: Glucokinase; HeK: Hexokinase; NO: nitric oxide).

Important remarks

Defining “non-insulin dependence”

Tissues exhibit a variable dependency on insulin and many mechanisms in glucose transport and metabolism have been shown to be able to operate independently of insulin. While necessitating no insulin, these processes are nevertheless influenced by the physiological presence of the hormone. For example insulin can exert priming effects on cell membranes, thereby affecting the glucose transport system even in insulin-insensitive cells such as erythrocytes [9, 10]. This priming effect can be seen in the case of metformin action, traces of insulin being sufficient–but requested–to observe its glycaemia lowering action [11, 12].

Basal insulin is claimed to represent about 12% of basal glucose uptake [13]. Other studies showed that insulin can also modify the metabolic fate of glucose by favouring its oxidation, even without modifying the amount of glucose transported [14] Importantly, therefore, the present review refers to mechanisms which can be activated–or inhibited–by processes not using insulin, its receptor or the immediate post-receptor signalling cascade.

Limitations

Most of our present knowledge about insulin-independent glucose uptake stems from in vitro studies, i.e. condi-
tions which are largely non physiological. The necessary caution for interpreting such data applies also to some artificial in vivo setups aimed at unravelling precise organ/tissue reactions, such as zero insulin or hyperglycaemia with maintenance of basal hormonal environment. Finally, differences between clamp settings [eu-vs hyperglycaemia], between clamps and oral glucose tolerance tests as well as between oral glucose tolerance test and mixed meals have major impacts on the pertinence of the data and their interpretation [15, 16].

Main mechanisms underlying glucose transport and not necessitating insulin

NIMGU (non-insulin mediated glucose uptake) and glucose effectiveness

The literature uses various words or expressions to describe specific aspects of this topic, which differ by varying degrees in their meaning. The frequent abbreviation NIMGU (non-insulin mediated glucose uptake) is the uptake of glucose at zero insulin, either basal or stimulated by glucose itself. NIMGU has been claimed to represent two thirds of basal glucose uptake in dogs [17]. Surprisingly this parameter has been poorly investigated, likely because of the inherent technical problems and protocol. Possibly for the same reasons, the available data appear divergent for situations linked to hyperglycaemia [18-20]. For example, in vitro measurements of glucose uptake in red blood cells from T2DM patients has generated conflicting results [10, 21-22]. Glucose effectiveness [GE], on the other hand, represents the change in glucose uptake when glycaemia rises in the presence of basal insulin [23]. Its quantitation is also subjected to specific limitations [24, 25]. In fact glucose elevation compensates for deficient hormonal stimulation of glucose transport and this emerging concept has been elegantly termed “allostasis” [26]. Indeed glucose can stimulate its own uptake in all tissues except the brain [27]. GE also involves allosteric activation of some enzymes like glucokinase [23].

One way whereby glucose may promote its uptake is “intrinsic activity”, which can also be activated by various compounds (Fig 2). Alternatively glucose transporters (GTrs) can be unmasked in the cell plasma membrane.

Glucose effectiveness

In contrast to the slowly-activated insulin-induced glucose uptake, GU mediated by glucose shows a rapid onset. Moreover it is a key component of the postprandial glucose delivery: it has been proposed to be responsible for 70% of glucose disappearance after oral glucose [28]. When insulin rises concomitantly the part played by GE diminishes [17] but remains important because the insulin effect rapidly saturates [28]. GE also contributes to the reduction in hepatic glucose production [17, 29]. Thus GE is considered to be an important component at low insulin levels such as in the fasting state or in patients with poor pancreatic responsiveness [30]. Furthermore GE is influenced by various factors playing important roles in insulin resistance/diabetes, such as cortisol [31] or free fatty acids [32-34].

GE essentially mediates the increase in GU induced by hyperglycaemia [35], a biological effect already observed as early as 1937 [36]. The physiological importance of this phenomenon is well illustrated by in vitro and in vivo observations in pathological situations. In T1DM the increase in glycaemia normalizes GU [37]. The same is seen in T2DM, where it can be shown that when glucose levels are maintained at their ambient levels both in vitro [38] and in vivo GU (iso-or hyperglycaemic clamps) is normal [39-41]. In vitro it was found to be additive to the effects of insulin or exercise [42], suggesting the use of different pathways. In diabetes the importance of GE is further highlighted by data showing that activation of glycogen synthase is higher in diabetic than in normal rats [43, 44]. Therefore activation of glycogen storage by hyperglycaemia might be an additional compensatory process for maintaining glucose homeostasis. When insulin is low, however, the ability of hyperglycaemia to activate glucokinase and inhibit glucose-6-phosphatase activity is impaired [44].

Studies in disease situation have again shown some conflicting results in T2DM: normal [45] or reduced GE [46]
was reported. Several data point to organ differences in the functioning of GE; thus normal GE was found in liver but GE was abnormal in skeletal muscle at glucose levels as low as 130 mg/dl [31]. This finding was corroborated by other studies and led to the notion of “glucose resistance” [47]. The opposite was, however, reported by Nagasaka [48]. Another study found defective GE in both organs [49]. In vitro both reduction [10, 27] and increase [26] of GU were found in red cells from T2DM patients. Similar discrepancies were reported in prediabetes; increased GE in normoglycaemic insulin-resistant offsprings of diabetic patients [46], while in another study those patients who later developed diabetes exhibited reduced GE (Sg value of 1.6 vs 2.3); moreover, a low Sg value in clamps was found to increase 15 fold the risk of developing diabetes [50].

The discrepancy among these findings might be reconciled in a tentative hypothesis of a basal defect in GE, which might be transiently improved when glycaemia starts to increase in the early phases of diabetes development [glucose intolerance or mild fasting hyperglycaemia]. Indeed experiments show that acute hyperglycaemia can stimulate glucose transport (GT) [10, 42, 51] while prolonged hyperglycaemia leads to glucose resistance [47, 52-54]. Acute hyperglycaemia is indeed able to translocate GLUT-4 [51] whereas reduction in GLUT-4 translocation to plasma membrane or to enhance the intrinsic activity of GLUT-1 [10], whereas hyperglycaemia is indeed able to translocate GLUT-4 [51]. Acute hyperglycaemia leads to glucose resistance [47, 52-54]. The stimulation of GLUT by myricetin [72] or metformin [73] appears to be linked to membrane fluidization. Similarly in vitro reduction of membrane cholesterol, which improves fluidity, increases transporter activity while an increase leads to insulin resistance [74, 75]. Occlusion or suppression of the exofacial sugar binding sites is a reversible phenomenon able to modify the transporter activity [76]; again, this process is expected to closely depend on membrane physical properties. Glucose transporters are also linked to the cell cytoskeleton by specific binding proteins, which make them further dependent on cell structure [77].

The question then is how GE [insulin-independent] proceeds, what are its mechanistic defects in disease and, most importantly, if promising therapeutic target[s] can be envisaged on this basis.

Activation of glucose transporters

In the following we will consider various mechanisms whereby glucose transport can be increased independently of insulin. Most belong to a “push” principle (direct stimulation of the transport), others to a “pull” principle (stimulation secondary to downstream metabolic processes).

Several mechanistic hypotheses have been proposed to explain glucose-induced GU; GE has been found to be calcium-dependent in vitro [42] and another study reported high glucose to stimulate the translocation of PKC-b2 to the plasma membrane [59], an effect which could be blocked by dantrolene. Other isoforms of PKC have been described in this process, for instance PKCα, which stimulated the insulin receptor kinase independently of insulin in L6 cells [60] and PKCQ which may be activated by an increase in oxidative stress [61]. In chronic studies an impairment of protein kinase B activation by hyperglycaemia was found, with no defect in insulin receptor signalling, suggesting that the impact of glucose might be at the level of intracellular GTr activation [62]. The subsequent analysis will deal with one of the potentially best suited mechanisms.

Acting on glucose transporters appears as an evident possibility to increase GU independently of insulin. Indeed the activity of membrane-inserted transporters (particularly the ubiquitous GLUT-1) can be modified by changes in the protein itself or in its immediate environment.

Unmasking of glucose transporters in cell plasma membrane

Experiments in red cells, a cell type having exclusively GLUT-1 isofrom and no capacity for protein synthesis, were particularly useful to unravel several aspects of how glucose transport activity may be modified. It was found that GLUT-1 are partly masked in membranes. They have been also described as “cryptic carriers” [63, 64]. Binding of GLUT-1 to a small protein could explain the inactive state [65]. Stomatin (band 7.2b in RBCs) associates with GLUT-1 and substances like EDTA or cytochalasin E could unmask these transporters. [66]. Thus activation of glucose transporters would be a derepression, occurring at the level of their C-terminals [67, 68]. This principle has been suggested to explain the activating effect of protein synthesis inhibitors [69] and of insulin [70].

It can be expected that the physical state of the cell membrane, notably fluidity, influences GTr activity [71]. Fluidity depends on membrane lipid composition and determines the mobility of the protein in the membrane. The stimulation of GTr by myricetin [72] or metformin [73] appears to be linked to membrane fluidization. Similarly in vitro reduction of membrane cholesterol, which improves fluidity, increases transporter activity while an increase leads to insulin resistance [74, 75]. Occlusion or suppression of the exofacial sugar binding sites is a reversible phenomenon able to modify the transporter activity [76]; again, this process is expected to closely depend on membrane physical properties. Glucose transporters are also linked to the cell cytoskeleton by specific binding proteins, which make them further dependent on cell structure [77].

The metabolic environment of the GTrs can also modify their activity: in avian RBCs metabolic depletion increased glucose transport by derepression [68]. In mammalian erythrocytes the ATP-GLUT1 interaction was shown to depend on the tetrameric structure of the transporter and
Intrinsic catalytic activity of glucose transporters

Structural properties of the GTrs determine their intrinsic activity (IA). In this context, Tryp 412 was found to play a cardinal role in GLUT-1 [86]. Glycation of the transporters also affects their IA, although data are conflicting [63,87,88]. Recent investigations suggest that p38 might be one novel mechanism increasing IA and enhancing GT [89,90]. Indeed p38 was found to be involved in the stimulating action of insulin [89-91], of IGF-I [89], vanadate [92], muscle contraction [93], arsenite [94], anisomycin [95], as well as of the AMPK activator AICAR [96] and metformin [97]. There is still debate as to whether only p38 alpha isoform [92] or both alpha + beta isoforms are stimulated [93,96]. p38 was also suggested to mediate GT in ischemic preconditioning [98]. Inhibition of p38 decreased GT without affecting the translocation process. These data provide strong support for an involvement of p38 in the activation of GTr independently of insulin as well as of the hormone action itself; they potentially provide a mechanistic explanation for the classical observation of concomitant transporter translocation and activation under insulin. Interestingly, an inverse relationship between p38 expression in skeletal muscle and BMI was recently reported: in obesity p38 was reduced in skeletal muscle and increased in fat tissue [99]. When p38 was highly phosphorylated, glycemia was reduced, whereas insulin did not affect this process, suggesting a role for p38 in the regulation of basal glucose metabolism regulation [99]. Whether modulation of p38 is a candidate for therapeutic intervention is debatable in view of the many harmful biological effects of this protein. Altogether these data show that several processes linking the transport of glucose to the protein itself but also to its regulatory environment (both physical and metabolic) can modulate the uptake of glucose by cells. Most are independent of insulin and could be potential targets for new drug development.

It has long been known that translocation of GTrs from cell interior to the plasma membrane was not able to fully explain the quantitative effect of insulin on GU [100]. It was indeed recognized that, once inserted into the cell membrane, GTrs can be subjected to further activation, i.e. they can convey more glucose molecules per unit time across the cell membrane [101]. Therefore, not only unmasking of preexisting transporters but also the activation of newly inserted transporters exists. It is usually manifested in biochemical measurements as an increase in Vmax. Other terms used are “turnover number” and “accelerated exchange”. Surprisingly very little attention – and even doubt – has been devoted to this mechanism once the mechanisms of insulin signalling were discovered. Indeed insulin itself also enhances GTr-IA in various cell types [89, 102-106]. However, according to some authors this effect was due to lowering of Km of GTrs in red cells [107] without modifications in Vmax. Recently AMPK activation was also shown to increase GT without changes in GTr translocation [108]. Substances like isoproterenol, glucagon, PGE1 and nicotinic acid also modulate IA [109]. Parathyroid hormone (PTH) suppresses IA, an effect which correlated inversely with insulin sensitivity [110]; interestingly PTH also inhibits the IA-activation by metformin [111]. The PTH effect may be due to phosphorylation of the transporter [7], which reduces IA at least in GLUT-4 [112]. Hormonal effects mediated via Gs-coupled receptors generally inhibit, while Gi-coupled receptors stimulate GT by increasing Vmax without changes in Km [64]. Some authors proposed an exciting hypothesis stating that normally over 90% of basal GLUT1-IA is suppressed in 3T3-L1 adipocytes, explaining their low basal GU and high sensitivity to insulin [113]. In this cell type, therefore, there exists clearly a possibility to reverse this inhibition and activate GT. When GLUT-1 is over expressed, insulin sensitivity is reduced despite normal GLUT-4 translocation [114]. Thus, IA might serve as an alternative mechanism to compensate for defects in insulin efficacy. A list of activators/inhibitors of IA is found in figure 2. Surprisingly little is known about the behaviour of IA in diabetes but a decline was reported in human RBCs [115] and in STZ rats [116], in pancreatectomised rats [117], in high fat-induced insulin resistance [118] and in obese Zucker rats [119]. It has been proposed that an increase in GLUT4-IA could explain the increase in basal GT in L6 myocytes [120].

Insulin mimetics

Pharmacological research has also considered how to bypass the insulin receptor (binding and/or early signalling steps) by stimulating proteins and their interactions at the postreceptor level or in the lower part of the signalling cascade. Some molecules can activate the insulin receptor kinase activity by modulating its phosphorylation, notably vanadium salts [121,122]. Such compounds act primarily through inhibition of the phosphatase PTB1B, thereby increasing the tyrosine phosphorylation of the receptor. However sustained stimulation of the insulin receptor
kinase bears an inherent risk of harmful side-effects (growth factor) and safety concerns also exist about the chronic use of vanadium salts. Inhibition of PTP1B is the main principle underlying their type of approach.

Insulin has been shown to generate molecules called IPGs (also known as PIGs or IPOs) which converge at the level of IRS-1 and would therefore represent true insulin mimetics (also known as PIGs or IPOs) which converge at the level of main principle underlying their type of approach.

NF Wiernsperger et al. recently described substances with this property but little neuregulin [133] or the fungal metabolite shikonin [134] are induced glucose transport [131]; D-xylose derivatives [132], and has been claimed to be involved in exercise-related cellular levels of AMP, could be this factor [135]. It was established that AMPK, a kinase stimulated by elevated cellular levels of AMP, could be this factor [135]. Although there is now good agreement that this mechanism is involved in these serious energetic situations, there is also increasing debate as to which extent AMPK activation can fully explain the increased glucose transport. Subsequently this novel principle has attracted much attention as a potential alternative to insulin-linked pathways for treating diabetes [136].

AMPK seems to derive glucose towards lactate formation since it reduces glycogen synthase activity [137] and, conversely, high levels of glycogen inhibit AMPK efficacy [138, 139]. This casts some doubts about its validity for treating hyperglycaemic states. Most of the current, limited knowledge on AMPK in this field is based on the experience with few drugs. Indeed AMPK mediates the effect of the ribose derivative AICAR [136] as well as of high doses of metformin in vitro [140] and possibly of anisomycin [95]. The recent observation that adiponectin can increase AMPK may provide an alternative explanation for its beneficial effects on glycaemia [141]. In vivo AICAR reduces hyperglycaemia [142]; it is partly metabolized to ZMP, a covalent activator of AMPK, but AICAR [or ZMP] have also properties not mediated by AMPK, making the interpretation of data possibly hazardous. AMPK might act by stimulating IA [143] and p38 rather than by translocating GLUTs [96]. In vitro high concentrations of metformin can stimulate AMPK but without concomitant changes in cellular AMP levels [144], pointing towards alternative underlying mechanisms which remain to be identified. In vivo tissue metformin concentrations are much lower, but animal and human data show that the drug nevertheless stimulates AMPK in skeletal muscle [145]. Due to the variety of mechanisms affected by metformin in vivo [146], it is likely, however, that this stimulation is indirect.

The transposition of AMPK stimulation into a therapeutic principle is a good illustration of what should be a constant concern in drug development, i.e. the balance between the pertinence of targets on one hand and their validity/feasibility in terms of chronic use. Indeed the present availability of information on AMPK in human diabetes is weak and it has been reported that there was no difference in AMPK expression/activity in obese vs. obese diabetic patients [147]. Actually controversy about possible side-effects exists with occasional reports about negative interferences with insulin-stimulated glucose transport [148] and pro apoptotic effects in certain cell types [149]. Moreover enhancement of lactate production is always a concern since it can promote gluconeogenesis and elevate glycaemia. This field clearly needs clarification and specific safety investigations before AMPK stimulation can be defined as a valuable therapeutic principle for treating long-term diabetes.

**Nitric oxide**

Another novel finding has been the possible action of nitric oxide (NO) which is well-known as an important endothelium-derived vasodilator. NO stimulates GT in exercise without affecting insulin-mediated GU [150-152] and has been proposed to be involved in the effect of insulin [153] as well as AMPK [154] on GT. The level of inducible NO synthase (iNOS) seems to be linked to insulin sensitivity: mice knocked-out for iNOS are protected from high fat-induced insulin resistance whereas iNOS is increased in obesity with insulin resistance [155]. In adipocytes, as well as in vascular smooth muscle cells and skeletal muscle, the NO-induced increase in GT involves cGMP, thus differing from insulin [156-158]. Interestingly NO was proposed as a mediator of AMPK [159]. iNOS may be specifically elevated in diabetes [induced by chronic cytokines?] since it appears to be almost absent in normal healthy skeletal muscle [160].
Glycogen storage

Surprisingly few data exist about absolute glycogen levels in diabetic skeletal muscle and the liver. Biopsies of human T2DM patients showed conflicting results; almost normal fasting glycogen levels were seen, possibly because the elevated basal GU in these patients was sufficient to activate glycogen synthase [167-169]. Others, in contrast, reported slightly reduced concentrations in diabetic monkeys [170] and T2DM [171, 172]. Moreover methodological aspects of glycogen determination as well as overnight subject’s metabolic situation are likely to explain such differences. In any case, the latter do not appear to be large. Eventually the differences become more pronounced in postmeal periods, when there is a larger amount of glucose to be stored. Glycogen concentrations determine the tissue sensitivity to insulin in white (not in red) skeletal muscle [173] and high glycogen can also inhibit AMPK [139]. Since skeletal muscle plays the most important quantitative role in whole-body non-oxidative glucose metabolism, the main question will be: is the defect in glycogen storage sufficient to constitute a sink for excessive plasma glucose, so that its filling would drastically lower hyperglycaemia in patients? Is the glycogen storage defect able to match the amount of excess glucose and/or does increased glycogen formation create or maintain a gradient sufficient to attract glucose across the cell membrane? Unfortunately there is no answer to these key questions at this moment. In fed situations the hepatic glycogen stores are likely normal or even elevated [174].

Glucose entering cells is either metabolized through the aerobic and anaerobic glycolysis or channelled towards glycogen storage; stimulation of glycogen synthase, the activity of which is considered to be reduced in diabetics due to insulin resistance, therefore appears as an interesting target. The enzyme can be activated by insulin, glucose-6P and substances which dephosphorylate the enzyme. Glycogen synthase is phosphorylated notably by GSK3; inhibition of GSK3 in vivo has shown some promising effects in ZDF rats [175, 176]. Although some authors have claimed GSK3 inhibition to be superior to the effects of insulin or IPGs and additive to insulin [177], others using lithium [the best GSK3 inhibitor actually known] reported increases in GU in vivo [178, 179] which were, however, limited to 20% of the maximal hormone effect [180]. Lithium has been proposed to reduce the β-isofrom of GSK3 but, although stimulating GU and glycogen synthesis, insulin sensitivity was not improved [181]. An alternative possibility could be so-called glycogen-targeting proteins [182].

Activation of skeletal muscle G6P could be achieved, independently but in the presence of insulin, by increasing the allosteric effector glucose-6P. However experiments aimed at overexpressing hexokinase II showed the rapid limit of this approach because of substrate feedback inhibition of hexokinase [183]. When experimentally a switch from hexokinase to glucokinase was performed (glucokinase not being substrate-inhibited) glycogen was indeed formed but rapidly saturated [183, 184]. In contrast, after glycogen synthase overexpression, glycogen was increased 10fold [185], confirming that the capacity of physiologically intact myocytes with basal enzyme equipment to store glycogen is limited and saturates [186, 187].

Finally and assuming that glycogen formation would be a therapeutic answer, one must consider at least two serious limitations. First a net increase in liver or muscle glycogen might induce glycogenosis, a harmful side effect. Glycogen could also be increased by interfering with glycogen phosphorylase, however this effect might require organ specificity and could only be done to a limited extent in order to avoid complete inhibition of glycogenolysis.

As a whole, increasing glycogen formation above the simple pharmacological correction of diabetic defects may appear both difficult to achieve and hazardous in its outcome.

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