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ORIGINAL RESEARCH

Risk of reduced intestinal absorption of myo-inositol caused by D-chiro-inositol or by glucose transporter inhibitors

Simone Garzon [a], Antonio Simone Laganà [a] and Giovanni Monastra [b]

[a]Department of Obstetrics and Gynecology, "Filippo Del Ponte" Hospital, University of Insubria, Varese, Italy; [b]Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy

ABSTRACT

Background: D-chiro-inositol (DCI) and glucose transporter inhibitors may inhibit myo-inositol (MI) transporters, and the aim is to clinically evaluate their effect on MI absorption.

Research design and methods: Fasting 18 healthy volunteers received orally 6000 mg MI, 6000 mg MI with 1000 mg DCI, and 6000 mg MI with SelectSIEVE® Apple PCQ and Sorbitol, Maltodextrin and Sucralose (PCQ-SMS), in three different phases with a washout period of 7 days. At each phase, blood samples were collected before administration, and every 60 minutes until 540 minutes after administration. MI plasma levels (μmol/L) were quantified by gas chromatography-mass spectrometry; maximum plasma concentration (Cmax), time to reach it (Tmax), and the area under the time-concentration curve of MI (AUC 0-540) were evaluated.

Results: The Cmax of MI alone (Tmax = 180min) was 1.29-fold higher than those of MI with DCI (Tmax = 180min) ($p < 0.001$) and 1.69-fold higher than those of MI with PCQ-SMS (Tmax = 240min) ($p < 0.001$). The AUC 0-540 was reduced by 19.09% in MI plus DCI ($p = 0.0118$) and by 31.8% in MI plus PCQ-SMS ($p < 0.001$) as compared to MI alone.

Conclusions: DCI, glucose transporter inhibitors and sugars, such as sorbitol and maltodextrin, seem to inhibit MI absorption, decreasing MI plasma concentration as compared to MI alone.

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1. Introduction

Inositols are a group of nine polyols with a 6-carbon ring and a hydroxyl group bound to each carbon; the epimerization of the six hydroxyl groups determines the different stereoisomeric structures of Inositols [1]. The most common stereoisomer in nature is Myo-Inositol (MI), which can be synthetized in humans from glucose-6-phosphate (primarily in the liver and in the kidneys) and converted to D-Chiro-Inositol (DCI) through an insulin-dependent intracellular epimerase [2–4]. The homeostasis of Inositols in tissues and cells is maintained by endogenous synthesis and catabolism, carrier-mediated transmembrane transport, and by intestinal absorption and renal excretion [2]. Although Inositols can be synthetized from glucose, in mammals they are primarily obtained from dietary sources as inositol-6-phosphate (fruits, cereals, nuts, and animal tissues) [5].

Inositols are primordial molecules involved in a great array of functions, such as cell proliferation, fertilization, contraction, metabolism, and vesicle and fluid secretion [6–8]. They play in cells both a structural and functional role and are present in a free form as well as components of the cell-membrane [5], where MI and DCI are found as phosphatidylinositols (PIs) [9]. PIs represent the starting point of different pathways mediated by the action of phospholipases (PLPs), phosphatases, and phosphatidylinositol 3-kinase (PI-3-K) [9], which can convert MI and DCI into inositolphosphoglycan (IPG) second messengers (MI-IPG and DCI-IPG) [10–12].

Overall, MI represents the predominant part of Inositols content (>99%), and DCI the remaining one (another stereoisomer, scyllo-inositol, was detected only in the Central Nervous System). Nevertheless, there is a considerable variability in MI and DCI concentrations in different tissues and organs, depending on the distinct functions played by these isomers in various environments of the organism. The intracellular epimerase enzyme activity regulated by insulin determines such tissue-specific intracellular MI:DCI ratio [12–14]. In insulin-sensitive tissues (muscle, liver, and fat) MI conversion to DCI is of paramount importance in the regulation of glucose metabolism. In these tissues, the insulin second messenger (INS-2) is a DCI-IPG, which stimulates glucose uptake, glycolysis, and glycogen synthesis. Based on this observation, DCI deficiency was found related to insulin resistance [15]. On the other hand, MI is involved primarily in cellular glucose uptake and its level is high in tissues with high glucose utilization, such as heart and brain [10].

Inositols are further involved in many other pathways that mediate cellular function in different organs and tissues, from fetal development to adulthood [2]: they regulate oocyte development, theca and granulosa cells function, and steroid production in the ovaries, where MI has a key role in the

CONTACT Antonio Simone Laganà antoniosimone.lagana@uninsubria.it Department of Obstetrics and Gynecology, "Filippo Del Ponte" Hospital, University of Insubria, Piazza Biroldi 1, Varese 21100, Italy

Supplemental data for this article can be accessed here.

gonadotropins receptors signaling [16]. Similarly, in the testis, MI is produced and released by Sertoli cells under the action of gonadotropins, and it is involved in the development of sperm cells motility, capacitation, and acrosome reaction [17]. Through the modulation of PI3K/AKT and Wnt/β-catenin pathways, MI exerts anti-inflammatory and anticancer effects, reducing cancer risk both in lung and breast [18]. Moreover, MI has been reported having an important role in the therapy of broncho-pulmonary disease and retinopathy in premature preterm infants, promoting the maturation of pulmonary surfactant phospholipids, phosphatidylcholine, and PIs [19]. Finally, downregulation of MI metabolism has been associated to several neurological and psychiatric diseases, such as Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease, suggesting a protective effect in different neurodegenerative disorders [20].

Based on these pieces of evidence, supplementation with Inositols became of high interest to achieve a modulation of different pathways, representing a possible treatment or a strategy to improve symptoms in several diseases [2]. One example is the Polycystic Ovary Syndrome (PCOS), where Inositols seem to have a key role in the physiopathology, representing a link between altered glucose metabolism and ovarian dysfunction, and a growing body of evidence demonstrates the effectiveness of Inositol supplementation in these patients [12]. Nevertheless, although DCI have a key role in PCOS through the improvement of glucose metabolism, excessive concentrations of DCI seem to have a detrimental effect on oocyte quality and ovarian response also in normal subjects [21]. At the same time, the combined treatment with MI and DCI was reported improving ovarian function as well as hormonal and metabolic state more than either MI or DCI treatment alone, probably due to the synergistic actions of MI and DCI. Particularly MI counteracts the effect of DCI on ovaries [22–28]. Therefore, in PCOS as well as in other pathologies, the correct posology of MI and DCI administered in therapy plays a pivotal role to provide the correct supplementation and the expected results, avoiding unwanted effects (currently, the administration of the 40:1 ratio between MI and DCI proved effective in PCOS) [24,27,29].

This important issue has to be managed considering that the two stereoisomers use the same transporter [30]. Therefore, when MI and DCI are administered together, their absorption can be decreased in an unequal way as consequence of this competition. MI and DCI have higher affinity for their transporter as compared to glucose; nonetheless, compounds that reduce glucose absorption at intestinal level may interfere with MI absorption if administered together. This causes an inadequate supplementation [30].

On these bases, our study aimed to investigate this issue in a clinical setting. MI alone, MI plus DCI, and MI plus SelectSIEVE® Apple PCQ (PCQ) and Sorbitol, Maltodextrin, Sucralose (SMS) were given in a single administration to healthy volunteers, and then their pharmacokinetic (PK) profiles were compared to highlight possible changes, which may guide a correction of MI and DCI posology in diet supplements.

2. Subjects and methods

2.1. Study population

The study included 18 healthy volunteers: 8 men and 10 women. We recruited healthy volunteers aged between 18 and 35 years and with a body mass index (BMI) ranging between 18 and 25 kg/m^2. Volunteers were evaluated based on medical history, physical examination and laboratory screenings (liver and kidneys function tests and glucose metabolism). The subjects affected by any diagnosed or suspected disease were excluded from the study. Moreover, we excluded subjects with regular intake of drugs for specific disease or any other reason, such as contraception (contraceptive pill), or psychoactive substances, or diet supplements.

2.2. Study protocol

Volunteers were enrolled in June 2018, and the pharmacokinetic (PK) study was carried out in October 2018. MI (purity 99%) (Zhucheng Haotian Pharm Co. Ltd. – Zhucheng, China) was obtained by extraction from corn phytin, the calcium magnesium salt of phytic acid, which is found in plants where it plays the role of main phosphorus reserve (70% of total phosphorus). DCI (purity 96.5%) (Catalent Pharma Solutions Inc – Somerset, NJ, USA) was obtained from carob (Ceratonia Siliqua) bean pod, using water and ethanol (fermented) as extractions solvents. SelectSIEVE® Apple PCQ (PCQ) (Roelmi HPC – Origgio, Varese, Italy) was naturally obtained through soft-processing of food grade apple pomace, which confers a standardized profile of bio phenols, including dihydrochalcones at 15–30%, flavanols at 15–25%, and hydroxycinnamic acid at 15–30%. Its main components are phlorizin, quercetin and chlorogenic acid. Sorbitol, maltodextrin, sucralose (SMS) were administrated with SelectSIEVE® Apple PCQ.

The study consisted in three phases, conducted after a washout period of 7 days between each phase, on the same group of subjects. In phase I, the subjects, all fasting for 12 hours, received orally 6000 mg MI in powder form dissolved in 80 mL H_2O in a single dose. In phase II, after a washout period of 7 days, the same subjects, fasting for 12 hours, received an oral dose of 6000 mg MI and 1000 mg DCI in powder form dissolved in 80 mL H_2O. In phase III, after a washout period of 7 days, the same subjects, fasting for 12 hours, received an oral dose of 6000 mg MI plus PCQ (25.5 mg phlorizin, 1.7 mg quercetin, 46.7 mg chlorogenic acid) and SMS (1193.1 mg sorbitol, 260.7 mg maltodextrin and 42.7 mg sucralose) in powder form, dissolved in 80 mL H_2O. The dose of 6000 mg MI was chosen empirically to obtain well detectable blood levels, although it was similar to the dosage used in the clinical practice for MI supplementation in different diseases, such as PCOS, for which the usually investigated dosage ranges between 2000 and 4000 mg per day [12]. Once the dose of MI was chosen, the volunteers received 6000 mg of MI and 1000 mg of DCI (ratio of 6:1). The ratio of 6:1 was also chosen empirically, in order to obtain well detectable blood levels of the two inositol isoforms and to highlight the potential inhibitory effect of DCI on MI absorption.

Volunteers were evaluated based on medical history, physical examination and laboratory screenings (liver and kidneys function tests) at each phase to confirm inclusion criteria and to exclude adverse effects. At each phase, blood samples were collected by venous puncture before oral dose administration (time point 0), and every 60 minutes until 540 minutes post-administration. The blood samples were collected in heparinized tubes and kept on ice. Subsequently, blood samples were centrifuged and stored at −80°C, before to be analyzed.

2.3. MI and DCI assay and pharmacokinetic analysis

Quantification of MI levels (µmol/L) was performed by Mérieux NutriSciences Italia (Resana – Treviso, Italy) and was carried out with the following procedure. After extraction with organic solvents and derivatization, sample analysis was made by gas chromatography-mass spectrometry with Agilent 6890 (Agilent, 5301 Stevens Creek Blvd, Santa Clara, CA 95051, USA). The injection (1.0 µl) was performed in a splitless mode at 270°C, using a capillary column Agilent 122-5532 DB-5 ms (0.25 mm x 30 m x 0.25 µm). The total run-time lasted 15 min: oven at 70°C from 0 to 1 min; 20°C/min to 150°C; 10°C/min to 240°C; 4 min at 320°C post-run. The flow rate was fixed at 1.2 mL/min, and the results were analyzed by a MS 5973 Network Series detector in sim mode.

PK parameters were subsequently evaluated following oral administration of MI alone, MI in combination with DCI, or MI in combination with SelectSIEVE® Apple PCQ and Sorbitol, Maltodextrin and Sucralose (PCQ-SMS), determining plasma MI and DCI concentrations at various time points up to 540 min post-administration. Maximum plasma concentration (Cmax) and time to reach it (Tmax), were calculated directly from the plasma concentration. The area under the time-concentration curve (AUC 0-540) was calculated by the trapezoidal method from 0 to 540 min.

2.4. Statistical analysis

The primary outcome was the detection of a difference in the AUC 0-540 of the time-concentration curve, and the secondary endpoint measures were the differences of Cmax and Tmax between the three compositions. With a resulted pilot AUC 0-540 for MI alone of 36,548 µmol·min/L, an expected standard deviation of 5%, and considering a drop-out of 10%, a sample size of 18 subjects would achieve 80% of power (α error: 0.05; β error: 0.2) to find a minimum change of AUC 0-540 of 5% between MI alone and MI plus DCI or MI plus PCQ-SMS. Statistical analyses were performed using STATA V.8.2 (StataCorp LLC, Texas, USA). Kolmogorov-Smirnov test was used to determine if the data were Gaussian distributed. Since all the quantitative data were normally distributed, they were presented as mean ± standard deviation (SD), while qualitative variables were expressed as numbers and percentages. Comparison of quantitative variables between the arms was performed using the Student's t-test, with the p value corrected with Bonferroni post-hoc analysis, while the comparison of qualitative variables was performed with the Chi-Square test. P value < 0.05 was defined as statistically significant.

2.5. Ethics and methodological standards

The design, analysis, interpretation of data, drafting and revisions conform the Helsinki Declaration, the Committee on Publication Ethics (COPE) guidelines (http://publicationethics.org/), the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement, available through the EQUATOR (enhancing the quality and transparency of health research) network (www.equator-network.org) The study was approved by the independent Institutional Review Board (IRB) of the study center (approval ID: 06/2018). Each patient enrolled in the study signed informed consent for all the procedures and to allow data collection and analysis for research purpose. The study was advertised, and remuneration was offered to the study subjects to enter and continue the study. An independent data safety and monitoring committee evaluated the results.

3. Results

All the 18 enrolled subjects completed the trial, and no adverse events were reported. The volunteers were aged between 23 and 34 years. The body mass index (BMI) ranged between 21 and 25 kg/m^2. The glucose metabolism was normal in all subjects, consistently with inclusion and exclusion criteria (Table 1 reports average glucose blood concentration values). The mean MI plasma concentration before dose administration at fast was 22 ± 4.7 µM.

The analysis of MI plasma concentrations reported a similar curve profile in the administered formulations. The Tmax was recorded at 180 minutes in MI alone and MI plus DCI, and at 240 minutes in MI plus PCQ-SMS. The time course of MI concentrations in the plasma of the 18 volunteers before and after oral administration of MI alone, MI combined with DCI, and MI combined with PCQ-SMS are graphically reported in Figure 1. The AUC 0-540 were significantly different between the MI alone and the other two formulations. The AUC 0-540 of MI plus DCI was found reduced of about 19.1% as compared to MI alone ($p = 0.0118$), and the AUC 0-540 of MI plus PCQ-SMS was found reduced of about 31.8% as compared to MI alone ($p < 0.001$). Conversely, no significant difference was reported between the AUC 0-540 of MI plus DCI and MI plus PCQ-SMS ($p = 0.1294$). Nevertheless, Cmax of the three formulations resulted significantly different: the average peak plasma concentration (Cmax) recorded at 180 min (Tmax) in MI administered alone was about 1.29-fold higher than MI combined with DCI ($p < 0.001$); similarly, Cmax of MI alone recorded at 180 min (Tmax) was about 1.69-fold higher than MI combined with PCQ-SMS at 240 min ($p < 0.001$). Moreover, Cmax of MI plus DCI recorded at 180 min (Tmax) was about 1.32-fold higher than MI combined with PCQ-SMS at 240 min ($p < 0.001$). PK parameters of the

Table 1. Average values of blood glucose in the subjects of the study.

	t = 0′	t = 360′ [a]	t = 540′
Mean (mg/dL)	85	92	86
SD	3.3	10.1	7.2

[a] 60′ after lunch. SD = Standard deviation.

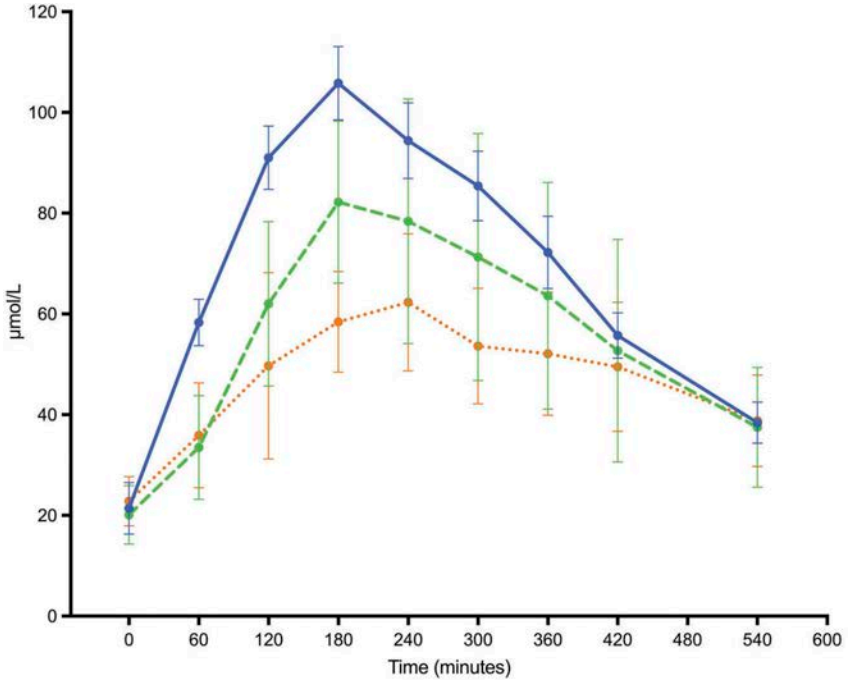

Figure 1. Comparison of myo-inositol (MI) concentrations (µmol/L) in plasma of 18 healthy volunteers at different time points after oral administration of MI alone (continuous blue line), oral administration of MI with D-chiro-inositol (DCI) (dashed green line), and oral administration of MI with SelectSIEVE® Apple PCQ (PCQ) plus sorbitol, maltodextrin and sucralose (SMS) (dotted orange line). For each point: mean ± standard deviation (SD).

absorption of MI alone, MI with DCI, and MI combined with PCQ-SMS are shown in Table 2.

4. Discussion

The aim of our study was to evaluate the changes in the PK profile of MI absorption in humans when administered combined with DCI or glucose transporter inhibitors, as compared to MI alone. Our study demonstrates that the absorption of MI is reduced when administered with DCI, with a reduction of the AUC 0-540 of 19.1% and a reduction of 22.3% of the Cmax. Similarly, the glucose transporter inhibitors, which are present in PCQ-SMS, can cause, at least in part, the reduction of MI absorption (AUC 0-540 decreases by 31.8% and Cmax by 41.1%).

Although the absorption of MI can occur by a diffusion process at high MI concentrations, the uptake of Inositols by cells is primarily carried out by a complex system of transporters, which mediate an active transport of Inositols. Na^+-coupled transport is exerted by Sodium/Myo-Inositol Transporter-1 (SMIT1) and Sodium/Myo-Inositol Transporter-2 (SMIT2), and H^+-coupled transport is exerted by H^+/myo-inositol transporter (HMIT) [30]. These MI transporters have different tissue distribution in human body and are of medical interest due to the growing body of evidence about their role in different diseases [30]. HMIT is primarily expressed in the brain, and at a lesser extent in the kidney, adipose tissue, and oocyte [31,32]. SMIT1 RNA was detected in the brain, heart, kidney, lung and bone tissue [33,34]. SMIT2 RNA was identified in brain, kidney, heart, skeletal muscle, spleen, liver, placenta, lung, leukocytes, neurons, oocytes, and small intestine [31,35–

Table 2. Pharmacokinetic parameters after oral administration of myo-inositol in healthy volunteers in the three formulations.

	1	2			3				
Phase	MI	MI + DCI	Δ 1–2 (%)	p-value 1–2	MI + PCQ SMS	Δ 1–3 (%)	Δ 2–3 (%)	p-value 1–3	p-value 2–3
Cmax (µmol/L)	105.8 ± 7.3	82.2 ± 16.1	−22.3	<0.001	62.3 ± 13.6	−41.1	−24.2	<0.001	<0.001
Tmax (min)	180 min	180 min			240 min				
AUC (0–540)	38,385 ± 812.9	31,056 ± 2,632	−19.09	0.0118	26,187 ± 1,699	−31.8	−15.7	<0.001	0.1294

Data are expressed as mean ± Standard deviation (SD). Δ = difference.
Cmax (µmol/L) = maximum observed plasma concentration during the 0–540 min dosing interval; Tmax = Time (min) to reach the peak concentration; AUC (0-540) (µmol·min/L) = area under the time-course curve of plasma concentration, from baseline to 540 min. MI = Myo-Inositol; DCI = D-Chiro-Inositol; PCQ = SelectSIEVE® Apple PCQ; SMS = Sorbitol, Maltodextrin and Sucralose.

37]. The detection of SMIT2 RNA in small intestine mucosa suggests that SMIT2 may be the primary transporter that mediates intestinal absorption of MI.

Based on the data analysis of our study, we may speculate about an inhibitory effect of DCI on MI absorption in humans. SMIT2 transports MI with an average K_m of 120–150 µM (spanning between 67 and 283 µM), which is consistent with MI human plasma concentration having a mean value of 32.5 ± 1.5 µM, with a range of 26.8–43.0 µM [38]. Conversely, although DCI is transported with an average K_m of 110–130 µM similar to that of MI, the average plasma level of DCI is less than 100 nM [35–38]. Therefore, DCI transport represents usually a minor physiological activity of SMIT2 due to the low concentration of DCI as compared to MI [30,35–37]. Nevertheless, when DCI is administered at high dosage and achieve higher concentration it is able to compete with MI. This mechanism may explain the reason why administration of DCI at high dosage seems to be able to interfere and inhibit the intestinal transport of MI as reported in our study, and this element should be carefully considered when it is necessary to achieve the correct diet supplementation of Inositols. Moreover, the DCI affinity represents the primary difference between the two SMIT transport systems: indeed, DCI is transported with high affinity by SMIT2, but is not transported by SMIT1 [35–37], our results might further clinically confirm the key role of SMIT2 in the intestinal absorption of Inositols [30]. Of note, an involvement of SMIT1 and HMIT cannot be excluded based on our study, and the use of selective inhibitors of these other two transporters are required to test their role in human small intestine [36].

SMIT1 and SMIT2 are members of the SLC5 human gene sub-family of the Sodium Substrate Symporter Gene Family (SSSF), of which only a few genes were identified so far. The sodium-coupled glucose cotransporters-1 (SGLT1) is the most studied member of the SLC5 family; SGLT1 is primarily expressed in the small intestine, and its natural substrates are glucose and galactose with a K_m of 0.5 mM [33]. Both SMIT1 and SMIT2 (SGLT6) show only a low affinity for glucose with an average K_m value of 50 mM for SMIT1 and 30 mM (36 ± 7 mM) for SMIT2, which is well above normal serum glucose levels and determines a limit of glucose transport through SMIT1 and SMIT2 [30,35–37]. On that basis, although diet supplementation of Inositols should be taken upon fasting, it is difficult that glucose achieves such concentration to inhibit MI absorption. Nevertheless, the similarities between SGLT1 and SMIT2, that are members of the same gene family, can determine the interaction with the same inhibitors [33]. Phlorizin is a dihydrochalcone naturally produced by different fruit trees. Phlorizin is a non-transported competitive inhibitor of sodium-coupled sugar co-transporters, and it is more potent on the external side of the membrane than on the internal side. Considering this point, it was used in the treatment of diabetes, obesity, and stress hyperglycemia because of the induction of renal glycosuria and the block of intestinal glucose absorption [39]. Phlorizin represents an important component of PCQ providing hypoglycemic activity in humans and improvement in glucose metabolism. Nevertheless, like SGLT1, SMIT2 is sensitive to phlorizin, which acts as a potent inhibitor with an average K_i of 15 ± 6 µM [35–37,39]. This data may explain part of our study results: indeed, the administration of PCQ-SMS interfered and inhibited the transport of MI with a reduction of AUC 0-540 of 31.8% as compared to MI alone, and this may be explained by the 25.5 mg of phlorizin. In addition, although

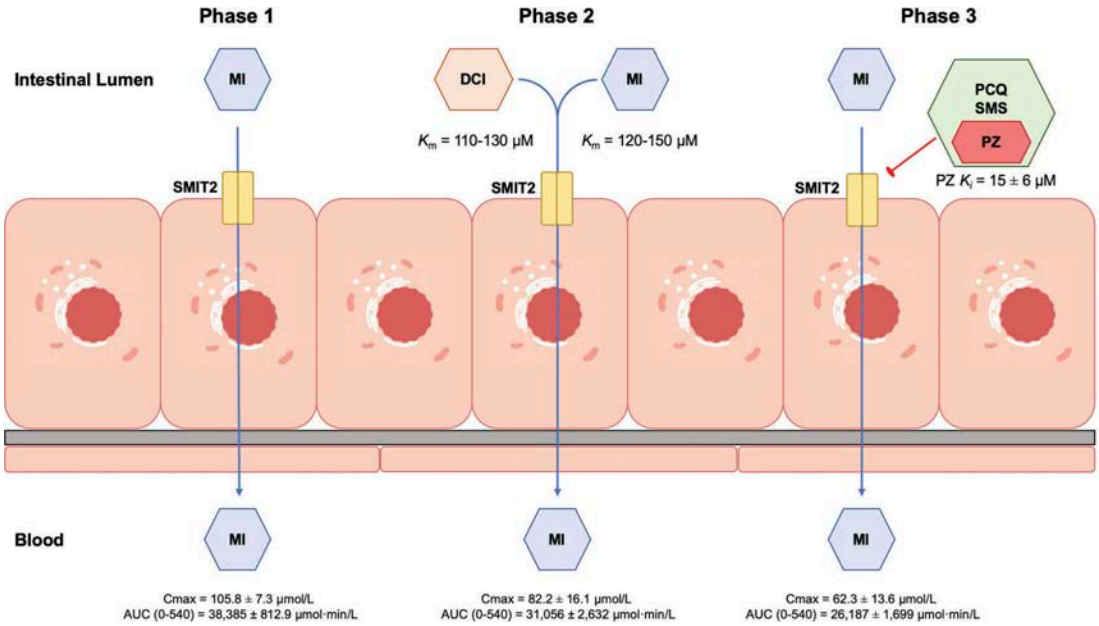

Figure 2. The figure describes the three phases of the study. Phase 1: administration of myo-inositol (MI) alone. Phase 2: administration of MI with D-chiro-inositol (DCI), which provides a competitive inhibition for the same transporter. Phase 3: administration of MI with SelectSIEVE® Apple PCQ (PCQ) plus sorbitol, maltodextrin and sucralose (SMS), which inhibits the transporter of MI.

Cmax (µmol/L) = maximum observed plasma concentration in the 0–540 min dosing interval; AUC (0–540) (µmol·min/L) = area under the time-course curve of plasma concentration, from baseline to 540 min. SMIT2 = Sodium/Myo-Inositol Transporter-2; PZ = Phlorizin.

some compounds may improve glucose metabolism, it should be taken into account that any molecule that counteracts glucose absorption may inhibit MI absorption as well, causing a reduced bioavailability of MI.

Interestingly, the administered 25.5 mg of phlorizin seems to be a small quantity as compared to the 6000 mg of MI to justify the 31.8% reduction of AUC 0-540. Nevertheless, this result may be explained by the small K_i of phlorizin as compared to the K_m of MI (K_i phlorizin 15 ± 6 μM vs K_m MI 120–150 μM) and by the non-transported competitive inhibitor role of phlorizin, which persists in intestinal lumen as compared to the transported competitive inhibitor role of DCI [35–37,39]. However, the inhibition of MI absorption in MI plus PCQ-SMS phase may be partially further explained by the other sugar molecules, such as sorbitol and maltodextrin. Maltodextrins are a variable source of rapidly absorbed glucose that may provide a local high concentration able to interfere with MI absorption due to an enzymatic digestion that takes place at a high rate [40]. Sorbitol is a sugar alcohol with slow metabolism that seems transported by GLUT2 and GLUT5, although a specific transport system is not clearly identified. The available evidence suggests that sorbitol provides an inhibitory effect on MI transport, particularly at high MI concentration [41,42].

Figure 2 provides a schematic explanation of the three phases and of the inhibitory effects of DCI and PCQ-SMS.

Despite our results are consistent with previously available pieces of evidence, this study has some limitations that should be taken into account for a proper interpretation. Although a sample size calculation was performed (see *'Statistical analysis'* section), the study population is small and based on healthy subjects, which do not represent the actual population that may require supplementation with Inositols. Therefore, the PK of Inositols could be different in the target population. The study was designed to evaluate the PK of MI as compared to MI plus DCI and MI plus PCQ-SMS, and not to compare MI plus DCI with MI plus PCQ-SMS; therefore, although results suggest a higher inhibitory effect of PCQ-SMS compounds as compared to DCI, any robust conclusion cannot be drawn about this point. Finally, the study does not provide any information about the clinical relevance of observed differences in PK profiles. Nevertheless, the strict inclusion criteria of healthy volunteers and the check of exclusion criteria before and after each phase of the study allow to exclude pathological alteration of MI PK and strength the physiological value of the model. Moreover, the evaluation of MI plasma concentrations every 60 minutes until 540 minutes provides an accurate description of MI time-concentration curve.

5. Expert opinion

Based on our study, the combined administration of oral DCI with MI and of glucose transporter inhibitors plus Sorbitol, Maltodextrin and Sucralose with MI seems to be able to inhibit MI absorption, leading to lower plasma concentration respect to the administration of MI alone. Moreover, the inhibitory effect of DCI on intestinal absorption of MI may confirm a potential role of SMIT2 as primary transporter of Inositols at intestinal mucosa in humans. Therefore, the diet supplementation of MI may require to be modulated based on the used combined formulation: the association of MI with DCI or with PCQ-SMS may require higher dosage of MI to match the reference dosage of MI alone, particularly when the aim is to achieve a specific MI plasma level. Further studies are required both to improve the knowledge about Inositol transport in humans and to better define the optimal dosage of MI and DCI in pathologies other than PCOS.

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Author contributions

G. Monastra: study conceptualization and organization, protocol planning, laboratory analyses, data collection, manuscript editing. S. Garzon and A.S. Laganà: data analysis, manuscript writing. All the authors conform to the International Committee of Medical Journal Editors (ICMJE) criteria for authorship, contributed to the intellectual content of the study and gave approval for the final version of the article.

ORCID

Simone Garzon http://orcid.org/0000-0002-5840-699X
Antonio Simone Laganà http://orcid.org/0000-0003-1543-2802
Giovanni Monastra http://orcid.org/0000-0003-2012-1435

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