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REVIEW

Pharmacodynamics and pharmacokinetics of inositol(s) in health and disease

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Abstract

Introduction: Inositol and its derivatives comprise a huge field of biology. Myo-inositol is not only a prominent component of membrane-incorporated phosphatidylinositol, but participates in its free form, with its isomers or its phosphate derivatives, to a multitude of cellular processes, including ion channel permeability, metabolic homeostasis, mRNA export and translation, cytoskeleton remodeling, stress response.

Areas covered: Bioavailability, safety, uptake and metabolism of inositol is discussed emphasizing the complexity of interconnected pathways leading to phosphoinositides, inositol phosphates and more complex molecules, like glycosyl-phosphatidylinositols.

Expert Opinion: Besides being a structural element, myo-inositol exerts unexpected functions, mostly unknown. However, several reports indicate that inositol plays a key role during phenotypic transitions and developmental phases. Furthermore, dysfunctions in the regulation of inositol metabolism have been implicated in several chronic diseases. Clinical trials using inositol in pharmacological doses provide amazing results in the management of gynecological diseases, respiratory stress syndrome, Alzheimer's disease, metabolic syndrome, and cancer, for which conventional treatments are disappointing. However, despite the widespread studies carried out to identify inositol-based effects, no comprehensive understanding of inositol-based mechanisms has been achieved. An integrated metabolomics-genomic study to identify the cellular fate of therapeutically administered myo-inositol and its genomic/enzymatic targets is urgently warranted.

Keywords: Myo-inositol; inositol phosphates; cell signalling; phosphoinositides; inositolphosphoglycans.

Article highlights box

• After enzymatic digestion of dietary IP6, MYO is actively absorbed and transported by intestinal cells by two transport systems: sodium ion coupled (SMIT1/2) and proton coupled (HMIT1) inositol transporters.

• MYO undergoes a complex metabolic transformation, leading to inositol isomers, inositol phosphates (IPs), phosphatidylinositol (PI), phosphatidylinositides (phosphatidylinositol-phosphates PIP), glycosyl-phosphatidylinositols (GPI), and many other derivatives as inositol-phosphoglycans (IPG).

• MYO and its derivatives participate in several physiological processes, including calcium metabolism, proliferation, endocrine modulation, phenotypic determination, developmental processes, and stress response.

• Dietary inositol has been shown to inhibit lung tumorigenesis in rats and humans

• MYO may play a relevant role in preventing Respiratory Distress Syndrome in preterm infant, as well as other developmental defects.

• MYO counteracts the main features of the PCOS-associated metabolic syndrome, meanwhile it improves several ovarian functions: oocyte quality, frequency of ovulation, increased pregnancy rate.

• MYO and its derivatives represent possible therapeutic agents for the treatment of neurological disorders, including Alzheimer disease.

• An integrated metabolomics-genomic study to identify the cellular fate of therapeutically added MYO and its genomic/enzymatic targets is urgently warranted.

1. Introduction

Inositol (Ins)[1] is among the oldest component of living beings, undergoing complex evolutionary modifications ultimately leading to the current multiplicity of functions for inositol-containing molecules in eukaryotes [1]. Besides the biological role sustained in its free form, Ins also serves as an important component of structural lipids and of secondary messengers. Inositol (cyclohexanehexol), a cyclic carbohydrate with six hydroxyl groups, was originally isolated from muscle extracts by Scherer in 1850 who called it *myo*-Inositol (myo-Ins), from the Greek word meaning 'muscle' [2]. Since then, interest in inositol and its derivatives has undergone mixed fortunes. Nevertheless, the last decades witnessed a sustained revival of studies in this field because of the discovery of new Ins derivatives and pervasive understanding of the pivotal role that Ins and its metabolites play in so many cellular and tissue processes, including development, metabolism regulation and regenerative medicine [3]. Although being the most abundant form in nature, myo-Ins is only one of nine possible structural isomers of inositol, as other naturally occurring stereoisomers have been found: *scyllo-, muco-, epi, neo-, allo-, cis-, D-chiro*-and *L-chiro*-inositol. It is worth noting that a few of those isomers also display significant physiological functions [4]. Yet, myo-Ins occupies a central position in cellular metabolism given that the inositol moiety is widely utilized by nature to biosynthesize several intermediate molecular components of the biochemical machinery, including inositol phosphates (IPs or InPs), phosphatidylinositol (PI), phosphatidylinositides (phosphatidylinositol-phosphates PIPs or PtdIns), glycosyl-phosphatidylinositols (GPI), and many other derivatives as inositol-phosphoglycans (IPG) and inositol ethers and esters.

2. Bioavailability and metabolism of inositol.

Myo-Ins homeostasis in cells and tissue is maintained at three different levels: 1) intestinal absorption and renal excretion; 2) carrier-mediated transport from plasma/interstitial fluids into cells; 3) endogenous synthesis and catabolism.

2.1 Dietary inositol.

Myo-Ins is the principal storage form of phosphorus in many plant tissues and it is principally found in bran and seeds [5]. It is consumed by humans in its free form, as inositol-containing phospholipid, but mostly as phytic acid (inositol hexakisphosphate, $InsP_6$). Bacterial phytases and phosphatases – homologous of the mammalian InsP6 phosphatase (MINPP) - are mainly

[1]Inositol isomers and inositol phosphate terminology continues to be misused, as complained by several scholars [Shears SB and Turner BL. Nomenclature and terminology of Inositol phosphates, in: Inositol phosphates: linking Agriculture and the Environment, BL Turner, AE Richardson, EJ Mullaney (Eds), CaB Intl , 2006, pp. 1-5]. Herein after denominations and abbreviations were reported according to IUPAC recommendation [IUPAC. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Compiled by AD McNaught and A Wilkinson. Blackwell Scientific Publications, Oxford (1997). XML on-line corrected version: http://goldbook.iupac.org (2006-) created by M Nic, J Jirat, B Kosata; updates compiled by A Jenkins.]

responsible for digesting dietary $InsP_6$, releasing myo-Ins and phosphate." [6,7]. A mixed western diet provides the human adult with approximately 1 g of total inositol per day [8]. However, despite the demonstration of signs of inositol deficiency in several animal species [9] no requirement for dietary inositol in man has been specifically determined. Dietary requirements may be higher depending on the person's age, the long-term use of antibiotics, or the regular consumption of coffee (higher than 100 mg/day, corresponding to 2 espresso cups approximately)[10]. In addition, from the 70s many foods have been processed to remove phytic acid, deemed as an anti-nutritional factor for its chelating affinity to some minerals, like calcium, iron and zinc [11]. Therefore, it may be reasonably to surmise that in western countries low-vegetable consumers may suffer from a relative deficiency of myo-Ins due to the reduced content of phytic acid in the diet. This is a controversial issue discussed in detail elsewhere [12,13]. Additionally, a reliable assessment of dietary requirements is further complicated by the fact that a noticeable amount of myo-Ins in mammals is endogenously synthesized from glucose [14].

2.2 Inositol transport and excretion

Myo-Ins is actively transported by intestinal cells. Uptake and accumulation occur against a concentration gradient in a Na^+-dependent manner [15]. This process, while unaffected by high dietary calcium intakes [16] (which hinders the absorption of phytic acid), is significantly inhibited in a non-competitive manner by glucose and other sugars. Conversely, glucose inhibits the reuptake of myo-Ins in the human renal tubule. Lipid composition of the meal may influence myo-Ins intestinal uptake and pharmacokinetic studies using different myo-Ins pharmaceutical formulations have demonstrated that oral myo-Ins availability is highly enhanced when myo-Ins is administered in soft gel capsules [17]. Filtered inositol is almost completely reabsorbed at physiologic plasma levels in normal subjects and urinary myo-Ins loss occurs only for higher plasma concentrations. However, large amounts of myo-Ins are excreted in diabetic patients, even in the presence of normal serum myo-Ins levels [18]. Moreover, a 3000 % increase in myo-Ins clearance was observed following glucose loading at normal plasma inositol levels, in both normal and diabetic patients [18]. In blood, myo-Ins circulates mainly as free myo-Ins, reaching concentrations ranging from 0.03 to 0.1 mM with a turn over half-life of 22 min [19]. *In utero*, early foetal serum myo-Ins levels are 2–10 times higher than in adults and decrease gradually toward term [20]. Plasma myo-Ins levels increases steadily in diabetic and uremic patients [21], while no data are yet available for other pathological conditions. However, a significant drop in myo-Ins levels has been observed in preterm infants at risk for respiratory distress syndrome [22].

2.3 Cell uptake

According to their mechanism, myo-Ins transport systems can be classified into two groups: sodium ion-coupled and proton-coupled transporters [reviewed in 23]. The Na^+/inositol transporters have been so far only identified in animals, while H^+/inositol symporters have been found in all eukaryotic kingdoms. The major transport system for myo-Ins uptake into several mammalian cells is a saturable carrier-mediated and active process (with a Km of about 30 μM) deriving its energy from the sodium gradient. Noticeably, myo-Ins is concentrated in these cells to levels well above the concentrations recorded in the blood or in the culture medium. An active transport system has been described in kidney, endothelial, epithelial and neuronal cells. Namely, two transporters, SMIT1 and SMIT2, have been identified in regulating brain and peripheral inositol levels, by co-transporting two sodium ions along the concentration gradient and generate enough energy to actively transport myo-Ins [24]. Myo-Ins transport ensured by SMIT1/2 increased after down-regulation of protein kinase C activity and decreased after activation of protein kinase-A in cultured human cells, indicating that the inositol uptake system is post-translationally regulated through phosphorylation [25]. On the contrary, in hepatic parenchymal cells a non-active, non-saturable, carrier-mediated transport system has been documented [26]. In hepatocytes, myo-Ins uptake is linearly related to Ins concentrations (without a clear defined half-maximal uptake rate) and it is decreased by drugs like Cytochalasin B [27], or inhibitors of the SGLT1/2 glucose transporter system (like Phloretin), indicating that myo-Ins share with glucose some transporter systems. Indeed, galactose and D-glucose both reduce myo-Ins uptake as well as its incorporation into phospholipids. It is worth of noting that a measurable inhibition of myo-Ins transport occurs even under physiologic concentrations of glucose [27]. Hyperglycemia induces myo-Ins depletion in nervous tissues, via competitive inhibition of sodium-dependent myo-Ins uptake by hyperglycemic concentrations of glucose [28]. On the contrary, proton-coupled inositol transporters (HMIT1) are insensitive to glucose, while other inositol isomers (*D-chiro-*, *scyllo-*and *muco-inositol*) actively compete with myo-Ins for HMIT1 binding. HMIT1 is encoded by SLC2A13, a member of the SLC2 gene family. HMIT1 is predominantly expressed in the brain, even if its precise subcellular localization is still controversial [29]. Indeed, it has been demonstrated that HMIT1 is embedded in intracellular vesicles but may appear on the cell surface in a P12 cell culture in response to increased Ca^{2+} concentrations [30]. In contrast, other reports identified HMIT only in the Golgi apparatus without detecting any HMIT-mediated myo-Ins uptake in neurons [31]. It can be argued that these contradictory results may reflect differences in the analytical approaches or different adaptive phenotypic neuronal changes under the specific experimental culture conditions.

Overall, those findings demonstrated that myo-Ins uptake by living cells is accurately tuned, leading

thus to significant differences in Ins content among different tissues [32]. The organs of the male reproductive tract (and epididymal, vesicular, and prostatic fluids) are rich in free myo-Ins [33]. High myo-Ins concentrations, several-fold greater than in blood, have also been confirmed in mammalian semen [34]. Unbound myo-Ins levels in the brain, cerebrospinal fluid, and choroid plexus are also higher than in plasma [35]. While in the liver, muscle and heart myo-Ins is predominantly represented in its phospholipid-bound form [36], high free myo-Ins levels have been observed in the small intestine, brain and kidney. Myo-Ins levels ten-fold higher than those recorded in blood have been observed during lactation in human breast milk. Generally, some tissues (kidney, organs of the reproductive tract, brain) seem to actively concentrate myo-Ins to levels from three to twenty-fold higher than those estimated in plasma. Noteworthy, despite the contribution of endogenous myo-Ins biosynthesis, myo-Ins content in those tissues is highly dependent on inositol provided by food supply. Indeed, myo-Ins levels in testis, liver, plasma, heart, lens, lung, kidney, and small intestine – with the noticeable exception of the brain -are significantly reduced when inositol depleted diets are fed as compared to controls [37] (Fig. 1).

2.4 Biosynthesis and catabolism.

Organisms may endogenously synthesize myo-Ins. Inositol is converted from glucose-6-phosphate (G6P) through two biochemical reactions: G6P is first isomerized by the NADH-dependent, cytosolic D-3-myoinositol-phosphate synthase (INO1 or MIPS1, encoded by ISYNA1 gene)[2] to Inositol-3-phosphate (Ins3P), which is then dephosphorylated by inositol monophosphatase-1 (IMPA-1) to yield free *myo*-inositol [38]. In yeast, both enzymes are induced by myo-Ins depletion and down regulated in presence of normal/high myo-Ins cellular content [39]. This tight regulation suggests a key biological role of myo-Ins as metabolic sensor and it is worth noting that many aspects of myo-Ins biochemistry and functions have been preserved in several yeast and mammalian pathways, including the action of the INO1 gene [40]. Biosynthesis of myo-Ins occurs primarily in the kidney, with a rate approaching 4 g/day. Extrarenal tissues, mainly the brain and testis, can also contribute to the endogenous production of inositol. It is noticeable that INO1 activity may be under hormonal control in the reproductive organs [41] and liver of male rats, thus providing an indirect proof of the importance of myo-Ins metabolism in such organs. In addition, GSK3 is required for optimal INO1 activity, as loss of GSK3 activity causes inositol depletion [42].

[2]The systematic name of this class of enzyme is 1D-myo-inositol-3-phosphate lyase. However, some confusion may arise, as other names are frequently in common use, including myo-inositol-1-phosphate synthase, D-glucose 6-phosphate cycloaldolase, inositol 1-phosphate synthetase, glucose 6-phosphate cyclase, inositol 1-phosphate synthetase, glucose-6-phosphate inositol monophosphate cycloaldolase, glucocycloaldolase.

Overall, myo-Ins biosynthesis in mammals has been considered of limited value until now, given that the activity of INO1, the rate-limiting enzyme in the synthesis of myo-Ins, is low in most animal tissues [43]. However, this assumption has recently been cast on doubt by the observation that inositol biosynthesis is essential for proliferation and neurite outgrowth of SK-N-SH human neuroblastoma cells [44]. As approximately one-half of the unbound myo-Ins in animal brain is obtained from glucose *in situ,* with the remainder being transported into the brain from the blood [45], it may be hypothesized that endogenous myo-Ins is really critical only for neural tissues. Indeed, the brain maintains a high level of free inositol (5–50 mM), which is about 100 times higher than that in blood and other tissues [46]. Therefore, further studies are warranted to ascertain the importance of inositol biosynthesis in other tissues.

Kidney is the only organ of relevance in inositol catabolism, given that nephrectomy in animals impairs myo-Ins degradation, while renal failure is associated with significant abnormalities in myo-Ins metabolism and increased plasma levels of inositol [47]. The initial committed step in inositol catabolism involves cleavage of the ring to yield O-glucuronic acid and, through further metabolic steps, oxylulose-5-phosphate that enters the pentose phosphate cycle.

2.5 Safety

Myo-Ins supplementation is well tolerated since myo-Ins LD50 in mouse is 10000 mg/kg body weight when orally administered [48]. In humans, myo-Ins safety has been assessed by several trials in which inositol was given for prolonged periods (from 1 to 12 months) at doses ranging from 4 to 30 g/day. Mild side effects (mostly represented by nausea or diarrhoea) are reported in a small fraction of subjects, only for doses up to 12 g/day [49]. In a limited number of psychiatric patients mild neurological discomfort (insomnia, dizziness, headache, nausea, sleepiness and a few case reports of mania) have been observed [50]. Myo-Ins is currently added to some infant milk powder at a level of 0.01%, as it has been given GRAS status, which indicates that no evidence implicates it as a dietary hazard to the public when used at current levels [51].

3. Myo-Inositol conversion to isomers and derivatives or incorporation into phospholipids

Myo-inositol is the precursor to the biosynthesis of inositol phospholipids and inositol phosphates, compounds that play an important role in signal transduction [52].

3.1 Inositol isomers

Myo-Ins can be transformed into numerous derivatives mainly through either epimerization or phosphorylation of one or several of its hydroxyl groups. Nonetheless, most of these compounds

cannot be recovered from mammal cells, while they are generally found in some plant species. Namely, myo-Ins can be converted into naturally occurring isomers - *D-chiro-, scyllo-, muco-, neo* - by means of specific epimerases. The conversion rate of myo-Ins to *D-chiro-inositol* (D-chiro-Ins) ranges from 7% to about 9%, as measured by the analysis of radiolabeled [^{3}H]-myo-Ins, whereas the production of other isomers is minimal, not exceeding 0.06% of total radiolabeled myo-Ins [53]. In its free form myo-Ins plays a relevant role as osmolyte, providing an important protection facing several environmental and metabolic stresses. Protective effects of myo-Ins include protein stabilization, osmotic compensation and freezing avoidance during supercooling of tissue water [54]. It is tempting to speculate that several of these mechanisms could help in explaining some unexpected effects displayed by both myo-Ins and *scyllo*-Ins on cytoskeleton, β-amyloid and chromatin rearrangement. Namely, the *scyllo*-isomer, which is not involved in PI signaling, is known to bind and inhibit Aβ42 peptide aggregation and formation of Aβ fibrils [55].

3.2 Phospholipids synthesis

It has generally been assumed that *myo*-inositol is the only inositol that is incorporated into phospholipids, but a few studies suggest that plant and animal cells may occasionally contain some phosphatidyl-*scyllo*-inositol or phosphatidyl-*chiro*-inositol [56,57]. The de novo biosynthesis of PI involves the reaction of myo-Ins with cytidine-diphosphate-diacylglicerol, promoted by the enzyme inositol phosphatidyltransferase (PI synthetase, PIS). Alternatively, myo-Ins may react with phospholipids, becoming incorporated through aMn^{2+}-dependent exchange reaction occurring in microsomes [58]. It must be emphasized that the Mn^{2+}-dependent exchange reaction, does not promote any de novo phospholipid synthesis, but exchanges free and phospholipid-associated inositol. PI can be phosphorylated to form phosphatidylinositol-4-phosphate (PI4P or PIP), phosphatidylinositol-biphosphate (PIP2 or PtdIns(3,5)*P* or PI(3,5)P_2) and phosphatidylinositol trisphosphate (PIP3 or Ptdins (3,4,5)P or PI(3,4,5)P_3). All phosphorylated PI-based lipids are collectively known as Inositides or Phosphoinositides (PIPs) and they are produced by a set of specific phosphoinositide-kinases (PIKs): phosphoinositides-3-kinases (PI3K, belonging to three main classes: I, II and III), PI4K, PI5K and lipid phosphatases that add to the synthesis of the inositol lipids [59-61]. Given that the myo-Ins ring can be phosphorylated on the 3,4, and 5 hydroxyl group (the 2 and 6 hydroxyl group cannot be phosphorylated due to steric hindrance provided by the diacylglycerol part of the structure) [62] PIPs can be found in seven different combinations. In experiments with [^{3}H]-myo-Ins in isolated hepatocytes, isotope distribution demonstrated that myo-Ins is mainly incorporate in PI (95% of the total radioactivity), PI4P and PI(4,5)P_2(~4%), according with a saturable process characterized by a maximum rate of myo-Ins

incorporation (~125 pmol/mg of cells in 30 min) and myo-Ins concentration for half-maximum incorporation (~ 0.4 mM). In presence of higher myo-Ins levels in the medium, total cell radioactivity in the lipid fraction decreases (-50% at 0.01 mM myo-Ins and 4 % at 100 mM myo-Ins), while the free myo-Ins-radiolabelled fraction increases, as only negligible quantities of inositol phosphates were recorded [26].

PIP3 and PI3K play a pivotal role in cell biochemistry. Acting as a second messenger, PI(3,4,5)P_3 recruits pleckstrin homology (PH) domain-containing proteins, such as Akt serine–threonine kinase (AKT) and PI-dependent kinase 1 (PDK1) to the plasma membrane. AKT is subsequently phosphorylated at Thr308 by PDK1 and at Ser473 by mTORC2 [63].

Activated AKT (pAKT) phosphorylates multiple downstream proteins and through phosphorylation of AKT modulates many aspects of cellular physiology. PTEN (*p*hosphatase and *ten*sin homolog deleted on chromosome *ten*)is a physiological negative regulator of this pathway, and it acts as a phosphatase, dephosphorylating the D3 position of PIP3 and thus reducing PIP3 levels [64] (Fig. 2). Like PTEN, SH2 domain-containing inositol phosphatase (SHIP) has also been shown to dephosphorylate PtdIns(3,4,5)P_3 and to inhibits the PI3K/Akt pathway [65]. However such finding was not confirmed by other studies in which it is shown that SHIP, unlike PTEN, does not affect Akt activity despite its ability to dephosphorylate a PI3K product [66]. Modulation of the aforementioned pathways is critical in switching cell fate from survival and proliferation, to apoptosis. Myo-Ins is therefore deemed to actively participate in such processes, namely in response to external stressors.

3.3 Inositol phosphates

In both plants and protozoa myo-Ins may be directly phosphorylated by an inositol kinase to yield Ins3P [67,68]. However, in humans inositol phosphates are produced from the dephosphorylation of more phosphorylated forms (including $InsP_6$) by specific phosphatases, and/or from phosphoinositides hydrolysis, since myo-inositol kinases do not exist in human cells.

Indeed, upon stimulation, phospholipase C (PLC) metabolizes PI-(4,5)-P_2 into the intracellular second messengers 1,2-diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (Ins-(3,4,5)-P_3 or IP3) [69]. DAG is well known as an activator of protein kinase C (PKC), while IP3 act as a pivotal regulator of calcium flux by binding to specific IP3 receptors (IP3R, constituted by type I, II and III distinct isoforms). Upon binding of four Ins(1,4,5)P_3 ligands, the receptor opens subsequently inducing the release of $Ca^{2+}$from the ER to the cytosol [70,71]. Upon depletion of these intracellular Ca^{2+} stores, extracellular Ca^{2+} must refill them by a process known as capacitative Ca^{2+} entry. Ins(1,4,5)P_3 has a short half-life within the cell [72] and is rapidly metabolised through one of two

pathways: removal of the 5-phosphate from the inositol ring by inositol polyphosphate 5-phosphatases results in the production of Ins(1,4)P_2. This species is then sequentially dephosphorylated to form free myo-ins. The other pathway of Ins(1,4,5)P_3 metabolism involves further phosphorylation of the inositol ring: the enzyme Ins(1,4,5)P_3 3-kinase catalyses the ATP-dependent phosphorylation of the hydroxyl groupon the 3-carbon of inositol, resulting in the formation of inositol (1,3,4,5)-tetrakisphosphate (Ins(1,3,4,5)P_4) [73]. Furthermore, other inositol phosphates and diphosphoryl inositol phosphates (commonly referred to as inositol pyrophosphates, PP-IPs or InsP7 and InsP8) are also found in living cells [74].

From the discovery of Ins(1,4,5)P3/Ca^{2+} signalling paradigm [75] "the number of known inositol phosphates has grown along with the appreciation that they are regulating every aspect of cellular physiology" [76]. Accordingly, the interest for inositol phosphates and polyphosphates raised increased attention in the last decade, as these metabolites appear to fulfil fundamental and unexpected biological functions: a) rapid recycling of IP3, and hence the control on IP3-mediated release of Ca^{2+} from the endoplasmic reticulum; b) synthesis of specific InsPs, which may have distinctive functions in participating to many signalling pathways; and c) ultimately, recycling of InsPs by a series of InsP-phosphomonoesterases to regenerate the intracellular pool of free myo-Ins [77]. The latter pathway is a critical one, as it represents the main source of myo-Ins during endocrine stimulation and it is efficiently blocked by Li^+ administration [78].

Indeed, besides its free form, myo-Ins exists under many phosphorylated forms within cells: inositol mono-phosphate (InsP or $InsP_1$), poly-phosphorylated forms ($InsP_2$, $InsP_3$,$InsP_4$, $InsP_5$, $InsP_6$) and pyrophosphate forms. Living beings actively maintain $InsP_4$ levels, given that animals feeding an InsP6-free diet show unchanged $InsP_4$ concentrations, while $InsP_6$ and $InsP_5$ decrease dramatically [79]. Furthermore, similar results have been obtained in mould, in which myo-Ins starvation does not significantly affect $InsP_4$ levels [80].

$InsP_4$ is actually a group of several different isomers – 3,4,5,6-$InsP_4$, 1,4,5,6-$InsP_4$, 1,3,4,5-$InsP_4$ and 1,3,4,6-$InsP_4$ - the first three of which have been shown to regulate chloride ion channels, histone acetylation and calcium signalling, respectively [3,81]. As anticipated by R. Irvine [52], these findings suggest that $InsP_4$ would fulfil relevant biological tasks, even if this evidence has still to be thoroughly assessed.

3.4 Phosphoglycans

Myo-Ins and D-chiro-Ins participate in the constitution of glycosyl-phosphatidylinositol (GPI) anchors and of inositol phosphoglycans (IPGs) that would constitute second messengers of insulin action in the GPI/IPG pathway [82]. IPG incorporating either myo-Ins or D-chiro-Ins are released

upon stimulation of insulin by hydrolysis of GPI lipids located on the outer leaflet of the cell membrane. IPGs affect intracellular metabolic processes, namely by activating key enzymes controlling the oxidative and non-oxidative metabolism of glucose. Both D-chiro-Ins and myo-Ins-containing IPG significantly reduce insulin resistance, improve glucose metabolism and many features belonging to the metabolic syndrome [83]. Recent discoveries showed that non-phosphoglycans- as IP7 and PI5P – also participate in modulating glucose metabolism. IP7 is required for efficient exocytosis of insulin containing secretory granules from pancreatic β cells [84], while PI5P has been demonstrated mimicking insulin effect in facilitating GLUT4 translocation to cell surface, this way enhancing glucose uptake [85].

4. Inositol and Human diseases

Myo-Ins and its derivatives participate in several physiological processes, including proliferation, endocrine modulation [86], phenotypic determination, developmental processes, stress response, to mention just a few. It is therefore not surprising that genetic mutations of enzymes involved in Ins signaling cause numerous pathologies [87]. Additionally, deregulation of myo-Ins metabolism has been shown to occur in several chronic human diseases, including cancer, metabolic syndrome [88], diabetes [89], thyroid dysfunctions [90], respiratory distress syndrome and infertility (Tab. I).

4.1 Cancer

In the wake of studies carried out on the anticarcinogenic action of IP6, some preliminary reports have highlighted the involvement of myo-Ins in carcinogenesis and its supportive role in cancer treatment. In *in vitro* studies, it has been shown that exogenously administered InsP6 is internalized through endocytosis and almost completely dephosphorylated to myo-Ins and lower inositol phosphates (mainly InsP5), which further affect signal transduction pathways resulting in cell cycle arrest [91]. *In vivo*, myo-Ins plus $InsP_6$ enhances the anticancer effect of conventional chemotherapy, controls cancer metastases, and improves the quality of life, as shown in a few pilot clinical studies [92]. Both myo-Ins and $InsP_6$ have been also proven to be effective as safe chemopreventive agents, by triggering anti-oxidant and anti-inflammatory effects [93]. Namely, it has been demonstrated that $InsP_6$ inhibits IGF-1 receptor (IGF-1R) pathway-mediated sustained growth in cancer [94]. Given that myo-Ins may efficiently counteract insulin resistance (IR), it is tempting to speculate that it may also prevent IGF-1 increase associated to IR. As both IR and IGF-1 are linked to increased cancer risk [95], it is conceivable that myo-Ins modulation of insulin activity may efficiently contribute in reducing cancer risk.

Several studies show that myo-Ins decreased both the incidence and multiplicity of malignant lung

tumors chemically induced in mice [96,97]. It has been hypothesized that such effects should be ascribed to the down-regulation of specific biochemical pathways. [98]. Indeed, myo-Ins has been shown to modulate both PI3K/AKT [99] and Wnt/β-catenin pathways [100], which are frequently involved in lung carcinogenesis. Indeed, dietary inositol has been shown to inhibit lung tumorigenesis in female A/J mice exposed to the carcinogen benzo(α)-pyrene or 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [101,102]. Myo-Ins was also effective in the post-initiation phase and when given for short periods of time before, during, and immediately post carcinogen exposure. A phase I study enrolling 26 smokers showed that myo-Ins in a daily dose up to 18 g/p.o. is safe and well tolerated, while inducing a significant regression of individual pulmonary dysplastic lesions (91% in the INS group versus 48% in the placebo group) [103]. As PI3K activation represents a critical and early step in lung carcinogenesis, inhibition of PI3K is likely to be a key factor in lung cancer chemoprevention. Indeed, a significant increase in a genomic signature of PI3K pathway activation in cells of normal bronchial airway of smokers with lung cancer and smokers with dysplastic lesions has been recorded, thus suggesting that PI3K is activated in the proximal airway before tumorigenesis. These preliminary results have been confirmed by two papers [104,105], in which both dysplastic lesions and PI3K activity in the airway of high-risk smokers showed a significant regression after myo-Ins treatment. Such preliminary findings deserve further investigation, namely through large, randomized trials. A recent clinical study promoted by the NIH [106] showed no benefit associated with myo-Ins supplementation in heavy smokers carrying bronchial dysplasia. Yet, even this survey is biased by the limited number of subjects (38 in the myo-Ins arm versus 36 placebo-treated controls) entering the study.

The anticancer effects displayed by myo-Ins have been also recorded in breast tumors. Breast cancer cells treated *in vitro* with myo-Ins showed increased E-cadherin, down-regulation of metalloproteinase-9, redistribution of β-catenin behind cell membrane, while motility and invading capacity were severely inhibited. Those changes were associated with a significant down-regulation of PI3K/Akt activity, leading to a decrease in downstream signaling effectors: NF-kB, COX-2, and SNAI1. Inositol-mediated inhibition of presenilin-1 (PSEN1) leads to lowered Notch 1 release, thus contributing to decrease SNAI1 levels. Furthermore, inositol-treated cells underwent a profound cytoskeleton remodeling. Overall, these data indicated that myo-Ins inhibits the principal molecular pathway supporting EMT in cancer cells [107].

Moreover, it has been demonstrated that increased biosynthesis of myo-Ins may have detrimental effects on cancer cells. Indeed, over-expression of ISYNA1 gene, increased INO1 activity and myo-inositol levels in HCT116 colon cancer cells and suppressed tumor cell growth, probably by re-

activating p53 activity [108]. Those data suggest that myo-Ins exerts a pivotal inhibitory control almost on two critical pathways – the PI3K/Akt and the PSEN1 pathway – both of them known to participate in modulating apoptosis, proliferation and phenotypic differentiation. Yet, it is still a matter of investigation if such effects are directly induced by myo-Ins or through indirect changes in its phosphate derivatives (PIPs and/or InsPs). Thereby, mechanisms through which myo-Ins and/or its phosphate derivatives antagonize cancer occurrence and development still deserve to be investigated in depth.

4.2 Respiratory distress syndrome and pulmonary functions

Interest has recently focused on the use of myo-Ins supplementation in preterm infants for the prevention of bronchopulmonary disease (including the Respiratory Distress Syndrome, RDS) and retinopathy of prematurity (ROP) [109]. Myo-Ins promotes maturation of pulmonary surfactant phospholipids, phosphatidylcholine and phosphatidylinositol. Namely, synthesis of PI in type II pneumocytes appears to be dependent on extracellular myo-Ins concentrations [110]. Compositional changes in fetal rat lung surfactant correlate with changes in plasma myo-Ins levels, and myo-Ins supplementation increases PI levels in the deprived rat pup [111]. In turn, surfactant enriched in myo-Ins content significantly improves the mechanical properties of alveoli. Myo-Ins and its phosphate derivatives recruit organic osmolytes and water within the alveolar space, and foster the reconstitution of a bio-film layer (featured by a hydrophobic tail and a hydrophilic head) at the interface, thereby decreasing surface tension and antagonizing collapsing forces [112]. Additionally, inositol promotes a mechanical stabilization of cell shape, mostly by modulating cytoskeleton dynamics, thus enabling alveolar cells to counteract collapsing forces [113]. Mechanical effects displayed by myo-Ins make this compound priceless in affording pulmonary protection against atelectasia-driven processes, namely in preterm infants. Indeed, human milk has a high myo-Ins concentration, with preterm milk being the richest source. Indeed, infants who are breastfed have higher serum myo-Ins levels compared to those that are not [114]. In infants with RDS a premature drop in serum myo-Ins levels predicts a more severe course [115]. On the contrary, myo-Ins supplementation increases inositol serum levels as well as the phosphatidylcholine/sphingomyelin ratio of surfactant. These data suggest myo-Ins may play a relevant role in preventing RDS in preterm infant. Although only few published trials of myo-Ins supplementation have been subjected to systematic review, the quality of the reports, as stated by Cochrane studies [22], has been deemed appropriate. Myo-Ins supplementation significantly reduces short-term adverse neonatal outcomes and the incidence of bronchi-pulmonary dysplasia. The effectiveness of inositol in reducing the severity of RDS is consistent with experimental data

indicating that myo-Ins serves as a substrate enhancing the synthesis and secretion of surfactant phospholipid in immature lung tissue [116].

4.3 Gynaecological disorders

Abnormalities of inositol metabolism, involving both myo-Ins and D-chiro-Ins isomers, have been noticed in women with polycystic ovary syndrome (PCOS) and may contribute to other gynaecological conditions [117]. Women affected by PCOS show reduced serum levels of D-chiro-Ins, increased urinary loss of D-chiro-Ins [118] and reduced myo-Ins/D-chiro-Ins ratio within the ovary [119]. An increasing body of clinical studies has provided compelling evidence that myo-Ins may be an effective therapeutic option for PCOS patients. Myo-Ins counteracts the main features of the metabolic syndrome, meanwhile it improves several ovarian functions including oocyte quality, frequency of ovulation, pregnancy rate, while reducing the intensity of FSH treatment required to trigger ovulation. Those results have been assessed by both pilot and randomized studies [120-123]. It is worth to outline that the frequency of both ovulation and pregnancy rate are significantly raised by myo-Ins treatment [124]. These effects are mainly ascribed to inositol-based phosphoglycans, which participate in insulin signaling transduction. Insulin-resistance (IR) is indeed a common feature of PCOS, affecting up to 80% of patients. Insulin enhances the synthesis and release of androgens from theca cells. Additionally, high insulin concentrations inhibit the hepatic synthesis of sex hormone-binding globulin [125], therefore causing a consequent increase of biologically circulating free-active androgens. When insulin binds to its receptor, IPGs are released by hydrolysis of glycosyl-phosphatidylinositol lipids on the outer side of cell membrane. However, women affected by PCOS show reduced serum levels of D-chiro-Ins and increased urinary loss of D-chiro-Ins. These preliminary hints fostered further investigations leading to evidence a severe deregulation of inositol metabolism in PCOS, thus enabling in establishing a clear mechanistic link between IR and inositol deficiency in PCOS patients [126]. Indeed, by treating PCOS patients with low doses of D-chiro-Ins a significant improvement of several PCOS features (reduced levels of lipid biomarkers, increased insulin sensitivity, decreased serum androgen levels and higher ovulation frequency) was recorded [127]. Those effects have been mainly ascribed to a D-chiro-Ins systemic activity, able to counteract the main consequences of the metabolic syndrome, which are associated to PCOS in a significant proportion of patients. Yet, whereas D-chiro-Ins benefits seem to be restricted to non-ovarian tissues, myo-Ins effects mostly involve the ovary. Myo-Ins participates in modulating LH/FSH activity [128], GnRH agonist-mediated LH inhibition [129] and probably it may modulate steroidogenesis by acting through an insulin-independent pathway, involving cytoskeleton rearrangement [130]. This possibility would explain why myo-Ins

supplementation has been demonstrated to be effective even in non-insulin resistant PCOS patients [131]. On the contrary, D-chiro-Ins, mostly when administered at high doses, seems to exert controversial effects on ovary tissues, leading to a decreased myo-Ins/D-chiro-Ins ratio due the insulin-induced epimerase activity [119]. The conversion of [^{3}H]myo-Ins to [^{3}H]D-chiro-Ins is markedly decreased in GK diabetic rats compared to controls in liver, muscle, and fat, insulin sensitive tissues. Decreases of 20-25% conversion to baseline levels of under 5% conversion were observed. Such findings are associated with decreased epimerase activity in insulin-sensitive tissues, thus explaining the decreased chiro-inositol to myo-inositol urine and tissue ratios observed here and in previous animal and human studies [132]. Indeed, the epimerase conversion of myo-Ins to D-chiro-Ins is under insulin control: in type 2 diabetes patients the reduced tissue insulin sensitivity leads to decreased epimerase activity and hence down-regulates D-chiro-Ins synthesis. However, ovary retains normal insulin sensitivity even when other tissues display insulin resistance. Thus, increased insulin levels are likely to paradoxically foster the activity of ovarian epimerase, raising D-chiro-Ins intracellular production and decreasing myo-Ins levels. Therefore, in hyperinsulinemic PCOS patients D-chiro-Ins levels are increased in the ovary, and further D-chiro-Ins administration cannot lead to any significant benefit, while myo-Ins depletion will in turn negatively affect oocyte quality. These findings provide the experimental evidence of the so called "D-chiro-Ins ovary paradox" [133], according to which treatment with high doses of D-chiro-Ins in hyperinsulinemic PCOS patients worsen the ovarian function. However, numerous questions regarding the mechanism of action of myo-Ins, particularly its role in glucose metabolism and insulin-dependent signalling, remain to be answered. Namely, we urgently need to ascertain the exact role sustained by myo-Ins and D-chiro-Ins. Indeed, both isomers positively affect glucose metabolism in several insulin-sensitive tissues, while in the ovary normal homeostasis require an appropriate myo-Ins/D-chiro-Ins ratio, given that high D-chiro-Ins levels showed to be detrimental [134]. These data provided the rationale in attempting to associate both Ins isomers according to their respective physiological levels. Yet, this assumption still awaits further confirmation. In a first place, myo-Ins metabolism must be thoroughly investigated in order to evidence if Ins-related effects on PCOS should be ascribed to myo-Ins or to its phosphate-derivative. Secondly, it would be useful to elucidate the participation of myo-Ins in non-insulin-related mechanisms, i.e. through cytoskeleton modification. Thirdly, epimerase activity is probably selectively regulated within different tissues and this aspect deserves to be appropriately investigated [135].

4.4 Sperm function

Myo-Ins concentration in the reproductive tracts of both male and female mammals is substantially

higher than in blood serum [33,34], suggesting that this molecule influences fertility positively with a role in reproduction. In the testis, myo-Ins is produced and released by FSH-responsive *Sertoli* cells and is involved in processes that include the regulation of motility, capacitation and acrosome reaction of sperm cells. Myo-Ins concentration increases from the caput to the *cauda epididymis* enriching the seminal fluid. Studies performed on pathological sperm samples have shown that myo-Ins is crucial in male spermatogenesis for at least two different functions. Sperm cells from oligoastenospermic patients display low motility and high levels of IMPA-1 [136], which catalyzes the dephosphorylation of InsP. This finding suggests that InsPs plays an important role in the signal transduction pathways that regulate and maintain sperm cell motility. Besides the reduced motility, sperm cells from these patients are characterized, among others, by morphological abnormalities in mitochondria and the midpiece. Electron microscopy imaging has recently demonstrated that treatment of these cells with myo-Ins improves midpiece volume and restores mitochondrial *cristae* morphology, suggesting a structural normalization of mitochondria [137]. Functionally, myo-Ins acts directly on mitochondria increasing the membrane potential [138]. This is an apoptotic marker clearly related to the functional parameters of the sperm cells, including motility, fertility potential and quality of the resulting embryos, and is therefore used as an index of fertility. High values of mitochondrial membrane potential indicate integrity of this structure and are associated with high cell viability. Confirming these morphological-functional data, treatment of sperm cells from both oligoastenospermic patients and normal subjects with myo-Ins increases total and progressive motility, improving the recovery of cells utilizable in IVF cycles after swim-up [139].

4.5 Oocytes and embryos

In women, myo-Ins content of the follicular fluid correlates positively with oocyte quality and pregnancy outcome [140]. As already stated, myo-Ins is actively imported into mammalian cells and this transport has been observed in growing and fully-grown oocytes and preimplantation embryonic blastomeres in virtue of two different membrane protein transporters. Uptake of myo-Ins appears essential for oocyte physiology, being this molecule strongly involved in both processes of meiotic maturation and fertilization [141]. Myo-Ins action is mainly ascribed to receptor-mediated IP3 modulation of intracellular Ca^{2+} ion concentration in response to the action of the hormones LH and FSH [142].This pathway has a key role during maturation. Upon fertilization and at later stages, IP3 plays a similar key role in egg activation and subsequent rapid blastomere divisions by modulating intracellular calcium ion fluxes [143]. After fertilization, myo-Ins uptake increases progressively between the one-cell stage and the blastocyst stage [144], suggesting a parallel increase in cellular requirement of the molecule. It has been shown that the majority of myo-Ins in

preimplantation blastomeres is rapidly incorporated into phosphoinositides [144]. Experiments performed in the mouse model on oocytes and early embryos have produced encouraging results for the employment of myo-Ins in the manipulation and culture of human embryos in assisted reproduction techniques. When oocytes matured in the presence of myo-Ins were fertilized in vitro and transferred to foster mothers, the implantation rate and post implantation viability of the resulting embryos was also increased [145]. More recently, we have shown that including myo-Ins in human embryo culture media increases the ability of in vitro produced and cultured embryos to complete preimplantation development [146]. Compared to embryos cultured in standard medium, embryos maintained in the presence of myo-Ins displayed a faster cleavage and developmental rate. These results confirm and extend previous observations on the positive effect of myo-Ins in preimplantation embryos, obtained on farming species [147].

4.6 Developmental defects

Myo-Ins is needed to afford normal developmental processes. Foetuses require myo-Ins during gestation and concentrate it from maternal blood. At term, serum myo-Ins levels had decreased, but they are still two-to three-fold higher than in maternal blood [148]. Both myo-Ins and D-chiro-Ins promote differentiation of the foetal lung and prevent neural tube defects [149]. It has been hypothesized that the competitive inhibition of myo-Ins uptake by glucose may explain the increased rate of congenital malformations recorded in infants of diabetic mothers [150], while myo-Ins supplementation is capable to counteract neural tube defects observed among the offspring of diabetic rats [151]. In addition, inadequate myo-Ins uptake has a detrimental effect on prenatal skeleton development and on postnatal bone remodeling. Therefore, myo-Ins is currently deemed essential for proper osteogenesis and extracellular matrix deposition in bone [152]. This effect can only be minimally ascribed to the calcium-modulating effects of IP3 derivative. Indeed, inefficient intracellular uptake of myo-Ins down-regulates several genes (osterix, Runx2 and NFATc1) and impairs the normal bone phenotypic determination, probably also involving complex cell-matrix interplay through cytoskeleton rearrangement. Similarly, proper myo-Ins uptake is required for nerve development and function [153]. It is worth noting that both bone and neural developmental abnormalities can be completely corrected by myo-Ins administration.

4.7 Neurological disorders

Inositol levels in the brain are several fold higher that in plasma. Myo-Ins content is tightly controlled and less dependent on dietary supply within the CNS [154]. Besides the central role played by PI and PIPs in signal transduction downstream neurotransmitter stimulation [155,156],

myo-Ins is credited in fulfilling specific tasks mainly through its phosphate derivatives, InsP5 and $InsP_6$ [157]. Deregulation of myo-Ins metabolism has been observed in several psychiatric and neurological diseases. Many evidences pointing out the possible use of myo-Ins in treating neurodegenerative disorders are also derived by the observation that lithium, by inhibiting IMPA1, shows neuroprotective effects in different neurodegenerative disorders, including Alzheimer's disease (AD), amyotrophic lateral sclerosis, and Parkinson's disease [158].

Furthermore, changes in the distribution of myo-Ins may reveal regional brain consequences of deregulated amyloid protein metabolism before the pathology could be detectable [159]. Increased intracellular calcium elicits the characteristic Alzheimer lesions, including the accumulation of β-amyloid, the hyperphosphorylation of TAU and neuronal death [160]. Consequently, as Ca^{2+} release from ER stores is mainly mediated by IP3, change in Ca^{2+} homeostasis has been thought for a while as the main process that should be mechanistically link neurological diseases and myo-Ins deregulated metabolism. In fact, mitochondrial function is essential for neuronal survival and mitochondrial activity tightly depends on Ca^{2+} availability from the extracellular space or from the endoplasmic reticulum (ER). Indeed, abnormalities in IP3 and IP3R activity are likely to be involved in Ca^{2+}deregulation, which are common in neurodegenerative disorders as well as in cerebral stroke and ischemia [161,162]. However, myo-Ins and its isomer *scyllo-inositol* have been suspected to be strongly associated with both production and physical aggregation of the AD-associated peptide, β-amyloid [58,163]. A critical step in β-amyloid production is constituted by the activation of γ-secretase [164] and it is worth of noting that the same enzymatic complex enhances Ca^{2+} release in neuronal cells by influencing the phosphoinositide calcium signaling [165]. Indeed, Presenilin-1, the main component of the γ-secretase complex interacts with type 1 inositol trisphosphate receptor (InsP3R1) in the ER and boosts its activity [166].

As myo-Ins has been proven to inhibit PSEN1 in breast cells [107], it can be hypothesized that myo-Ins may affect the amyloidogenic pathway by inhibiting γ-secretase activity. Taken together, these observations stress the idea that myo-Ins and its derivatives represent possible therapeutic agents for the treatment of CNS-associated diseases.

5. Conclusions

Myo-inositol and its derivatives play many relevant biological functions, including modulation of glucose metabolism, calcium release in cell signaling, chromatin and cytoskeleton remodeling, gene transcription, proliferation, apoptosis and proper structural development. Namely, the inositol network seems to display a key role during phenotypic transition and developmental processes, enabling the cells to properly respond to many different stress conditions. Deregulation of myo-Ins

metabolism has been observed in several illnesses. Conversely, treatment with myo-Ins or other inositol isomers has been proven to induce appreciable clinical results in so different diseases such as PCOS, cancer, respiratory distress syndrome and neurological disorders. Studies on inositol functions have therefore gained momentum. Yet, despite the impressive body of research witnessed in the last decades, some key issues need to be properly addressed in order to achieve a full understanding of the inositol *modus operandi*.

6. Expert Opinion

6.1 Key findings

Myo-Ins is the key molecule of important intracellular signaling pathways, where it participates as component of complex derivatives or in its free form. The fact that myo-Ins and its derivatives modulate the PI3K/Akt pathway, the intracellular signaling of several hormones (Insulin, LH, and FSH), the remodeling of CSK proteins, just to mention a few processes, allows us to recognize inositol as a key player in biological activities. In addition, it has been recently reported that, in yeast, hundreds of genes are affected in response to myo-Ins addiction or removal [167]. This finding strongly indicates that, besides being a simple structural element, or chiefly acting as an osmolyte, myo-Ins may exert a wide range on unexpected functions, mostly still unknown. However, data hitherto gathered suggest that myo-Ins may play a key role during transition and developmental phases involving both cells and tissues. This conclusion comes principally from studies carried out in pre-term infants, in women with PCOS as well as in cancer. The ultimate goal in this field is to carefully ascertain how myo-Ins and its derivatives contribute in driving key-transition processes in order to preserve cell homeostasis at both structural and functional levels. Indeed, a recent simulation carried out with systemic perturbation analysis revealed that inositol-based pathways are among the most influential components affecting each other when cells are exposed to different stressors [168]. Namely, the combinatorial perturbation consisting of PI3K inactivation and over-activation of IP3R can lead to increased activity levels of apoptosis-related components and tumor-suppressor genes, suggesting that the inositol network is at the core of fundamental cellular phenotypic transition processes.

6.2 What potential does this research hold?

Dysfunctions in the regulation of myo-Ins metabolism, frequently coupled with PIPs and InsPs abnormalities, have been implicated in several chronic diseases [169]. Empirical clinical trial using inositol at pharmacological doses (from 2 to 20 g/day) provided promising, preliminary results in different disorders like PCOS, respiratory stress syndrome, Alzheimer, metabolic syndrome, and

cancer. It is worth noting that in these conditions myo-Ins can represent a significant step ahead, given that conventional treatments for such diseases are still disappointing. Additionally, a new emergent field of investigation is constituted by the use of myo-Ins in the anticancer chemo-preventive field. Indeed, several studies provided evidence supporting the beneficial role sustained by myo-Ins supplementation in reverting bronchial dysplasia among heavy smokers, as well as in improving the clinical outcome of the respiratory distress syndrome in pre-term newborns. Insofar as promising those results could be, we must outline that a fundamental, clear-cut understanding of the mechanism(s) through which myo-Ins influences biological functions is warranted. Furthermore, it is now time to plan well-designed, wide randomized clinical trial to unequivocally assess myo-Ins effectiveness in those conditions (namely in PCOS) where inositol supplementation has already proven to induce consistent medical benefits.

6.3 What is the biggest challenge in this goal being achieved?

While there is little uncertainty that changes in intracellular myo-Ins levels could indirectly interfere with the complex cross talk occurring among PIPs, IPGs and IPs, it is still a matter of investigation if myo-Ins could modulate gene or biochemical functions in its free form. How addition or removal of myo-Ins may actually modify PIPs and InPs pattern in different cells or in pathological conditions require an in depth study. In order to understand the possible role exerted by myo-Ins in its free form, a key argument is how Ins participates in water–solute–macromolecule interactions to stabilize proteins and membranes. The distinguishing pattern of polar and non-polar surfaces represented by inositol may allow the interaction with soluble proteins to form a template either for fibril growth or for inhibition. Indeed, myo-Ins and *scyllo-inositol* have been shown to bind to β-amyloid proteins and allosterically prevent target interactions [170], whereas $InsP_6$has been shown to be tightly linked to the catalytic domain of the RNA adenosine deaminase being required for proper protein folding [171]. In addition, myo-Ins, mainly through its phosphate derivatives, induces actin synthesis [172] and F-actin ring formation at cell-cell contacts through ROCK-dependent myosin II activation [173]. However, it is not clearly established if those effects could be ascribed to myo-Ins itself or to some of its phosphate derivatives. Moreover, inositol-based effects are not limited to cytosol components given that InsPs also localize within the nuclei where they exert numerous specific regulations on nuclear processes, including modulation of gene expression, mRNA export, DNA repair and telomere maintenance [174]. An intriguing finding is the recent discovery of nuclear InsP kinases and both InsP and PIPs receptors (including nuclear hormone receptors, nucleosome mobilization and chromatin remodeling complexes) [175]. These data reinforce the relevance of myo-inositol based signaling pathways within the nucleus, highlighting

their involvement in epigenetic mechanisms. Indeed, changes in InsP4, InsP5 and InsP6 levels have been proven to interfere with chromatin remodeling and eventually reduce gene transcription [176]. Yet, many mechanistic questions still remain on how and where this regulation occurs. Namely, critical in our understanding of InsPs regulation will be the identification of InsP nuclear receptors. Furthermore, despite the widespread studies carried out to identify inositol-based effects on biological pathways, no comprehensive metabolomics investigations have been so far performed. Only an integrated metabolomics-genomic study would indeed provide the basic information required to identify the cellular fate of therapeutically added myo-Ins and its genomic/enzymatic targets.

6.4 Next frontier

Besides the integrated metabolomics studies focused in addressing the cellular fate of myo-Ins, a specific issue that deserves great attention is the participation of myo-Ins and its derivatives in modulating cytoskeleton and nucleoskeleton (NSK) remodeling. Studies on the dynamics of CSK-NSK have gained momentum during the last decade. Changes in the synthesis and spatial arrangement of CSK-NSK proteins play a pivotal role in several biological processes, namely during phenotypic transition occurring both in physiological and pathological (PCOS, cancer) conditions [177]. How myo-Ins or components of its biochemical pathway (including specific kinases and phosphatases) intervene in CSK-NSK remodeling in response to a wide range of stresses is currently under investigation but it will presumably lead to fruitful insights.

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Tab. I Involvement of Inositol and derivatives in biological functions and human diseases

Condition	**Molecular pathways and mechanisms**	**Effects**	**References**
Reproduction and endocrine system	LH/FSH Modulation	Restoring ovulation	117, 118, 119, 120, 128, 129, 133
	Insulin modulation (IPGs release)	↑ Oocyte quality ↑ Embryo quality	120, 124, 126, 127, 140, 142,143, 144, 146, 147
		↑ sperm motility	137,138,139
	CSK modulation	Steroidogenesis (?)	172
	↑IPs release	Normalizing thyroid function	86, 90
Metabolism	↓ Triglycerides, LDL cholesterol, ↑ HDL	↓HOMA Homeostasis Model Assessment	88
	↓ insulinemia, ↓ glycaemia, ↑ GLUT4 translocation induced by PI5P	Insulinmodulation, ↓ IR	82, 88, 89, 119
Developmental defects	↑ Gene expression (osterix, Runx2 and NFATc1)	Proper bone remodelling and osteogenesis	152
	↑IP3 and PKC	Proper nerve development and function	153, 154
	?	Prevention of neural tube defects	149,151
	?	Normal lung development	115, 116
	Surfactant composition	Respiratory Distress Syndrome	22, 109, 110, 116
Neurological diseases	↑ binding and inhibition of Aβ42 peptide aggregation and formation of Aβ fibrils	β-amyloidinhibition	55, 163, 164, 170
	↑IP3, IP3R	Mitochondrial function and Ca^{2+} flux in neuronal cells	158, 161
Cancer	Inhibition PI3K/Akt and Wnt pathways	↓proliferation, ↑ apoptosis	99, 100
	Lung cancer chemoprevention		92, 101, 102, 103
Inflammatory pathway	TGFβ modulation	↓ fibrosis, ↓ chronic inflammation	93

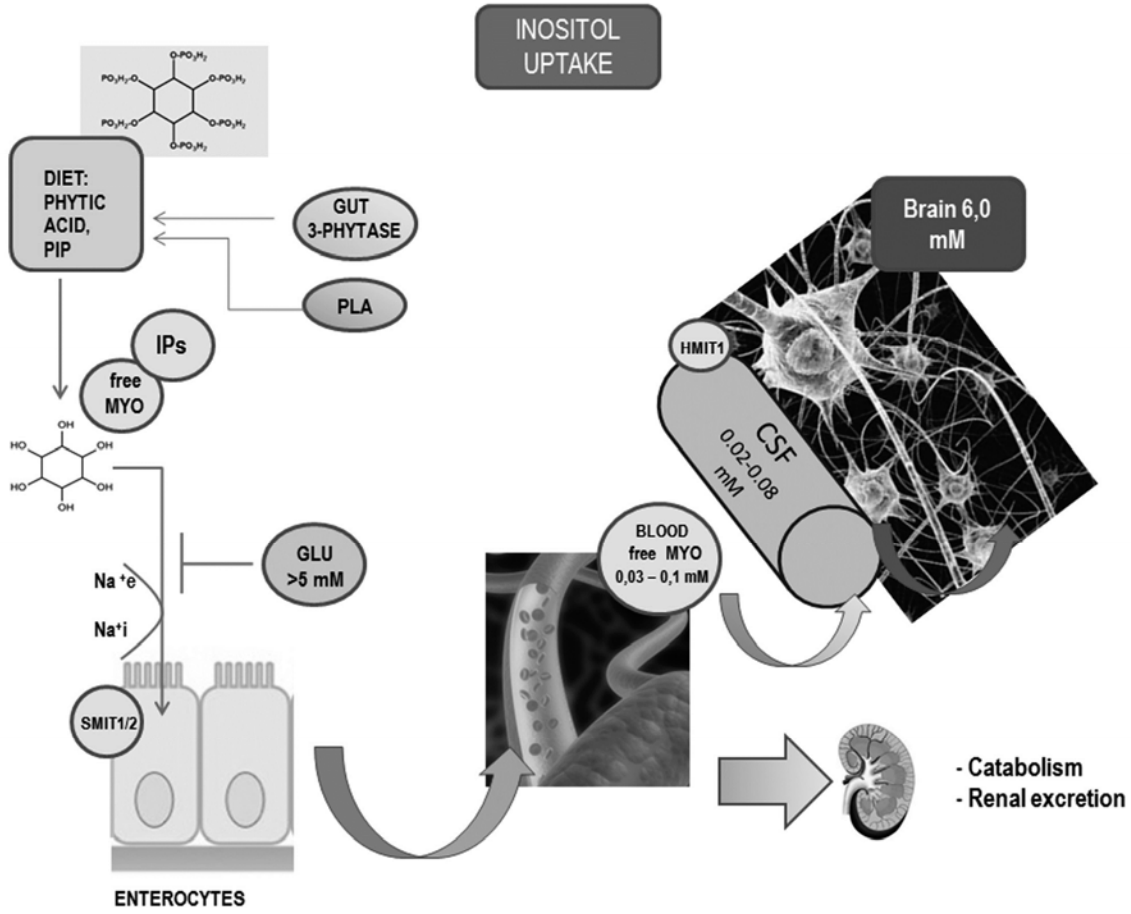

Fig. 1. Myo-Inositol uptake and tissue distribution.

Dietary MYO is released from intestinal digestion of phospholipids (through the phospholipase A, PLA) and IP6. IP6 enzymatic degradation is performed by a gut-3-phytase (multiple inositol-polyphosphate phosphatase-1, MINPP1). MYO enters the circulation mainly as such. Little amounts of inositol phosphates (IPs) or phosphatidyl-inositol phosphate (PIP) are also absorbed into the circulation. Cell uptake is performed by two groups of MYO transport systems: sodium ion coupled (SMIT1/2) and proton coupled (HMIT1) inositol transporters. D-glucose (GLU) both reduce MYO uptake by SMIT1/2 as well as its incorporation into phospholipid. MYO is found in

blood in concentrations ranging from 0,03 to 0,1 mM. Some tissues ((kidney, organs of the reproductive tract, cerebrospinal fluid [CSF], brain) actively concentrate MYO in concentrations from three to twenty-fold higher than plasma levels. The kidney is the only organ of relevance in inositol excretion and catabolism.

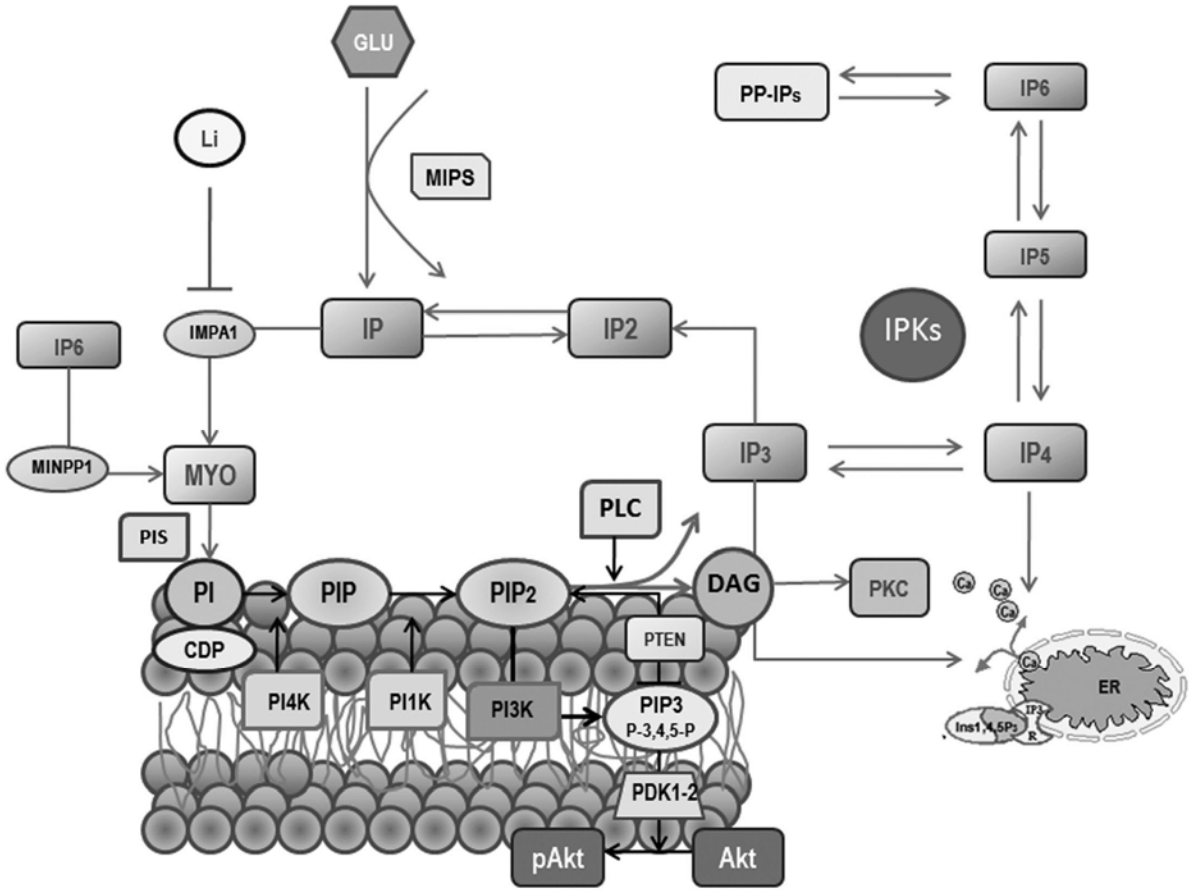

Fig. 2. Myo-Inositol conversion to inositol phosphates and incorporation into phospholipids.

De novo biosynthesis of phosphatidyl-inositol (PI) involves the reaction of MYO with cytidine-diphosphate-diacylglicerol (CDP), promoted by the enzyme inositol phosphatidyltransferase (PI synthetase, PIS). Phosphatidyl-inositol-4-kinase (PIP4K) converts PI to PI4P (PIP). Thus, PI(4,5)P2 (PIP2) is generated by PIPK-1 isoforms, which utilize PI4P as substrate. PIPK-1 localizes on the plasma membrane and is thought to account for the majority of PI(4,5)P2 synthesis. PIP2 is further phosphorylated by PI3K into PI(3,4,5)P (PIP3). This step is antagonized by PTEN, acting as a phosphatase, thus dephosphorylating PIP3. PIP3 recruits AKT and PI-dependent kinase 1 (PDK1) to the plasma membrane. AKT is activated through phosphorylation by PDK2. Phospholipase C (PLC) metabolizes PIP2 into the intracellular second messengers 1,2-diacylglycerol (DAG) and

inositol 1,4,5-trisphosphate (IP3). DAG is an activator of protein kinase C (PKC), while IP3 act as a pivotal regulator of calcium flux by binding to specific IP3 receptors (IP3R) and subsequently inducing the release of Ca2+ from the endoplasmic reticulum (ER). IP3 is rapidly phosphorylated by inositol-kinases (IPKs) to form a number of more polar inositol phosphates (IP4, IP5, IP6) and inositol pyrophosphates (PP-IPs). IP3 may be dephosphorylated by inositol phosphatases yielding IP2 and IP. Inositol-monophosphate is also obtained from glucose conversion catalyzed by D-3-myoinositol-phosphate synthase (MIPS). In turn IP is dephosphorylated by inositol monophosphatase-1 (IMPA1) to yield ultimately free myo-inositol. This step can be pharmacologically inhibited by Lithium (Li), which blocks the reconstitution of MYO pool, thus leading to accumulation of phosphorylated inositol forms. However, free MYO is mainly provided by enzymatic digestion of dietary IP6 performed by the multiple inositol-polyphosphate phosphatases 1 (MINPP1).