Nutrilab

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Scientific Justification for Nutri ENDO 1 & 2 in Endometriosis

Risk assessment

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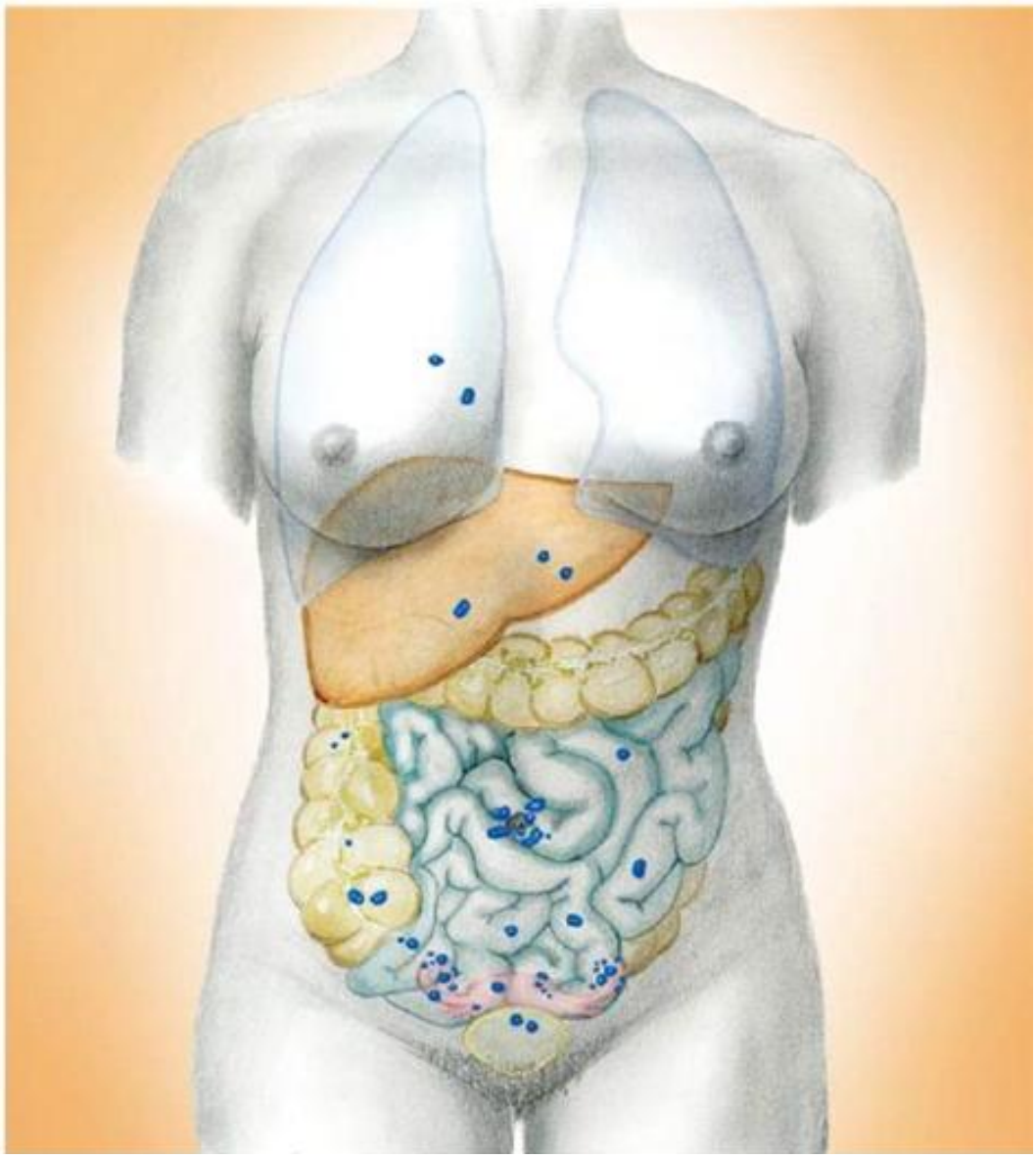
1 Scientific justification

Endometriosis is defined as the presence of endometrial tissue, comprising both the glandular epithelium and the stroma, outside the uterine cavity. It is one of the most common benign gynaecological disorders, affecting 10-15% of all women of childbearing age and 0.3% of infertile women (1.2). This disease is associated with various distressing symptoms such as dysmenorrhea, dyspareunia, pelvic pain and hypofertility. Despite an increase in the number of studies on endometriosis, its etiology remains elusive due, in part, to its multifactorial characteristics. Indeed, a growing number of studies suggest that a combination of genetic, hormonal, environmental, immunological and anatomical factors play a role in the pathogenesis of this disorder (3-6).

Normal uterus	***Retrograde menstruation***	***Mild endometriosis***
The uterus is a pear-shaped organ, almost as big as a fist. The uterine lining, called "endometrium", performs an important role in the occurrence of endometriosis	The uterine lining is formed cyclically in the uterine cavity and it is expelled every month during a woman's period. In most women, a little blood containing these mucous cells passes into the abdominal cavity during the woman's period, via the fallopian tubes. This phenomenon is called "retrograde menstruation" (flows backwards).	Depending on manifestations, we distinguish between various stages of endometriosis. In the case of slight endometriosis, a few endometriosis sites can be seen which attach to the external wall of the uterus, the fallopian tubes and the ovaries.



A disease which spreads

To place this disorder in a broader context we can cite Barker's hyposthesis. Barker's hyposthesis (7) has been of considerable interest to find the first origins of the disease (8). This hypothesis postulates that the first exposures, including those arising from parent lifestyle during the sensitive period of human development that is pregnancy, can permanently reprogramme embryonic development, from the foetal stage until life outside of the womb. This reprogramming is largely caused by epigenetic mechanisms (9). This reprogramming of human fertility, defined as the biological capacity of men and women to reproduce, irrespective of pregnancy intentions, has also suggested that there could be a link with early environmental exposures such as trans-generational effects (10.11).

It is in this context that the Pronutri laboratories plan to intervene in this gynaecological disorder through a nutripuncture approach. The rationale of nutripuncture will be described in later chapter.

We can however announce in this scientific chapter, that it intervenes by means of trace metal sequences (NUTRI 1 to 40) which aim to transmit subtle information, via membrane signals, which manifests itself at psychological and organic levels. What we are interested in, in the context of demonstrating a scientific rationale for this approach, is the intervention of the membrane and the trace metals in the etiology of this disorder.

Endometriosis is a chronic oestrogen-dependent inflammatory disease. The etiological factors that contribute to the survival of the extra-uterine tissue include oestrogenic compounds (12). They contribute to the prolonged survival of these tissues which therefore assume importance. Convincing in vitro, in vivo evidence in animals and humans (12) and irrefutable evidence, molecular (13), in vitro (14) and in vivo (15), have demonstrated the capacity of metal ions to activate oestrogen receptors (16). They are defined as "xenoestrogens", called "metalloestrogens" (17). A study (18) conducted among 50 women of childbearing age, recruited at the Colombo gynaecology unit in Sri Lanka, among female patients whose endometriosis is viewed through a laparotomy or laparoscopy, has been conducted on their blood samples to test for the presence of metals known as "metalloestrogenes". The average age of the women recruited and the control group was 33 ± 5.4 and 32.7 ± 5.4 years respectively, their BMI was similar and they were smokers.

Lead, cadmium and nickel were detected in these participants.

Table 1 Levels of metalloestrogens in whole blood (µg/L) of cases and controls

Metalloestrogen	Cases (n=50)	Controls (n=50)	*P* value†
Nickel	2.6 (1.9-3.3)	0.8 (0.7-0.9)	0.016
Lead	11.0 (8.6-13.3)	6.9 (5.7-8.0)	0.389
Cadmium	0.7 (0.7-0.9)	0.8 (0.6-1.0)	0.423

Data expressed as geometric mean (95% CI).
† *t*-test between blood levels of cases and controls.

The cadmium rate was lower, and nickel and lead were higher in patients than among the control group, although these results are not statistically significant. One possible cause for the prevalence of endometriosis is industrialisation and its environmental pollution.
However, the increase in nickel does not seem to be only related to pollution, since the doses detected are not toxic, and similar to the rate observed in other countries. These high levels may contribute to its presence in endometrial tissue. Martin and al. (2003) (19) measure the oestrogenic potential of nickel, and describe it with a relative potency of 1 compared to oestradiol, indicating a "metalloestrogen" potential. Although only a small number of subjects took part in this study, a link can be suggested.
Cadmium and other metals considered to be metalloestrogens have been associated with adverse effects on the prognosis of the reproduction (20) and may play a role in the etiology of endometriosis.
Another study suggests a part played by copper (21). Other evidence suggests that iron (22, 23) as well as oxidative stress (24, 25) play a part in endometriosis.

Copper is a metal with a very important vital redox activity. It is both an antioxidant and a pro-oxidant, effects linked to oxidative stress and inflammation (26, 27). In the serum of mammals, copper is predominant and contains proteins such as Cp, a glycosylated protein multi-Cu ferroxydase mainly synthesised in the liver, which carries 95% of total serum Cu (28). This is a protein linked to acute responses similar to Cu, it is linked to inflammation and oxidative stress, with both an antioxidant and pro-oxidative effect (26, 27). Endometriosis is a disease that manifests itself both in inflammation and oxidative stress. In view of the insufficient number of studies on Cu and Cp levels and endometriosis, Turgut et al. (2013) (21) studied this link among 81 women selected. At the end of the selections by laparoscopy or laparotomy, 72 women, 31 with stage III/IV endometriosis and 41 control women without endometriosis were recruited at the Faculty of Medicine, Gynaecology and Obstetrics Clinic of the University of Dicle. The biochemical analysis of various parameters has been studied among these women with an advanced stage of endometriosis and among their controls 31.3 ± 4.6 and 30.4 ± 7.8 years respectively. This population is described in the table hereafter.

Table I. Surgical approach and preoperative indications of the patients included in the study.

Parameters	Distribution among total subjects (n=72)
Surgical approaches	
Laparoscopy	54 (75%)
Laparotomy	18 (25%)
Preoperative indications	
Infertility	35 (48.6%)
Pelvic mass	19 (26,4%)
Pelvic pain	15 (20.8%)
Tubal ligation	3 (4.2%)

And the following table describes the biochemical results:

Table II. Comparison of mean level of parameters in women with advanced-stage endometriosis and controls.

Parameters	Advanced-stage endometriosis (n = 31) (mean ± SD)	Controls (n = 41) (mean ± SD)	*p* value
TAS (mmol Trolox Equivalent/L)	1.01 ± 0.10	1.15 ± 0.17	< 0.001
TOS (μmol H_2O_2 Equiv/L)	25.40 ± 9.35	15.98 ± 6.97	< 0.001
OSI (H_2O_2/Trolox)	25.07 ± 9.21	14.05 ± 6.69	< 0.001
PON-1 (u/l)	73.38 ± 44.34	98.47 ± 44.46	0.020
MDA (mmol/L)	220.87 ± 41.84	205.49 ± 43.57	0.136
Cu (μg/ml)	1088.00 ± 273.58	811.20 ± 265.77	< 0.001
Cp (mg/dl)	38.41 ± 9.58	26.50 ± 8.63	< 0.001
TG (mg/dl)	121.65 ± 43.11	97.83 ± 33.47	0.014
TC (mg/dl)	183.13 ± 23.98	164.80 ± 31.59	0.009
HDL (mg/dl)	49.58 ± 9.06	56.20 ± 11.72	0.011
LDL (mg/dl)	108.52 ± 23.12	89.09 ± 28.33	0.003

SD = standard deviation.

In this study, the rate of copper and Cp in patients with advanced stages of endometriosis are significantly higher than in the control group. Similar and common mechanisms are found in arteriosclerosis and endometriosis. In addition, these same parameters are found to be as high in arteriosclerosis, suggesting that these parameters could be markers during therapeutic investigations. Associations have also been found with other oxidative stress markers, indicating an oxidant/antioxidant imbalance in endometriosis. Antioxidants may confer some protection against endometriosis lesions (29) and trace metals can change oxidative stress levels with an impact on endometriosis (30).

As said above, the important role played by oxidative stress is accepted (31-35). Reactive oxygen species (ROS) are molecules containing oxygen produced during normal metabolism. In the body, conditions of stress lead to anomalies in the enzymatic and non-enzymatic systems that neutralise the deleterious effects of these endogenous molecules. A break in the oxidant/antioxidant balance leads to destructive changes in circulating lipoproteins, proteins, carbohydrates, nucleotides and membranes (36). For example, low intra-follicular concentrations of ROS are 'prognostic' markers of good fertility. In the clinic, one of the important effects of endometriosis is the tendency to cause infertility. For the purpose of protecting the follicle from oxidation risks, these are naturally filled with an effective antioxidant system (37) and trace metals are major components of the antioxidant system (38).

Table 2
Levels oxidative stress parameters and antioxidants (enzymatic and non-enzymatic) in follicular fluid of women with endometriosis and tubal infertility group.

Parameters	Endometriosis; $n = 200$	Tubal infertility; $n = 140$
NO (μM)	41.61 ± 3.89	36.92 ± 4.12
ROS (cps)	101.23 ± 10.52	96.37 ± 11.83
MDA (μM)	0.92 ± 0.23	0.68 ± 0.22
TAC (μM Trolox equivalent)	658.32 ± 56.52	896.25 ± 78.47
SOD (mIU/mg protein)	31.21 ± 4.12	45.37 ± 3.81
Catalase (mIU/mg protein)	10.36 ± 2.14	19.56 ± 3.28
GPx (mIU/mg protein)	0.82 ± 0.21	2.14 ± 0.43
GR (mIU/mg protein)	0.08 ± 0.006	0.2 ± 0.1
Vitamin A (μg/l)	3.12 ± 0.16	4.13 ± 0.14
Vitamin C (mg/l)	4.21 ± 0.13	5.52 ± 0.17
Vitamin E (mg/l)	9.53 ± 0.17	12.53 ± 0.19

Abbreviations: NO, nitric oxide; ROS, reactive oxygen species; MDA, malondialdehyde; TAC, total antioxidant capacity; SOD, superoxide dismutase; GPx, glutathione peroxidise; GR, glutathione reductase.
All values are significant, $P < 0.001$.

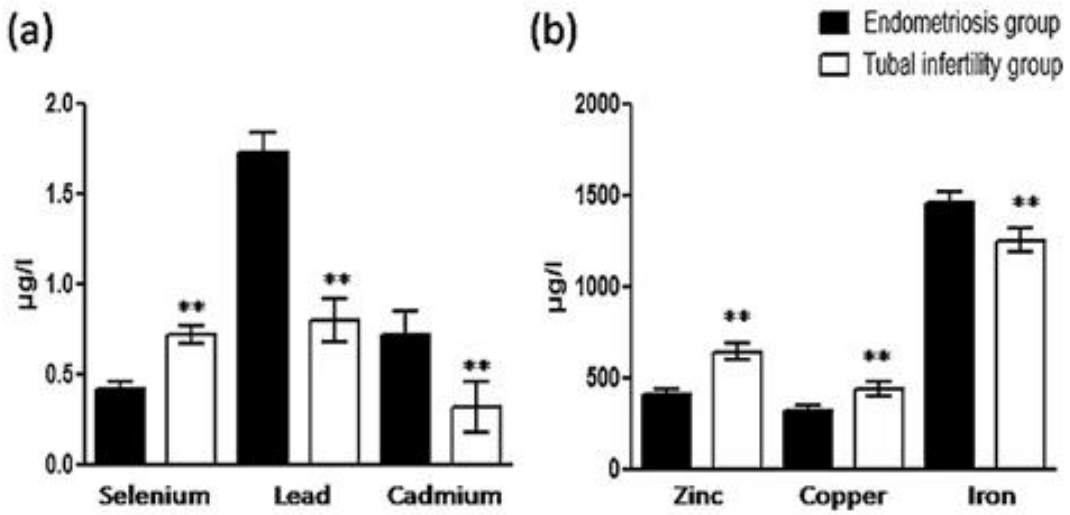


Fig. 4. Concentrations of trace elements measured in follicular fluid of women with endometriosis and tubal infertility. $^{**}P < 0.001$.

Increased concentrations of reactive oxygen species (ROS), nitric oxide (NO), lipid peroxidation (LPO), iron, lead, cadmium, and reduced levels of total antioxidant capacity (TAC), superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GR), vitamins A, C, E, copper, selenium and zinc have been observed in relation to tubal sterility. Increases in ROS and NO in endometriosis and infertility are associated with poor oocytes in relation to embryo quality. The increase in ROS, NO, LPO, cadmium and lead levels has been observed among women who have not fallen pregnant, compared to women suffering from endometriosis, who subsequently fell pregnant by IVF and in whom the intra-follicular zinc level was high.

In another study (39), the ENDO study, a population of 473 women aged 18 to 44 years were recruited in an operative population which also comprised a similar population of 127 women. The table hereafter describes these populations.

TABLE 1
Reproductive history by cohort and endometriosis diagnosis, ENDO Study (n = 600)

Characteristic	Operative cohort n = 473		Population cohort n = 127	
	Endometriosis n = 190 n (%)	No endometriosis n = 283 n (%)	Endometriosis n = 14 n (%)	No endometriosis n = 113 n (%)
Age, y				
<20	5 (2.6)	7 (2.5)	0 (0)	4 (3.5)
20-24	22 (11.6)	26 (9.2)	4 (28.6)	21 (18.6)
25-29	48 (25.3)	55 (19.5)	1 (7.1)	22 (19.5)
30-34	44 (23.2)	58 (20.6)	2 (14.3)	18 (15.9)
>35	71 (37.4)	136 (48.2)	7 (50)	48 (42.5)
Mean (SD)	31.98 (6.75)	33.61 (7.09)[a]	33.14 (8.33)	32.07 (7.76)
Ever sexually active				
No	27 (14.2)	37 (13.2)	1 (7.1)	14 (12.4)
Yes	163 (85.8)	244 (86.8)	13 (92.9)	99 (87.6)
Age at first consenting sex				
≤17	92 (48.4)	157 (55.5)	4 (28.6)	55 (48.7)
18-20	50 (26.3)	67 (23.7)	6 (42.9)	38 (33.6)
≥21	48 (25.3)	59 (20.8)	4 (28.6)	20 (17.7)
Mean (±SD)	19.19 (4.26)	18.33 (3.63)	19.08 (2.47)	18.49 (2.99)
Ever use oral contraceptives				
No	21 (11.1)	45 (15.9)	1 (7.1)	17 (15)
Yes	169 (88.9)	238 (84.1)	13 (92.9)	96 (85)
Gravidity				
Nulligravid (0)	81 (42.6)	74 (26.3)[b]	5 (35.7)	46 (40.7)
Gravid (≥1)	109 (57.4)	207 (73.7)	9 (64.3)	67 (59.3)
Mean (±SD)	1.85 (1.98)	2.28 (2.12)[c]	2.21 (2.08)	1.65 (1.80)
Parity (no. of live births)				
Nulliparous	21 (19.4)	25 (12.1)	1 (11.1)	10 (14.9)
Parous	87 (80.6)	182 (87.9)	8 (88.9)	57 (85.1)
Mean (±SD)	1.81 (1.27)	2.19 (1.44)[d]	2.56 (1.59)	2.21 (1.45)
Age at first pregnancy, y				
<20	42 (38.5)	79 (38.2)	0 (0)	14 (20.9)
20-24	42 (38.5)	71 (34.3)	5 (55.6)	27 (40.3)
25-29	20 (18.3)	33 (15.9)	4 (44.4)	20 (29.9)
30-34	4 (3.7)	22 (10.6)	0 (0)	4 (6)
35-39	1 (0.9)	1 (0.5)	0 (0)	1 (1.5)
≥40	0 (0)	1 (0.5)	0 (0)	1 (1.5)
Mean (±SD)	21.83 (4.19)	21.98 (5.13)	23.56 (2.79)	23.64 (4.99)
History STIs				
No	160 (84.2)	219 (77.4)	13 (92.9)	91 (80.5)
Yes	30 (15.8)	64 (22.6)	1 (7.1)	22 (19.5)

Peterson. Risk factors associated with endometriosis. Am J Obstet Gynecol 2018.

(continued)

In these populations, 20 trace metals were studied in the urine and 3 in the blood. They were quantified by mass spectrophotometry.

Table 2
Comparison of geometric mean blood and urine element concentrations by endometriosis status and cohort, ENDO Study.

Trace elements	Operative cohort ($n = 473$)		Population cohort ($n = 127$)	
	Endometriosis $n = 190$ Mean (95% CI)	None $n = 283$ Mean (95% CI)	Endometriosis $n = 14$ Mean (95% CI)	None $n = 113$ Mean (95% CI)
Blood				
Cadmium (µg/L)	0.28 (0.25, 0.31)	0.34 (0.31, 0.37)**	0.25 (0.18, 0.33)	0.30 (0.26, 0.34)
Lead (µg/dL)	0.61 (0.57, 0.66)	0.67 (0.63, 0.71)*	0.63 (0.49, 0.81)	0.63 (0.58, 0.70)
Mercury (µg/L)	0.65 (0.56, 0.77)	0.59 (0.51, 0.68)	0.37 (0.13, 1.07)	0.71 (0.57, 0.88)
Urine (µg/L)				
Antimony	0.06 (0.05, 0.07)	0.06 (0.06, 0.07)	0.09 (0.06, 0.13)	0.07 (0.06, 0.08)
Arsenic	8.37 (7.41, 9.46)	8.37 (7.50, 9.33)	7.74 (4.88, 12.25)	8.69 (7.26, 10.39)
Barium	1.92 (1.72, 2.15)	2.13 (1.92, 2.36)	2.72 (1.84, 4.02)	2.28 (1.96, 2.64)
Beryllium	0.05 (0.04, 0.06)	0.04 (0.03, 0.04)	0.05 (0.03, 0.10)	0.06 (0.05, 0.08)
Cadmium[a]	0.22 (0.20, 0.25)	0.25 (0.23, 0.27)	0.27 (0.18, 0.40)	0.25 (0.22, 0.28)
Cesium	4.50 (4.20, 4.83)	4.73 (4.46, 5.01)	4.44 (3.54, 5.56)	4.76 (4.37, 5.17)
Chromium	1.04 (0.89, 1.20)	0.99 (0.85, 1.16)	1.26 (0.46, 3.43)	1.01 (0.80, 1.29)
Cobalt	0.51 (0.47, 0.56)	0.54 (0.50, 0.58)	0.51 (0.42, 0.63)	0.56 (0.50, 0.63)
Copper	10.64 (9.90, 11.45)	10.33 (9.85, 10.83)	12.58 (9.72, 16.29)	11.66 (10.66, 12.76)
Lead	0.35 (0.28, 0.44)	0.34 (0.29, 0.41)	0.58 (0.23, 1.45)	0.26 (0.19, 0.34)
Manganese	1.41 (1.30, 1.54)	1.39 (1.28, 1.50)	1.21 (0.66, 2.23)	1.68 (1.51, 1.88)
Mercury	0.31 (0.25, 0.40)	0.36 (0.30, 0.44)	0.43 (0.02, 7.79)	0.48 (0.33, 0.72)
Molybdenum	44.49 (40.86, 48.45)	44.56 (41.24, 48.15)	45.64 (32.59, 63.93)	47.99 (42.59, 54.08)
Nickel	4.48 (4.04, 4.96)	4.01 (3.65, 4.40)	7.54 (5.24, 10.84)	6.59 (5.75, 7.56)
Tellurium	0.13 (0.09, 0.18)	0.09 (0.07, 0.12)	0.12 (0.04, 0.33)	0.10 (0.07, 0.13)
Thallium	0.15 (0.14, 0.16)	0.15 (0.14, 0.16)	0.14 (0.10, 0.18)	0.16 (0.14, 0.18)
Tin	0.68 (0.60, 0.78)	0.70 (0.62, 0.78)	0.67 (0.47, 0.97)	0.59 (0.51, 0.68)
Tungsten	0.08 (0.06, 0.09)	0.09 (0.08, 0.10)	0.08 (0.02, 0.24)	0.07 (0.05, 0.10)
Zinc	265.64 (237.35, 297.31)	283.95 (257.99, 312.53)	393.12 (216.63, 713.40)	327.79 (259.66, 413.80)

Note: Urinary trace element concentrations are creatinine adjusted. Nonparametric Wilcoxon test were performed to test significance of endometriosis status for each metal within cohort.
LOD, limit of detection.
[a] Cadmium corrected for molybdenum interference
* $p < 0.05$.
** $p < 0.01$.

The regressions were analysed with the diagnosis of endometriosis. In these analyses, it was observed that cadmium, chromium and copper were correlated.

Table 4
Simultaneous modeling of significant metals and odds of an endometriosis diagnosis in the operative cohort, ENDO Study.

Metals	Adjusted[a] OR (95% CI)	Adjusted[b] OR (95% CI)
Blood		
Cadmium (μg/L)		
<0.21	Reference	Reference
0.21–0.36	0.75 (0.46, 1.24)	0.78 (0.47, 1.29)
≥0.37	0.52 (0.29, 0.93)	0.52 (0.29, 0.94)
Urine (μg/L)		
Chromium		
<0.47	Reference	Reference
0.47–1.30	2.32 (1.42, 3.79)	2.34 (1.43, 3.86)
≥1.31	0.99 (0.59, 1.66)	0.97 (0.57, 1.63)
Copper		
<7.08	Reference	Reference
7.08–13.80	0.96 (0.53, 1.74)	0.97 (0.53, 1.77)
≥13.81	1.15 (0.73, 1.80)	1.12 (0.71, 1.78)

[a] Adjusted for age (years), body mass index (kg/m^2), smoking (yes/no), site (CA/UT), race (non-Hispanic white, non-Hispanic black, Hispanic, Asian/Islander/Native American, other), vitamin use (yes/no). Urine metals were standardized by creatinine.

[b] Adjusted for age (years), body mass index (kg/m^2), smoking (yes/no), site (CA/UT), race (non-Hispanic white, non-Hispanic black, Hispanic, Asian/Islander/Native American, other), vitamin use (yes/no) and parity conditional on gravidity (never pregnant/pregnant without births/pregnant with births). Urine metals were standardized by creatinine.

The exposure of women to trace metals in this study is generally lower than those of the NHANES study (2003-2004).

This is the only epidemiological approach study on the possible relationships between traces of nutrients and endometriosis. This ENDO study from 2013 supports development in the next few years, and encourages us in this direction

By this description of the literature in these areas which is of interest to us we can highlight the following points:

- A role played by trace metals
  - On oestrogen receptors
  - Antioxidant system
- A role played by the antioxidant system
  - Protection of membranes
  - Impact on receptor function

Our approach in this project, of a weakly dosed intake of trace metals, besides their actions on the meridians of Chinese Medicine (nutripuncture), rests on the fact that they can intervene in modulating oestrogen receptor and thus act on the tropism and on the survival of the cells put into play. By modulating the antioxidant system, they can interact on the membranes influencing various tissue biochemistry parameters.

1.1 Risks/Benefits

Benefits

The main benefit is expected to be a decrease in endometriotic tissue and improved comfort for the patient, revealed by the questionnaires and by the reduction of the concomitant intake of analgesics.

Risks

The Nutri Endo 1 and 2 sequences are food supplements that have been marketed under their unit forms since 1995 and no side effects have been reported. They have benefited from a tacit marketing agreement from the Directorate General for Competition Policy, Consumer Affairs and Fraud Control (DGCCRF) ENDO 1 and ENDO 2 contain nutrients in their intake forms authorised by European regulation (REGULATION (EC) No. 1170/2009 OF THE COMMISSION of 30 November 2009 amending directive 2002/46/EC of the European Parliament and of the Council and Regulation (EC) No. 1925/2006 of the European Parliament and of the Council as regards the list of vitamins and minerals and that of their forms, which can be added to food, including food supplements). These intake forms have safety guarantees from ANSES and EFSA. The composition of the product is strictly controlled from a microbiological and allergic safety point of view: the manufacture and packaging sites are ISO 9001 certified: 2008 (food safety guidelines).

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1.3 Results of the study

1.3.1 ENDO 1 and 2

Composition

NUTRI ENDO1

1 set of pills (28/30/26/37/23-08/12/25/33/30) a day

Name of the vitamin or mineral	Quantity per DRD (maximum daily recommended dose)	% of the maximum permitted level, as defined in the Decree of 9 May 2006	% of daily recommended allowance as defined in Regulation 2008/100/EC
Calcium	21.9 mg	2.70%	2.70%
Magnesium	4.37 mg	1.50%	1.20%
Zinc	0.8 mg	5.40%	8%
Manganese	0.11 mg	3.10%	5.50%
Potassium	0.07 mg	< 0.1%	< 0.1%
Sodium	0.02 mg	-	-
Copper	53 µg	2.60%	5.30%
Iron	0.26 µg	< 0.1%	< 0.1%

Zinc acetate, calcium carbonate, magnesium carbonate, manganese carbonate, sodium carbonate, calcium chloride, magnesium chloride, copper gluconate, iron gluconate, manganese gluconate, potassium gluconate, zinc gluconate, sodium iodide, trimagnesium phosphate, zinc sulphate.

NUTRI ENDO2

1 set of pills (12/22/37/08/30-04/22/08/09/23) a day

Name of the vitamin or mineral	Quantity per DRD (maximum daily recommended dose)	% of the maximum permitted level, as defined in the Decree of 9 May 2006	% of daily recommended allowance as defined in Regulation 2008/100/EC
Calcium	22.94 mg	2.80%	2.80%
Magnesium	5.5 mg	1.90%	1.50%
Zinc	0.54 mg	3.60%	5.40%
Manganese	0.18 mg	5.10%	9%
Potassium	0.12 mg	< 0.1%	< 0.1%
Copper	52 µg	2.60%	5.20%
Iron	4.8 µg	< 0.1%	< 0.1%
Sodium	0.08 mg	-	-

Calcium carbonate, magnesium carbonate, manganese carbonate, calcium chloride, sodium chloride, copper gluconate, manganese gluconate, potassium gluconate, zinc gluconate, sodium iodide, tricalcium phosphate, trimagnesium phosphate, zinc sulphate, ferrous sulphate

1.3.2 How they are taken

- Route of administration: oral.
- Dosage and how they are taken: 1 sequence of Endo 1 tablets in the morning and Endo 2 in the evening.

1.3.3 Expected adverse effects

There are no expected adverse effects linked to taking the Nutris with low doses of these nutrients. In addition, in view of the number of years the Nutris used in the study have been marketed (since 1995) without any adverse effects been reported, it is reasonable not to expect any adverse effects.