

EXPERT OPINION

1. Introduction
2. Materials and methods
3. Results
4. Discussion
5. Study limitation
6. Conclusions

Myo-inositol soft gel capsules may prevent the risk of coffee-induced neural tube defects

Sara De Grazia, Gianfranco Carlomagno[†], Vittorio Unfer & Pietro Cavalli

[†]A.G.UN.CO., Obstetrics and Gynecology Center, Gennaro, Rome, Italy

Objective: Neural tube defects (NTDs) are classified as folate sensitive (about 70%) and folate resistant (about 30%); although folic acid is able to prevent the former, several data have shown that inositol may prevent the latter. It has recently been proposed that coffee intake might represent a risk factor for NTD, likely by interfering with the inositol signaling. In the present study, we tested the hypothesis that, beside affecting the inositol signaling pathway, coffee also interferes with inositol absorption.

Research design and methods: In order to evaluate coffee possible negative effects on inositol gastrointestinal absorption, a single-dose bioavailability trial was conducted. Pharmacokinetics (PK) parameters of myo-inositol (MI) powder and MI soft gelatin capsules swallowed with water and with a single 'espresso' were compared. PK profiles were obtained by analysis of MI plasma concentration, and the respective MI bioavailability was compared.

Results: Myo-inositol powder administration was negatively affected by coffee intake, thus suggesting an additional explanation to the interference between inositol deficiency and coffee consumption. On the contrary, the concomitant single 'espresso' consumption did not affect MI absorption following MI soft gelatin capsules administration. Furthermore, it was observed that MI soft gelatin capsule administration resulted in improved bioavailability compared to the MI powder form.

Conclusions: Myo-inositol soft gelatin capsules should be considered for the preventive treatment of NTDs in folate-resistant subjects due to their higher bioavailability and to the capability to reduce espresso interference.

Keywords: bioavailability, caffeine, myo-inositol, neural tube defects, soft gel

Expert Opin. Drug Deliv. (2012) 9(9):1033-1039

1. Introduction

Neural tube defects (NTDs) are severe birth defects with a multifactorial aetiology involving both genetic and environmental factors [1,2] that arise from failure of embryonic neural tube closure occurring within 8 weeks from conception. Despite periconceptional administration of high doses, folic acid can prevent approximately 70% of all NTDs cases (MRC trial), whereas 30% of NTDs appear unresponsive to folic acid intake, suggesting that a proportion of human NTDs should be considered folate-resistant. Due to the absence of a preventive therapy for this class of subjects, an alternative therapeutic solution is needed to prevent those NTDs that do not respond to folic acid supplementation.

The role of myo-inositol (MI) in neural tube development has been shown in several studies [3-5], and the hypothesis that the disruption of inositol signaling might be responsible for the NTDs has been suggested by experimental models; among these, the curly-tail strain is a genetic model of folate-resistant NTDs; the mutation

informa
healthcare

Article highlights.

- Maternal caffeine intake increases the risk of neural tube defects.
- Coffee consumption impairs myo-inositol absorption.
- There are evidence that myo-inositol is able to prevent folate-resistant NTD.
- Soft gel caps are able to prevent myo-inositol impaired absorption induced by espresso.

This box summarizes key points contained in the article.

induced in this strain closely resembles human NTDs in form and structure, axial location, sex bias and high levels of alpha-fetoprotein in the amniotic fluid [4,5]. Furthermore, folic acid supplementation (or several of its metabolites) is not able to prevent NTDs in curly-tail mice [4,5].

Studies performed on the murine model of folate-resistant NTDs showed that the majority of NTDs in this animal model can be prevented by MI administration [6,7]. Moreover, MI has been found to be effective in preventing NTDs in diabetic rats [8].

In humans, significantly lower MI blood levels have been reported in mothers carrying NTD fetuses compared to healthy pregnancies and mothers with low blood levels of MI have an increased risk of an affected offspring [9-11]. Furthermore, MI supplementation has been associated with a consistent reduction of the recurrence risk for NTDs in humans [12,13], thus suggesting that at least some types of human NTDs can be prevented by MI supplementation.

Two pilot studies performed on patients with a high risk of recurrence for NTDs have shown that periconceptional treatment with 500 – 1000 mg/day of MI (three months before conception and two months after) can reduce the risk of recurrence for NTDs [12,13].

Moreover, it was recently proposed the hypothesis that caffeine, another risk factor for NTDs, may exert that specific teratogenic effect by a mechanism that involves the intracellular pathway of inositol [14].

If so, it could be important to find and describe the possible interactions between inositol and espresso intake, thus explaining at least part of the complex pathogenesis of multifactorial NTDs.

Caffeine intake is considered among the risk factors connected with NTDs. Indeed, a population study [15] identified a positive association between total maternal caffeine intake and spina bifida. Caffeine is rapidly absorbed and because of the absence of cytochrome P450 1A2 in the placenta and the fetus, the amount of caffeine and metabolites available to the fetoplacental unit depends entirely on maternal metabolism [16,17].

Although genetic variability in metabolizing enzymes and environmental factors are considered as a basis to clarify the association between increased occurrence risk of NTDs and caffeine intake, an additional explanation involving

inositol metabolism has also been suggested. Inositol isomer MI is a precursor in the phosphatidylinositol cycle, in which the second messengers diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3) regulate calcium release from intracellular stores.

It was reported that caffeine inhibits the activity of IP3-gated calcium channels in neuronal tissues in a non-cooperative fashion [18] as well as the activity of IP3-generated calcium receptors in smooth muscle cells and cultured mammalian cells [19]. Therefore, caffeine intake was supposed to be associated with an effect mimicking inositol deficiency [13].

It has been reported that caffeine intake interferes with the absorption of several substances and impairs the bioavailability of many drugs [20-22].

For instance, L-throxine was recently added to the list of compounds whose intestinal absorption is tempered by the concomitant consumption of coffee [23].

It is still unknown, however, whether pre-conception caffeine intake interferes with prophylactic therapy for NTDs.

The aim of the present study was to investigate whether coffee intake, which is one of the factors that increases NTDs risk, might be related to MI deficiency or malabsorption.

To get more information about the possible negative effect of a single 'espresso' coffee consumption, a single-dose relative trial on healthy volunteers was performed.

A secondary endpoint of the study consisted in the evaluation of a possible different interference related to MI pharmaceutical forms. In particular, MI powder and a newly developed MI soft gel capsule form were administered and variations of the relative bioavailability after espresso intake were compared.

2. Materials and methods

2.1 Patients and methods

The study involved 12 healthy volunteers enrolled at the AGUNCO Obstetrics and Gynecology Center (Rome, Italy). All the volunteers were women aged between 20 and 40 years with a body mass index (BMI) value between 18 and 24. Subjects were evaluated on the basis of medical history, physical examination and laboratory screenings. Women who were either pregnant, affected by chronic disease and/or received pharmacological treatment in the previous 2-week period were excluded. The study was approved by SIFIOG (Italian Society of Phytotherapy and Dietary Supplements in Obstetrics and Gynecology) ethical committee and before entering the trial all the volunteers gave their written consent.

The study consisted of four different phases in which subjects received a single-dose of MI powder (LO.LI.pharma Rome, Italy) or MI soft gel capsules (patent pending, LO.LI.pharma Rome, Italy), with water or a concomitant consumption of one 'espresso' coffee (corresponding to about 100 mg of caffeine; espresso group).

During Phase I, volunteers were kept for 15 days under an inositol-poor diet and at the end of this period, 4 g of MI

powder was administered in a single dose. In order to highlight the relative pharmacokinetic parameters, the MI plasma concentration was analyzed. Blood samples were collected by venous puncture at predose (0), and at 30, 60, 90, 120, 180, 300, 420, 540, 1440 min post administration.

Phase II consisted of 15 additional days of an inositol-poor diet: basal levels of MI were again measured and then 3.6 g of MI soft gelatin capsules were administered. The same procedure was followed in Phase III and Phase IV, when MI administrations (powder and soft gelatin capsules, respectively) were concomitant to caffeine exposure through a single 'espresso' consumption.

Chelab Pharma Division (Resana, Italy) performed the procedure for the quantification of MI in plasma. A gas chromatography-mass spectrometry (GC-MS) (GC Agilent 6890, Agilent Milano, Italy) analysis was used after extraction and derivatization of the samples. A (1,0 µl) injection was performed on a splitless mode at 270°C and a capillary column Agilent 122-5532 DB-5 ms (0.25 mm × 30 m × 0.25 µm Agilent Milano, Italy) was used. The flow rate was fixed to 1.2 ml/min. Total run time was 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 in post run. A MS 5973 Network Series detector (Agilent Milano, Italy) in sim mode was used to analyze the results.

2.2 PK parameters

The main pharmacokinetic parameters such as C_{max} and T_{max} were obtained from the plasma concentration data and the area under the curve ($AUC_{(0-1440)}$) value referred to the last measured concentration was calculated using GraphPad Prism software (GraphPad Software, Inc., La Jolla USA).

One-way ANOVA followed by Bonferroni correction was used in order to identify statistical differences.

3. Results

All enrolled subjects completed the study. Arithmetic means for AUC, C_{max} and T_{max} related to each of the four phases of the trial are shown in Table 1.

Plasma concentrations related to MI powder administration (Phase I and Phase III) were compared, and variations of the PK profile, due to a concomitant 'espresso' administration, are shown in Figure 1. The C_{max} value for MI powder form was 30% lower in the 'espresso' group (Table 1), suggesting that the coffee intake strongly interferes with MI adsorption rate. Indeed, when MI administration was concomitant with coffee, MI serum concentrations were significantly lower after 120, 180, 300, 420 and 540 min (p value < 0.05 and 0.01, Figure 1).

Different results were obtained when the MI plasma concentrations related to soft gelatin capsule administration (Phase II and Phase IV, Figure 1) were compared. No relevant variation in the PK profile was registered and a nearly equal

C_{max} value was observed (89.2 vs 84.4 µmol/l; Table 1), thus suggesting that the new formulation is able to prevent coffee intake interference.

The T_{max} parameter did not exhibit any variation indicating that the time to peak concentration is equally reached in the four phases.

An interesting difference in MI bioavailability was also observed when the two pharmaceutical forms (powder and soft gel capsules) were compared. In particular, administration of MI in soft gelatin capsules resulted in enhanced bioavailability, with C_{max} value > 90% higher than the one of MI powder.

Subjects that received MI soft capsules did not report any adverse side effect, whereas two cases of mild gastrointestinal discomfort were registered when 4 g of MI powder was administered.

4. Discussion

Primary prevention with folic acid offers a possible universal solution to the problem of morbidity and mortality caused by NTDs. Neural tube defect risk is not only related to folic acid intake, as background serum folate is also a crucial parameter [24]. Despite high dose (4 mg daily) of folic acid administered in the periconceptional period, 1% of pregnancies still have recurrence of NTDs (MRC trial) [24]. This strongly suggests that a proportion of NTD cases is fundamentally resistant to folate supplementation. The MRC study found that about 70% of NTDs are prevented by high-dose folic acid supplementation [24], suggesting that around 30% of NTDs may be unresponsive to folate therapy.

Reports of NTD recurrence in families despite high dose of folate intake [12,25] also strengthen the hypothesis that certain human NTDs are essentially resistant to folic acid preventive therapy. This idea is further supported by the observation that some NTDs are not prevented even when the intake of folic acid is increased [26]. Novel therapies are needed to improve NTD primary prevention, by encompassing folate-resistant cases that currently cannot be prevented by periconceptional supplementation.

Among micronutrient deficiencies that have been associated with an increased risk for NTDs, inositol deficiency seems to play an important role [27]. The relation between NTDs and inositol was first described in 1988 in rat embryos [28] and confirmed in curly-tail mice a few years later [29]. More recently, the hypothesis that inositol deficiency and/or the disruption of inositol signaling might be responsible for at least some subtypes of NTDs has been proved in several murine experimental models [30].

Inositol 1,3,4-triphosphate 5/6-kinase (ITPK1) plays a pivotal role in inositol metabolism, and represents a key regulatory enzyme for highly phosphorylated inositol phosphates including hexakisphosphate (IP6), that drives intracellular regulation of ion channels, transcription and

Table 1. Pharmacokinetic parameters.

Parameter	Powder			
	Baseline	95% CI	Caffeine	95% CI
C _{max} (μmol/l)	45.69	42.95 – 48.43	33.45	31.32 – 35.58
T _{max} (min)	127.6	118.3 – 136.8	133.1	121.4 – 144.8
AUC _(0 – 1440)	47,394	45,736 – 49,052	36,774	35,335 – 38,231

	Soft Gel			
	Baseline	95% CI	Caffeine	95% CI
C _{max} (μmol/l)	88.2	85.89 – 90.52	83.64	81.71 – 85.58
T _{max} (min)	120.6	111.7 – 129.4	121.7	113.3 – 130.1
AUC _(0 – 1440)	79,475	78,034 – 80,915	70,684	69,301 – 72,067

Data are presented as mean ± standard deviation (SD).

AUC_(0 – 1440): (μmol·min/l) area under the plasma concentration–time curve to the last measured concentration; C_{max}: (μmol/l) maximum observed plasma concentration during the 0 – 1440 min dosing interval; T_{max}: Time to peak concentration.

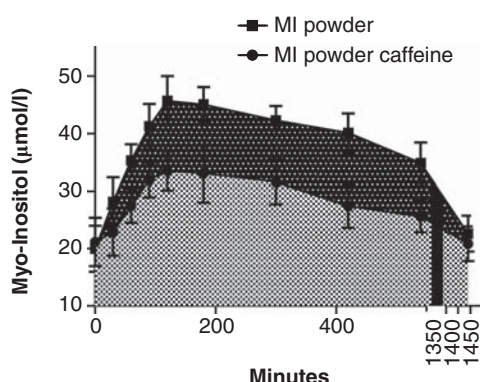


Figure 1. Comparison of the plasma concentration–time data profile for Phase I and Phase III (MI powder and MI powder swallowed with a single espresso).

DNA repair [5,31]. As ITPK1 is expressed in the brain, and is required for proper development of the neural tube, it has been suggested that a disruption of inositol signaling might be responsible for NTDs in the genetically engineered mouse with a hypomorphic allele at ITPK1 [4].

Type I phosphatidylinositol-4-phosphate 5-kinase (PIP5KI) catalyzes the synthesis of PIP2 by phosphorylating phosphatidylinositol 4 phosphate. PIP5KIgamma-null mouse embryos are characterized, among other congenital abnormalities, by NTDs, further linking the disruption of inositol signaling to NTDs [3].

Myo-inositol can prevent a large proportion of spinal NTDs in the curly-tail mutant mouse, whereas folic acid is ineffective [7,32].

Dietary MI supplementation is also effective in reducing the frequency of NTDs in diabetic rats [8], thus suggesting that the mechanisms involved in folate-resistant NTDs can be prevented by MI supplementation. Exogenous MI enters the inositol phospholipid cycle and is incorporated into

phospholipids, including phosphatidylinositol diphosphate (PIP2), that hydrolyzes into inositol triphosphate (IP3) and DAG, acting as second messenger in signal transduction pathways. Inositol triphosphate induces calcium release from the endoplasmic reticulum, whereas DAG activates protein kinase C (PKC) that produces the phosphorylation of specific substrates. Myo-inositol prevents folate-resistant NTDs in the curly-tail mutant mouse by reversing the defective proliferation of hindgut cells that depends on activation of specific PKC isoforms [32].

In humans, significantly lower MI blood levels were found in mothers with a NTD affected pregnancy and low maternal MI blood concentrations are associated with the risk of spina bifida in the offspring [10].

It is well known that maternal diabetes is a risk factor for NTDs in offspring, and the relative risk for an affected NTD pregnancy in diabetic women is 3 – 4 times higher than in non-diabetic mothers [33]. In those cases, NTDs would originate from an impaired maternal metabolism leading to defective turnover of phosphatidylinositol [34].

Interestingly, no affected pregnancy was reported in a cohort of women at high risk of recurrent NTDs undergoing MI plus folic acid periconceptional supplementation [13].

Inositol is a constituent of living cells, is widespread in many foods and is an essential nutrient included in the culture medium of many cell lines. No side effects were reported in adults under MI treatment for psychiatric disorders, polycystic ovary syndrome, psoriasis, patients on lithium therapy, nor in children treated for autism and newborns affected by respiratory distress [35–39].

Periconceptional inositol supplementation up to 4 g per day was never associated with any collateral side effects to the mother or the fetus [13,40,41].

All these data suggest an important role of MI in preventing some subtypes of human NTDs, and its use in the periconceptional period is under close observation [42].

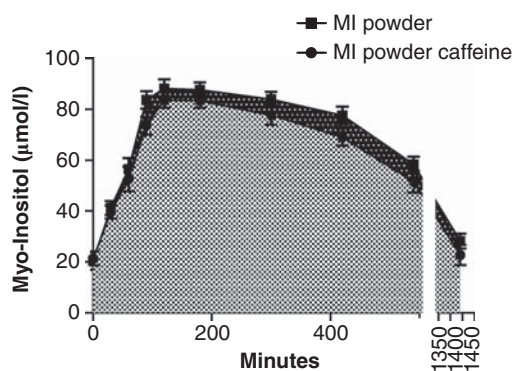


Figure 2. Comparison of the plasma concentration-time data profile for Phase II and Phase IV (MI soft gelatin capsules and MI soft gelatin capsules swallowed with a single espresso).

Maternal caffeine consumption has been associated with unfavorable effects on the fetus and the newborn. Beside being related to fetal growth restriction [16], caffeine intake has also been considered as a risk factor for congenital malformations, particularly NTDs [15,17,42].

Higher homocysteine blood levels will follow caffeine intake [43] and hyperhomocysteinemia is associated with NTDs [44], suggesting a possible explanation for the association between caffeine exposition and increased risk for NTDs.

However, given the inconsistencies often seen in the relationship between caffeine intake and the risk for NTDs [45-49], other possible explanations involving different mechanisms need to be explored.

Caffeine is a methylxanthine that can cross the placenta during pregnancy, and exposure to high doses of caffeine during pregnancy results in teratogenic effects, including NTDs, in animal studies [50].

Caffeine is rapidly absorbed and because of the absence of cytochrome P4501A2 (CYP1A2) in the placenta and the fetus, the amount of caffeine and metabolites available to the fetoplacental unit depends entirely on the maternal metabolism [16] [17].

Genetic variability in metabolizing enzymes CYP1A2 and N-acetyltransferase 2 (NAT2) was proposed as an alternative explanation for the association between the increased risk for NTDs and caffeine intake [17]. However, caffeine inhibits IP3-related calcium channels in neural tissue [18], thus suggesting the hypothesis that caffeine affects a biological pathway involved in calcium regulation [13].

Beside that specific mechanism, it is well known that caffeine may interfere with the bioavailability of several drugs [23].

The concomitant exposure to other dietary risk factors, for example, low folate intake or alcohol consumption, results in an amplification of the teratogenic effects of caffeine [51]. That mechanism might be involved even in the complex interplay between caffeine, inositol and the risk for NTDs.

Indeed, NTDs etiology includes not only genetic and environmental factors but, more likely, a combination of both. As both caffeine intake and inositol deficiency might increase the risk of occurrence and recurrence for NTDs in experimental models and in humans, their combined effect should be studied more in depth at the population level.

Our results clearly showed that MI powder administration is negatively affected by 'espresso' coffee concomitant consumption. Due to the reported positive effects of inositol in preventing NTDs in folate-resistant subjects, this interference could help to clarify the association between caffeine intake and the increased risk for NTDs.

Coffee impairs inositol(s) absorption; indeed, it was also able to interfere with D-chiro-inositol absorption (data not shown). The fact that coffee intake affects inositol bioavailability further strengthens the hypothesis of inositol deficiency as a risk factor for NTDs in humans.

Caffeine is likely to be one of the most used pharmacologic agents in the world, and even a small increase in risk for NTDs would have a great impact at the population level.

Therefore, to avoid caffeine interference during a prophylactic therapy for NTDs based on MI supplementation, women should be advised about the effects of caffeine exposure in the periconceptional period and counseled to reduce their caffeine intake before conception. Moreover, the concomitant intake of caffeine and inositol supplementation should be discouraged in women seeking for pregnancy and in the first months of pregnancy.

However, a very small variation of the PK profile was observed when the newly developed MI soft gelatin capsule form was swallowed with water or with coffee. This result suggests that the pharmaceutical form may play an important role in preventing the interactions with food and/or other substances.

Moreover, given the reduced interference on MI absorption, administration of MI soft gelatin capsules should be preferred to MI powder.

Despite the equivalence of MI concentration in the two pharmaceutical forms, the C_{max} value of the MI soft gelatin capsules was > 90% higher than that of the MI powder, suggesting that the new MI form is characterized by an enhanced bioavailability.

In conclusion, with the aim to search for new clinical treatments able to prevent the development of NTDs, recent studies reported that MI supplementation might prevent folate-resistant NTDs. Caffeine intake has been found to be a risk factor for NTDs. In order to evaluate its possible negative effects on MI gastrointestinal absorption, a single-dose bioavailability trial was conducted on 12 healthy volunteers. Pharmacokinetics (PK) parameters of MI powder and MI soft gelatin capsules swallowed with water and with a single 'espresso' were compared. MI powder administration was negatively affected by caffeine intake, thus suggesting an additional explanation to the interference between MI deficiency and coffee consumption. On the contrary, the concomitant single 'espresso' consumption did not affect MI absorption after MI soft gelatin capsules

administration. Furthermore, it was observed that MI soft gelatin capsule administration results in an improved bioavailability when compared to the MI powder form.

5. Study limitation

The present study has some limitations: for instance, the use of a complex drink such as an espresso rather than simply caffeine; therefore, we cannot undoubtedly conclude that caffeine impairs MI absorption.

Bibliography

- Blom HJ, Shaw GM, den Heijer M, Finnell RH. Neural tube defects and folate: case far from closed. *Nat Rev Neurosci* 2006;7(9):724-31
- Tinker SC, Hamner HC, Berry RJ, et al. Does obesity modify the association of supplemental folic acid with folate status among nonpregnant women of childbearing age in the United States? *Birth Defects Res A Clin Mol Teratol* 2012
- Wang Y, Lian L, Golden JA, et al. PIP5KI gamma is required for cardiovascular and neuronal development. *Proc Natl Acad Sci USA* 2007;104(28):11748-53
- Majerus PW, Wilson DB, Zhang C, et al. Expression of inositol 1,3,4-trisphosphate 5/6-kinase (ITPK1) and its role in neural tube defects. *Adv Enzyme Regul* 2010;50(1):365-72
- Wilson MP, Hugge C, Bielinska M, et al. Neural tube defects in mice with reduced levels of inositol 1,3,4-trisphosphate 5/6-kinase. *Proc Natl Acad Sci USA* 2009;106(24):9831-5
- De Castro SC, Leung KY, Savery D, et al. Neural tube defects induced by folate deficiency in mutant curly tail (Grhl3) embryos are associated with alteration in folate one-carbon metabolism but are unlikely to result from diminished methylation. *Birth Defects Res A Clin Mol Teratol* 2010;88(8):612-18
- Greene ND, Copp AJ. Inositol prevents folate-resistant neural tube defects in the mouse. *Nat Med* 1997;3(1):60-6
- Reece EA, Khandelwal M, Wu YK, Borenstein M. Dietary intake of myo-inositol and neural tube defects in offspring of diabetic rats. *Am J Obstet Gynecol* 1997;176(3):536-9
- Beemster P, Groenen P, Steegers-Theunissen R. Involvement of inositol in reproduction. *Nutr Rev* 2002;60(3):80-7
- Groenen PM, Peer PG, Wevers RA, et al. Maternal myo-inositol, glucose, and zinc status is associated with the risk of offspring with spina bifida. *Am J Obstet Gynecol* 2003;189(6):1713-19
- Groenen PM, Wevers RA, Janssen FS, et al. Are myo-inositol, glucose and zinc concentrations in amniotic fluid of fetuses with spina bifida different from controls? *Early Hum Dev* 2003;71(1):1-8
- Cavalli P, Tedoldi S, Riboli B. Inositol supplementation in pregnancies at risk of apparently folate-resistant NTDs. *Birth Defects Res A Clin Mol Teratol* 2008;82(7):540-2
- Cavalli P, Tonni G, Grosso E, Poggiani C. Effects of inositol supplementation in a cohort of mothers at risk of producing an NTD pregnancy. *Birth Defects Res A Clin Mol Teratol* 2011;91(11):962-5
- Cavalli P, Cavallari U, Unfer V, Tonni G. Caffeine intake and risk of neural tube defects. *Birth Defects Res A Clin Mol Teratol* 2011;91(1):67
- Schmidt RJ, Romitti PA, Burns TL, et al. Maternal caffeine consumption and risk of neural tube defects. *Birth Defects Res A Clin Mol Teratol* 2009;85(11):879-89
- CARE Study Group. Maternal caffeine intake during pregnancy and risk of fetal growth restriction: a large prospective observational study. *BMJ* 2008;337:a2332
- Schmidt RJ, Romitti PA, Burns TL, et al. Caffeine, selected metabolic gene variants, and risk for neural tube defects. *Birth Defects Res A Clin Mol Teratol* 2010;88(7):560-9
- Bezprozvanny I, Bezprozvannaya S, Ehrlich BE. Caffeine-induced inhibition of inositol(1,4,5)-trisphosphate-gated calcium channels from cerebellum. *Mol Biol Cell* 1994;5(1):97-103
- Hume JR, McAllister CE, Wilson SM. Caffeine inhibits InsP3 responses and capacitative calcium entry in canine pulmonary arterial smooth muscle cells. *Vascul Pharmacol* 2009;50(3-4):89-97
- Berkowitz BA, Spector S, Pool W. The interaction of caffeine, theophylline and theobromine with monoamine oxidase inhibitors. *Eur J Pharmacol* 1971;16(3):315-21
- Lasswell WL Jr, Weber SS, Wilkins JM. In vitro interaction of neuroleptics and tricyclic antidepressants with coffee, tea, and gallic acid. *J Pharm Sci* 1984;73(8):1056-8
- Morck TA, Lynch SR, Cook JD. Inhibition of food iron absorption by coffee. *Am J Clin Nutr* 1983;37(3):416-20
- Benvenia S, Bartolone L, Pappalardo MA, et al. Altered intestinal absorption of L-thyroxine caused by coffee. *Thyroid* 2008;18(3):293-301
- Wald NJ, Law MR, Morris JK, Wald DS. Quantifying the effect of folic acid. *Lancet* 2001;358(9298):2069-73
- Cavalli P, Copp AJ. Inositol and folate resistant neural tube defects. *J Med Genet* 2002;39(2):E5
- Heseker HB, Mason JB, Selhub J, et al. Not all cases of neural-tube defect can be prevented by increasing the intake of folic acid. *Br J Nutr* 2009;102(2):173-80
- Copp AJ, Greene ND. Genetics and development of neural tube defects. *J Pathol* 2010;220(2):217-30
- Cockcroft DL. Changes with gestational age in the nutritional requirements of postimplantation rat embryos in culture. *Teratology* 1988;38(3):281-90

6. Conclusions

Myo-inositol soft gelatin capsules should be considered for the preventive treatment of NTDs in folate-resistant subjects due to their higher bioavailability and to the capability to reduce coffee interference.

Declaration of interest

V Unfer is a consultant for LOLI Pharma.

29. Seller MJ.. Vitamins, folic acid and the cause and prevention of neural tube defects. *Ciba Found Symp* 1994;181:161-73; discussion 73-9
30. Harris MJ, Juriloff DM. An update to the list of mouse mutants with neural tube closure defects and advances toward a complete genetic perspective of neural tube closure. *Birth Defects Res A Clin Mol Teratol* 2010;88(8):653-69
31. Wilson KL, Sullivan KM, Wiese C. Inositol 1,4,5-trisphosphate receptor activation and nuclear envelope assembly. *Soc Gen Physiol Ser* 1996;51:189-93
32. Cogram P, Hynes A, Dunlevy LP, et al. Specific isoforms of protein kinase C are essential for prevention of folate-resistant neural tube defects by inositol. *Hum Mol Genet* 2004;13(1):7-14
33. Platt LD, Golde SH. Neural tube defects in infants of diabetic mothers. *Am J Obstet Gynecol* 1983;145(3):384-6
34. Goto MP, Goldman AS. Diabetic embryopathy. *Curr Opin Pediatr* 1994;6(4):486-91
35. Belmaker RH, Bersudsky Y, Benjamin J, et al. Manipulation of inositol-linked second messenger systems as a therapeutic strategy in psychiatry. *Adv Biochem Psychopharmacol* 1995;49:67-84
36. Colodny L, Hoffman RL. Inositol-clinical applications for exogenous use. *Altern Med Rev* 1998;3(6):432-47
37. Howlett A, Ohlsson A. Inositol for respiratory distress syndrome in preterm infants. *Cochrane Database Syst Rev* 2003(4):CD000366
38. Levine J.. Controlled trials of inositol in psychiatry. *Eur Neuropsychopharmacol* 1997;May7(2):147-55
39. Palatnik A, Frolov K, Fux M, Benjamin J. Double-blind, controlled, crossover trial of inositol versus fluvoxamine for the treatment of panic disorder. *J Clin Psychopharmacol* 2001;21(3):335-9
40. Rizzo P, Raffone E, Benedetto V. Effect of the treatment with myo-inositol plus folic acid plus melatonin in comparison with a treatment with myo-inositol plus folic acid on oocyte quality and pregnancy outcome in IVF cycles. A prospective, clinical trial. *Eur Rev Med Pharmacol Sci* 2010;14(6):555-61
41. Papaleo E, Unfer V, Baillargeon JP, et al. Myo-inositol may improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial. *Fertil Steril* 2009;91(5):1750-4
42. De Marco P, Merello E, Cama A, et al. Human neural tube defects: genetic causes and prevention. *Biofactors* 2011;37(4):261-8
43. Urgert R, van Vliet T, Zock PL, Katan MB. Heavy coffee consumption and plasma homocysteine: a randomized controlled trial in healthy volunteers. *Am J Clin Nutr* 2000;72(5):1107-10
44. Steegers-Theunissen RP, Boers GH, Trijbels FJ, et al. Maternal hyperhomocysteinemia: a risk factor for neural-tube defects? *Metabolism* 1994;43(12):1475-80
45. Fedrick J.. Anencephalus and maternal tea drinking: evidence for a possible association. *Proc R Soc Med* 1974;67(5):356-60
46. Kurppa K, Holmberg PC, Kuosma E, Saxen L. Coffee consumption during pregnancy and selected congenital malformations: a nationwide case-control study. *Am J Public Health* 1983;73(12):1397-9
47. Linn S, Schoenbaum SC, Monson RR, et al. No association between coffee consumption and adverse outcomes of pregnancy. *N Engl J Med* 1982;306(3):141-5
48. Olsen J, Overvad K, Frische G. Coffee consumption, birthweight, and reproductive failures. *Epidemiology* 1991;2(5):370-4
49. Rosenberg L, Mitchell AA, Shapiro S, Slone D. Selected birth defects in relation to caffeine-containing beverages. *JAMA* 1982;12247(10):1429-32
50. Wilkinson JM, Pollard I. In utero exposure to caffeine causes delayed neural tube closure in rat embryos. *Teratog Carcinog Mutagen* 1994;14(5):205-11
51. Ross CP, Persaud TV. Neural tube defects in early rat embryos following maternal treatment with ethanol and caffeine. *Anat Anz* 1989;169(4):247-52

Affiliation

Sara De Grazia¹, Gianfranco Carlomagno^{†1}, Vittorio Unfer¹ & Pietro Cavalli²

[†] Author for correspondence

¹A.G.UN.CO., Obstetrics and Gynecology Center, Gennaro Cassiani 15, 00155 Rome, Italy

Tel: +39 335 1422615;

E-mail: gianfranco.carlomagno@agunco.it

²Clinical Genetics, Azienda Istituti Ospitalieri, Via Concordia,

1 26100 Cremona, Italy