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# Potential role and therapeutic interests of *myo*-inositol in metabolic diseases

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### ABSTRACT

Several inositol isomers and in particular *myo*-inositol (MI) and D-*chiro*-inositol (DCI), were shown to possess insulin-mimetic properties and to be efficient in lowering post-prandial blood glucose. In addition, abnormalities in inositol metabolism are associated with insulin resistance and with long term microvascular complications of diabetes, supporting a role of inositol or its derivatives in glucose metabolism. The aim of this review is to focus on the potential benefits of a dietary supplement of *myo*-inositol, by far the most common inositol isomer in foodstuffs, in human disorders associated with insulin resistance (polycystic ovary syndrome, gestational diabetes mellitus or metabolic syndrome) or in prevention or treatment of some diabetic complications (neuropathy, nephropathy, cataract). The relevance of such a nutritional strategy will be discussed for each context on the basis of the clinical and/or animal studies. The dietary sources of *myo*-inositol and its metabolism from its dietary uptake to its renal excretion will be also covered in this review. Finally, the actual insights into inositol insulin-sensitizing effects will be addressed and in particular the possible role of inositol glycans as insulin second messengers.

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### 1. Introduction

*myo*-Inositol is a cyclitol naturally present in animal and plant cells, either in its free form or as a bound-component of phospholipids or inositol phosphate derivatives. It plays an important role in various cellular processes, as the structural basis for secondary messengers in eukaryotic cells, and in particular as inositol triphosphates (IP<sub>3</sub>), phosphatidylinositol phosphate lipids (PIP<sub>2</sub>/ PIP<sub>3</sub>) and possibly inositol glycans. For this reason, *myo*-inositol is

essential or important for the smooth running of a wide range of cell functions, including cell growth and survival [1], development and function of peripheral nerves [2], osteogenesis [3] and reproduction [4–9] (See Fig. 1). *myo*-Inositol and *D*-*chiro*-inositol, another inositol isomer, could be also implicated in glucose homeostasis since abnormalities in their metabolism were associated to insulin-resistance and long-term diabetes microvascular complications in diabetic subjects. Furthermore, given as dietary supplements, both *myo*- and *D*-*chiro*-inositol showed insulin-mimetic

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Review





*Abbreviations*: AC, adenylyl cyclase; ACC, acetyl-coenzyme A carboxylase; AGEs, advanced glycation end products; AMP, adenosine monophosphate; cAMP, cyclic AMP; AMPK, 5' AMP-activated protein kinase; AUC, area under the curve; BMI, Body Mass Index; CDP-DAG, cytidine diphosphate-diacylglycerol; DCI, D-chiro-inositol; ECM, extracellular matrix; FA, folic acid; FSH, follicle-stimulating hormone; GFR, glomerular filtration rate; GK, Goto Kakizaki (rat); GLUT-4, glucose transporter 4; GMD, gestational diabetes mellitus; G3PAT, glycerol-3-phosphate acyltransferase; GPI, glycosyl phosphatidylinositol; GS, glycogen synthase; GSK3, glycogen synthase kinase 3; HDL, high density lipoprotein; HK, hexokinase; HMIT, H<sup>+</sup>/myo-inositol transporter; HOMA-IR, homeostasis model assessment of insulin resistance; IMPase, inositol monophosphates (including in particular: Ins-P: inositol monophosphate, IP3, inositol triphosphates, IP6, inositol hexakisphosphates or phytic acid); IPG, inositol phosphogycan; IR, insulin receptor; IRS, insulin receptor; substrate(s); LD50, median lethal dose; LDL, low density lipoprotein; LH, luteinizing hormone; LysoPI, lysophosphatidylinositol; MES, necabolic syndrome; MI, *myo-*inositol-phosphate synthase; MIOX, *myo-*inositol oxygenase; MNCV, motor nerve conduction velocity; mTOR, mammalian target of rapa-mycin; OGTT, oral glucose tolerance test; PCOS, polycystic ovary syndrom; PDH, pyruvate dehydrogenase; PDHP, pyruvate dehydrogenase; PDK, phosphatidylinositol (3.4.5)-trisphosphate); PKA, cyclic AMP-dependent protein kinase; PKB, Protein Kinase B; PKC, protein kinase; C PLC, phospholipase D; PP2Cø, phosphotein phosphates 2C alpha; PP-InsPs, pyrophosphate forms of inositol phosphates; RCT, randomized controlled trial; SHR, spontaneously hypertensive rat; SHBG, Sex Hormon Binding Globulin; SMIT1/2, sodium-dependant *myo-*inositol transporter 1/2; STZ, streptozotocin.

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effects in several animal models of insulin resistance [10-12] and in women with polycystic ovary syndrome [13], a metabolic and endocrine disorder associated with insulin resistance.

The aim of this review is to compile and discuss the results of the randomized controlled trials that tested the potential benefit of a dietary *myo*-inositol supplement in contexts of insulin-resistance or long-term diabetic complications. As an introduction and to further discuss the therapeutic interest of a *myo*-inositol supplement in those contexts, the dietary sources of *myo*-inositol, its metabolism from its oral intake to its catabolism by the kidney, and the abnormalities in inositol metabolism associated with insulin-resistance will be addressed. Finally, the putative and actually unclearly defined mechanisms of action of inositol derivatives as insulin sensitizers will be discussed on the basis of animal and clinical studies.

### 2. Biological forms and dietary sources

Inositol or cyclohexane-1,2,3,4,5,6-hexol is a polyol existing under nine stereoisomeric forms depending on the spatial orientation of its six hydroxyl groups (Fig. 2). myo-Inositol, or cis-1,2,3,5trans-4,6-cyclohexanehexol, is the predominant isomeric form of inositol that we can find in nature and in our food. myo-Inositol was once considered to belong to the vitamin B family, however, because it is produced in sufficient amount by the human body from D-glucose, it is no more regarded as an essential nutrient. Human diet from animal and plant sources can contain myoinositol in its free form, as inositol-containing phospholipid (phosphoinositides) or as phytic acid (inositol hexaphosphate or IP<sub>6</sub>) [14]. Indeed, all living cells (animal, plant, bacteria, fungi) contain inositol phospholipids in their membranes, and phytic acid is the principal storage form of phosphorus in many plant tissues, especially bran and seed. Hence, the greatest amounts of myoinositol in common foods are found in fresh fruits and vegetables, and in all foods containing seeds (beans, grains and nuts). Especially high phytic acid contents are found in almonds, walnuts and Brazil nuts (9.4, 6.7 and 6.3% of dry weight, respectively) [15] and oats and bran contain more myo-inositol than cereals derived from



**Fig. 2.** Structures of the nine stereoisomers of inositol. Inositol exists under 9 stereoisomeric forms through epimerization of its hydroxyl groups. *myo*-Inositol (framed) is the most common isomer of inositol in foodstuffs and animal tissues.

other grains. Among the vegetables, the highest contents are observed in the beans and peas, leafy vegetables being the poorest vegetable sources. Among the fruits, cantaloupe and citrus fruits (with the exception of lemons) have extraordinarily high contents of *myo*-inositol: for example, a portion of grapefruit juice (120 g) contains about 470 mg of *myo*-inositol [16]. The amount of



Fig. 1. Functions and benefits of a myo-inositol diet supplement for human health.

*myo*-inositol present in the 2500 kcal American diet is approximately 900 mg, of which 56% is lipid-bound. However, the *myo*-inositol intake provided by common foods can range from 225 to 1500 mg/day per 1800 kcal depending on the composition of the diet [16].

#### 3. Dietary MI uptake and metabolism

### 3.1. Digestion and absorption

*myo*-Inositol from phytic acid can be released in the gut of monogastric animals by the enzymes phytases, which occurs in the intestinal mucosa. Phytases (*myo*-inositol hexaphosphate phosphohydrolase, EC 3.1.3.8 and EC 3.1.3.26) are found in plants, microorganisms and in animal tissues [15]. These enzymes are capable of releasing free inositol, orthophosphate, and intermediary products including the mono-, di-, tri-, tetra- and penta-phosphate forms of inositol. Much of the ingested inositol hexaphosphate is hydrolyzed to inositol. A considerable fraction of the ingested *myo*-inositol is consumed in the form of phosphatidylinositol (PI) that may be hydrolyzed by a pancreatic phospholipase A in the intestinal lumen. The resulting lyso-phosphatidylinositol (lysoPI) may be then reacylated via acyltransferase activity upon entering the intestinal cell or further hydrolyzed with the release of glycerylphosphorylinositol [14].

Virtually all of the free *myo*-inositol ingested (99.8%) is absorbed from the human gastrointestinal tract, through an active transport system involving a Na<sup>+</sup>/K<sup>+</sup>-ATPase [14]. In normal and healthy subjects, the circulating fasting plasma *myo*-inositol concentration has been found to be approximately 30  $\mu$ M and it turns over with a half-life of 22 min Refs. [14,16]. *myo*-Inositol is also present in small but significant amounts in phospholipids in association with the circulating serum lipoproteins, and as phytic acid at a level of about 0.1–0.4  $\mu$ M.

### 3.2. Organ and tissue incorporation

Lewin et al. [17] followed the distribution of radiolabeled myoinositol after intraperitoneal injection in male rats. Radiolabeled myo-inositol accumulated rapidly (within 1 h) and in large amounts in the thyroid, coagulating gland and seminal vesicles. Other tissues, such as the pituitary, prostate gland, liver and spleen, also concentrated myo-inositol quite actively. Of note, all the organs of the male reproductive tract (the vas deferens, epididymis, coagulating gland, seminal vesicle and prostate) except testis had radioactivity levels that were approximately 10-30 fold those of blood serum. The muscle tissues studied (diaphragm and heart) concentrated little inositol and adipose tissue (epididymal fat pad) was apparently unable of concentrating it from the blood, as well as the brain and testis which are, however, organs with high levels of endogenous inositol. Most of the radioactivity was found in the aqueous trichloroacetic acid extract, largely as free myo-inositol in most organs, with the exception of the liver where the lipid fraction contained most (approximatively 60%) of the radio-labeled myoinositol accumulated.

Lewin et al. also reported that in bilaterally nephrectomized rats, *myo*-inositol catabolism did not occur since the sole pathway of inositol catabolism in the rat takes place in the kidney. As expected, nephrectomized rats were essentially unable to convert inositol into CO<sub>2</sub>, whereas the sham-operated rats catabolized about 16% of the injected inositol to CO<sub>2</sub>. The nephrectomized rats accumulated more radioactivity in most of the organs tested, pre-sumably because significant amounts of the administered inositol are normally metabolized or excreted by the kidney. An interesting exception to the rule was the brain, which accumulated more

radioactivity in sham-operated than in nephrectomized animals. This may be due to the presence of metabolites of inositol, produced by the kidney, which are more prone to cross the blood/brain barrier than is inositol [14].

### 3.3. Cellular uptake

Cells normally derive inositol from three sources: (1) *de novo* biosynthesis from glucose-6-phosphate by 1-D-*myo*-inositol-phosphate synthase (MIPS) and inositol monophosphatase (IMPase), (2) dephosphorylation of inositol phosphates derived from breakdown of inositol-containing membrane phospholipids; or (3) uptake from the extracellular fluid via specialized *myo*-inositol transporters [18].

Inositol can be transported from extracellular fluid via three specialized myo-inositol transporters: sodium-dependent myoinositol transporters 1 and 2 (SMIT1/2), and H<sup>+</sup>-myo-inositol transporter HMIT, that co-transports myo-inositol with H<sup>+</sup> [19]. SMIT1 and SMIT2, co-transport two sodium ions along the concentration gradient, to generate enough energy to actively transport myo-inositol. SMIT1 and SMIT2 are both expressed in the brain and may be responsible for regulating brain myo-inositol level that is about 100-fold greater than those found in the periphery. Active myo-inositol transport through SMIT2 also mediates myo-inositol uptake in apical membrane of rat small intestine (although SMIT1 is present) and is responsible for myo-inositol reabsorption in rabbit kidney [20,21]. This active transport is inhibited by D-glucose and phlorizin and accounts for the inosituria occurring in diabetes mellitus. Of note, SMIT2 (but not SMIT1) also transports D-chiroinositol.

### 3.4. Metabolism

#### 3.4.1. MI de novo biosynthesis

*myo*-Inositol can be synthesized endogenously from D-glucose in rat testis, brain, kidney and liver [22,23] in three steps: first glucose is phosphorylated by hexokinase, second, glucose-6phosphate is converted to *myo*-inositol-1-phosphate by MIPS, and finally, *myo*-inositol-1-phosphate is dephosphorylated by IMPase to produce free MI (See Fig. 3). The second step is the rate limiting step of MI biosynthesis in most organisms [24]. In human, this endogenous biosynthesis of inositol is rather important in the kidney since it produces about 2 g/day so the endogenous daily production is about 4 g in the binephric human, which is significantly above the daily dietary intake (about 1 g/day). Extrarenal tissues can also contribute to the endogenous production of inositol in human and animals. Indeed, one half of the free inositol content of the rabbit brain comes from endogenous production *in situ*, the other half being transported from the blood.

### 3.4.2. MI conversion to isomers and derivatives or incorporation into phospholipids

*myo*-Inositol can lead to numerous derivatives through either epimerization, phosphorylation or methylation of one or several of its hydroxyl groups. Nonetheless, several of these derivatives cannot be obtained from *myo*-inositol in animal cells. For example, the inositol isomers *allo*-, *cis*-, and *epi*- are synthetically prepared compounds. Methylated inositol derivatives such as *D*-pinitol, sequoyitol or quebrachitol can be found in some plant species but these compounds are unlikely produced from *myo*-inositol in human body. In cells, *myo*-inositol exists under many phosphorylated forms from monophosphorylated forms (Ins-1-P, Ins-3-P or Ins-4-P) to the hexaphosphorylated form (IP<sub>6</sub> or phytic acid) and even to pyro-phosphate forms (PP-InsP<sub>4</sub>, PP-InsP<sub>5</sub>, [PP]<sub>2</sub>-InsP<sub>3</sub> or [PP]<sub>2</sub>-InsP<sub>4</sub>). Even so, the mono-, di- and tri-phosphorylated forms



Fig. 3. myo-Inositol de novo biosynthesis, incorporation into phospholipids and catabolism. Abbreviations: HK, hexokinase; MIPS, 1-D-myo-inositol-phosphate synthase; IMPase, inositol monophosphatase; CDP-DAG, cytidin diphosphate-diacylglycerol; CMP, cytidin MonoPhosphate; MIOX, myo-inositol oxygenase.

cannot come directly from the phosphorylation of *myo*-inositol by kinases, since such enzymes do not exist in human cells, but they can come from the dephosphorylation of more phosphorylated forms by specific phosphatases, and/or from phosphoinositide hydrolysis (i.e. inositol-1,4,5-triphosphate comes from the hydrolysis of phosphatidyl-inositol-(4,5)-biphosphate by Phospholipase C) (see Fig. 4).

The naturally occurring inositol isomers are *myo-*, *chiro-* (L-*chiro* and D-*chiro*), *scyllo-*, *muco-* and *neo-*. *In vivo* conversion of *myo*-inositol to D-*chiro-*inositol can occur in tissues expressing the specific epimerase. Pak et al. measured a conversion rate of radio-labeled [<sup>3</sup>H]-*myo*-inositol to [<sup>3</sup>H]-D-*chiro-*inositol of about 7.6% in rat blood and 8.8% in rat muscle and liver [25]. An epimerase interconverts *myo-* and *scyllo-*inositol with simultaneous production of *neo-*inositol in bovine brain [26]. However, in the study of Pak et al., labeling of inositol, was minimal, approximately 0.06% of radiolabeled *myo-*inositol [25].

Finally, only a small amount of myo-inositol or none is converted to other isomers or methylated derivatives in mammalian tissues and cells and it is primarily found as free myo-inositol or bound covalently to phospholipids, as the structural basis for a number of secondary messengers, including inositol triphosphates (IP<sub>3</sub>), phosphatidylinositol (PI) and polyphosphoinositides (i.e. PI(4)P,  $PI(4,5)P_2$  and  $PI(3,4,5)P_3$ , in much lower concentrations than phosphatidylinositol). Phosphatidylinositols (PI) are synthesized in vivo from myo-inositol and cytidine diphosphate-diacylglycerol (CDP-DAG) (Fig. 3). This synthesis is catalyzed by phosphatidylinositol synthase with a relatively high  $K_m$  (1.5–2.5 mM) [27,28] for myo-inositol making intracellular MI homeostasis potentially important to numerous cell functions. Phosphatidylinositol phosphate lipids (PIPs) are a product of class I, II and III phosphoinositide 3-kinases (PI 3-kinases) acting on phosphatidylinositol. As explained above, many inositol phosphates are produced through the hydrolysis of phosphatidylinositol phosphates by phospholipase C and may also be synthesized or remodeled by many kinases and phosphatases (See Fig. 4).

*myo*-Inositol and p-*chiro*-inositol can also be bound components of glycosyl-phosphatidylinositol (GPI) anchors and of inositol phosphoglycans (IPGs) that would constitute second messengers of insulin action in the GPI/IPG pathway. See Section 5.3 of this review for further details on this putative secondary signaling pathway of insulin.

### 3.4.3. MI catabolism

The kidney is the sole organ of importance in the catabolism of *myo*-inositol since  $[2^{-14}C]$ -inositol was not degraded to  $^{14}CO_2$  in nephrectomized rats in contrast with sham operated rats [17,29]. *myo*-Inositol is catabolized to D-glucuronic acid by *myo*-inositol oxygenase (MIOX) exclusively in the kidney. Through subsequent metabolic steps, D-glucuronic acid can lead to D-xylulose-5-phosphate which can enter the pentose phosphate cycle (See Fig. 3). In human subjects, urinary excretion accounts for a small fraction of the disposal of inositol by the kidney. Therefore, the kidney appears to be an important regulator of plasma inositol concentration in human subjects.

### 3.5. Tolerance

*myo*-Inositol supplementation is well tolerated and relatively safe since *myo*-inositol LD50 in mouse is 10 000 mg/kg body weight when orally administered [30]. In human, *myo*-inositol in a daily dose up to 18 g per os for 3 months or 2 g/day for 1 year is safe and well tolerated. Side effects, when present, are mild and mainly gastrointestinal in nature (nausea, flatus and diarrhea) [31,32].

### 4. Inositol metabolism abnormalities associated with insulin resistance

MI and DCI are involved in an array of cellular functions and abnormalities in their metabolism have been involved in the development of several diseases states (e.g. Bipolar, Panic and Obsessive Compulsive Disorders, Depression, Alzheimer's Disease) and in particular in the development of insulin resistance and



Fig. 4. Phosphoinositides metabolism in eukaryotic cells. Figure adapted from K. Abel, et al., J. Cell Sci. 114 (2001) 2207-2208.

diabetic complications. Indeed, in primary sites for the development of diabetic microvascular complications (kidney, sciatic nerve, retina and lens) a concomitant depletion of intracellular *myo*inositol and accumulation of intracellular sorbitol is commonly observed in diabetic animal models and human subjects [33,34]. In addition to this tissue-specific *myo*-inositol depletion, type 2 diabetic human subjects [35] and experimental models (rhesus monkeys [35], Goto Kakizaki (GK) rat [36]) excrete excessive amounts of MI and decreased amounts of DCI in urine (a phenomenon called inosituria). This urinary excretion pattern leads to a decrease in DCI to MI urinary ratio. The same inositol abnormal pattern is observed in insulin sensitive tissues (liver, muscle, fat and kidney) of human [37] and animal [36] diabetic subjects.

### 4.1. Intracellular MI depletion

### 4.1.1. Putative mechanisms of MI intracellular depletion

MI intracellular concentration is regulated through processes such as extracellular MI uptake, *de novo* biosynthesis, regeneration (phosphoinositide cycle), efflux and degradation (See Fig. 5). Alteration of one or several of these processes can lead to inositol intracellular abnormalities. In diabetes mellitus, inhibition of cellular MI uptake, altered MI biosynthesis, enhanced MI efflux due to sorbitol intracellular accumulation and increased MI degradation are putative mechanisms of MI intracellular depletion [34].

Indeed, a reduction of MI uptake was observed in cells (aorta, nerve cells and brush border vesicles) cultured in medium containing ambient glucose [38–40]. This glucose-induced MI uptake inhibition results from a competition between MI and glucose for MI transporters since MI and glucose exhibit structural similarities [41]. Therefore, under hyperglycemic conditions, high glucose ambience could impair extracellular MI uptake and so contribute to the MI intra-tissular depletion observed in diabetes. However, hyperglycemia *per se* is not sufficient to explain this intra-tissular MI depletion since the use of aldose reductase inhibitors (which selectively inhibit the conversion of glucose to sorbitol) corrected sorbitol intracellular accumulation and concomitantly inositol intracellular depletion, without affecting hyperglycemia [34].

In tissues possessing osmolyte efflux systems such as neuronal tissues, a rapid intracellular sorbitol accumulation can result in an osmotic stress that may favor the net efflux of osmolytes such as MI through the volume-sensitive organic osmolyte anion channels and thus reduce intracellular MI levels [34]. This sorbitol-induced osmotic stress was important in diabetic lens but may not mediate MI depletion in other tissues [42,43].

In the testes of diabetic animals, a significant reduction (50%) in the activity of MIPS, the enzyme regulating the first and critical step of MI biosynthesis, was observed [44]. However, no changes in MIPS activity were observed in the other organs (kidney, brain, and nerves) and even if the rate of MI biosynthesis is intrinsically greater in testes, the contribution of this MIPS activity reduction in MI intracellular depletion remains unclear [34].

Finally, an up-regulation of MIOX, the enzyme that breaks down MI, was observed at both mRNA and protein levels in the kidney of animal models of diabetes (STZ-diabetic rat [34], db/db mice [45]), insulin resistance (high fat diet-induced insulin resistant C57BL6 mice [34]) or hypertension (SHR rat). In all cases (normoglycemic hypertensive rat, insulin resistant mice or hyperglycemic STZ-rat), MIOX up-regulation was associated with an intra-renal MI deficiency [34]. These findings suggest that MI depletion in the kidney

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**Fig. 5.** *myo*-Inositol intracellular level regulation. *myo*-Inositol intracellular level depends on its extracellular uptake through specific transporters (HMIT/SMIT1/SMIT2), its *de novo* biosynthesis from glucose (HK, MIPS and IMPase), its regeneration or entry into the phosphoinositide cycle, its efflux and/or its catabolism by MIOX (in kidney). Figure adapted from H.H.G. Chang PhD Thesis, 2011. Abbreviations: HK, hexokinase, HMIT, H<sup>+</sup>/*myo*-inositol transporter, IMPase, inositol monophosphatase, MIOX, myo-inositol oxygenase MIPS, 1-*p*-*myo*-inositol-phosphate synthase, SMIT1/2, sodium-dependant *myo*-inositol transporter 1/2.

is not directly attributable to hyperglycaemia *per se* and may instead reflect in one aspect the up-regulation of the glucuronatexylulose pathway as indicated by the elevated MIOX expression and activity. In addition, the activation of MIOX and its subsequent glucuronate-xylulose pathway have been implicated in the development of diabetic nephropathy through the activation of fibronectin [46].

# 4.1.2. Consequences of MI depletion: possible role in diabetic microvascular complications

As explained above, inositol is involved in many cell functions, especially as a precursor of phophatidylinositol and phosphoinositides. Since the K<sub>m</sub> for the biosynthesis of PI from MI is relatively high (i.e. in the millimolar range), a depletion of intracellular myo-inositol could have a negative impact on the synthesis and availability of PI and PIPs in cells. Indeed, altered PI metabolism associated to MI deficiency has been observed in the sciatic nerve of streptozotocindiabetic rat model [47]. Since altered PI turnover is associated with impaired Na<sup>+</sup>/K<sup>+</sup>-ATPase activity, abnormal Na<sup>+</sup>/K<sup>+</sup>-ATPase activity may be a direct consequence of intracellular MI deficiency and a possible mechanism of diabetic microvascular complication development. Indeed, in neuronal cells, Na<sup>+</sup> and K<sup>+</sup> ions are essential for the maintenance of membrane potential for neurotransmitterinduced excitement and altered Na<sup>+</sup>/K<sup>+</sup>-ATPase activity has been associated to impaired nerve conductivity [48,49] and could be linked to the pathological changes observed in diabetic neuropathy (axonal degeneration and demyelination) [50] through a possible inhibition of cell growth, transformation and differentiation [51].

The hyperglycemia-induced MI depletion is also associated with the hemodynamic disturbances in the diabetic kidney, which are believed to be directly responsible for the development of glomerulosclerosis and its attendant proteinuria [34]. On the other hand, the depletion of MI may also affect the normal physiological function of renal tubular epithelial cells, resulting in increased accumulation of extracellular matrix (ECM), which may lead to renal tubulointerstitial fibrosis. Therefore, the depletion of MI plays an important role in the development and progression of diabetic nephropathy [46,52]. Despite a lack of well-defined aetiological mechanisms, the MI depletion observed under hyperglycemic conditions in insulin insensitive tissues seems to contribute to the development of diabetic microvascular complications, together with the four major and more recognized pathways, namely: increased Advanced Glycation End products (AGEs) formation, activation of protein kinase C (PKC), increased hexosamine and sorbitol pathways [34].

## 4.2. Inosituria and decreased DCI to MI ratios in insulin target tissues

### 4.2.1. Putative mechanisms of inosituria and altered inositol profiles

Larner and colleagues described a decreased urinary excretion of DCI and an increased urinary excretion of MI in human subjects and rhesus monkey with Type 2 Diabetes (10 times higher than in healthy subjects) [35]. A similar urinary excretion profile was observed in studies of the Goto Kakizaki rat [53]. Although this fact was known since 1859 (Neukomm et al., 1859 guoted by Ref. [54]), the cumbersome analytic procedures for inositol prevented a thorough study of the mechanism of this abnormality at this time. In 1954, the increased inositol clearance observed in diabetes mellitus was related to glycosuria rather than polyuria [55]. In monkeys the inositol excretion pattern became more marked with the progression of the disease from normal to obese non-diabetic to diabetic [56] and additional studies on humans and monkeys demonstrated that this altered inositol profile in urine was more directly related to the underlying insulin resistance rather than to the type 2 diabetes per se (with a correlation between the decrease in urinary DCI and the severity of insulin resistance measured by five distinct parameters [10]). Altered ratios of increased myoinositol to decreased chiro-inositol in urine have even been proposed as an index of insulin resistance in human subjects [57].

An altered DCI to MI ratio was also found in autopsy and biopsy muscles of type II diabetic subjects. In autopsy muscle, urine and hemodialysate samples, *chiro*-inositol was decreased about 50% compared to control subjects [37]. In the muscle biopsy specimens, no DCI was detected in the type II diabetic samples either before or after insulin administration. MI, in contrast, was present in the type II diabetic samples in increased amounts over controls and was further increased with insulin administration [35]. To explain this inositol imbalance associated with insulin resistance, a defect in MI to DCI epimerization activity was postulated. To test this hypothesis, the existence of such a MI to DCI conversion was demonstrated in vivo in rats [25] and in vitro in fibroblasts [58] in a process stimulated by insulin. It was then shown that this epimerase activity is dependent on time, pH, tissue (liver and kidney being more active enzymes sources) and co-factors availability, full activity being obtained with the co-factors NADH and NADPH [36]. In keeping with this hypothesis, a strikingly decreased conversion of [<sup>3</sup>H]-MI to [<sup>3</sup>H]-DCI was observed in muscle, liver and fat cytosolic extracts of Goto Kakizaki type 2 diabetic rats compared to Wistar control rats (conversions of 20-25% in controls were reduced to basal levels of 5% or less in GK rat) [36]. This 2–3 fold decreased epimerase activity in insulin target tissues of GK rat is consistent with the decrease in DCI content (and so in DCI to MI ratio) observed in the same tissues and also seen in human muscle autopsy of type 2 diabetics [37]. Finally, the decreased MI to DCI epimerase activity observed in GK rat insulin target tissue extracts may play a role in explaining the decreased urine and tissue DCI content (and decreased DCI to MI ratios) related to insulin resistance.

### 4.2.2. Consequence of inosituria and altered tissues DCI to MI ratios in diabetes

Excessive urinary MI excretion could reduce MI plasma level and consequently emphasize MI intracellular depletion, particularly in tissues heavily dependent on extracellular MI import. Decreased production of DCI from MI reduces the availability of intracellular DCI for its incorporation into IPGs, putative downstream second messengers of insulin. Indeed, type 2 diabetes mellitus patients display decreased IPG levels in muscle biopsies as compared to healthy controls [35]. Therefore, the decreased DCI content in insulin target tissues could reduce insulin signal transduction involving IPGs and so further enhance or contribute to the insulin resistance in those tissues. Depleted plasma levels of DCI observed in PCOS (a syndrome characterized by insulin resistance and hyperinsulinemia, see Section 5.1) patients further emphasize the correlation between impaired plasma DCI and insulin resistance.

To sum up this section, insulin resistance and diabetes are associated with 1) abnormally low levels of DCI in urine, plasma and insulin target tissues (liver, muscle, fat); 2) excessive MI urinary excretion and 3) intracellular MI deficiency in insulin insensitive tissues (kidney, sciatic nerve, lens and retina). DCI deficiency could emphasize insulin resistance in liver, muscle and fat while MI depletion in specific tissues could play a role in the development or aggravation of diabetic microvascular complications (neuropathy, nephropathy and retinopathy). Therefore, it seems reasonable to speculate on a possible beneficial effect of MI and/or DCI supplementation in diabetes to restore depleted MI and/or DCI intratissue levels.

### 5. MI supplementation benefits for some metabolic disorders associated with insulin resistance

### 5.1. Polycystic ovary syndrome (PCOS)

Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder affecting 5–10% of women in reproductive age and characterized by hyperandrogensim, polycystic ovaries and ovulatory dysfunction. According to the 2003 Rotterdam Consensus Criteria, PCOS can be diagnosed after exclusion of other conditions that causes hyperandrogenism and if at least two of the following three criteria are present: chronic oligo- or anovulation (manifesting as oligoamenorrhea or amenorrhea), elevated serum androgen levels or clinical manifestations of hyperandrogenism (hirsutism, acne or androgenic alopecia) and polycystic ovaries on ultrasonography [59]. The cause of PCOS is unknown but studies suggest a strong genetic component that is affected by gestational environment, lifestyle factors or both.

Insulin resistance with compensatory hyperinsulinemia and central obesity are frequent metabolic features associated with PCOS and are key factors in the pathogenesis of anovulation and hyperandrogenism. Indeed, hyperinsulinemia could produce hyperandrogenism in PCOS women via two distinct and independent mechanisms: 1) by stimulating androgen production by the ovary and 2) by directly reducing the liver secretion of testosterone transporter (SHBG for Sex Hormon Binding Globulin) thereby reducing its serum level [60]. The net result of these actions is to increase circulating levels of free (active form) testosterone. In addition to promoting hyperandrogenism, recent evidence indicates that hyperinsulinemia contributes to the anovulation of PCOS. Indeed, hyperinsulinemia could adversely affect folliculogenesis and impede ovulation by increasing intra ovarian androgen production, altering gonadotropin secretion, or directly affecting follicular development. Confirming the important role of hyperinsulinemic insulin resistance in the pathogenesis of PCOS, insulin reduction, whether achieved by inhibition of pancreatic insulin release (diazoxide or octreotide) or improvement in peripheral insulin sensitivity (metformin, troglitazone, DCI), is associated with a reduction in circulating androgens, an improvement in ovulatory function, and enhanced fertility in women with PCOS.

myo-Inositol, through its insulin-sensitizing effect, was also found to be effective in improving metabolic and hormonal parameters in PCOS women. Previous studies have demonstrated that MI supplementation can restore spontaneous ovarian activity (spontaneous ovulation, menstrual cyclicity restored), and consequently fertility in most women patients with PCOS [13,61-65]. A significant improvement of typical hormonal parameters was observed in PCOS women after MI treatment: decreased LH, FSH, and testosterone circulating levels, and increased SHBG, estrogens and progesterone circulating levels [62–64,66]. Insulin peripheral sensitivity and insulinemia were improved (reduced HOMA-IR index and/or reduction of the AUC of glucose and insulin during an oral glucose tolerance test). Markers of cardiovascular risk were also improved with a decrement in systolic and diastolic blood pressure, a decrease in plasma triglycerides, an increase in HDL cholesterol, and a decrease in LDL and total cholesterol concentrations [64,67]. In some studies [64,68], anthropometric measurements showed a significant decrease in the Body Mass Index (BMI) and a decrease in circulating leptin concentration in the MI group after at least 16 weeks of treatment. Unfer et al. [13] reviewed and analyzed the six Randomized Controlled Trials (RCTs) focused on MI supplementation to improve PCOS hormonal and metabolic disturbances and they provided level Ia evidence of myo-inositol effectiveness (with a dosage of 2-4 g/day for 12-16 weeks in those studies and no side effects reported in these conditions). myo-Inositol mechanism of action appears to be mainly based on improving insulin sensitivity of target tissues, resulting in the reduction of insulinemia which has a positive effect on the reproductive axis (ovulation restoration and oocyte quality improvement) and hormonal functions (reduction of clinical and biochemical hyperandrogenism and of dislipidemia). Of note, myo-inositol positive effect on the reproductive axis could also be related to the pivotal role of  $I(1,4,5)P_3$  in the regulation of  $Ca^{2+}$ release during oocytes development which conditions the acquisition of meiotic competence and drives oocytes to the final stages of maturation [4].

In conclusion, MI supplementation seems to be a simple, safe and effective first-line treatment for women with PCOS (See for review Unfer et al., 2012 [13]). 1818

### 5.2. Diabetes, gestational diabetes and metabolic syndrome

### 5.2.1. Lessons from clinical studies

Since inositol, under the isoform MI or DCI, has been reported to improve insulin sensitivity and ovulatory function in young women affected by polycystic ovary syndrome, the ability of a MI supplementation to prevent or reduce insulin resistance was investigated in post-menopausal women with metabolic syndrome and in pregnant women with gestational diabetes or at risk of developing one [69-73] (See Table 1). A supplement of MI (2 g/day) to a controlled diet for 8 weeks in gestational diabetes [73] and for 6–12 months in post-menopausal women with metabolic syndrome [71,72] further improved fasting serum insulin and blood glucose levels, and consequently the HOMA-IR index compared to the diet treatment alone (-75%) at 6 and 12 months compared to baseline in post-menopausal women with MI vs. -42% for the placebo group with the diet only; about -50% at 8 weeks with MI in women with gestational diabetes vs. -29% in the placebo group with diet only). In pregnant women with a family history of Type 2 Diabetes [70], a 4 g/day MI supplement throughout the pregnancy also significantly reduced the fasting and 1 h-glycemia at OGTT, and reduced the incidence of gestational diabetes by 40% (6% cases vs. 15.3% in the placebo group). A reduction of 65% of the risk for gestational diabetes (odd ratios 0.35) with MI was registered in this study. The improvement in glucose control obtained in the women supplemented with MI resulted in a significant reduction of some hyperglycemia-related pregnancy outcomes, in particular fetal macrosomia and high mean fetal weight. In postmenopausal women, the cardiovascular risk parameters were also further improved with the MI supplement with a reduction in blood pressure, in total and LDL-cholesterol, in serum triglycerides (-34%) and an enhancement of the HDL-cholesterol (+21%). Finally, after 6 months or one year of supplementation, the MI added to the diet of the post-menopausal women improved significantly almost all the metabolic parameters studied compared to the placebo group and even treated the metabolic syndrome of 20% of the women of the study group (8 on 40) while only one patient on 40 (2.5%) had no longer a metabolic syndrome in the placebo group with the diet alone.

Comparing MI with other insulin-sensitizing substances, MI is more effective than rosiglitazone in reducing serum triglycerides but less effective than pioglitazone (Pioglitazone (-50%) [74] > myo-inositol(-34%) > Rosiglitazone(-20%)[75]). In a study performed with metformin for 12 months [76], no change in triglycerides was noted, but only a slight improvement of HDL cholesterol (+2.4%) while in the study of Santamaria et al., one year of MI supplement enhanced HDL cholesterol concentrations by 21% which was also better compared to others obtained with pioglitazone [74] and rosiglitazone [75]. Hence MI supplementation seems to be a valuable and effective method to reduce cardiovascular risk in a context of metabolic syndrome or PCOS. The most important result of Santamaria and Giordanno et al. studies is the critical reduction in serum insulin and, consequently, insulin resistance (HOMA-IR index), which was about double compared to other insulin-sensitizing substances such as pioglitazone [74], rosiglitazone [75] and metformin [76] that are or were, at the moment, the gold standard of therapy for patients with impaired glucose tolerance.

Finally, according to those four randomized controlled trials (and to the 6 RCTs on PCOS), MI dietary supplementation (2–4 g/day) seems to be a safe and effective mean of fighting insulin resistance and associated cardiovascular risk in women in a context of gestational diabetes, post-menopausal metabolic syndrome or PCOS. However, those studies only include women and more precisely women with a special hormonal status (gestation, menopause or

PCOS) which enables us to conclude on the effect of MI supplementation in other contexts since the true mechanism of action is still unclear. Moreover, those four studies have been done only on Caucasian women and mostly (3 on 4) in open-label trials. Additional studies on larger populations, from different origins, sex and in double-blind trials should be done to really and powerfully prove the efficacy of *myo*-inositol supplementation as an insulinsensitizing treatment.

### 5.2.2. Lessons from animal studies

The effectiveness of certain inositol isomers or derivatives, especially DCI and D-pinitol, in lowering post-prandial blood glucose level had been reported in several cases of diabetes mellitus (STZ-diabetic rat [77], rhesus monkey [11], ob/ob mice [77] and human [78,79]). Studies on DCI showed that this effect was related to its insulin sensitizing activity. The herbal constituent sequovitol, the 5-O-methyl form of myo-inositol, also exerts anti-diabetic effects in mice when administered chronically. Indeed, both subcutaneous and oral administrations of sequoyitol (80 mg/kg per day) for 8-10 weeks improved hyperglycemia, glucose intolerance and enhanced insulin signaling in liver of ob/ob insulin resistant mice [80]. The blood glucose lowering effect of high doses of MI in postprandial condition was first established in the insulin resistant Rhesus monkey [12] and gave rise to a patent in 1998 [81] for the treatment of hyperglycemia in diabetes. Earlier studies on streptozotocin diabetic rats had however failed to show any improvement in hyperglycemia with dietary MI [82]. This could be explained by the fact that MI hypoglycemic effect passes through an improvement in insulin sensitivity [83] and so could not counteract hyperglycaemia in animal models of type 1 diabetes wherein insulin is missing. In addition, MI cellular uptake is competitively inhibited by glucose so its action is probably mitigated under hyperglycemic conditions. Later studies on healthy mice confirmed the ability of high doses of MI (given acutely [84] or chronically [83]) to reduce blood glucose level after a glucose load. This effect was associated with an improvement in peripheral insulin sensitivity established in vivo during an insulin tolerance test and further confirmed by the observation of an enhanced GLUT-4 translocation to the plasma membrane in response to hyperglycemia in the skeletal muscle [84].

# 5.3. Insight into the MI mode of action on insulin sensitivity – possible role of inositol glycans as second messengers of insulin

The exact mechanisms of action of MI and other inositol isomers (DCI) or derivatives (e.g. *D*-pinitol, sequoyitol) with insulin-mimetic activities are still unclear. A putative mechanism of action implies inositol phosphoglycans (IPGs) containing MI or DCI as insulin mediators.

The discovery of IPGs has emerged from the observation that the canonical model of insulin signaling invoking phosphorylation of insulin receptor substrates (IRS), phosphoinositide 3 kinase (PI3K) and protein kinase B/Akt (PKB/Akt) accounts for most, but not all, intracellular actions of insulin. Non-oxidative and oxidative glucose disposal by activation of glycogen synthase (GS) and mitochondrial pyruvate dehydrogenase (PDH) remain indeed incompletely explained by such model. Moreover, insulin stimulates both cellular glucose uptake and glycogen synthesis but these actions sometimes occur in a disconnected manner suggesting the possible existence of not only one but also two parallel signaling pathways connecting the insulin receptor to the activation of glucose uptake and its metabolic intracellular disposal. Consequent research on second messengers of insulin action led to the discovery of two IPGs as putative insulin mediators, extracted from rat liver and released in response to insulin. Their chemical nature was revealed later: the

Reference	Study design	Duration	Treatment	No of subjects	Inclusion criteria	Exclusion criteria	Assessment of the response	Results
D'Anna et al., 2013 [70]	Randomized, controlled vs. placebo (FA 200 µg twice/day), open- label	From the 1st trimester through the whole pregnancy	2 g MI + 200 μg FA twice/day	N = 220 Placebo: 110 MI: 110	1) First-degree relatives (mother, father or both) affected by T2D; 2) prepregnancy BMI < 30 kg/m <sup>2</sup> ; 3) fasting plasma glucose <126 mg/ dL and random glycemia <200 mg/ dL; 4) single pregnancy; 5) Caucasian race	<ol> <li>Prepregnancy BMI ≥ 30 kg/m<sup>2</sup>; 2) previous GDN; 3) pre-gestational diabetes; 4) first trimester glycosuria; 5) first- degree relatives not affected by T2D: 6) fasting and random glycemia ≥126 and 200 mg/dL respectively 7) twin pregnancies; 8) associated therapy with corticosteroids; 9) not Caucasian race; 10) PCOS women</li> </ol>	Main outcome: Incidence of gestational diabetes (diagnosed with IADPSG recommendations); Secondary outcomes: prevalence of fetal weight > 4000 g at delivery), caesarean section, gestational hypertension, preterm delivery, neonatal hypoglycemia (<45 mg/dL), shoulder distocia and distress respiratory syndrome	Incidence of gestational diabetes significantly reduced in the MI group compared with the placebo group: 6 vs. 15.3%, respectively ( $P = 0.04$ ) and reduction of gestational diabetes risk occurrence (odds ratio 0.35). Significantly reduced fasting ( $p < 0.001$ ) and 1 h- glycemia ( $p < 0.02$ ) at OGTT in the MI group. A statistically significant reduction of mean fetal weight at delivery in MI group and absence of fetal macrosmia (vs. 7 cases in placebo group). No difference between the groups for the other secondary
Matarrelli et al., 2013 [69]	Randomized, controlled vs. placebo, double- blind	For the entire pregnancy period	2 g MI + 200 μg FA twice/day taken with at least 6 h interval	N = 75 Placebo: 39 Ml: 36	Consecutive singleton pregnant women with an elevated fasting glucose (glycemia ≥5.1 mmol/L or 92 mg/dL and ≤7.0 mmol/L or 1.26 mg/dL) in the 1st or early 2nd trimester	Pregestational obesity (BMI above 35) and refusal to participate were the only exclusion criteria	OGTT at 24–28 weeks' gestation. BMI, need for maternal insulin therapy, macrosomia, polyhydramnios, neonatal birth weight and hypoglycaemia	onicome studied The incidence of gestational diabetes in mid pregnancy was significantly reduced (p = 0.001) in women who received MI compared to placebo (relative risk 0.127). Women supplemented with MI required less insulin therapy, delivered at a later gestational age, had significantly smaller bables with fewer episodes of neonatal hypoglycaemia.

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Table 1 (continued)

Reference	Study design	Duration	Treatment	No of subjects	Inclusion criteria	Exclusion criteria	Assessment of the	Results
							response	
Santamaria et al., 2012 [71]	Randomized, controlled vs. placebo	12 months	MI 2 g/day	N = 80 Placebo: 40 MI: 40	Postmenopausal women with MetS (at least 3 criteria of the ATP III of the National Cholesterol Education Programme); age between 50 and 60 years old and at least a 12-month period from the last menstruation	Use of glucose- lowering drugs and/or lipid- lowering drugs	Serum glucose, insulin, HOMA-IR, TG, total and HDL- CST, and BP at baseline and after 6 and 12 months of treatment	Serum glucose, insulin, HOMA-IR, TG, total and HDL- CST and BP significantly improved with MI compared to placebo. A significant difference from basal values was highlighted only in the MI group ( $p < 0.0001$ ) for both BMI and WC at 12 months. In the MI group, the number of women without MetS was eight (20%) vs. only one in the control group after 12 months of diet
Giordano et al., 2011 [72]	Randomized, controlled vs. placebo	6 months	MI 2 g/day	<i>N</i> = 80 Placebo: 40 MI: 40	Postmenopausal women with MetS (at least 3 criteria of the ATP III of the National Cholesterol Education Programme): Age between 50 and 60 years old and at least a 12-month period from the last menstruation	Use of glucose- lowering drugs and/or lipid- lowering drugs	Serum glucose, insulin, HOMA-IR, TG, total and HDL- CST and BP at baseline and after 6 and 12 months of treatment	In the group treated with MI, significant improvements in diastolic BP (-11%), HOMA index (-75%), serum TG (-20%) and in HDL cholesterol (+22%) were observed
Corrado et al., 2011 [73]	Randomized, controlled vs. folic acid (FA) 400 µg/ day, open label	8 weeks	2 g MI + 200 µg FA twice/ day—Inofolic <sup>®</sup> (MI + FA)	N = 69 Placebo: 45 Ml: 24	Gestational diabetes (diagnosed with an OGTT performed between 24 and 28 weeks of gestation)	Insulin therapy: premature delivery (before 35 weeks of gestation)	Fasting HOMA-IR and adiponectin blood level	Fasting glucose and insulin, and consequently HOMA-IR, decreased in both groups (50% in the MI group vs. 29% in the control group), but the decline in the MI group was significantly greater than that in the control group ( $P = 0.0001$ ). Adiponectin increased in the MI group while it decreased in the control group ( $P = 0.009$ ).

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**Fig. 6.** New model of insulin-signaling proposed by Larner and co-workers – Inositol glycans as putative second messengers (INS-2) of insulin (inspired from the review of Larner et al, 2010). In this model, insulin binding to its receptor (IR) leads to autoactivation of the receptor and the activated IR can transduce the signal through two parallel signaling pathways. The first and well-accepted pathway of insulin implies the recruitment and activation of substrates of insulin receptor (IRS) by the activated IR. Subsequent protein activations (PI3K, PDK-1) finally lead to PKB/Akt recruitment and activation at the plasma membrane. Activated PKB/Akt induces GLUT-4 translocation to the plasma membrane and so enhances glucose entry into the cell. In the second putative pathway, the IR is coupled to a G protein itself coupled to a phospholipase (possibly PLD or PLC) that catalyzes the hydrolysis of a GPI. The insulin-induced hydrolysis of the GPI releases an inositol phosphoglycan containing *p*-*chiro*-inositol which acts as a putative second messenger of insulin (INS-2) mediating insulin effects on glucose oxidative and non-oxidative disposal. INS-2 binds and allosterically activates two Mg<sup>2+</sup>-dependent protein phosphatases: PP2C $\alpha$  in the cytosol and PDHP in the mitochondria. Activated PP2C $\alpha$  stimulates glycogen synthase directly and also indirectly through possible activation of PI3K, Akt and subsequent inhibition of GSK3. In the mitochondria, activated PDH and so glucose oxidative use. Those two pathways act together to mediate insulin action in a complementary and synergistic manner. Abbreviations: IR, insulin receptor, GLUT-4 glucose transporter 4, GPI, glycosyl phosphatidylinositol, GSK3, glycogen synthase kinase 3, INS-2, insulin second messenger, PDH, pyruvate dehydrogenase, PDHP, pyruvate dehydrogenase phosphatase, PDK-1, phosphoinositide-dependent kinase 1, PI3K, phosphoinositid 3 kinase, PKB/Akt, protein kinase B/Akt, PLC, phospholipase C, PLD, phospholipase D, PP2C $\alpha$ , phosphoprotein phosphatase 2C

first glycan termed IPG-P (Inositol Phosphoglycan-Phosphatase stimulator) contained methylated DCI (i.e. D-pinitol) and galactosamine and activated PDH phosphatase; the other one termed IPG-A (Inositol Phosphoglycan-AMP kinase inhibitor) contained MI and glucosamine and inhibited cAMP-dependent protein kinase (PKA) and adenylate cyclase (AC) [85]. Both types of IPG have been shown to additionally contain neutral sugars and phosphate residues. IPG-A and IPG-P were both insulin mimetic when administered in vivo in normal or diabetic rats: they reduced hyperglycemia dosedependently by intravenous injection in low-dose STZ type 2 diabetic rat and stimulated glucose incorporation into glycogen in rat diaphragm muscles by intraperitoneal injection [86]. The origin of their insulin-stimulated production was first brought to light in 1986 by the discovery that inositol glycans release from hepatic plasma membranes in response to insulin was reproduced by addition of a phosphatidylinositol-specific phospholipase C [87]. Numerous reports then confirmed that insulin, other growth factors and classical hormones, stimulated the hydrolysis of glycosyl-phosphatidylinositol (GPI) generating water-soluble inositol phosphoglycan (IPG) second messenger. The origin of IPG-A is thought to be myo-inositol-containing GPI, as both PLC and PLD mediated hydrolysis of GPI yield biologically active IPG molecules (reviewed in Ref. [88]). Point-mutated and kinase-deficient insulin receptors fail to couple the generation of IPG-A through GPI hydrolysis, implying in some way that IPG-A release from GPI is controlled by insulin-stimulated tyrosine phosphorylation events. For DCI glycans (IPG type P like), Larner and colleagues propose that they are hydrolyzed from membrane phospholipids and/or GPI linked proteins such as alkaline phosphatase [89]. Indeed, bovine liver has four GPI lipid species with 1:1 M ratios of chiro-inositol and galactosamine like IPGs of P type.

Further evidence for a possible role of IPGs as insulin second messengers comes from studies on isolated rat adipocytes, in which IPGs released from GPI extracted from rat liver or purified from hemodialysate (Actovegin<sup>®</sup>) mimicked the anti-lipolytic and lipogenic effects of insulin [90,91]. The anti-lipolytic effect of such IPGs was associated with a reduction in the cAMP production stimulated by isoproterenol [90]. This effect is probably related to an inhibitory effect of these IPGs on adenylate cyclase and/or cAMP kinase, likely to IPG-A type. The stimulation of lipogenesis from glucose might be explained by PDH activation, similarly to IPG-P type; PDH and ACC are indeed two important control points for *de novo* lipogenesis from glucose. In addition, a purified *chiro*-inositol-containing IPG mediator (IPG-P type) from beef liver directly activated G3PAT in cell-free preparations of BC3H1 myocytes and Wistar rat adipocytes and is probably a mediator of this insulin action [92].

Further efforts allowed the identification of a novel putative mediator of insulin purified from beef livers and termed INS-2 [93]. Its exact chemical structure was determined and further confirmed by its chemical synthesis: it was a 4-O-(2-amino-2-deoxy-beta-D-galactopyranosyl)-3-O-methyl-D-*chiro*-inositol. This unique pinitol  $\beta$ -1,4-galactosamine structure is contrasted with the more common

*myo*-inositol  $\alpha$ -1,6-galactosamine structure determined in other *myo*-inositol glycans. The bioactivity of this galactosamine *chiro*-inositol pseudo-disaccharide Mn<sup>2+</sup> chelate was studied. This allowed the proposition of a new model of insulin signaling depicting the production of INS-2 in response to insulin and its role as second messenger of insulin action.

This new model proposed by Larner and colleagues (Fig. 6) (Reviewed in Ref. [89]) incorporates how insulin activates GS and PDH via a chiro-inositol glycan second messenger like INS-2 and how insulin activates GLUT-4 translocation to the plasma membrane. As depicted in Fig. 6, binding of insulin activates insulin receptor (IR) tyrosine kinase that autophosphorylates, recruits IRS proteins and phosphorylates them on Tyr residues to serve as scaffolds. A principal IR/IRS target is PI3K that generates PIP<sub>3</sub> to activate the phosphorylation of PKB/Akt by the PDK. After several steps. Akt activation leads to the translocation of GLUT-4 vesicles to the plasma membrane to increase glucose transport into the cells. In parallel of this IRS/PI3K/Akt pathway, IR activation would be also coupled to the heterotrimeric G protein Gq itself coupled to a GPIphospholipase (possibly GPI phospholipase D, PLD) (See the review of Larner et al., 1999 [94] for further details). Activation of the phospholipase would release an inositol glycan second messenger INS-2 from a GPI lipid precursor in the inner and/or outer leaflets of the plasma membrane. INS-2 could be released either directly into the cytosol or outside the cell and then transported back into a neighboring cell or into the original cell via an ATP-dependant inositol glycan transporter (anti-inositol glycan antibodies inhibition studies [95], cultured cell dilution experiments [96] and impermeant fluorescent tag experiments [97] all supporting the extracellular generation/release of IPGs hypothesis). Inside the cell, INS-2 would bind and allosterically activates two members of the  $Mg^{2+}$ -dependent protein phosphatase family: cytosolic PP2C $\alpha$  and mitochondrial PDHP. In the cytosol, activated PP2Ca stimulates glycogen synthase directly and indirectly via PI3K/PDK/Akt/GSK3 pathway. Indeed, it has been reported that PP2Ca dephosphorylates the Ser-608 residue of PI3Ka p85 regulatory subunit, resulting in activation of PI3Ka p100 catalytic subunit. The consecutive activation of PKB/Akt leads to the inactivation of glycogen synthase kinase 3 (by phosphorylation on its Ser-9 residue) resulting in activation of glycogen synthase. Other signaling events occur downstream of activated Akt and lead in particular to the activation of mTOR kinase and GLUT-4 translocation to the plasma membrane. Not depicted in Fig. 6 but possibly important, it has been observed that PP2Ca also inactivates AMPK by dephosphorylation of its Thr-172 in hepatocytes, heart and hypothalamus. In the mitochondria, allosteric activation of PDHP by INS-2 as a manganese chelate dephosphorylates Pyruvate dehydrogenase (PDH) thereby enhancing the oxidative glucose disposal.

The model depicted in Fig. 6 and the studies on IPGs provide a possible explanation for the observed effect of insulin on GS and mitochondrial PDH activation that were not fully explained by the conventional model. It also proposes a conceptual framework for the origin, production and actions of *chiro*-inositol-containing glycans as second messengers working in a complementary and synergistic manner with the better-accepted pathways of insulin signaling. However, this model does not integrate *myo*-inositol-containing glycans second messengers and their mode of action still remains elusive. In addition, the exact structure–activity relationship of IPGs is not precisely defined and a recent study yielded conflicting results since over a wide range of synthetic IPGs, none were insulin mimetic in both *in vivo* and *in vitro* studies [98] contrary to previous studies [99–102].

The insulin sensitizing effect of a MI, DCI or pinitol supplementation could possibly results from their intracellular enhanced availability for the production of membrane IPGs precursors, but real evidence for this phenomenon is lacking. Moreover, a recent study showed an enhanced GLUT-4 translocation to the plasma membrane in baseline conditions (e.g. in the absence of a direct or glucose-induced insulin stimulation) in mice skeletal muscle in vivo as well as in rat muscle and L6 myotubes in vitro with several inositol isomers [84,103]. Unless a baseline production of IPGs can be obtained with inositol supplement and independently of insulin stimulation, this result cannot be explained by the production of second messengers induced by insulin. In addition, 12 h pretreatment of HepG2 hepatocytes or 3T3-L1 adipocytes with 100 µM myo-inositol or sequoyitol (5-O-methyl-myo-inositol) directly enhanced IR, IRS-1 and Akt activation in response to insulin stimulation (10 nM, 5 min) [80]. Considering that IPGs act on insulin signaling downstream of IR and IRS-1 phosphorylation events, these results cannot be explained by an enhanced production of IPGs.

It is worth noting that part of the MI supplementation effect on insulin sensitivity may come from its partial *in vivo* epimerization to DCI. However, the MI to DCI epimerase activity was reported to be reduced in insulin-resistant tissues. Hence, if MI efficiency mainly relies on its *in vivo* conversion to DCI, the MI supplementation effects will be mitigated in contexts of insulin resistance (unless a supplement of MI epimerase substrate could enhance its activity).

To conclude, MI and other inositol isomers insulin mimetic properties are still not fully understood. Numerous evidences support the hypothesis of a role of inositol glycans insulin-second messengers in insulin mimetic properties of some inositol isomers. However, many questions remain unanswered and deserve further investigations and/or explanations.

### 5.3.1. Some outstanding issues

- Why a GLUT-4 translocation and a glucose uptake enhancement were observed in response to several inositol isomers without insulin stimulation in some animal and *in vitro* studies [84,103]? Is there a baseline production of IPGs independently of insulin stimulation?
- How sequoyitol (a potential *myo*-inositol precursor) and *myo*-inositol pretreatments (100 μM, 12 h) of hepatocytes and adipocytes cell lines directly enhance IR, IRS-1 and Akt in response to insulin (10 nM, 5 min)?
- What is the phospholipase releasing inositol glycans and from which precursor lipids and proteins?
- How many inositol glycan second messenger of insulin exist? Are they different depending on the species, tissue or cell type? Is the structure of plasma IPGs different from that of tissular IPGs?
- Are inositol glycans released extracellularly and/or intracellularly?
- If existing, what is/are the inositol glycan transporter(s) in plasma and mitochondrial membranes.
- Does a MI or DCI supplementation increase IPGs production and how?
- Is MI conversion to DCI essential for its efficiency on glucose homeostasis?

### 6. MI supplementation effects on diabetic complications

In diabetes, tissues likely to develop long-term microvascular complications (kidney, sciatic nerve, lens and retina) are depleted in MI and this MI intracellular deficiency could play a pivotal role in the development and progression of these complications. Restoring MI intracellular levels with dietary MI supplement could then be a suitable strategy to prevent or delay the development of diabetic neuropathy, nephropathy or retinopathy.

### 6.1. MI supplementation and neuropathy

Several animal and human studies showed a beneficial effect of a MI supplementation in the diabetic nerve. First, Green et al. demonstrated that a 1% (w/w) MI supplemented diet (vs. 0.011% or 0.069% free MI in normal diets) restored MI intracellular level in the nerve of STZ-diabetic rat model. On this supplemented diet, the development of impaired Motor Nerve Conduction Velocity (MNCV) by the 14th day after STZ administration was moderated or totally prevented, despite persistent hyperglycemia and elevated nerve intracellular levels of sorbitol and fructose [82]. In this type 1 diabetes rat model, insulin treatment from day 3 after STZ administration failed to prevent impaired MNCV in the sub-group of diabetic rats in which hyperglycemia and weight loss were improved. However, insulin treatment prevented the development of MNCV in the other sub-group of diabetic rats in which the tail vein glycemia never exceeded 160 mg/dl and average glycemia was 75 mg/dl during the days 6-14 after STZ injection. In this latter group of STZ-diabetic rats, insulin treatment also prevented MI nerve depletion. This study suggests that insulin deficiency and possibly hyperglycemia are primary factors in the development of impaired MNCV and that this MNCV impairment appears to be related to a disruption of MI intracellular level regulation in the nerve.

Although some discrepancies were published on MI supplement benefit for MNCV in diabetes [104,105], Greene et al. finally proposed a partial explanation to those differences showing that dietary MI supplementation ameliorated the diabetes-induced MNCV impairment in both sciatic and tibial motor nerves but with different time courses (suggesting metabolic or physiologic heterogeneity among populations of large myelinated motor fibers).

Finally, beneficial effects of MI supplementation on nerve function were confirmed in latter studies. Mayer and colleagues demonstrated that a dietary supplement of MI (0.667 g/kg per os, daily) or an aldose reductase inhibitor treatment (ICI 105552; 50 mg/kg per os, daily) prevented defects of both axonal transport and MNCV in STZ-diabetic rat [106]. Aldose reductase inhibitor treatment also normalized sorbitol levels in motor nerve of diabetic rats. Altered Na<sup>+</sup>/K<sup>+</sup>-ATPase activity was also corrected by 1% (w/w) MI supplementation, such as nerve conductivity [48]. The effect of MI content in diet (low, normal or high) was studied on patients with symptomatic distal symmetrical diabetic polyneuropathy. It was found that ingestion of high-MI diet increased significantly the median sensory and the sural sensory nerve conduction velocities (+1.92 m/s and +6.67 m/s respectively, p < 0.001). In contrast, ingestion of low-MI diet led to a decrease in the median and peroneal MNCV (p < 0.001). Thus ingestion of diet enriched in MI may have a positive effect upon peripheral nerve function in the patients with symptomatic diabetic neuropathy.

### 6.2. MI supplementation and nephropathy

In an *in vitro* model of diabetic renal disease, it has been previously demonstrated that elevated glucose levels stimulate procollagen transcription and secretion in proximal tubule cells in culture while inducing cellular hypertrophy and reducing cellular proliferation. MI supplementation (800  $\mu$ M) in a high glucose culture medium (4.5 g/L) reverses the glucose-induced reduction in cell proliferation and the increase in pro-collagen transcription and secretion [107]. On the other hand, it did not prevent the glucoseinduced cellular hypertrophy. In animal models of diabetes (STZ rat), Na<sup>+</sup>/K<sup>+</sup>-ATPase activity was significantly increased in the cortex of untreated diabetic rats compared with nondiabetic control rats at both 1 and 2 weeks after STZ injection. This increased Na<sup>+</sup>/K<sup>+</sup>-ATPase activity was prevented by MI (0.65 g/kg, p.o.) or Sorbinil (an aldose reductase inhibitor) treatment [108]. The effect of MI supplements on Na<sup>+</sup>/K<sup>+</sup>-ATPase appeared independent of glomerular filtration rate (GFR), since the increase in inulin clearance measured in diabetic rats was unaffected by MI. A long term supplementation (4 months) with 1% MI to the Cohen diabetic (type 2 diabetes) rat also reduced the increased renal Na<sup>+</sup>/K<sup>+</sup>-ATPase activity but had no effect on blood glucose levels, body weight, increased kidney weight, or creatinine clearance and did not prevent or reduce the development of renal glomerular pathology [109]. There was no correlation between the level of Na<sup>+</sup>/ K<sup>+</sup>-ATPase activity and the degree of nephropathy. It is then possible that some renal pathological changes are due to metabolic and humoral factors resulting from hyperglycaemia, other than MI depletion.

### 6.3. MI supplementation and cataracts

MI supplementation restored intracellular MI content in the lens of STZ-induced diabetic rats (otherwise undetectable after 14 weeks post STZ injection) [34,110]. The MI supplemented STZ group displayed minor structural changes and early stages of cataract formation while the untreated group displayed apparent structural changes and well-established cataracts [34].

To conclude, MI supplementation seems to be efficient to counteract the diabetes-induced MI depletion in kidney, nerves and lens. Correction of MI deficiency by dietary MI supplement prevented or delayed the development of some microvascular complications of diabetes in the motor nerves and lens in type 1 diabetes animal models. The fact that MI treatment was shown to have a beneficial effect on restoring impaired conduction velocity and on the disruption of structural elements in the nerve but had no effect on the development of renal pathological changes indicates that the effect of the biological changes ensuing from hyperglycemia vary in different tissues depending on local conditions. Additional clinical studies would be useful to evaluate the efficiency of a MI supplementation against long-term complications of diabetes in human.

### 7. Concluding remarks

myo-Inositol is a polyol naturally present in eukaryotic cells and is a component of numerous biological molecules, including second messengers like IP<sub>3</sub>, PIP<sub>2</sub>/PIP<sub>3</sub> and IPGs which makes it essential for numerous biological processes. Abnormalities in its metabolism are associated to pathological states and in particular, MI intracellular deficiency in sciatic nerve, kidney, lens and retina of diabetic subjects probably contribute to the development or aggravation of some diabetes complications in those tissues. Correction of MI intracellular depletion by MI supplement prevented or delayed the development of some microvascular complications of diabetes in the motor nerves and lens in animal models but was inefficient for diabetic nephropathy. Insulin resistance is also associated to excessive urinary excretion of MI and decreased urinary level of DCI. A correlation has even been observed between the decrease in urinary DCI and the severity of insulin resistance. Insulin resistant tissues like skeletal muscles also presented altered DCI to MI ratios and decreased IPGs content, activity and production in response to insulin. Since IPGs are putative mediators of insulin action, their deficit in insulin target tissues probably participate to the development or progression of insulin resistance. Dietary supplement of inositol isomers DCI, p-pinitol or MI were found to be efficient in lowering post-prandial plasma glucose in several animal models of diabetes or insulin resistance. The insulin-mimetic properties of dietary inositol supplements is mainly believed to be related to the production of inositol glycan secondary messengers containing

either MI or DCI. However further investigations are required to unravel the exact molecular mechanisms of action of MI and to confirm or infirm this IPGs hypothesis. Randomized control trials on MI dietary supplement gave positive results in fighting insulin resistance and reducing cardiovascular risk in women with PCOS, gestational diabetes mellitus or metabolic syndrome in postmenopause. However, larger studies, in double-blind trials, including populations with other than a Caucasian origin and also including men would be necessary, 1) to confirm the previous results for women with GDM, PCOS or post-menopausal MetS; 2) to test a possible application for a more generalized population of subjects already presenting an insulin resistance or at risk of developing one because of genetic predisposition.

#### Disclosure

The authors declare no conflict of interest.

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