

Finding the best therapeutic approach for PCOS: the importance of inositol(s) bioavailability

B. ORRÙ¹, R. CIRCO¹, P. LOGOTETA¹, S. PETOUSIS², G. CARLOMAGNO¹

¹R&D Department, Lo.Li. Pharma, Rome, Italy

²IAKENTRO, Infertility Treatment Center, Thessaloniki, Greece

Abstract. – Broadening clinical evidence has markedly designated inositol(s) as a common and effective therapeutic approach for PCOS and infertility. Although considerable research has been focused on the use in clinical practice of myo-inositol (myo-ins) and D-chiro-inositol (D-chiro-ins), the two major inositol stereoisomers, less attention has been paid to their bioavailability. Therefore, the aim of this paper is to gather and analyze information on inositol(s) bioavailability, to better delineate its optimal concentration for scientific and clinical purposes. Throughout the search in PubMed, Google Scholar, and ResearchGate we identified only two studies that investigated the pharmacokinetic (PK) profile of different myo-ins administrations. This analysis found no advantage in terms of PK for single 4 g dosing of myo-ins compared to 2 g twice a day, which allowed to get a 24-hour coverage, contrary to the singular dose. Indeed, the differences regarding the area under the curve (AUC) between the two PK profiles are linked only to the maximum concentration (Cmax) but not to the time variable. In conclusion, splitting the therapeutic dosage of 4 g myo-ins in two distinct administrations seems to be the best approach for a full-day coverage.

Key Words:

Inositol, Myo-inositol, Bioavailability, Pharmacokinetics, Polycystic ovary syndrome.

Introduction

Myo-inositol (myo-ins) is a natural molecule involved in cell morphogenesis and cytogenesis, lipid synthesis and cell growth. It regulates many important cellular processes including cell proliferation and development, gametogenesis, fertilization, secretion, contraction and neural activity^{1,2}. Myo-ins is a precursor for many ino-

sitol-containing compounds that play different roles in signal transduction, vesicle trafficking, membrane biogenesis and chromatin remodeling³.

The PREIS school (Permanent International and European School in Perinatal, Neonatal and Reproductive Medicine) has promoted and organized an International Consensus Conference on myo-ins and D-chiro-inositol (D-chiro-ins), the two major inositol stereoisomers, to better clarify and define their role in the clinical application of Obstetrics and Gynecology⁴. The scientific committee was formed by experts in the field of cell biology, embryology, human endocrinology, obstetrics and gynecology with deep knowledge of physiology, biochemistry, and pharmacology of inositol(s).

Results from the International Consensus Conference (ICC) have further stated that myo-ins and D-chiro-ins are involved in insulin-signaling and mediate different insulin-dependent processes; while the first one is critical to the glucose uptake, the second one mediates glycogen synthesis. Furthermore, in the ovaries, myo-ins regulates follicle stimulating hormone (FSH) signaling, whereas D-chiro-ins the insulin-mediated androgen production. Due to their versatile actions, applied researchers have become increasingly interested in the possible role and use of inositol(s) in clinical practice⁴⁻⁶. In fact, several clinical trials have shown how myo-ins and D-chiro-ins can be effectively used as a treatment for polycystic ovary syndrome (PCOS)⁷, infertility⁸⁻¹⁰ and metabolic syndrome¹¹. However, even though many studies have reported the effectiveness of such use, there has been very little research reported on the bioavailability of myo-ins. With a high oral bioavailability, the drug dosage can be reduced, diminishing the risk of side effects and toxicity, although maintaining the desired pharmacological effect.

Therefore, the purpose of the present review is to gather and analyze information on myo-ins bioavailability, to better understand and define its optimal concentration for clinical practice.

Myo-ins Bioavailability

Throughout the search in PubMed, Google Scholar, and ResearchGate only two articles on the bioavailability of myo-ins were identified^{12,13}. In one study the plasma concentration – time data profile after oral administration of myo-ins in powder or softgel capsules was compared¹². Interesting results highlighted how 2 g of myo-ins in powder has the same pharmacokinetic (PK) profile as 0.6 g in softgel capsules. Same results were obtained for 4 g of myo-ins in powder and 1.2 g in softgel capsules. Using the softgel capsules, several advantages such as improved bioavailability, lower dosages, reduced gastrointestinal side effects, masking odors and protection of compounds against external agents were observed. Moreover, the area under the curve (AUC) after administration of 2 g and 4 g of myo-ins in powder seemed to be remarkably similar. The only difference regarding the AUC between the two PK profiles is linked to the maximum concentration (C_{max}) but not to the time variable.

The second study included in our review demonstrated that coffee intake strongly interferes with myo-ins absorption rate¹³. The administration of myo-ins in soft gelatin capsule improved the bioavailability compared to the myo-ins powder form, besides reducing caffeine interference.

The pharmacological interaction between molecules has gained wide importance in the practice of medicine. On occasion, these interactions may be sought to obtain an improved therapeutic effect, but they may also increase the risk of side effects or overdoses. On the other hand, if the action of a molecule is reduced it may weaken or even cease its therapeutic effect because of lower absorption.

Many food supplements containing a different concentration of myo-ins, often combined with other molecules like folic acid, melatonin, glucomannan, vitamin D, cinnamon or alpha lipoic acid are available in the market. For many of these compounds, PK studies and/or data on their interaction not always are available. It is worth noting that in some products myo-ins is mixed with high molecular weight molecules such as glucomannan¹⁴. This well-known water-soluble fiber impairs nutrients absorption, through its mechanism

of action in the intestinal epithelium, promoting weight loss¹⁵. Therefore, even though glucomannan is generally well tolerated and has a favorable safety profile, additional studies on interference with other molecules might be required. As vitamin D deficiency has been correlated with insulin resistance and infertility, this molecule is often combined with myo-ins to improve ovulatory function, menstrual cyclicity and egg quality in PCOS women. In fact, it has been shown that vitamin D modulates several regulatory pathways in human reproduction^{16,17}. Furthermore, it aids normalizing the blood glucose levels and reducing the incidence of gestational diabetes¹⁸. However, the potential adverse effects related to an overconsumption are still controversial, due to the lack of data regarding the long-term use of high doses vitamin D in women of childbearing age. Likewise, it is still unclear which regimen of vitamin D supplementation is most effective during pregnancy¹⁹. Therefore, long-term studies are required either on the correlation between vitamin D supplementation during pregnancy and the adverse health outcomes in the offspring as well as on its interference with other molecules. This might avoid erroneous over-dosages that can be toxic to mother and fetus. Indeed, oral absorption can be influenced by a variety of factors such as the physiochemical properties, formulation, and compound-dosing, as well as the physiology and pathology of the gastrointestinal tract.

One interesting study on PK analysis of melatonin identified an improved bioavailability of 1 mg melatonin encapsulated in softgel compared to higher doses in powder. Considering the numerous physiological and pathological conditions in which melatonin supplementation is recommended, these findings highlight a significant clinical advantage for the treatment of such disorders²⁰. Indeed, a study showed how supplementation of melatonin combined with myo-ins in a softgel formula, improved in vitro fertilization (IVF) outcomes in PCOS patients²¹. Likewise, several trials showed the remarkable efficacy of inositol(s) combined with the folic acid in PCOS treatment¹¹. Folate is a vitamin that plays important health benefits. Its use for prevention of neural tube defects²²⁻²⁴, cardiovascular diseases^{25,26}, anemia²⁷ or cognitive impairment²⁸ is well recognized. The preventive effect of high folic acid intake by women of childbearing age against neural tube defects has been considered one of the most important nutritional breakthrough²⁹. Findings from early bioavailability studies have shown that

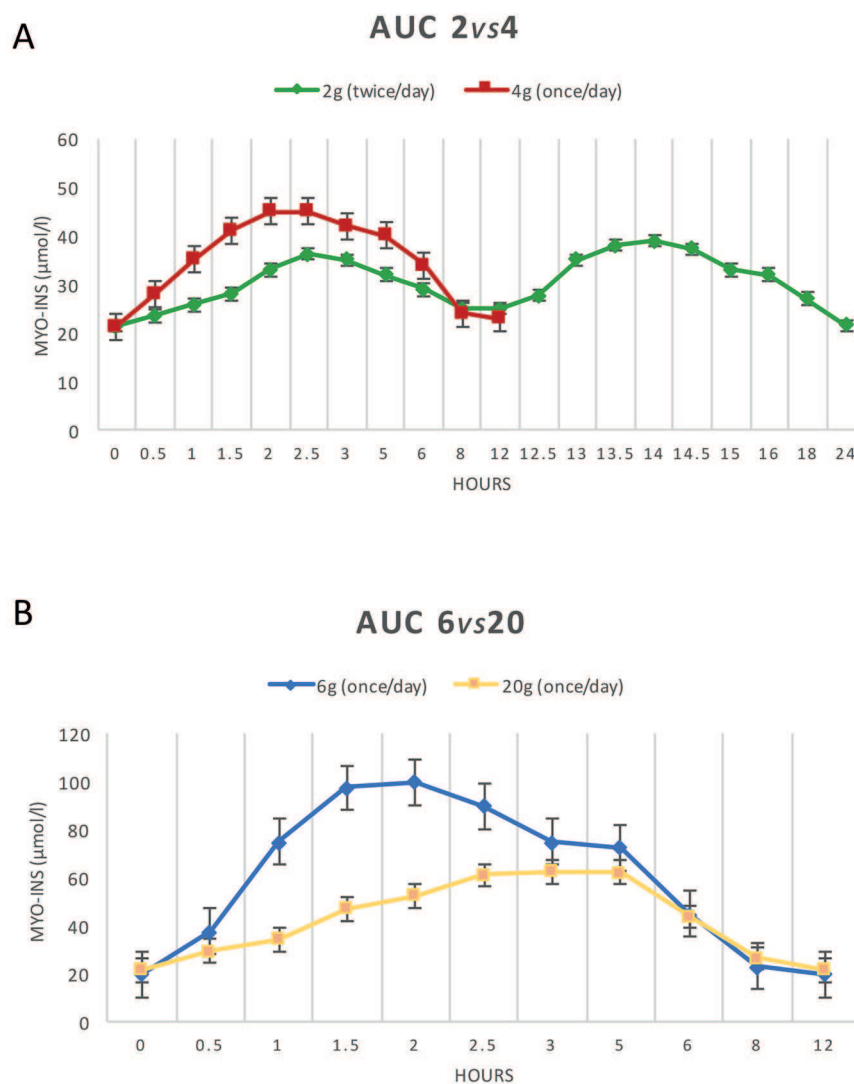


Figure 1. *A*, Comparison of the plasma concentration-time values after administration of 2 g (twice a day) or 4 g myo-ins (once a day) in powder. A washout between the two different administrations (2 g and 4 g) was carried out. *B*, Comparison of the plasma concentration-time values after administration of 6 g or 20 g myo-ins (once a day) in powder. A washout between the two different administrations (6 g and 20 g) was carried out.

the plasma/serum folate concentration increased by 44% after administration of folic acid dietary supplement³⁰. On the other hand, further investigation is needed to delineate the potential interferences with inositol(s) absorption and bioavailability for an optimal therapeutic approach^{13,31}.

Interesting Unpublished Data

Considering the existing data, it is worth sharing unpublished data that might provide useful reflections about the PK parameters of myo-ins dosages. Results were obtained from the analysis of myo-ins plasma concentration of 23 healthy volunteers (10 men and 13 women, aged between 20 and 40

years, with a body mass index ranging between 21 and 25 kg/m²) after myo-ins dosing of 2 g (twice a day) or 4 g (once a day) in powder. A washout between the two-different administrations (2 g and 4 g) was carried out. The C_{max} was dose-dependent, marking a higher peak after administration of 4 g myo-ins (Figure 1A). After 8 hours (T_{min}), both dosages reached the same concentration. However, the second administration of 2 g myo-ins after 12 hours gave another peak, like the first one, guarantying almost 24-hour coverage. Similar results were observed after 6 g and 20 g of myo-ins in powder (once a day) (Figure 1B). Surprisingly, C_{max} was greater after 6 g of myo-ins compared to

20 g. Furthermore, there was no difference in the PK parameters between genders (data not shown). Some severe gastrointestinal (GI) adverse effects were reported in all participants receiving 20 g of myo-ins, as expected. Mild GI side effects were observed in five subjects after administration of 6 g of myo-ins. It is already known that high doses of myo-ins (12 g/day) may cause GI side effects such as diarrhea, nausea, and flatus decreasing patient's compliance³². These results have emphasized that a single-dose of 4 g has no PK advantage compared to the administration of 2 g. The difference between the two PK profiles regarding the AUC is only linked to the C_{max} but not to the time variable. Therefore, from the PK data available it can be deduced that 2 g of myo-ins twice a day is the preferable therapeutic approach to guarantee almost 24-hour coverage.

Discussion

The history of myo-ins is now well structured, and its proven insulin-sensitizing role has expanded horizons for the prevention and treatment of common endocrine-metabolic disorders^{5,6,11}. PCOS, as an example, is characterized by metabolic and reproductive alterations, due to an unbalanced myo-ins and D-chiro-ins ratio^{33,34}. This condition involves insulin signaling and FSH pathway aberrations impairing follicular growth. Positive evidences have featured the use of myo-ins in two different commercial formulations for the treatment of PCOS or improvement in IVF outcomes¹¹. One formula comprises 4 g of myo-ins in powder, usually reported as a dosage of 2 g twice a day (sachets)^{8,35-37}. The other combines the physiologic plasma ratio of myo-ins and D-chiro-ins (40:1) with the innovative technology of softgel capsules. Recently, some clinical trials adopted this new formulation and provided promising results^{10,38,39}. It has been shown also that this combined therapy, myo-ins plus D-chiro-ins in their physiological ratio, reduces the cardiovascular risk by improving the lipid profile in PCOS women⁴⁰.

In October 2016, the Italian Ministry of Health indicated 4 g as the maximum daily intake of inositol. Even though the data presented here showed a higher peak with the administration of 4 g myo-ins, the bioavailability is not covered for the full day. Considering the Italian Ministry of Health recommendations and the side effects correlated with high doses of inositol, it is obvious that taking

4 g twice a day would not be the right choice either for patient's compliance and ethical reasons.

The presence of side effects, like diarrhea and nausea, might diminish the absorption of higher dosages of myo-ins and this may lead to a lower bioavailability. This is seen after administration of 20 g myo-ins compared to 6 g, which showed a greater C_{max} . Such difference might be caused also by oversaturation of the single dose of 20 g myo-ins, that consequently may trigger inflammation in the gastrointestinal tract. Myo-ins has been used at high dosages as a potential chemo-preventive agent in smokers with bronchial dysplasia⁴¹. Eighteen grams per day in two divided doses were the maximum tolerated, with mainly mild gastrointestinal adverse effects. In our study, in terms of bioavailability, there is an ample gap between 6 g and 20 g per day; therefore, it could be useful to investigate how the myo-ins PK profile varies in this dose range.

A poor oral bioavailability may impair drug efficacy. Thus, for drug design and development, PK analysis are important steps in the research phase, to understand the mechanism of drug action and identify PK properties. Therefore, the PK studies available in literature on myo-ins bioavailability, and the unpublished data that have been reported in this review confirmed: 1) the optimal therapeutic approach for PCOS therapy is the administration of 2 g myo-ins twice daily; 2) the softgel capsules improve molecule bioavailability, representing a clinical advantage; 3) evidence of myo-ins bioavailability when combined with other molecules is mandatory.

Conclusions

Proven PK profile, as well as clinical evidences, are critical steps to support the effectiveness of treatment. In this specific analysis, the administration of 2 g myo-ins twice a day seems to be the best therapeutic approach in PCOS women for a 24-hour coverage guaranteed. Extended studies on the bioavailability of the numerous molecules combined with myo-ins are strongly recommended to avoid bias on the valuation of their dosages and efficacy.

Conflict of interest

Beatrice Orrù, Rita Circo, Patrizia Logoteta and Gianfranco Carlomagno are employees at L.O.L.I. Pharma, Rome, Italy. The other author declares that has no conflict of interest regarding the publication of this paper.

References

- 1) PAPALEO E, UNFER V, BAILLARGEON JP, CHIU TT. Contribution of myo-inositol to reproduction. *Eur J Obstet Gynecol Reprod Biol* 2009; 147: 120-123.
- 2) BIZZARRI M, FUSO A, DINICOLA S, CUCINA A, BEVILACQUA A. Pharmacodynamics and pharmacokinetics of inositol(s) in health and disease *Expert Opin Drug Metab Toxicol* 2016; 12: 1181-1196.
- 3) AGRANOFF BW. Turtles all the way: reflections on myo-Inositol. *J Biol Chem* 2009; 284: 21121-21126.
- 4) FACCHINETTI F, BIZZARRI M, BENVENGA S, D'ANNA R, LANZONE A, SOULAGE C, DI RENZO GC, HOD M, CAVALLI P, CHIU TT, KAMENOV ZA, BEVILACQUA A, CARLOMAGNO G, GERLI S, OLIVA MM, DEVROEY P. Results from the International Consensus Conference on Myo-inositol and d-chiro-inositol in Obstetrics and Gynecology: The link between metabolic syndrome and PCOS. *Eur J Obstet Gynecol Reprod Biol* 2015; 195: 72-76.
- 5) BIZZARRI M, CARLOMAGNO G. Inositol: history of an effective therapy for Polycystic Ovary Syndrome. *Eur Rev Med Pharmacol Sci* 2014; 18: 1896-1903.
- 6) UNFER V, ORRÙ B, MONASTRA G. Inositols: from physiology to rational therapy in gynecological clinical practice. *Expert Opin Drug Metab Toxicol* 2016; 12: 1129-1131.
- 7) UNFER V, CARLOMAGNO G, DANTE G, FACCHINETTI F. Effects of myo-inositol in women with PCOS: a systematic review of randomized controlled trials. *Gynecol Endocrinol* 2012; 28: 509-515.
- 8) UNFER V, CARLOMAGNO G, RIZZO P, RAFFONE E, ROSEFF S. Myo-inositol rather than D-chiro-inositol is able to improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial. *Eur Rev Med Pharmacol Sci* 2011; 15: 452-457.
- 9) PAPALEO E, UNFER V, BAILLARGEON J-P, FUSI F, OCCHI F, DE SANTIS L. Myo-inositol may improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial. *Fertil Steril* 2009; 91: 1750-1754.
- 10) COLAZINGARI S, TREGLIA M, NAJJAR R, BEVILACQUA A. The combined therapy myo-inositol plus D-chiro-inositol, rather than D-chiro-inositol, is able to improve IVF outcomes: results from a randomized controlled trial. *Arch Gynecol Obstet* 2013; 288: 1405-1411.
- 11) UNFER V, NESTLER JE, KAMENOV ZA, PRAPAS N, FACCHINETTI F. Effects of Inositol(s) in women with PCOS: a systematic review of randomized controlled trials. *Int J Endocrinol* 2016; 2016: 1849162.
- 12) CARLOMAGNO G, DE GRAZIA S, UNFER V, MANNA F. Myo-inositol in a new pharmaceutical form: a step forward to a broader clinical use. *Expert Opin Drug Deliv* 2012; 9: 267-271.
- 13) DE GRAZIA S, CARLOMAGNO G, UNFER V, CAVALLI P. Myo-inositol soft gel capsules may prevent the risk of coffee-induced neural tube defects. *Expert Opin Drug Deliv* 2012; 9: 1033-1039.
- 14) GONZÁLEZ CANGA A, FERNÁNDEZ MARTÍNEZ N, SAHAGÚN AM, GARCÍA VIENTEZ JJ, DIEZ LIÉBANA MJ, CALLE PARDO AP, CASTRO ROBLES LJ, SIERRA VEGA M. Glucomannan: properties and therapeutic applications. *Nutr Hosp organo Of la Soc Esp Nutr Parenter y Enter* 2004; 19: 45-50.
- 15) KEITHLEY JK, SWANSON B, MIKOLAITIS SL, DEMEO M, ZELLER JM, FOGG L, ADAMJI J. Safety and efficacy of glucomannan for weight loss in overweight and moderately obese adults. *J Obes* 2013; 2013: 610908.
- 16) LUK J, TORREALDAY S, NEAL PERRY G, PAL L. Relevance of vitamin D in reproduction. *Hum Reprod* 2012; 27: 3015-3027.
- 17) JOHNSON LE, DELUCA HF. Vitamin D receptor null mutant mice fed high levels of calcium are fertile. *J Nutr* 2001; 131: 1787-1791.
- 18) ASEMI Z, HASHEMI T, KARAMALI M, SAMIMI M, ESMAILZADEH A. Effects of vitamin D supplementation on glucose metabolism, lipid concentrations, inflammation, and oxidative stress in gestational diabetes: a double-blind randomized controlled clinical trial. *Am J Clin Nutr* 2013; 98: 1425-1432.
- 19) SOWELL KD, KEEN CL, URIU-ADAMS JY. Vitamin D and reproduction: from gametes to childhood. *Healthc (Basel, Switzerland)* 2015; 3: 1097-1120.
- 20) PROIETTI S, CARLOMAGNO G, DINICOLA S, BIZZARRI M. Soft gel capsules improve melatonin's bioavailability in humans. *Expert Opin Drug Metab Toxicol* 2014; 10: 1193-1198.
- 21) PACCHIAROTTI A, CARLOMAGNO G, ANTONINI G, PACCHIAROTTI A. Effect of myo-inositol and melatonin versus myo-inositol, in a randomized controlled trial, for improving in vitro fertilization of patients with polycystic ovarian syndrome. *Gynecol Endocrinol* 2015; 3590: 1-5.
- 22) GENETICS C ON. Folic Acid for the Prevention of Neural Tube Defects. *Pediatrics* 1999; 104.
- 23) MRC VITAMIN STUDY RESEARCH GROUP. Prevention of neural tube defects: Results of the Medical Research Council Vitamin Study. *Lancet* 1991; 338: 131-137.
- 24) WALD NJ. Folic acid and the prevention of Neural-tube Defects. *Annu Rev Nutr* 1996; 16: 73-97.
- 25) YANG HT, LEE M, HONG KS, OVBIAGELE B, SAVER JL. Efficacy of folic acid supplementation in cardiovascular disease prevention: an updated meta-analysis of randomized controlled trials. *Eur J Intern Med* 2012; 23: 745-754.
- 26) WANG X, QIN X, DEMIRTAS H, LI J, MAO G, HUO Y, SUN N, LIU L, XU X. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. *Lancet* 2007; 369: 1876-1882.
- 27) THORADENIYA T, WICKREMASINGHE R, RAMANAYAKE R, ATUKORALA S. Low folic acid status and its association with anaemia in urban adolescent girls and women of childbearing age in Sri Lanka. *Br J Nutr* 2006; 95: 511-516.
- 28) MA F, WU T, ZHAO J, SONG A, LIU H, XU W, HUANG G. Folic acid supplementation improves cognitive function by reducing the levels of peripheral inflammatory cytokines in elderly Chinese subjects with MCI. *Sci Rep* 2016; 6: 37486.
- 29) KATAN M, BOEKSCHOTEN M, CONNOR W, MENSINK RP, SEIDELL J, VESSBY B, WILLET W. Which are the greatest recent discoveries and the greatest future challenges in nutrition? *Eur J Clin Nutr* 2009; 63: 2-10.
- 30) OHRVIK VE, WITTHOFT CM. Human folate bioavailability. *Nutrients* 2011; 3: 475-490.
- 31) WILLIAM H, DAUGHADAY J, LARNER EH. The renal excretion of inositol in normal and diabetic human beings. *J Clin Invest* 1954; 33: 1075-1080.

- 32) CARLOMAGNO G, UNFER V. Inositol safety: Clinical evidences. *Eur Rev Med Pharmacol Sci* 2011; 15: 931-936.
- 33) CARLOMAGNO G, UNFER V, ROSEFF S. The D-chiro-inositol paradox in the ovary. *Fertil Steril* 2011; 95: 2515-2516.
- 34) UNFER V, CARLOMAGNO G, PAPALEO E, VAILATI S, CANDIANI M, BAILLARGEON J-P. Hyperinsulinemia alters Myoinositol to d-chiroinositol ratio in the follicular fluid of patients with PCOS. *Reprod Sci* 2014; 21: 854-858.
- 35) CIOTTA L, STRACQUADANIO M, PAGANO I, CARBONARO A, PALUMBO M, GULINO F. Effects of Myo-Inositol supplementation on oocyte's quality in PCOS patients: A double blind trial. *Eur Rev Med Pharmacol Sci* 2011; 15: 509-514.
- 36) COSTANTINO D, MINOZZI G, MINOZZI F, GUARALDI C. Metabolic and hormonal effects of myo-inositol in women with polycystic ovary syndrome: a double-blind trial. *Eur Rev Med Pharmacol Sci* 2009; 13: 105-110.
- 37) GERLI S, PAPALEO E, FERRARI A, DI RENZO GC. Randomized, double blind placebo-controlled trial: effects of myo-inositol on ovarian function and metabolic factors in women with PCOS. *Eur Rev Med Pharmacol Sci* 2007; 11: 347-354.
- 38) BENELLI E, DEL GHIANDA S, DI COSMO C, TONACCHERA M. A combined therapy with Myo-Inositol and D-Chiro-Inositol improves endocrine parameters and insulin resistance in PCOS young overweight women. *Int J Endocrinol* 2016; 2016.
- 39) NORDIO M, PROIETTI E. The combined therapy with myo-inositol and D-Chiro-inositol reduces the risk of metabolic disease in PCOS overweight patients compared to myo-inositol supplementation alone. *Eur Rev Med Pharmacol Sci* 2012; 16: 575-581.
- 40) MINOZZI M, NORDIO M, PAJALICH R. The combined therapy myo-inositol plus D-chiro-inositol, in a physiological ratio, reduces the cardiovascular risk by improving the lipid profile in PCOS patients. *Eur Rev Med Pharmacol Sci* 2013; 17: 537-540.
- 41) LAM S, McWILLIAMS A, LERICHE J, MACAULAY C, WATTENBERG L, SZABO E. A Phase I Study of myo-Inositol for Lung Cancer Chemoprevention. *Cancer Epidemiol Biomarkers Prev* 2006; 15: 1526-1531.