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# Comparaison de nutritionnelles stratégies ciblant la voie nitrique oxyde pour améliorer la fonction vasculaire macrovasculaire : une revue systématique et une méta-analyse

Myrtille Beau Guillaumot

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UNIVERSITÉ GRENOBLE ALPES  
UFR DE PHARMACIE DE GRENOBLE

Année : 2018-2019

COMPARAISON DE STRATEGIES NUTRITIONNELLES CIBLANT LA VOIE DU  
MONOXYDE D'AZOTE AFIN D'AMELIORER LA FONCTION VASCULAIRE :  
REVUE SYSTEMATIQUE ET META-ANALYSE

MÉMOIRE DU DIPLÔME D'ÉTUDES SPÉCIALISÉES DE  
Pharmacie hospitalière Pratique et Recherche

Conformément aux dispositions du décret N° 90-810 du 10 septembre 1990, tient lieu de

THÈSE  
PRÉSENTÉE POUR L'OBTENTION DU TITRE DE DOCTEUR EN PHARMACIE  
DIPLÔME D'ÉTAT

Myrtille BEAU GUILLAUMOT

[Données à caractère personnel]

MÉMOIRE SOUTENU PUBLIQUEMENT À LA FACULTÉ DE PHARMACIE DE  
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Le : 28/09/2018

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Président du jury : Monsieur le Professeur Christophe RIBUOT

Directeur de thèse : Monsieur le Docteur Charles KHOURI

Membres : Monsieur le Professeur Jean-Luc CRACOWSKI

Madame le Docteur Nathalie FOUILHE SAM-LAI

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 LAPM : Laboratoire Adaptation et Pathogenèse des Microorganismes  
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 TIMC-IMAG : Laboratoire Technique de l'Imagerie, de la Modélisation  
 UMR: Unité Mixte de Recherche  
 UVHCI: Unit of Virus Host Cell Interactions

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## Table des matières

Remerciements .....	7
Index des figures .....	10
Liste des abréviations .....	11
Introduction .....	12
COMPARAISON OF NUTRITIONNAL STRATEGIES TARGETING THE NITRIC OXYDE PATHWAY TO IMPROVE MACROVASCULAR FUNCTION: A SYSTEMATIC REVIEW AND A META ANALYSIS .....	15
Introduction .....	15
Methods .....	16
Interventions and search strategy .....	17
Eligibility criteria .....	17
Data extraction .....	18
Risk of bias assessment .....	19
Statistical analysis .....	19
Subgroup and sensitivity analyses .....	20
Results .....	21
Studies selection .....	21
Quality of included studies .....	22
Acute effect of nutritional strategies .....	24
Chronic effect of nutritional strategies .....	30
Summary results .....	37
Sensitivity analysis .....	38
Placebo effect .....	38
Discussion .....	38
Conclusion .....	47
Discussion .....	47
Conclusion .....	51
Bibliographie .....	53
Annexes .....	60

## Index des figures

Figure 1: Flow chart .....	21
Figure 2: quality of studies on acute supplementation .....	23
Figure 3: quality of studies on chronic supplementation .....	23
Figure 4: Acute effect of NO donors on FMD (Forest plot) .....	25
Figure 5: Z-curve of acute effect of NO donors on FMD .....	25
Figure 6: Acute effect of NOS substrates on FMD (Forest plot) .....	26
Figure 7: Acute effect of polyphenol on FMD (Forest plot).....	27
Figure 8:Z curve acute effect of polyphenols on FMD .....	28
Figure 9: funnel plot of polyphenol acute effect .....	29
Figure 10: chronic effect of nitrates on FMD (Forest plot).....	31
Figure 11: Z-curve of chronic NO donors on FMD .....	31
Figure 12: Chronic effect of NOS substrates (Forest plot) .....	32
Figure 13: Z-curve chronic NOS substrates on FMD .....	32
Figure 14: Chronic effect of folic acids on FMD (Forest plot).....	33
Figure 15: Z-curve on chronic folic acids on FMD .....	33
Figure 16: Chronic effect of polyphenols on FMD (Forest plot).....	34
Figure 17: Z-curve chronic effect of polyphenols on FMD .....	35
Figure 18: Funnel plot chronic effect of polyphenols on FMD .....	35
Figure 19: results summary .....	37
Figure 20: acute effect of NO donors on NMD .....	67
Figure 21: acute effect of NOS substrates on NMD .....	67
Figure 22: acute effect of polyphenol on NMD .....	67
Figure 23: chronic effect of NOS substrates on NMD.....	67
Figure 24: chronic effect of folic acids on NMD .....	68
Figure 25: chronic effect of polyphenols on NMD .....	68
<i>Figure 26: placebo effect on acute supplementation .....</i>	<i>69</i>
<i>Figure 27: placebo effect on chronic supplementation.....</i>	<i>70</i>

## Liste des abréviations

OMS : organisation mondiale de la santé

NO: monoxyde d'azote

NOS: NO synthase

BH4 : tétrahydrobioptérine

SGC: guanylate cyclase soluble

GMPc: guanosine mono phosphate cyclique

GMP: guanosine mono phosphate

PKG: protéine Kinase G

Ca: calcium

RCT : essais clinique contrôlé randomisé

BMI: body mass index

MD: mean difference

95% CI: intervalle de confiance à 95%

TSA : trial sequential analysis

Ros : espèces réactives de l'oxygène

H<sub>2</sub>S : sulfure d'hydrogène (hydrogen sulfide)

## Introduction

Selon l’OMS les pathologies cardiovasculaires sont la première cause de mortalité mondiale (1). Vu l’importance du phénomène, une large pharmacopée est développée pour ce sujet. Cependant, toujours selon l’OMS : « Il est possible de prévenir la plupart des maladies cardiovasculaires en s’attaquant aux facteurs de risques comportementaux – tabagisme, mauvaise alimentation et obésité, sédentarité et utilisation nocive de l’alcool – à l’aide de stratégies à l’échelle de la population. » Une notion qui combinée au discrédit des médicaments, conduit de plus en plus de patients à chercher une thérapeutique alternative, principalement en phytothérapie ou par une modification du régime alimentaire. En réponse à la demande, des produits et recommandations circulent, sans solide support scientifique, soutenant un marché lucratif et potentiellement dangereux pour certains. Quant aux données scientifiques, elles sont hétérogènes, parfois contradictoires si on les prend isolément, donc difficilement exploitables pour les patients comme pour les autorités de santé. Et pourtant, cet engagement des patients n’est pas à refreiner. Premièrement car comme nous le dit l’OMS, changer de mode de vie, et en particulier de régime alimentaire est l’un des meilleurs moyens d’inverser la tendance de ces pathologies, à moindre coût, et sans iatrogénie majeur. Ensuite, car faute d’évidence scientifique diffusée par les systèmes de santé, ce sont les recommandations à visée commerciales qui seront appliquées par les patients.

De plus, il faut pouvoir adapter les recommandations aux capacités de changement du patient. Par exemple, une méta-analyse (2) conclue que l’orientation vers un régime méditerranéen, mais sans restriction de l’apport lipidique, pourrait réduire les événements cardiovasculaire. Ceci permettrait d’avoir un objectif plus réaliste ou un premier pas chez certains patients pour qui une réduction des apports lipidiques est difficile à obtenir, et accompagnée d’atteinte psychologique. A partir de cela, et en fonction des voies connues pour impacter la fonction

cardio-vasculaire, il convient d'identifier les stratégies potentiellement efficaces pour les étayer par des études de large ampleur, permettant alors à des recommandations en termes d'orientation nutritionnelles de voir le jour.

Nous nous sommes intéressés à la voie du monoxyde d'azote (NO) qui aboutit à la vasodilatation. Dans les cellules endothéliales, le NO est produit par la NO synthase (NOS) à partir de L-arginine et en présence de son cofacteur la tétrahydrobioptérine (BH4). Le NO ainsi formé diffuse ensuite dans les cellules musculaires lisses où il active la guanylate cyclase soluble (sGC) qui produit la guanosine mono phosphate (GMP) cyclique (GMPc). S'en suit une activation des protéines kinases G (PKG) permettant la diminution de la concentration cytosolique du calcium (Ca) soit par pompage vers le réticulum sarcoplasmique soit par efflux de la cellule. Ce phénomène aboutit à la relaxation des cellules musculaires lisses vasculaires, donc leur vasodilatation. Etudier cette voie nous paraissait important car la biodisponibilité du NO a été décrite comme diminuée par certaines pathologies cardiovasculaires, de plus, plusieurs aliments ou produits de pharmaco-nutrition peuvent l'impacter. Nous avons donc réalisé une revue systématique de la littérature, suivie d'une méta-analyse des résultats disponibles.

Afin d'avoir des effets comparables, nous avons restreint notre analyse au sujet sain évitant ainsi des confusions avec les effets vasculaires des pathologies. La dilatation dépendante du flux (FMD) a été choisie comme critère de jugement principal car elle est le reflet de la vasodilatation dépendante du NO. Les stratégies nutritionnelles étudiées sont les substrats de la NOS : la L-arginine et son précurseur la L-citrulline que l'on trouve en grande quantité dans la pastèque ; les cofacteurs de la NOS : la BH4 et les folates qui en augmente sa régénération du BH4 ; les donneurs de NO, tous des nitrates ou nitrites inorganiques apportés sous forme de sel ou par des aliments de haute teneur en nitrate, les polyphénols, les sulfures

d'hydrogène. Les nitrates organiques étant des médicaments, ils ont été exclus de cette étude. Pour obtenir un niveau de preuve conséquent, nous avons choisi d'inclure que les essais contrôlés, randomisés, en double aveugle. Nos résultats étant le reflet de la qualité des essais cliniques inclus dans notre analyse, nous avons porté une attention particulière sur la qualité de chaque étude comme sur la qualité de l'analyse globale.

L'ensemble de ce travail est présenté dans le document ci-joint qui sera soumis à publication.



# COMPARAISON OF NUTRITIONNAL STRATEGIES TARGETING THE NITRIC OXYDE PATHWAY TO IMPROVE MACROVASCULAR FUNCTION: A SYSTEMATIC REVIEW AND A META ANALYSIS

## Introduction

Nowadays the interest of the whole population for non-drug approaches to improve their health is rapidly growing. This led to the development of a multitude of approaches, from way of life measures to dietary supplementations, botanical other natural products. Several lifestyle modifications and dietary regimens have showed a positive impact on the onset and severity of chronic diseases and mortality (2). One of the most popular measures is dietary supplementation and changes in dietary behavior. The market quickly expanded, promoted by plenty of suppliers carrying plethora of therapeutics benefits.

One of the most widely targeted physiological signaling pathway by dietary supplements is the nitric oxide (NO) pathway. Increasing NO bioavailability has been effective to treat several diseases such as pulmonary arterial hypertension (PAH), heart failure (3,4). The main therapeutic effect is mediated by the improvement of endothelial vascular function, thus leading to a cardiovascular mortality and morbidity reduction. Nitric oxide is produced by nitric oxide synthetases (NOS) from L-arginine, diffuses to the underlying smooth muscle cells and, through soluble guanylate cyclase (sGC), induce the production of cyclic guanosine monophosphate (cGMP), leading to vasodilatation. This pathway has been shown to be down-regulated in several diseases (3,5,6). Beyond drugs, several nutritional and pharmaco-nutritional approaches have shown the ability to modulate this pathway. Notably, two NOS substrates, L-arginine and its precursor L-citrulline, which is found in large amount in water melon, have been studied. The NOS activity may be also improved by folates in green leafy vegetable, legumes, beans, mushrooms, yeast, some fruits and liver (7,8), or like

synthetic folic acids; through the increase of tetrahydrobiopterin (BH4) recycling, a NOS cofactor (4). Furthermore, owing to antioxidant properties, folic acids may prevent NOS uncoupling(4). Polyphenols, a large chemistry family of compounds, are contained in fruits and vegetables (e.g. grape and red wine, cocoa, colored berries, onions, tea, soy, citrus...) (9) and are believed to directly interact with several enzymes involved in the NO pathway (10). NO can also be produced from nitrate and nitrite by commensal bacteria from the gastrointestinal tract (3,4). Lastly, inorganic nitrate (and nitrite) are contained in food, especially vegetables or beetroot, and NO release compounds like sodium or potassium salt could also be used (11).

Whereas pharmaceutical drugs are used to treat diseases, such dietary supplementations are mainly used by healthy people to improve several conditions claimed by manufacturers (e.g. exercise, cardiovascular ...)

To appraise and compare the efficiency of nutritional or pharmaco-nutritional strategies to increase the NO bioavailability on healthy participants, we performed a systematic review and a meta-analysis. We therefore included randomized clinical trials (RCTs) assessing the efficacy of such interventions on flow-mediated dilatation (FMD), a non-invasive vascular test highly correlated to NO bioavailability (12).

## **Methods**

The meta-analysis was conducted following a predefined protocol (registered on PROSPERO as CRD42018090965) and followed the preferred reporting items for systematic reviews and meta-analyses (PRISMA) for the reporting of our study (13).

## Interventions and search strategy

We searched Medline and clinicaltrial.gov up to July 4th, 2018 with the following evaluated strategies (nitrite, nitrate, citrulline, arginine, polyphenol, flavonol, resveratrol, cocoa, catechin, coffee, folic acid, hydrogen sulfide) and flow mediated-dilatation (FMD).

We selected the following strategies to modulate the NO pathway:

- NOS substrates and cofactors: L-arginine, L-citrulline and water melon, tetrahydrobiopterin, and separately submitted folic acids.
- NO donors sub-group, we selected inorganic nitrates and nitrites contained in food or in compounds like sodium (14,15) or potassium (16,17) salt. We restricted the dietary nitrate to vegetables with a very high nitrate content (>250 mg/ 100 g fresh weight): beetroot, celery, cress, chervil, lettuce, spinach, rocket (rucola) (11).
- Polyphenol: coming from extracts or dietary ingredient. Then results were classified in sub-groups using Phenol explorer database (18–20). We used keywords searches general (polyphenol, flavonol) and some products that we well-knew to provide polyphenol (i.e., cocoa, resveratrol, catechin and coffee).
- Hydrogen sulfide: and precursor allicin coming from garlic (4)

Only English and French articles were included.

## Eligibility criteria

We included only double-blind, randomized, controlled trials (RCT), assessing the efficacy of one of the above-mentioned strategies versus placebo. Moreover, only RCT on healthy adults were included, so without co-morbidities (e.g. hypertension, diabetes, cancer, any cardio-vascular disease). Post-menopause status and smoking was not considered as co-

morbidity. Heterogeneous groups containing healthy and non-healthy participants were included only if the results of a sub-group analysis on healthy volunteers were available.

In this meta-analysis we paid special attention to the placebo arms. Indeed, in dietary trials, producing a suitable placebo with a similar taste, color and galenic form should be challenging. Frequently, such placebo was obtained from the studied dietary ingredient depleted of the active substance. However, the processes of depletion are seldom total. Therefore, most of placebo presented as depleted products contained a small amount of active substance. We considered a depleted product like a placebo if it contained less than 10% of the substance contained into the active arm.

### **Data extraction**

One reviewer (MBG) screened titles and abstracts for inclusion, then reviewed the full-text of potentially relevant articles to determine inclusion using a standardized form according to predefined criteria. The same reviewer (MBG) performed the data extraction on the included full text articles. Other reviewers (JLC, MR, CK) checked the included studies and the extracted data. Discrepancies were resolved through discussion with the team.

The following data were extracted: year, sample size, methodology, participant demographics and baseline characteristics (age, percentage of women, percentage of menopause women, smoker, BMI, cholesterol, LDL, HDL, triglyceride, co-treatment use), follow-up period, wash-out period, details of the intervention and control conditions (type of nutritional or pharmacologic strategy, dosage and treatment duration), outcome (vascular test: FMD, NMD and results), funding.

## **Risk of bias assessment**

We assessed the risk of bias of the included studies according to Cochrane risk of bias tool (19). Finally, we rated their quality as low, medium or high for the following items: random sequence generation; allocation sequence concealment; blinding (participants, personnel); blinding of outcome assessment; completeness of outcome data, selective outcome reporting; and other sources of bias. An item “supported by industrial companies” was added to explain GRADE rating, involving funding, supplementation providing or conflict of interest. We added the overall risk of bias for each trial defined as low-risk if more or equal than 5 high-risk criteria were met, medium-risk between 3 and 4 and low-risk with 2 or less high-risk criteria were met. Then we calculated a quality score (number of low risk items per study) to weighting each study in a sensitivity analysis.

Moreover, we appraised the quality of each therapeutic class using the Grading of Recommendation Assessment, Development and Evaluation (GRADE) recommendations for meta-analysis (21–26). Finally, confidence in quality of results was summarized as very low, low, moderate or high. Details on GRADE evaluation are available in Supplementary Material

## **Statistical analysis**

We performed two separate meta-analyses to assess the effect of acute (<24h) and chronic supplementations (>24h) of nutritional strategies.

The primary outcome was the mean difference in endothelial vascular function assessed by flow-mediated dilatation of brachial or radial artery after the intervention and control arm. The secondary outcome was change in endothelial-independent vascular function Nitrate-mediated dilatation (NMD). If several FMD were performed in a trial, we

extracted the results of FMD on the time-point defined in the primary outcome; if no time point was defined we selected the peak FMD in both arms. When several dosages of the same intervention were tested against a control arm, the results were pooled in a single arm. If a trial presented results for acute and chronic modalities, it was included in both analyses.

A direct meta-analysis using inverse variance fixed effect model or DerSimonian and Laird random effect model was first performed to assess pooled mean difference (MD) and 95% confidence interval of flow-mediated dilatation between groups. Random effect model was used when substantial heterogeneity was detected,  $I^2$  statistic >50% or p value of the Q statistics < 0.10 indicating heterogeneity. If more than 10 studies were available, the funnel plot asymmetry was explored using Egger's regression test as recommended by Cochrane handbook for systemic reviews (27), with a p-value <0.05 suggesting publication bias. All statistical analyses were performed using R statistical software (version 3.2.4).

We secondly performed a Trial Sequential Analysis (beta 0.9.5.10) to adjust the threshold for statistical significance (O'Brien and Flemming method) on the available information for each therapeutic class and estimation of required information size (27,28).

### **Subgroup and sensitivity analyses**

We performed a pre-planned sensitivity analysis excluding high risk of bias studies. Moreover, we assessed the effect of control arms on FMD depending if the control intervention was a placebo or a low dose active substance. We therefore performed a subgroup meta-analysis of pre-post mean differences in the control arms (using a correlation of 0.5).

Several meta-regressions were also performed to assess the potential influence of gender, age, body mass index, total cholesterol, high-density lipoprotein cholesterol, low-

density lipoprotein cholesterol, triglycerides, proportion of active smokers, FMD before intervention, funding and duration of supplementation (only for chronic intake) on meta-analysis.

## Results

### Studies selection

1033 references were identified through database searching. After removal of duplicates, titles and abstracts screening, 98 reports proved potential eligibility. Finally, 55 RCTs were included in the quantitative synthesis after full text screening as present in Figure 1.

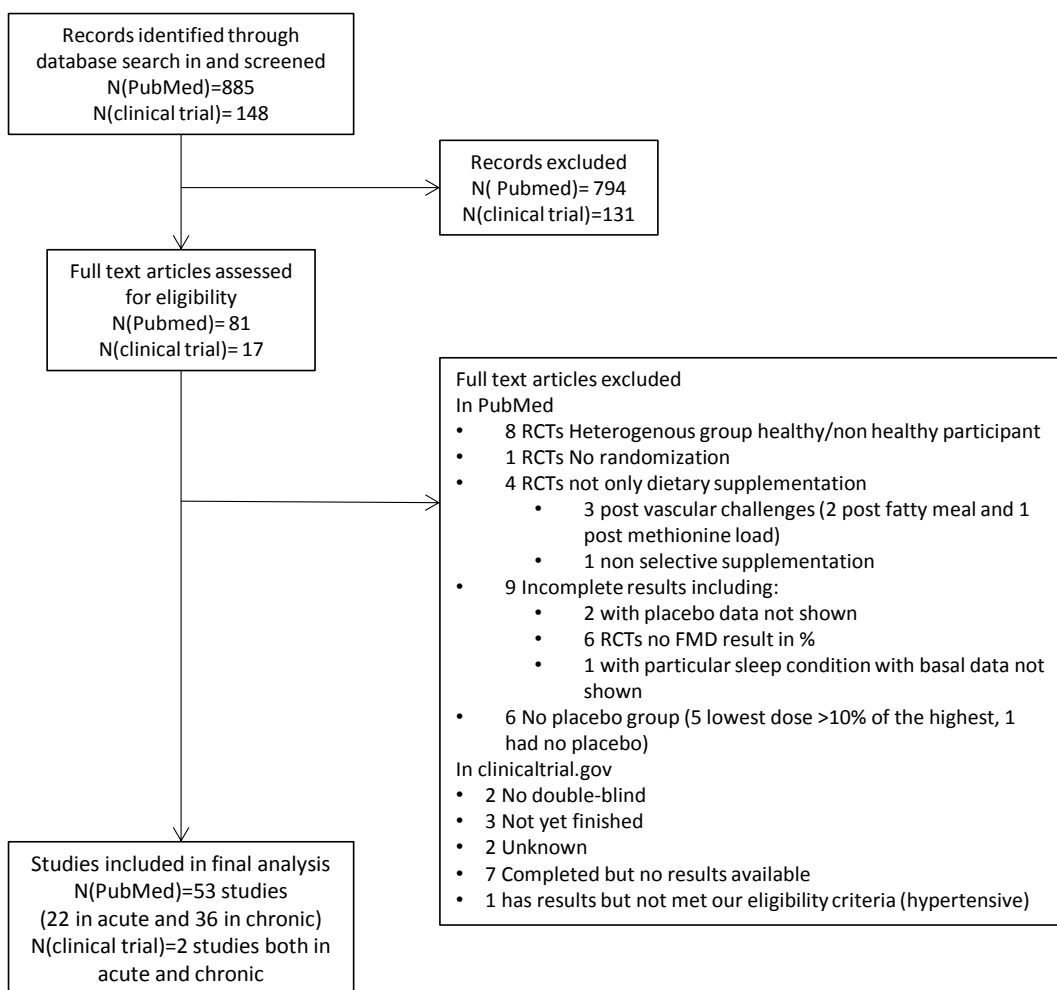


Figure 1: Flow chart

## Quality of included studies

Among the 55 included studies, the risk of bias was highly heterogeneous, only 13 (23.6%) reported an adequate randomization process and 5 (9%) supplied enough information to assess allocation concealment. Even though all selected studies were double blinded, 3 (5.4%) had questionable blinding. Only 17 (30.9%) studies explained how they performed blinding outcome assessment. Moreover, incomplete outcome data and selective reporting were detected in 24 (43.6%) and 17 (30.9%) studies respectively. The last domain-specific bias led to the exclusion of numerous studies (notably results of placebo arm not shown).

Overall, 5 (9.09%) studies were considered of low risk of bias, 30 (54.5%) of medium risk of bias, 20 (36.3%) of high risk of bias. “Industry funding” item was added on quality assessment with 24 studies supported by an industry. Results according to acute and chronic intake are summarized on figures 2 and 3.

We also performed a GRADE evaluation for each subgroup quality of evidence. Results are provided on Supplementary material.



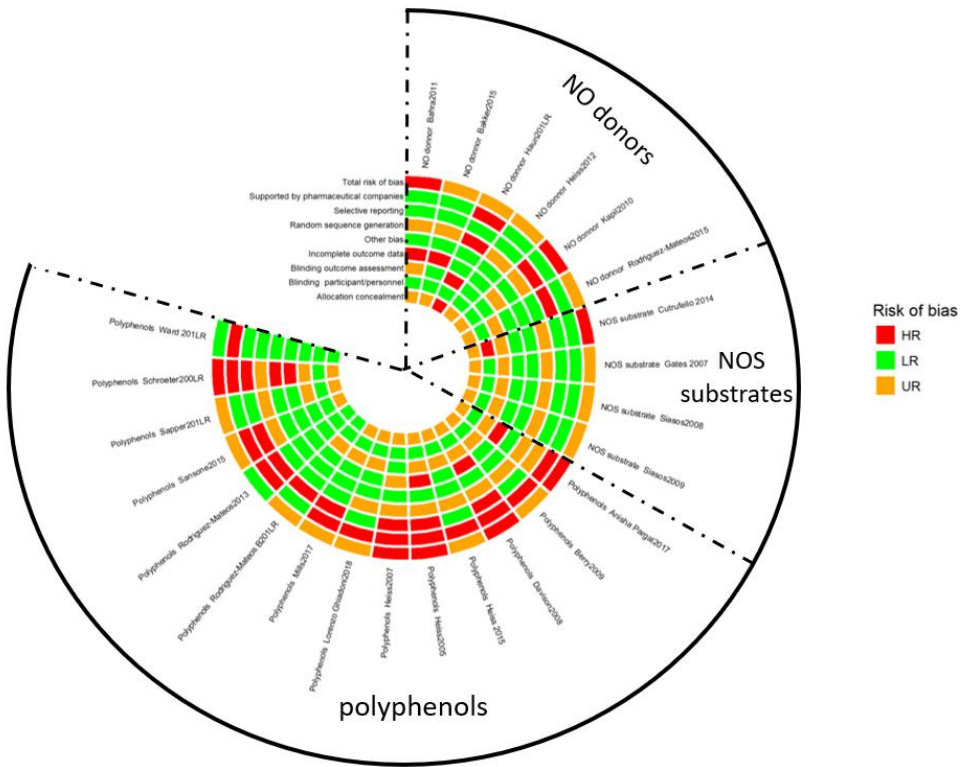


Figure 2: quality of studies on acute supplementation

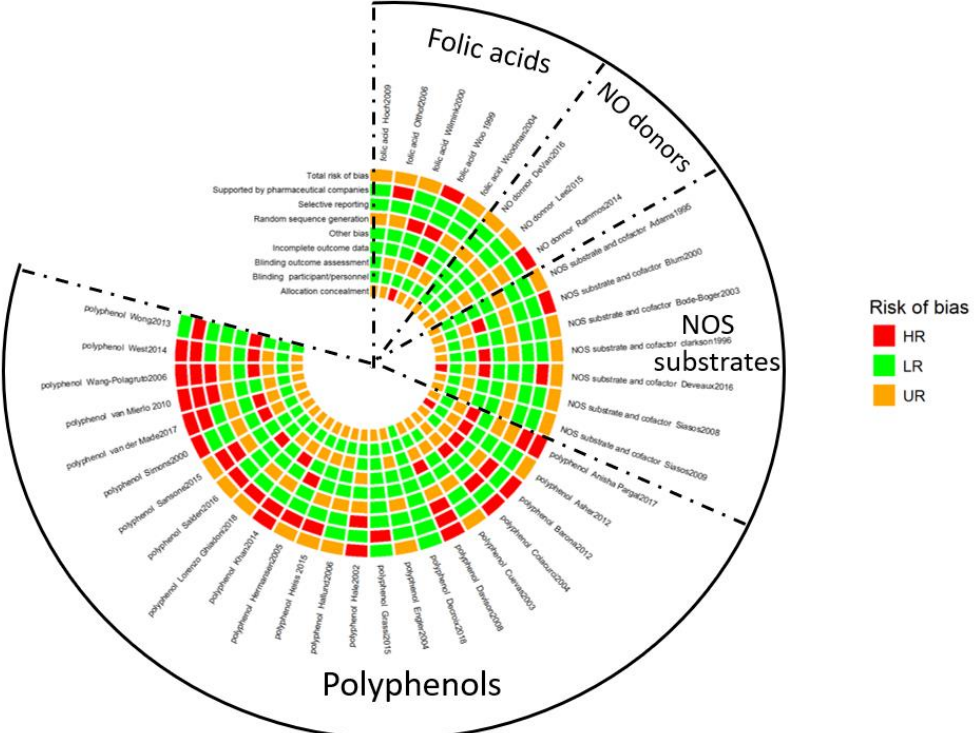


Figure 3: quality of studies on chronic supplementation

## **Acute effect of nutritional strategies**

### ***Characteristics of studies and patients***

Analysis of the acute effect of selected strategies was conducted on 24 RCTs including 503 volunteers. Mean age was  $36.6 \pm 13.2$  years and mean BMI  $23.58 \pm 2.94$  kg.m<sup>2</sup>

In the NO donor sub-group, 6 studies were included, 2 challenging dietary ingredients (beetroot juice (29) and red spinach extract (30)) and 4 other using potassium or sodium nitrate salt (supplementary material). In the NOS substrates sub-group, 1 study of l-citrulline (bring as molecule and into watermelon)(31) , 1 with intra-venous arginine (32) ant 2 other using per os arginine were included. For polyphenol sub-group, 14 studies divided in cocoa supplementation (8 studies), mix flavonoids and phenolic acids coming from colored berries (2 studies), 3 testing tea extract (2 black and 1 green tea), epicatechin (1 study) (33). All of them are flavonoids active compound, but we also included 2 studies with chlorogenic acids (i.e. other polyphenol non-flavonoid) (33,34). No study on acute effect of tetrahydrobiopterin, folic acids and hydrogen sulfide met eligibility criteria.

### ***Primary outcome***

Overall efficacy of inorganic nitrates on FMD was significant, mean difference (MD) =1.07 (95% CI 0.68, 1.45, I<sup>2</sup>=47%). Among inorganic nitrate, nitrate salts MD=1.07 (95% CI 0.64-1.49, I<sup>2</sup>=0%) displayed the larger effect size (Figure 4).

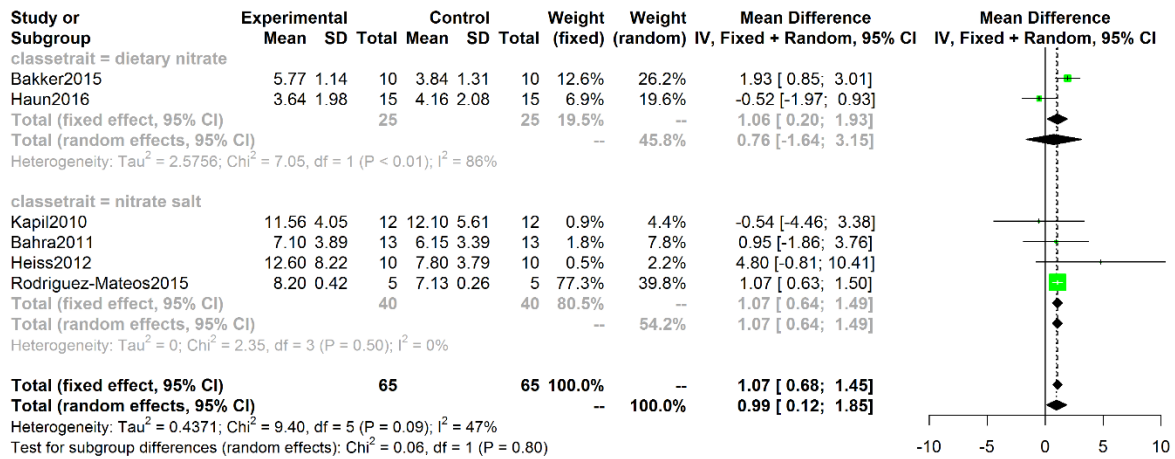


Figure 4: Acute effect of NO donors on FMD (Forest plot)

Although requested information size is not reach for acute NO donors effect, z-curve showed a positive effect of NO donors in acute effect on the adjusted threshold (Figure 5).

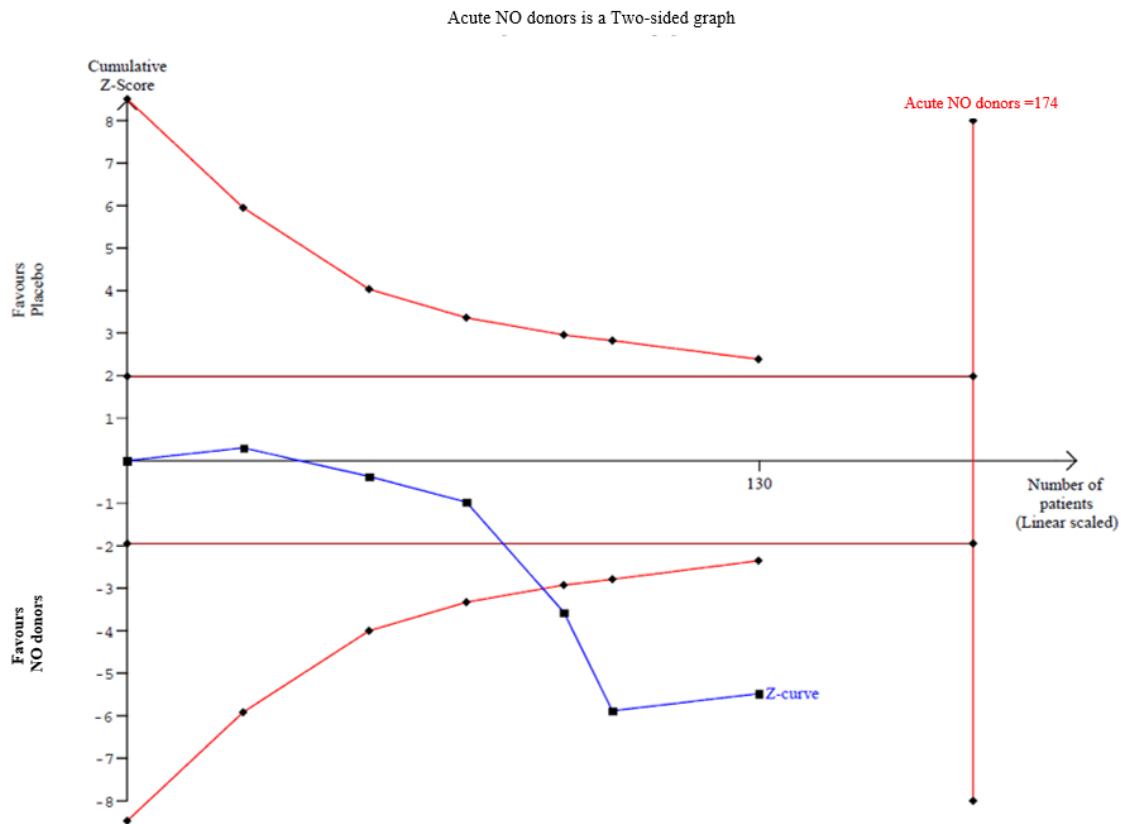


Figure 5: Z-curve of acute effect of NO donors on FMD

In the NOS substrate group, no result was significant, and no trend was apparent (Figure 6). Because less of 5% of requested information was available, the TSA was not performed.

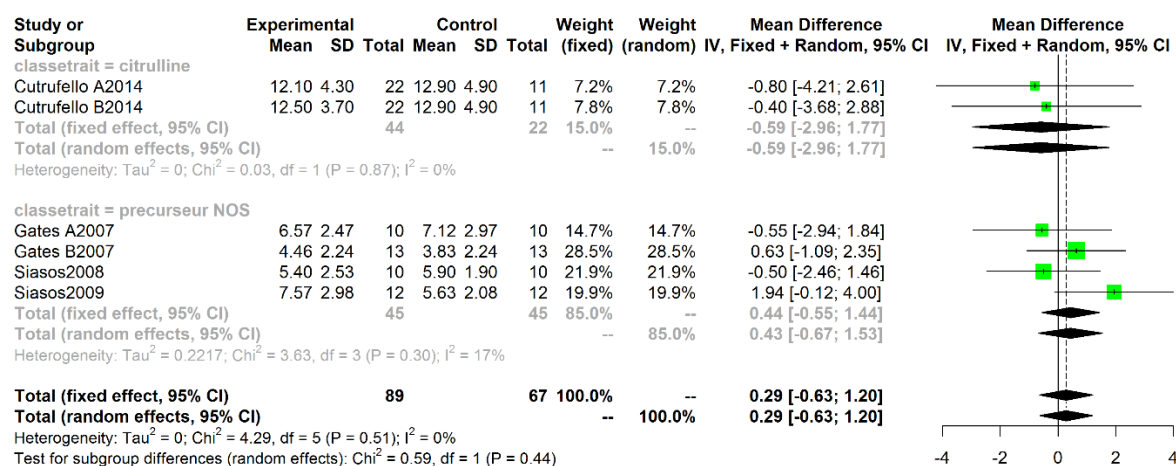


Figure 6: Acute effect of NOS substrates on FMD (Forest plot)

For polyphenol analysis, overall efficacy was significant with a MD= 1.45 (95% CI 0.84-2.05, I<sup>2</sup>=0%) Figure 7. Among this subgroup, overall effect was mainly driven by cocoa products (MD= 2.43, 95% CI 2.45-3.4, I<sup>2</sup>=89%).

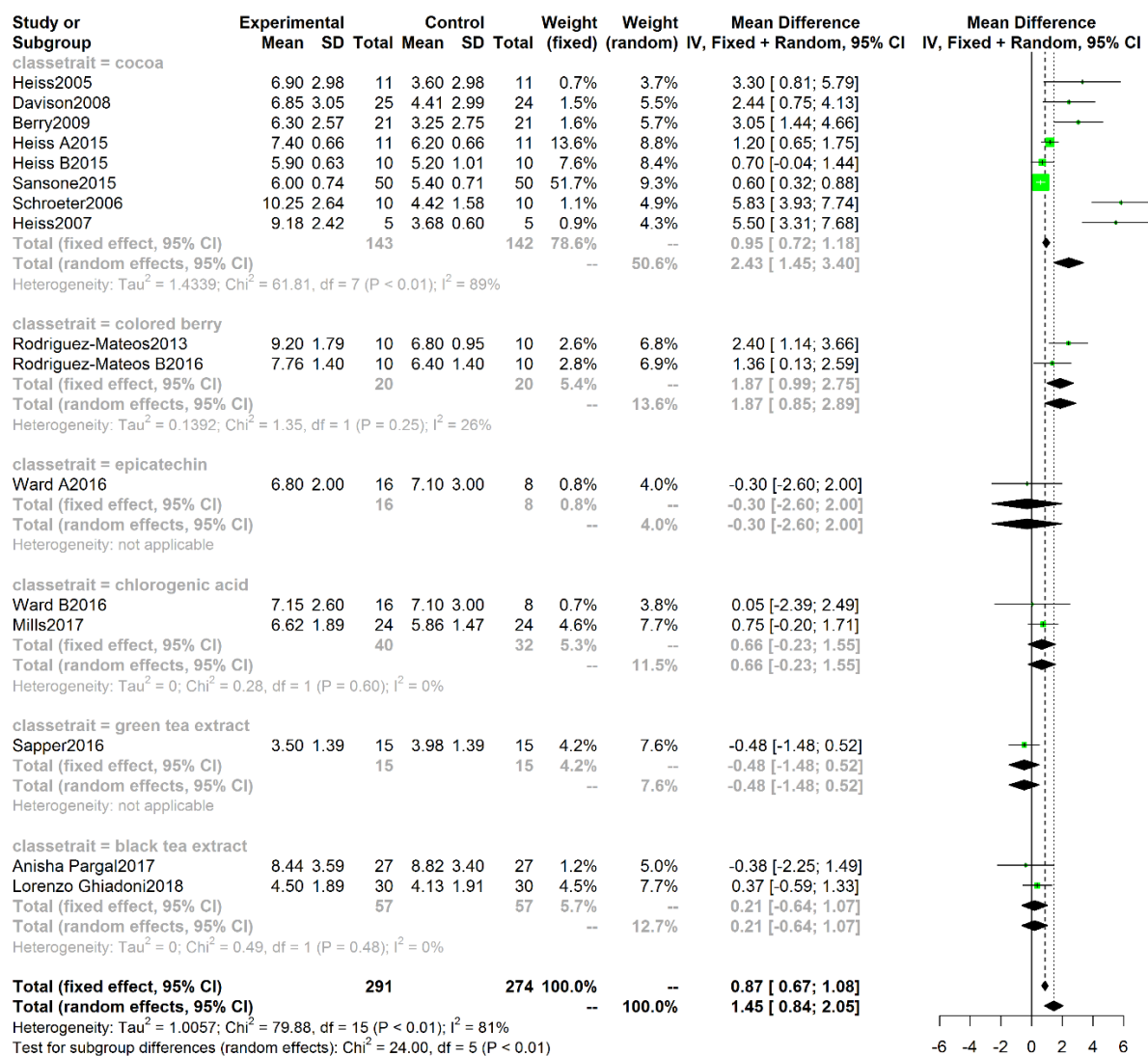


Figure 7: Acute effect of polyphenols on FMD (Forest plot)

In both length of supplementation, polyphenol effect was positive and significant and the requested information size was reached, thus improving the confidence on these results

(Figure 8).

Acute polyphenol 2 is a Two-sided graph

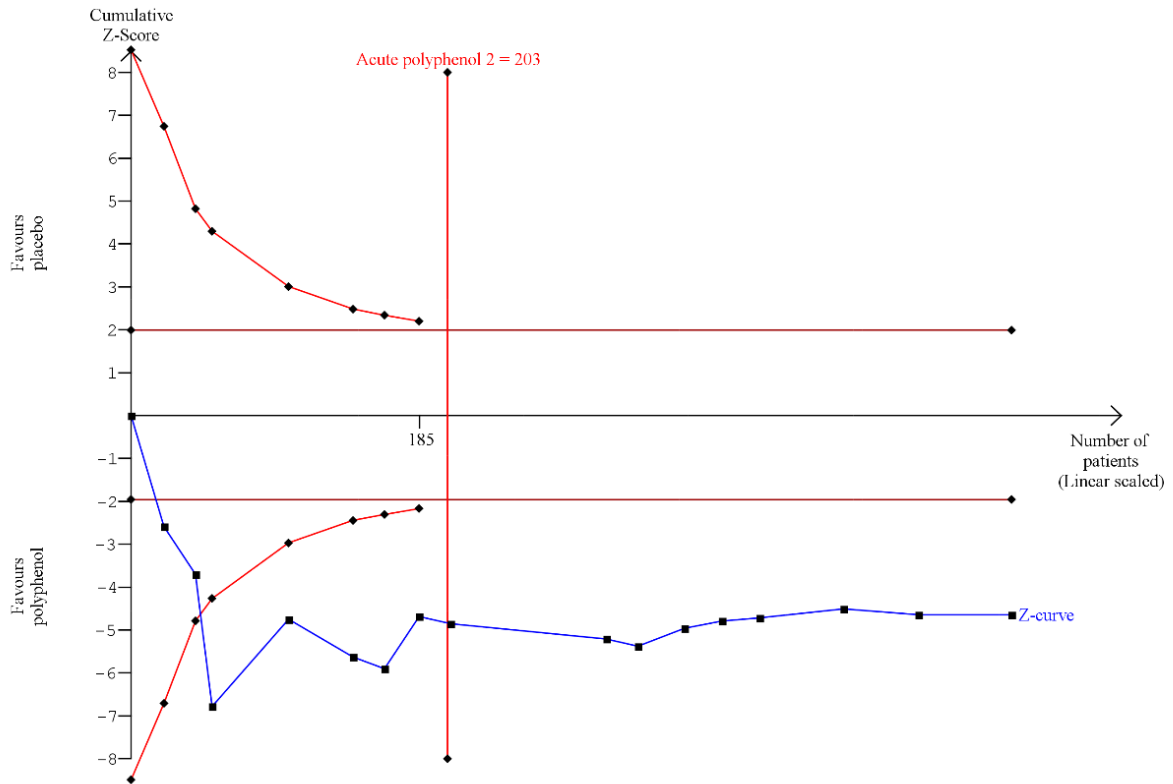


Figure 8: Z curve acute effect of polyphenols on FMD

NOS precursors and NO donors sub-groups funnel plots were not explored because of a too low number of studies. In the polyphenol sub-group, two outliers were visually detected (Figure 9) but the Egger test for funnel plot asymmetry was not significant (p-value= 0.05476).

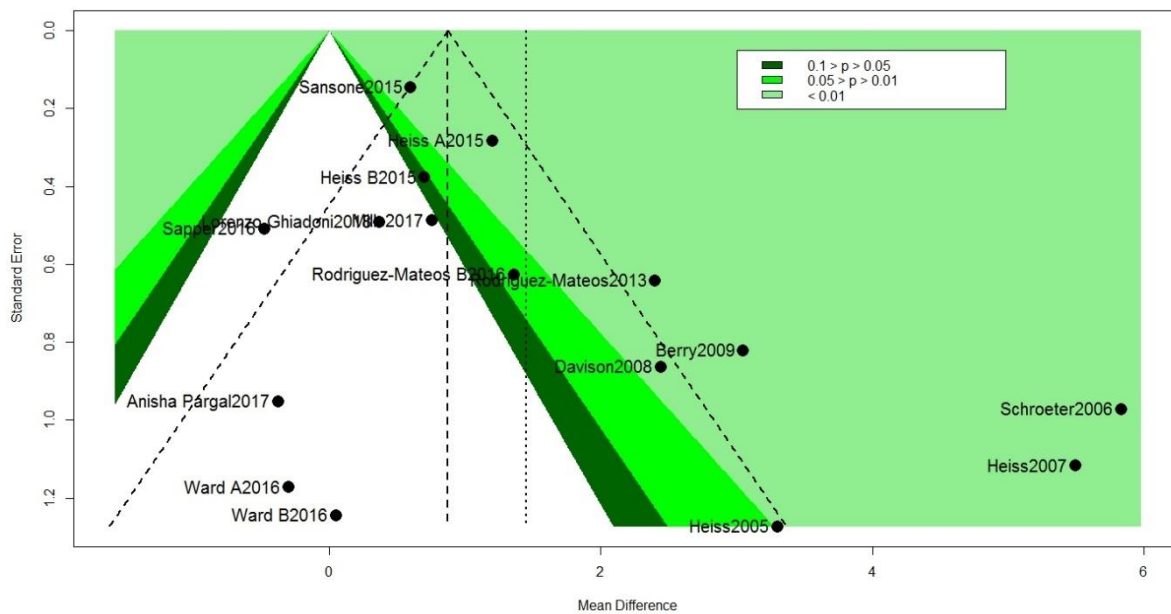


Figure 9: funnel plot of polyphenols acute effect

### Secondary outcome

Only 4 studies provided information on NMD. No effect was detected for any subgroup (Figure on supplementary material).

### Meta-regressions

Meta-regression showed no influence of the studied covariate except for smoking (p-value = 0.0357), which was significantly associated to a lower effect of nutritional strategies (Table on supplementary material).

## Chronic effect of nutritional strategies

### *Characteristics of studies and patients*

Data of 38 RCTs were included in the meta-analysis of chronic effect, median supplementation time is 28 (interquartile at 25 and 75%: 14-42) days (from 3 to 365 days), on 1076 volunteers, including 46.8 % of women, 8.8% active smokers, mean age was  $48.3 \pm 14.1$  years and mean BMI  $23.7 \pm 2.92$  kg.m<sup>2</sup>.

12 RCTs selected only post-menopausal women, and 2 RCTs only on active smokers.

For chronic effect analysis, only 3 RCTs constituted the NO donors sub-group, 2 using sodium nitrate salt (35,36) and 1 beetroot juice (37). We did not find any RCT using l-citrulline meeting our inclusion criteria, therefore NOS precursor were constituted only by arginine per os (7 RCTs), one of which using sustained-release L-arginine (38). In contrast, the polyphenol group was composed of 23 RCTs themselves subdivided in 8 categories. The main category was constituted by cocoa (8 RCTs), followed by isoflavones which are another flavonoid class, coming from soy (6 RCTs). Then, 2 RCTs assessed the effect of wine an grape extract (39,40); 2 RCTs of stilbene, a non-flavonoid polyphenol, using only stereoisomer trans-resveratrol 99.9% (41,42); 2 RCTs assessing the effect of black tea extract (43,44); one RCT on blackcurrant juice (45), expected to bring anthocyanins, flavanol and flavonols; one on citrus hesperidin which is a flavanone (46), and one on hawthorn extract (47). We search folate supplementation, but all of 5 RCTs we included provided non dietary ingredient folic acid supplementation. Any RCT about hydrogen sulfide and BH<sub>4</sub> was included because expression of FMD was not in percentage.



## Primary outcome

For NO donors sub-group, the mean difference was significant (MD= 0.78, 95% CI: 0.16; 1.4,  $I^2=47\%$ ).

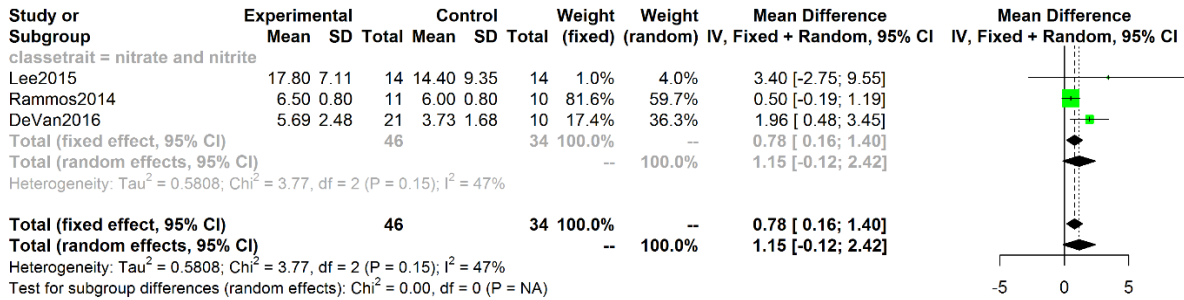


Figure 10: chronic effect of nitrates on FMD (Forest plot)

In chronic supplementation, included studies on NO donors neither reach requested information size, nor adjusted significance nor futility.

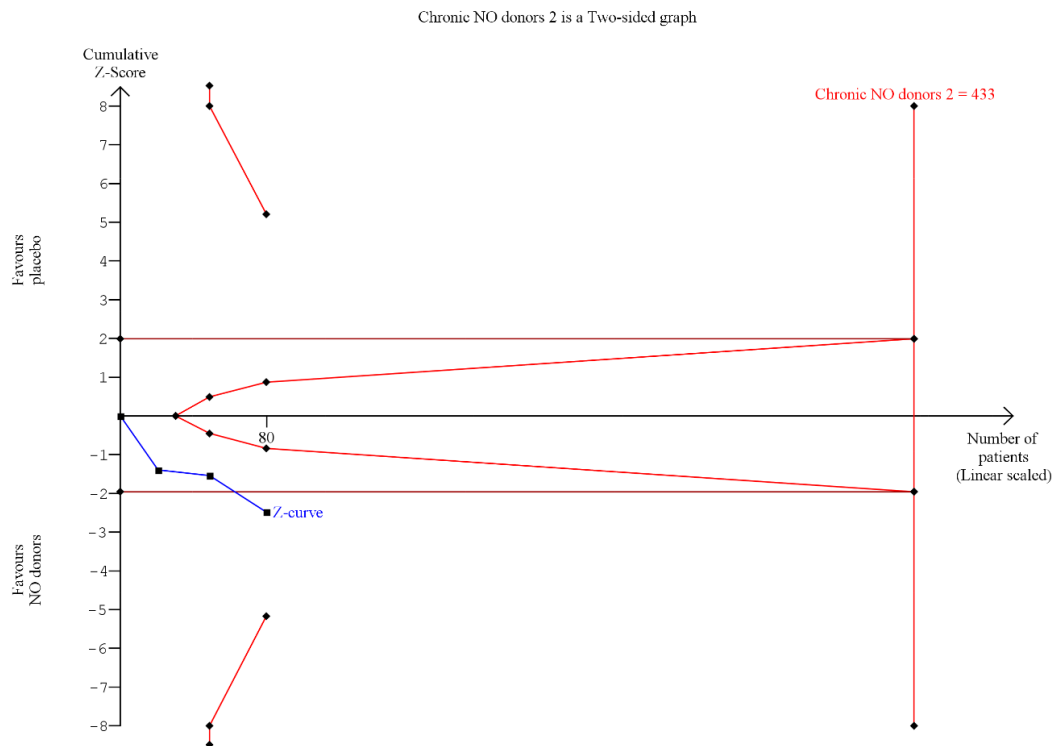


Figure 11: Z-curve of chronic NO donors on FMD

No significant effect was found for NOS substrates (MD= 1.22, 95% CI -0.64; 3.7, I<sup>2</sup>=85%).

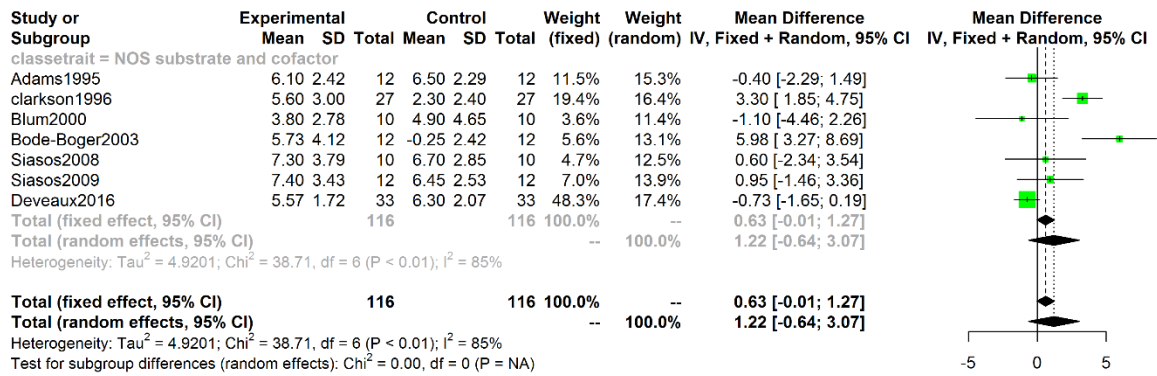


Figure 12: Chronic effect of NOS substrates (Forest plot)

While a little more informative than in acute supplementation, chronic effect didn't show any significance or futility. Requested information size stayed far away from available data.

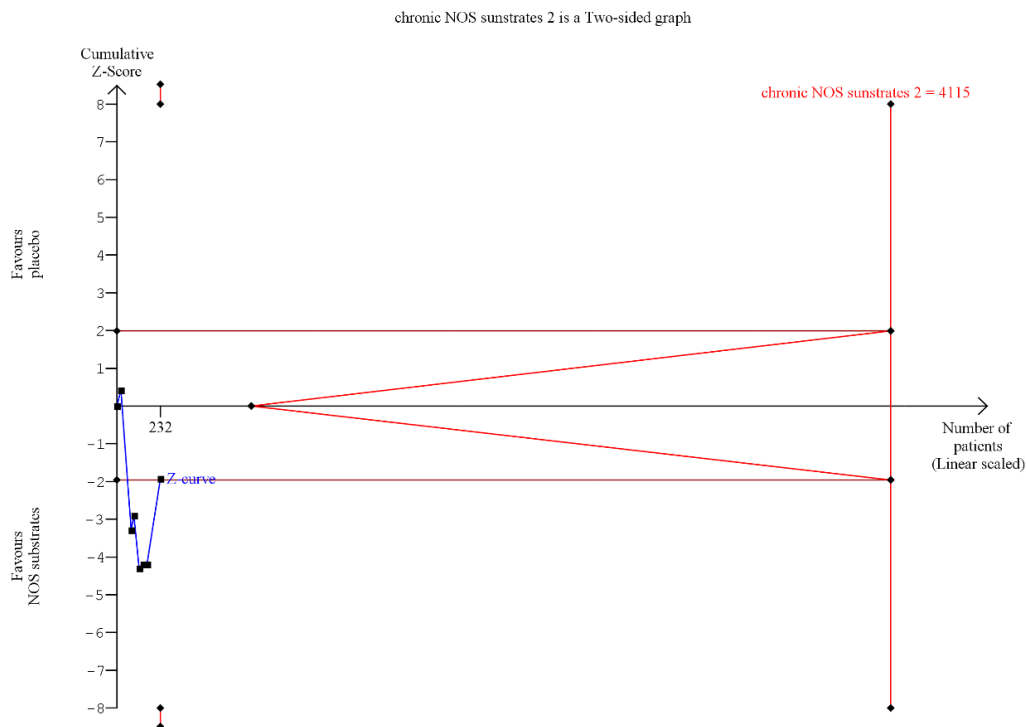


Figure 13: Z-curve chronic NOS substrates on FMD

Overall effect of folic acids group was not significant (MD= 1.1, 95% CI -0.34; 2.54, I<sup>2</sup>=79%). Requested information size (765) was not reached and the adjusted result was close of the fertility margin.

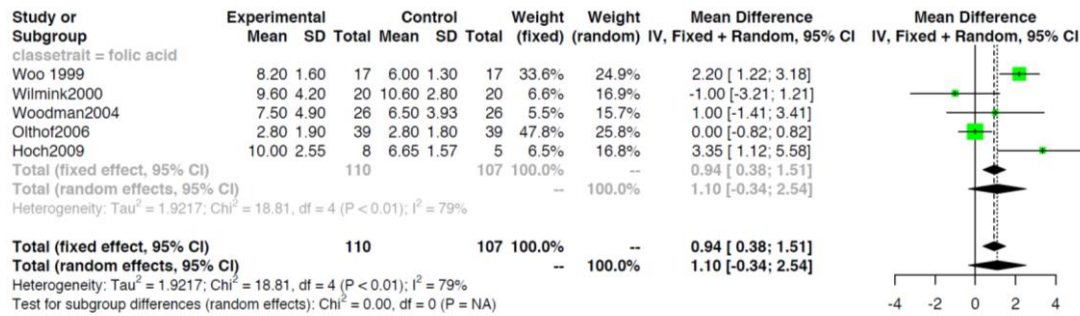


Figure 14: Chronic effect of folic acids on FMD (Forest plot)

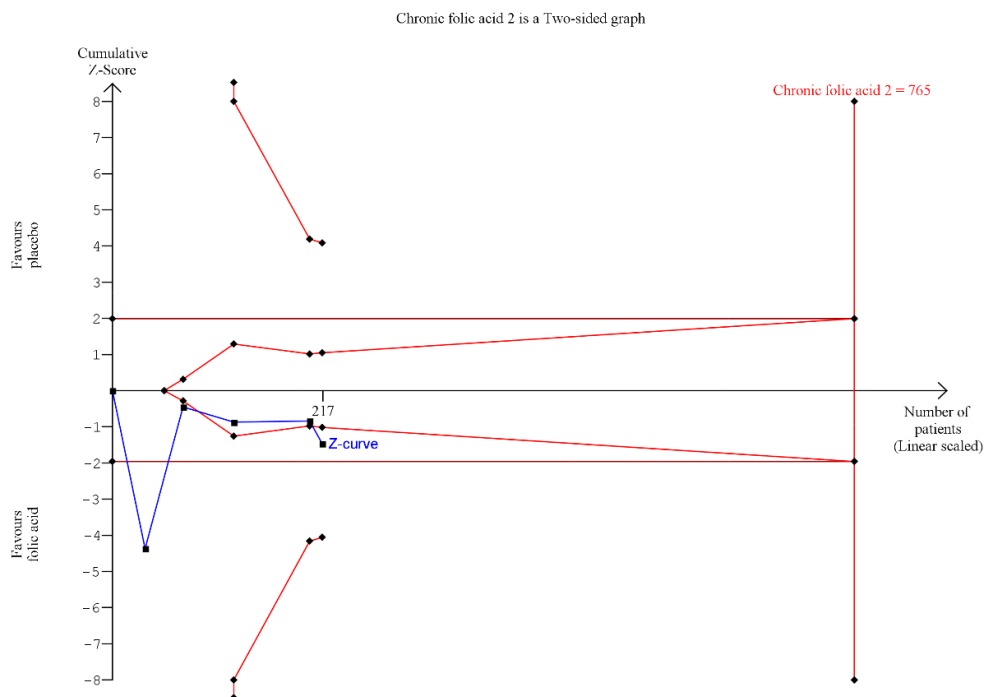


Figure 15: Z-curve on chronic folic acids on FMD

Polyphenols showed a significant improvement of FMD with a MD= 0.83 (95% CI 0.36-1.3, I<sup>2</sup>= 77%). Two polyphenol groups: cocoa and isoflavones presented the larger effect sizes.

Adjusted threshold analyses corroborate to a polyphenol effect by a significant positive effect and exceeded requested information size.

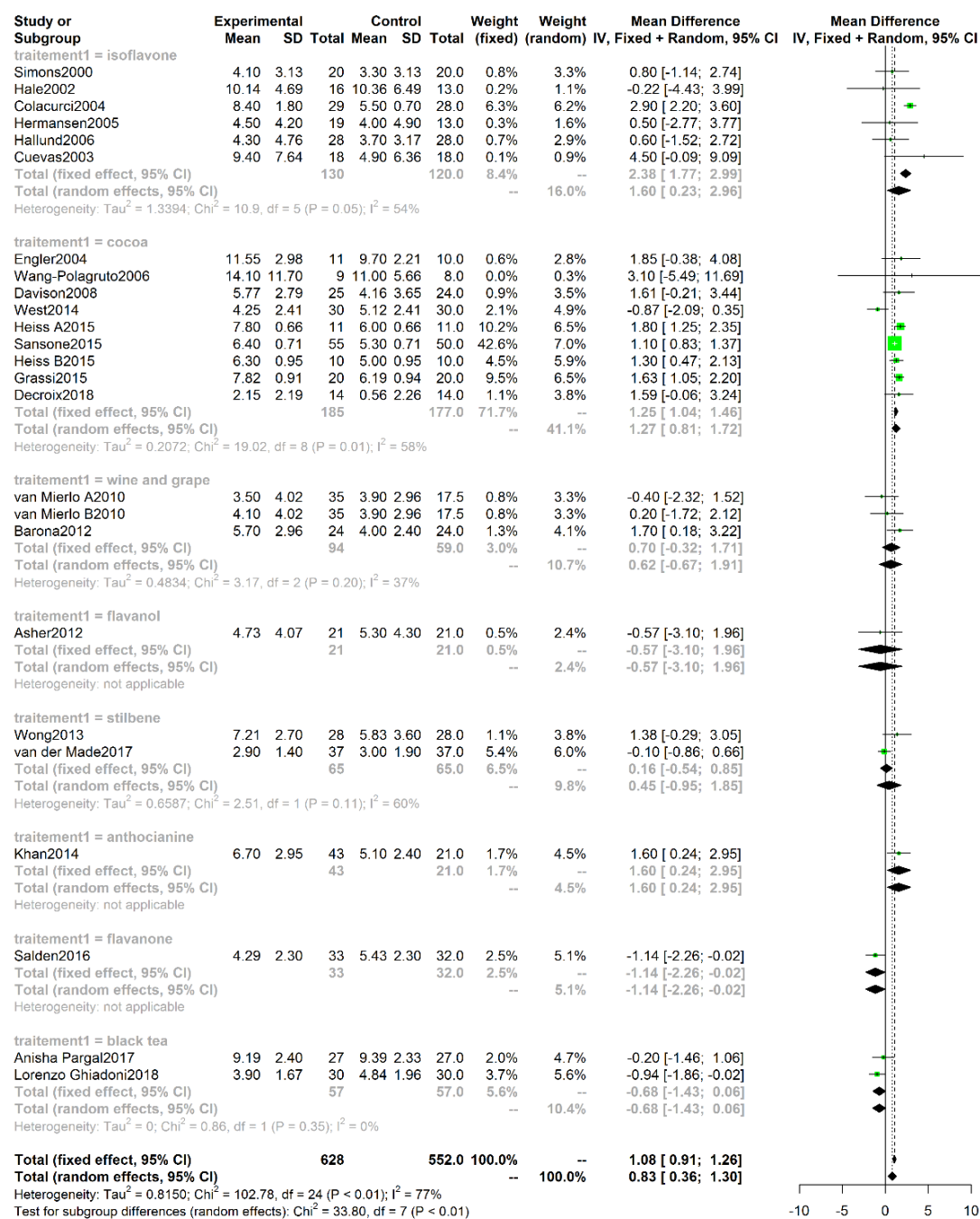


Figure 16: Chronic effect of polyphenols on FMD (Forest plot)

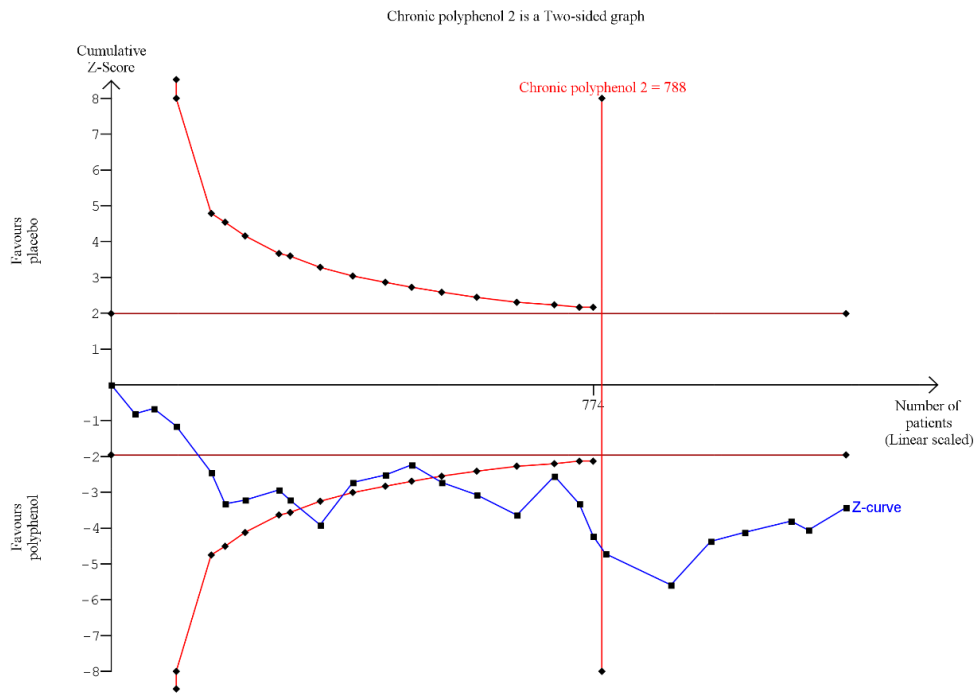


Figure 17: Z-curve chronic effect of polyphenols on FMD

According to the funnel plot, publication bias was not detected, as highlighted by a non-significant Egger test on polyphenols ( $p=0.41$ ).

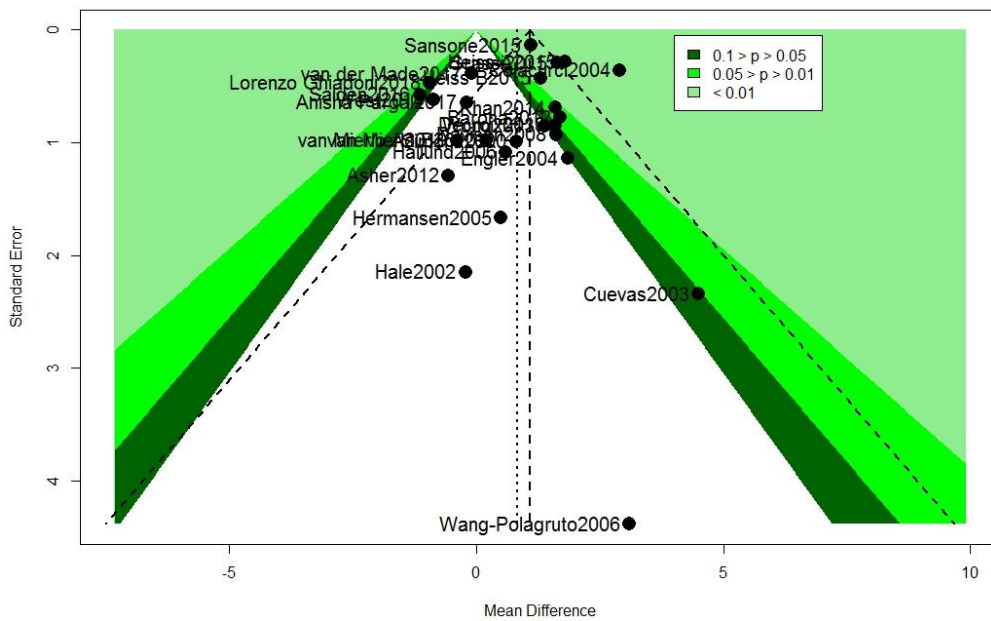


Figure 18: Funnel plot chronic effect of polyphenols on FMD

### *Secondary outcome*

Data retrieved for chronic effect allowed analysis for arginine (5RCTs), for folic acid (3RCTs) and polyphenol (with 6 RCTs for isoflavones, 2 for cocoa, 1 for black tea) only. No significance or trend was detected for any subgroup (Figure on supplementary material).

### *Meta-regressions*

Only 2 meta-regressions were significant. The supplementation duration which any day added improve FMD by 0.01% ( $p=0.0381$ ). Moreover industrial funded studies presented inferior results by 1.3 % on FMD compared to academic funded ( $p=0.003$ ).

## Summary results

The results are summarized in Figure 19: results summary .

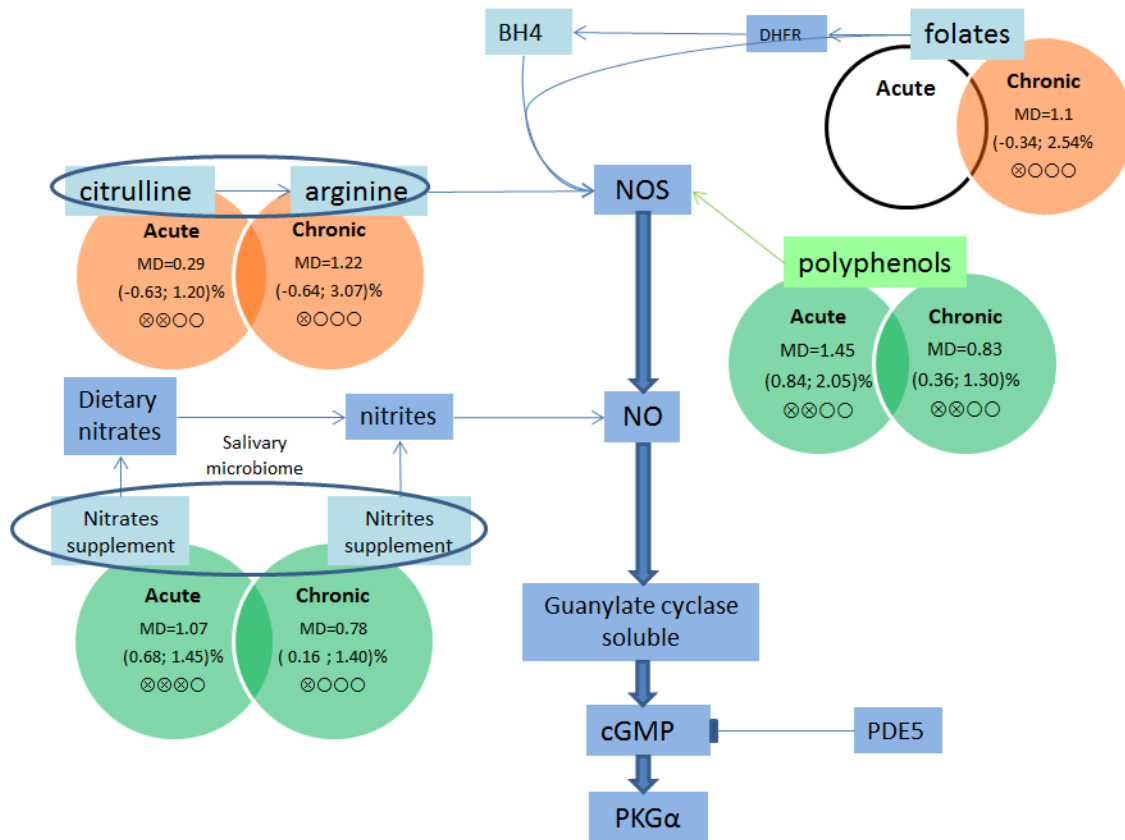


Figure 19: results summary

Constituents on NO pathway are in blue boxes. Light blue ones represent studied supplementations. In two circle above, effect are summarize by mean difference ( confidence intervals at 95 %) for acute and chronic supplementation, color varies according to the significance (green if result is significant and orange if it is not). For each ones, GRADE quality of evidence assessments are indicated by ⊗⊗⊗○: intermediate, ⊗⊗○○: low and ⊗○○○: very low.

## Sensitivity analysis

Sensitivity analysis excluding high risk of bias studies was performed. In the acute effect meta-analysis, only the polyphenol sub-group contained high quality studies, all study in NOS substrate and NO donors were rated as low or medium quality. The results were similar when excluding high risk of bias studies (presented on GRADE table supplementary material).

## Placebo effect

Overall, acute effect of the control interventions on FMD corroborated with a placebo effect MD= 0.11, 95% CI = 0.06; 0.16,  $I^2=0\%$  with no subgroup difference ( $p=0.15$ ). Likely explained by low dose placebo (<10%), MD= -0.13, 95% CI= -0.19; -0.7). Whereas total placebo effect was non-significant: MD= -0.04, 95% CI= -0.15; 0.08. (Forest plot on supplementary). No placebo/low dose effect was detected in chronic supplementation nor subgroup difference ( $p=-0.06$ ; 0.13).

## Discussion

We conducted this systematic review and meta-analysis to identify the best nutritional compounds able to improve vascular function in healthy volunteers without history, signs or symptoms of cardio-vascular diseases. Our primary conclusion is the limited number of low risk of bias studies despite an abundant literature and numerous trials performed on this topic. Then, we highlighted that, among nutritional strategies targeting the NO pathway, polyphenols showed the larger effect size on FMD and the robustness level of evidence. Inorganics nitrates might also be efficient but the level of evidence was lower on chronic effect and did not allow to conclude with certainty, especially in this pathway where tachyphylaxy is well-described. Contrariwise, increasing NOS substrates (L-arginine) or NOS



cofactors (folic acids) seemed not an efficient strategy to improve FMD on healthy individuals.

Despite the low number of selected studies, our results on inorganic nitrates group are in accordance with a previous meta-analysis (48). Proposed as a more appropriated alternative to organic nitrates for vascular modulation, inorganic nitrates have shown a dose-dependent beneficial effect on endothelial function, sustained in time with no evidence of tolerance (49). Inorganic nitrates can be absorbed from vegetables, drinking water, cured meat and nitrate salt supplementation (11). Nitrates are reduced to nitrites by the oral commensal bacteria (50), then the remaining nitrates and 95% of nitrites are rapidly absorbed into the systemic circulation by the upper gastro-intestinal tract (51). Absorbed nitrites are thereafter reduced to NO in the blood stream and tissues by several possible mechanisms, which include iron of haem-proteins, myoglobin, xanthine oxidoreductase (XOR), endothelial nitric oxide synthase (eNOS), aldehyde oxidase, ALDH-2, carbonic anhydrase, protons, ascorbate and polyphenols (49). NO could easily diffuse to the close environment, form iron-nitrosyl complex with haem molecules. Such binding activates soluble guanylate cyclase (sGC), which generates cyclic guanosine monophosphate (cGMP), leading to vasodilatation, nerve signaling, mitochondrial biogenesis and angiogenesis (3). This mechanism could explain the protecting effects of inorganic nitrites and nitrates as anti-inflammatory and antioxidant on hypertension, diabetes, ischemia/reperfusion, atherosclerosis and thrombosis (50). A key mechanism preventing the sudden effect, toxicity of the vascular effect of inorganic nitrites and nitrates is the entero-salivary circulation. In contrast to organic nitrates, 25% of the inorganic nitrates absorbed are concentrated in salivary gland and re-secreted into the mouth (49,51). Finally, even if this is out of the scope of our study, inorganic nitrates should be particularly interesting in pathologic conditions associated with a dysfunctional NOS, because they can increase NO production in a NOS independent manner (4,51).

Accordingly to previous meta-analysis (52–54), we found an improvement of vascular function in pooled analysis with polyphenols both acutely and chronically with an heterogeneous response depending on polyphenolic constituents. From cocoa to chlorogenic acids, the diversity of included supplementations could explain the heterogeneity  $I^2=81\%$  within this group. Cocoa was the only compound showing an acute and a chronic significant effect improving FMD. Concordantly, the blood pressure lowering effect described with flavanol-rich cocoa might also be mediated by the NO pathway (55). Trials on soy products only provided data on chronic supplementation with a significant positive impact. Chronic effect of red wine or grape did not show significant efficacy. Polyphenols are well known to improve vascular function, notwithstanding the mechanism of action is not fully understood (51). Traditionally described as antioxidant scavenging reactive oxygen species (ROS), their bio-availability about 5 to 10% of total intake, seems too low to support this hypothesis (3,56,57). An additional effect is inhibition of ROS-generating NADPH oxidase, preventing NO destruction by radicals which can interact with. Moreover, polyphenols stimulate eNOS, through phosphorylation, by increasing calcium concentration, PI3 kinase/AKT pathway and p38 mitogen-activated protein kinase (3,58). Furthermore, other mechanisms are hypothesized : anti-inflammatory (decrease of adhesion molecules (58), part by xanthine oxidase modulation (51), action on inflammatory cell and mediator), decrease of platelet aggregation, improvement of circulating lipid profile reducing atherogenesis(58). Nevertheless, numerous compounds with variable activity are pooled under the “polyphenol” designation. Vascular effect is mostly described for flavonoids but an effect of other polyphenol could not be excluded (stilbene, chlorogenic acid). Given that, one dietary ingredient may contain several polyphenols in different amounts, further complicated by interaction and possible additive effects, to identify the compound inducing the therapeutic effect is challenging.

Moreover, an additive effect was also recently described between nitrites/nitrates and flavonoids (15). As described above, nitrite can be metabolized in NO by eNOS, which can be stimulated by polyphenols. In the stomach, reduction of nitrite to NO is enhanced by the presence of vitamin C or polyphenol (by reductive hydroxyl group on the phenol ring) (3,51). Despite a lack of evidence in tissues, effects of inorganic nitrates and cocoa flavonols could be additive to improve vascular function in healthy humans (15). Furthermore nitration or nitrosation on polyphenols could result in longer NO donors half-life (3). So, the positive effect of inorganic nitrates contained in beetroot should be reconsidered. Indeed polyphenols (flavonoids: luteolin and quercetin and lignans) are present in beetroot, especially in beetroot juice (59)(18). Likewise, co-ingestion of methyl xanthines (caffeine or theobromine) in cocoa, coffee and tea, could enhance flavonol absorption leading to a synergic effect on vascular function (60).

The NOS precursor group was mostly represented by per os L-arginine intervention trials (except one on intravenous arginine and another one on citrulline). This semi-essential amino-acid seemed to be an attractive way of supplementation as a substrate of NOS in epithelial cells, increasing its concentration enhancing NO production. Our results constituted a good reflect of the very inconsistent literature on this topic and are concordant with a previous meta-analysis (61). In their work, L-arginine intake (from 3 to 24 g/d) improved FMD only if baseline FMD was low but has no effect of medium FMD. Thus, L-arginine might restore a dysfunctional endothelium by normalizing impaired NO production. Nevertheless, normal endothelial function could not be further improved in that way. One way could be to rebalance the L-arginine/Asymmetric dimethyl arginine (ADMA) ratio; given that ADMA is an endogen NOS inhibitor. ADMA is overproduced in cardiovascular disease (62). In the first instance, their conclusion seems to explain our results, because our population is overall healthy. But, the larger observed effect size was not observed in the lower FMD

group, maybe coincidence because seldom studies. The only consistent findings that L-arginine is on the cross way of number physiological processes: precursor of several other amino-acid, part of urea cycle as substrate for arginase, substrate for NOS (3). So, in a complex integrated system, with sprawling regulation.

Several NOS co-factors could be increase as tetrahydrobiopterin supplementation had also been considered. Especially because when NOS substrate or cofactor have limited bioavailability, NOS can be uncoupled (63), resulting in the production of superoxide radical rather than NO (3,4). Folates also named vitamin B9 are not synthesized de novo by mammals and depend of dietary intake or supplementation. Green leafy vegetable, legumes, beans, mushrooms, yeast, some fruits and liver, provide the majority of nutritional intake (7,8). Because bio availability of folates is rather low, around 50%, pharmaco-nutritional supplementation as synthetic folic acids is usual (8). After a dose dependent absorption (4), folates are bio-activated in 5-methyl tetrahydrofolate (5MTHF) by the liver (7) ; and if saturated, folate can be reduced in cell by dihydrofolate reductase (DHFR) (8). Mechanistically, two main actions are described on vascular function, a direct antioxidant effect by ROS scavenging (7) or an increase of NOS coupling through direct and indirect ways. 5MTHF is presumed to mimic the BH4 conformation and bind the NOS promoting NO production (64). Moreover, tetrahydrofolate upregulate DHFR activity, recycling BH4 from inactive BH2 (4,8). A previous meta-analysis on this topic (65) found a dose dependent improvement of FMD, especially with folic acid supplementation above 5 mg/j. They also conclude to an improvement independent to the homocysteine lowering effect. The FMD improvement impact larger on participants with high cardiovascular risks, consistent with literature (4). Our results were different. Nevertheless, on the 5 studies included in our analysis, one used less than 5 mg/d (0.8 mg/d) (66), and another just 5 mg/d (67). Adding our

population had no cardiovascular disease. These two characteristics could explain the non-significant tendency of FMD improvement we concluded.

Unfortunately we were not able to assess the efficacy of two identified strategies because of the absence of trials fitting our inclusion criteria. Tetrahydrobiopterin, a NOS cofactor, showed promising results on animals (68) but inconsistent results in clinical studies (4,7). An hypothesis is that BH<sub>4</sub> is immediately oxidized in dihydrobiopterin (BH<sub>2</sub>) (4), so ratio BH<sub>4</sub>/BH<sub>2</sub> remains unchanged even in supplementation group (69). Lastly, we planned to study hydrogen sulfide (H<sub>2</sub>S). This promising compound can be supplied by allicin contained in garlic (4). H<sub>2</sub>S act on 3 level in NO-sGC pathway, up to reducing oxidative stress by activating eNOS; enhancing nitrites reduction by xanthine oxidoreductase (XOR), leading to a NOS independent production of NO; stimulating sGC activity during oxidative stress and inhibiting PDE5 (4). On the 2 studies selected in our literature review, none were included due to results expression. One of them (70), conducted on young, trained and healthy men with acute garlic supplementation, didn't find improvement of FMD. However, 7 of 18 participants were excluded of the analysis, leading to a major suspected bias. In chronic supplementation with aged garlic extracts (aged for better concentration of sulfur), FMD were improved after vascular challenge of methionine compared with placebo (71). This result cannot be interpreted in regard to our analysis, because vascular challenges were excluded.

Almost all dietary source compositions are variable, complex and partially unknown. In dietary ingredients, other chemicals are presents. Dietary fiber, vitamins, amino-acids, ions (like nitrite or nitrates) in vegetables or fruits can affect the effect of the studied component. In addition, the composition of the same type of food could vary depending on growth conditions (soil, temperature, sunlight, water, genotype...) storage, transportation or processing (washing, peeling, cooking) (11,58). For example, in vegetable, the level of

nitrates are higher if they grew with nitrogen containing fertilizers than in organic vegetables (11). About nitrite/nitrate and polyphenol, the microbiota should also be considered. Biotransformation of polyphenols by the gut microbiota increase bio-availability of polyphenol (breaking the large structures to cross intestinal barrier) but also increase the diversity in absorbed polyphenols (57). In a study, the change in microbiome by prebiotics induced an improvement in NO dependent endothelial function (72). The last point to consider is the importance of supplementation versus the intakes in daily food. For polyphenol, coffee consumer ingest near up to triple amount of polyphenol (caffeic acid) than non-coffee consumer (9). Of course, it can lead to a major bias if supplementation intake is too low regarding daily food consumption or in inhomogeneous population. In the meta-analysis on L-arginine (61), in front of the same efficacy of low and high dose, authors supposed that daily food intake might be enough to bring maximal effect.

Our analysis presented several limitations. First, the low number of studies included, combined with the small sample size of studies led to less conclusive results, notably for inorganic nitrates, NOS substrates and folates.

Secondly, methodological heterogeneity between the selected studies could interfere with analysis. Techniques of measurement of FMD differed, well reflected by the range of FMD value from 2.3 % to 17.8% with an unexplained value at -0.25 at baseline (73). In one unpublished study (Anisha Pargal 2017 (43)), artery measure was unknown. Cuff placement was unknown in 5 studies, in arm in 7 studies and 43 in the forearm. However, meta-regressions on baseline FMD were not significant thus the impact of such heterogeneity is probably limited on the results. Another issue was the expression of FMD, some studies expressed in mean difference of dilatation against baseline (in percentage or millimeter), some other expressed overall effect without baseline and few presented mean diameters of the

artery before and after dilatation in millimeter. The result expressions restricted statistical performed of previous meta-analysis on non-far away topic (74). In contrast, we preferred excluded some RCTs without FMD in percentage, to keep coherent comparison despite information loss.

Although we tried to have a homogenous healthy population, smoking, BMI, lipid profile, sex-ratio, post-menopausal status for women, age, could influence the vascular function. However, only smoking significantly impacted the results on acute effect, according to meta-regression.

Duration of supplementation varied from 3 days to 1 year; median supplementation time was 28 (interquartile range at 25 and 75%: IQR 14-42) days. The meta-regression was significant and corroborate that each added day of supplementation increase FMD by 0.011 % (p-value= 0.0381). In acute administration, a potential bias could be produced by the measurement time of FMD regarding to the plasmatic peak of the studied molecule.

Placebo also differed by form and composition among the included studies, moreover placebo were mixed with low dose substances. In our meta-analyses, placebo increased mean FMD from 0.11% (95% CI 0.06, 0.16) on acute effect and was not significant in chronic supplementation. No difference in subgroup analysis between placebo and low dose compounds was detected. In our study, to be considered as a placebo the composition of the control arm must be composed of less than 10 % active molecule contained in the active arm. We based this choice on a batching of beetroot juice and nitrate-depleted beetroot juice which nitrate concentration were 3.5 mmol/l and 0.34 mmol/l respectively (75,76). Nevertheless, these studies also shown that compound of interest could be lower than reported by the manufacturer. Several studies didn't perform independent chemical analysis or only partially and selectively. We therefore pooled polyphenols by food origin rather than by major active

compounds. We agreed that this choice may have biased our analysis because same dietary food can supply different active molecules, leading to a mismatch between substantial intake and sub-group results (53).

A major limitation of our analysis was the quality of included RCTs, although we paid special attention to it. Included studies had to be double blinded, randomized and placebo controlled. Indeed, the overall Cochrane quality score was at maximum of 6 for only 2 studies (33,77) and of 5 in 2 studies (42,78,79) Notwithstanding, the sensitivity analyses excluding high risk of bias studies was consistent with the main analysis. Actually, dissenting opinions are expressed on study quality in nutrition, in particular versus study quality required for drug trials (74,80). However, all of them highlight the importance of quality given that conclusions of nutrition research are highly publicized and impact feeding behavior and policies on diet and health (80).

Funding is often quote to impact either results or quality of studies (80,81). However, in chronic supplementation, industry funded studies seem to support a smaller effect size in meta-regression ( $\beta = -1.55$ ,  $p\text{-value} = 0.0030$ ). In contrast, Chartres and al. (82) depicted a non-significant tendency of favorable conclusions in industry sponsored RCTs compared to academic sponsored. Nevertheless, funding sources were not often disclosed (82). In this meta-analysis, industries sponsored RCTs displayed equal or better quality score, although score do not assess the whole risk of bias (82). For example, publication bias and restricted publication of negative industrial studies should be suspected for polyphenol in our meta-analysis.



## Conclusion

In this study, we compared and assessed the effect of several nutritional strategies targeting the NO pathway on vascular function of healthy volunteers. We found that polyphenols had the larger effect size and the best evidence to support a positive effect on FMD. Moreover, while less robustly, inorganic nitrates also showed a positive effect. Contrariwise evidence is lacking to support an effect of NOS substrates and folic acid supplementation. More high-quality trials are mandatory to precisely assess the impact of the different strategies acting on the NO pathway, notably tetrahydrobiopterin or hydrogen sulfide. Meanwhile the active substances contained in each dietary supplement should be thoroughly identified as their mechanisms, factoring overall composition and interaction in.

## Discussion

Sur 1 033 références identifiées, nous avons finalement inclus dans notre analyse 55 essais cliniques (53 venant de Pub Med et 2 de clinicaltrial.gov) permettant une analyse sur 24 essais en supplémentation aigue et 38 en chronique.

Selon notre analyse, les nitrates inorganiques montrent un effet positif et significatif en supplémentation aigue comme chronique. Cependant si ce résultat semble confirmé avec les seuils ajustés pour la supplémentation aigue, ceci n'est pas le cas pour la supplémentation chronique qui est loin de l'effectif informatif (433 patients pour 80 inclus). Il paraît donc difficile d'affirmer formellement un effet des nitrates en chronique, en particulier dans cette voie pour laquelle une forte tachyphylaxie est déjà décrite pour les médicaments. De plus notre analyse porte particulièrement sur les sels sodiques ou potassiques de nitrate (6 études), contre seulement 3 études pour le jus de betterave et 1 pour les épinards rouges. Pourtant ce type de supplémentation est spécialement intéressant car augmente la biodisponibilité de NO

de manière indépendante de la NOS puisque les nitrates sont transformés en nitrites par la flore buccale puis en NO par différentes enzymes des cellules endothéliales.

Concernant les substrats de la NOS, les résultats ne sont jamais significatifs, et l'information apporté est très loin des effectifs informatifs. Similairement aux nitrates, seulement une étude portait sur l'aliment représentatif de cette classe (la pastèque), et c'était aussi la seule portant sur la L-citrulline. Ceci ne nous permet donc de conclure que sur la potentielle inefficacité du substrat de la NOS : la L-arginine à améliorer la FMD. La biodisponibilité de la L-arginine étant mauvaise, ce résultat n'est pas surprenant. Cependant, un résultat sur la L-citrulline précurseur de la L-arginine, dont la biodisponibilité est bien meilleure, eut été intéressant.

Au même niveau de régulation de la voie du NO, il faut considérer ici les stratégies visant à augmenter les cofacteurs de la NOS. Leur importance vient du fait qu'un déficit en substrat ou cofacteur de la NOS entraîne son découplage, et donc une production délétère de ROS, plutôt que celle de NO, protectrice de la fonction vasculaire. Pour la BH4 aucune étude n'a été incluse dans notre analyse, la littérature étant restreinte sur ce sujet. Ensuite nous sommes intéressés aux folates qui peuvent soit activer la NOS directement par liaison à la place de la BH4, soit augmenter la régénération de la BH4, en plus de leur effet antioxydant. Le résultat pour ces compléments alimentaires (aucun aliment inclus dans ce groupe) est décevant car non significatif, et proche de la futilité selon les courbes de seuil ajusté. La notion de futilité indique qu'une absence d'effet est significativement démontrée.

Quel que soit la durée de supplémentation, les polyphénols ont montré une amélioration significative de la FMD, appuyé par un effectif informatif largement dépassé. L'importante hétérogénéité dans ce groupe est en partie expliquée par la diversité des suppléments rassemblés que ce soit en terme d'aliment (du cacao à l'aubépine) ou en

terme de molécules actives qui comprend différentes catégories de polyphénol : le majoritaire étant les flavonoïdes, puis viennent les acides chlorogéniques et les stilbènes. Parmi ces composés, le cacao est l'aliment le plus testé, présentant un effet significatif en aigu comme en chronique, qui est à relier aux différents bénéfices vasculaires connus pour ce produit. Les isoflavones (polyphénols issus du soja) semblent elles aussi avoir un effet positif, mais analysées uniquement en supplémentation chronique, faute d'étude en supplémentation aiguë. Pour les polyphénols, qui est le groupe le plus conséquent de notre analyse, nous avons pu réaliser des funnel plots qui orientent sur un possible biais de publication en effet aigu, qui n'est pas retrouvé en effet chronique.

Enfin nous avons prévu l'analyse d'un dernier groupe, composé par les sulfures d'hydrogène, dont l'allicine présente d'en l'ail sert de précurseur, mais ceci n'a pas été réalisée, faute d'inclusion.

Aucun effet significatif n'a été retrouvé sur notre critère de jugement secondaire : la NMD, or le faible nombre d'études sur lesquelles reposent cette analyse peut l'expliquer.

Les analyses de l'effet placebo ont retrouvé un effet placebo significatif mais faible en supplémentation aiguë, et aucun effet significatif en supplémentation chronique.

Les méta-régressions de nos résultats de FMD ont été effectuées sur plusieurs critères pouvant expliquer des hétérogénéités, à savoir : la proportion de femmes et de fumeurs actifs, l'âge, l'indice de masse corporelle, la concentration sanguine de cholestérol totale, de LDL, de HDL, de triglycéride, la valeur de FMD avant intervention, la durée de supplémentation et le type de financement de l'étude. Les seules significatives étaient en aigu la proportion de fumeurs, et en chronique la durée de supplémentation et la source de financement. Pour la durée de supplémentation chaque jour de supplémentation augmenterait la FMD de 0.011%.

Quant au financement, les études industrielles présentent des résultats de taille d'effet inférieur de 1.3 % par rapport aux études académiques.

Afin d'apprécier la cohérence de nos résultats en fonction de la qualité des études incluses, des analyses de sensibilité ont été menées en excluant les essais à haut risque de biais. Tous les résultats ainsi obtenus étaient cohérents avec les précédents. Ces analyses, associées au financement et à nos résultats de la méta-analyse principale ont aussi servies à établir le niveau de preuve de nos résultats via le système GRADE. La qualité conclue est donc très faible pour toutes les analyses (nitrates inorganiques, substrats de la NOS et acide folique) en supplémentation chronique sauf pour les polyphénols pour lesquels elle est faible. En supplémentation aigue la qualité estimée est dans l'ensemble meilleure : faible pour les substrats de la NOS et les polyphénols et intermédiaire pour les nitrates inorganiques.

## Conclusion

**THÈSE SOUTENUE PAR :** Myrtille BEAU GUILLAUMOT

**TITRE :** COMPARAISON DE STRATEGIES CIBLANT LA VOIE DU MONOXYDE D'AZOTE AFIN D'AMELIORER LA FONCTION VASCULAIRE : REVUE SYSTEMATIQUE ET META-ANALYSE

### CONCLUSION :

La voie du monoxyde d'azote (NO) est une voie centrale de la régulation cardiovasculaire, dérégulée dans de nombreuses pathologies. Le monoxyde d'azote produit dans la cellule endothéliale par la NO synthase à partir d'arginine et de tétrahydrobioptérine diffuse vers les cellules musculaires lisses, où il active la guanylate cyclase soluble. L'augmentation de GMP cyclique qui en résulte permet la vasorelaxation. De nombreuses stratégies thérapeutiques sont basées sur la modulation et la restauration de cette voie. Plusieurs traitements pharmacologiques (sildénafil ou riociguat par exemple) ont montré leur efficacité mais certaines approches nutritionnelles ou de pharmaco-nutrition peuvent aussi impacter la voie du NO. Nous avons conduit une revue systématique et une méta-analyse afin d'identifier et de comparer les niveaux de supplémentation efficaces, afin d'orienter les recommandations.

Pour éviter les confusions et comme première analyse, nous avons restreint notre population aux individus non atteints de pathologies chroniques. La vasodilatation dépendant du flux (FMD) étant un critère de jugement intermédiaire de la fonction vasculaire, très dépendant de la libération endothéliale de NO, nous l'avons choisi comme critère de jugement principal. Notre critère de jugement secondaire était la vasodilatation médiée par les nitrates (NMD), étant quant à elle indépendante du NO. Nous avons donc recherché des essais cliniques contrôlés, randomisés (RCTs) en double aveugle dans Medline et clinicaltrial.gov jusqu' 4 juillet 2018, testant l'efficacité d'une des approches suivantes contre un placebo : les substrats de la NOS : L-arginine et L-citrulline, les acides foliques, les nitrates inorganiques et les polyphénols ; utilisé sous forme d'aliment ou de complément nutritionnel. Nous avons réalisé 2 méta-analyses afin d'évaluer l'effet aigu et l'effet d'une supplémentation chronique par ces substances sur la FMD.

Nous avons inclus 55 essais cliniques portant sur 1321 volontaires. Les nitrates inorganiques et les polyphénols ont montré une efficacité pour augmenter la FMD en

supplémentation aigüe et chronique. Cependant, le peu d'études sur les nitrates inorganiques ne permet pas de conclusion avec certitude sur leur effet à long terme, en particulier pour cette voie où la tachyphylaxie est déjà bien décrite avec les nitrates organiques. Les groupes de polyphénols étant hétérogènes quant à la nature des composés, il paraît délicat de conclure à leur effet en général. Cependant les résultats pour le cacao et les isoflavones semblent robustes et probants. Augmenter les substrats de la NOS ou son cofacteur par des folates, ne semble pas être efficace, aucun résultat n'étant significatif. Pour autant, nos résultats portent majoritairement sur des suppléments en L-arginine, dont la mauvaise biodisponibilité est connue. Seule une étude portait sur la L-citrulline qui est un précurseur de la L-arginine et pourrait être une meilleure option. Mais ce résultat peut aussi s'expliquer par l'absence de carence en substrats et cofacteur de la NOS chez l'individu sain, ces suppléments ne prenant un intérêt qu'en cas de déplétion.

Un point clef de notre étude est la mise en évidence du peu d'essais cliniques de bonne qualité (randomisé, contrôlé, en double aveugle) dans cette thématique. Trois orientations de recherche restent donc nécessaires afin de pouvoir apprécier avec justesse l'impact de ces substances. Premièrement la réalisation d'essais cliniques de faible risque de biais afin de confirmer l'efficacité de certains composés. Deuxièmement, la caractérisation précise des mécanismes d'action de chaque famille de composés. Troisièmement, la prise en compte de l'alimentation dans son ensemble, notamment des interactions possibles entre composés intra et inter aliment.

**VU ET PERMIS D'IMPRIMER**

**Grenoble, le : 3 septembre 2018**

**LE DOYEN**  
**Pr. Michel SÈVE**

**LE PRÉSIDENT DE LA THÈSE**  
**Pr. Christophe ABUOT**



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## Annexes

### COMPARAISON OF NUTRITIONNAL STRATEGIES TARGETING THE NITRIC OXYDE PATHWAY TO IMPROVE MACROVASCULAR FUNCTION: A SYSTEMATIC REVIEW AND A META ANALYSIS

#### *Supplementary material*

Table 1: summary of studies included in meta-analysis on acute supplementation

Studies	Design	number of subjects	Supplementation	Group	Cuff	NMD	wash out
Anisha Pargal2017	crossover	27	Black tea extract	polyphenol	forearm	no	6
Bahra2011	crossover	13	potassium nitrate	NO donors	forearm	no	7
Bakker2015	crossover	10	beetroot juice	NO donors	forearm	no	1
Berry2009	crossover	21	cocoa	polyphenol	forearm	no	3
Cutrufello2014	crossover	22	citrulline and watermelon	NOS substrates	arm	no	7
Davison2008	parallel	49	cocoa	polyphenol	forearm	no	NA
Gates A2007	crossover	10	Arginine IV	NOS substrates	forearm	yes	MD
Gates B2007	crossover	13	Arginine IV	NOS substrates	forearm	yes	MD
Haun2016	crossover	15	red spinach extract	NO donors	forearm	no	3
Heiss A2015	parallel	22	cocoa flavonol	polyphenol	MD	yes	NA
Heiss B2015	parallel	22	cocoa flavonol	polyphenol	MD	yes	NA
Heiss2005	crossover	11	Cocoa	polyphenol	arm	no	MD
Heiss2007	crossover	5	Cocoa	polyphenol	forearm	no	0
Heiss2012	crossover	10	sodium nitrate	NO donors	forearm	yes	MD
Kapil2010	crossover	12	potassium nitrate	NO donors	forearm	no	NA
Lorenzo Ghiadoni2018	crossover	30	black tea extract	polyphenol	forearm	yes	7
Mills2017	crossover	24	chlorogenic acid	polyphenol	forearm	no	7
Rodriguez-Mateos2013	crossover	10	blueberry drink	polyphenol	forearm	no	MD
Rodriguez-Mateos2015	crossover	5	Nitrate	NO donors	forearm	no	7
Rodriguez-Mateos2016	crossover	10	cranberry juice drink	polyphenol	forearm	no	7
Sansone2015	parallel	100	cocoa flavonol	polyphenol	forearm	no	NA
Sapper2016	crossover	15	catechin rich green tea extract	polyphenol	forearm	no	7
Schroeter2006	crossover	10	Cocoa	polyphenol	forearm	no	2

Siasos2008	crossover	10	L-arginine PO	NOS substrates	forearm	yes	28
Siasos2009	crossover	12	L-arginine PO	NOS substrates	forearm	no	28
Ward2016	crossover	15	chlorogenic acid et epicatechin	polyphenol	forearm	no	7

Table 2: subject characteristics on acute effect meta-analysis

studies	% women	% smoker	age	BMI	Cholesterol mmol/l	LDL mmol/l	HDL mmol/l	Triglyceride mmol/l
Anisha Pargal2017	0	0	36, SD :MD	23,20	MD	MD	MD	MD
Bahra2011	MD	0	27,9, SD :6,49	24,50	MD	MD	MD	MD
Bakker2015	36	0	25, SD :5	22,22	MD	MD	MD	MD
Berry2009	38 (100 % post menopause)	0	55, SD :10,08	31,60	MD	MD	MD	MD
Cutrufello 2014	50	MD	20,8, SD :1,25	23,55	MD	MD	MD	MD
Davison2008	64,89 (100% post menopause)	MD	45,12, SD: MD	33,47	5,14	3,50	1,52	1,51
Gates A2007	50	0	21, SD :3,16	22,00	4,36	2,44	1,38	1,20
Gates B2007	50	0	60, SD :7,21	25,00	5,01	3,20	1,38	1,32
Haun2016	46	MD	23,7, SD :3,1	27,20	MD	MD	MD	MD
Heiss A2015	0	0	26, SD :4,69	24,90	4,45	3,33	1,37	1,09
Heiss B2015	0	0	60, SD :9,38	26,50	5,35	4,06	1,39	1,33
Heiss2005	45	100	31, SD :3,32	21,80	4,96	2,99	1,73	MD
Heiss2007	0	100	27, SD :2,24	22,00	4,19	MD	MD	MD
Heiss2012	50	50	26 , SD :3,16	MD	3,72	2,09	1,34	0,72
Kapil2010	MD	0	24,7 , SD 4,85	MD	MD	MD	MD	MD
Lorenzo Ghiadoni2018	0	0	43 , SD :MD	25,00	MD	MD	MD	MD
Mills2017	0	100	23,8 , SD 6,86	23,20	MD	MD	MD	MD
Rodriguez-Mateos2013	0	0	27 , SD :4,11	25,00	4,30	MD	1,20	0,80
Rodriguez-Mateos2015	0	MD	25 , SD :2,24	24,10	4,80	MD	MD	1,53

Rodriguez-Mateos2016	0	MD	24 , SD :2	24,00	3,85	2,32	1,26	0,75
Sansone2015	47,5	MD	44,5 , SD :8,5	25,50	4,80	3,05	1,55	1,20
Sapper2016	0	0	25,3 , SD 3,87	22,40	4,13	MD	1,39	0,99
Schroeter 2006	0	MD	MD , SD :MD	MD	MD	MD	MD	MD
Siasos2008	70	100	24,3 , SD 2,31	MD	MD	MD	MD	MD
Siasos2009	58	100	24,4, SD:10,08	22,31	4,59	MD	1,43	0,75
Ward2016	62,5	0	59,9 , SD :8,2	24,70	MD	MD	MD	MD

Table 3: summary of studies included in meta-analysis

Studies	Design	Number of subjects	Supplementation	Group	Duration	wash out	Cuff	NMD
Adams1995	crossover	12	L-arginine PO	NOS substrates	3	7	forearm	yes
Anisha Pargal2017	crossover	27	Black tea extract	polyphenol	7	6	forearm	no
Asher2012	crossover	21	hawthorn standardized extract	polyphenol	3	1,5	forearm	no
Barona2012	crossover	24	grape polyphenol powder (GRAPE)	polyphenol	30	21	forearm	no
Blum2000	crossover	10	L-arginine PO	NOS substrates	30	30	forearm	yes
Bode-Boger2003	crossover	12	L-arginine PO	NOS substrates	14	14	forearm	yes
clarkson1996	crossover	27	L-arginine PO	NOS substrates	28	28	forearm	yes
Colacurci2004	parallel	57	isoflavone	polyphenol	180	NA	arm	yes
Cuevas2003	crossover	18	soy isoflavone	polyphenol	28	0	forearm	yes
Davison2008	parallel	49	cocoa	polyphenol	84	NA	forearm	no
Decroix2018	crossover	14	cocoa flavonol	polyphenol	6	7	forearm	no
DeVan2016	Parallel	31	nitrite	NO donors	70	NA	MD	no
Deveaux2016	crossover	33	sustained-release L-arginine	NOS substrates	28	28	forearm	yes
Engler2004	Parallel	21	dark chocolate bars	polyphenol	14	NA	MD	no
Grassi2015	crossover	20	cocoa	polyphenol	7	7	forearm	no
Hale2002	Parallel	29	soya isoflavone	polyphenol	14	NA	MD	no
Hallund2006	crossover	28	soya isoflavone	polyphenol	56	56	forearm	yes
Heiss A2015	Parallel	20	cocoa flavonol	polyphenol	14	NA	MD	yes
Heiss B2015	Parallel	20	cocoa flavonol	polyphenol	14	NA	MD	yes



Hermansen2005	Parallel	32	Abalon soy	polyphenol	168	NA	forearm	yes
Hoch2009	Parallel	13	folic acid	folic acid	42	NA	forearm	no
Khan2014	Parallel	64	blackcurrant juice drink	polyphenol	42	NA	forearm	yes
Lee2015	crossover	14	beetroot juice	NO donors	15	14	arm	no
Lorenzo Ghiadoni2018	crossover	30	black tea extract	polyphenol	7	7	forearm	yes
Olthof2006	crossover	39	folic acid	folic acid	42	42	forearm	no
Rammos2014	crossover	11	sodium nitrate	NO donors	28	NA	MD	no
Salden2016	Parallel	48	hesperidin	polyphenol	42	NA	forearm	no
Sansone2015	Parallel	100	cocoa flavonol	polyphenol	29	NA	forearm	no
Siasos2008	crossover	10	L-arginine PO	NOS substrates	3	28	forearm	yes
Siasos2009	crossover	12	L-arginine PO	NOS substrates	3	28	forearm	no
Simons2000	crossover	20	isoflavone	polyphenol	56	56	forearm	yes
van der Made2017	crossover	37	trans-resveratrol	polyphenol	28	28	forearm	no
van Mierlo2010	crossover	35	wine grape extract	polyphenol	14	7	arm	no
Wang-Polagruto2006	parallel	17	cocoa	polyphenol	42	NA	MD	yes
West2014	crossover	30	cocoa	polyphenol	28	14	forearm	no
Wilmink2000	crossover	20	folic acid	folic acid	14	56	forearm	yes
Wong2013	crossover	28	resveratrol	polyphenol	42	0	forearm	no
Woo 1999	crossover	17	folic acid	folic acid	56	28	MD	yes
Woodman2004	crossover	26	folic acid	folic acid	56	28	forearm	yes

Table 4: characteristics of subjects included in meta-analysis

Studies	% women	% smoker	Age (years)	BMI	Cholesterol mmol/l	LDL mmol/l	HDL mmol/l	TG mmol/l
Adams1995	0	0	34, SD: 3,46	MD	5,43	4,34	1,11	1,4
Anisha Pargal2017	0	0	36, SD: MD	23,2	MD	MD	MD	MD
Asher2012	57,2	0	50,9, SD: 10,5	29,3	MD	MD	MD	MD
Barona2012	0	MD	51,3, SD: 9,6	MD	MD	MD	MD	MD
Blum2000	100 (100 % post menopause)	0	55, SD: 5	MD	5,14	3,2	1,55	1,09
Bode-Boger2003	33 (100 % post menopause)	0	73,8, SD: MD	MD	MD	MD	MD	MD
clarkson1996	33	0	29, SD: 5	MD	7,6	6,15	1,29	1,36
Colacurci2004	100 (100 % post menopause)	0	55,15, SD: 3,85	25,8	MD	3,65	1,55	1,54
Cuevas2003	100 (100 % post menopause)	0	59, SD: MD	29,3	7,39	5,03	1,39	2,14
Davison2008	64,89	MD	45,12, SD: MD	33,4	5,13	3,49	1,51	1,51
Decroix2018	0	0	30,7, SD: 3,1	22,5	MD	MD	MD	MD
DeVan2016	45 (100 % post menopause)	0	61,71, SD: 7,45	24,6	4,47	2,58	1,49	0,842
Deveaux2016	27	0	45, SD: 8,9	28	2,1	1,2	0,4	2,4
Engler2004	47,6428571	0	32,13, SD: 9,93	22,5	4,53	2,29	1,59	1,2
Grassi2015	45	0	53,8, SD: 8,9	25,4	MD	3,4	1,43	1,29
Hale2002	100 (100 % post menopause)	0	57,17, SD: 5,71	24,9	5,05	3,97	1,63	1,21
Hallund2006	100(100 % post menopause)	0	57, SD: 5	24	5,98	3,91	1,53	1,18
Heiss A2015	0	0	26, SD: 4,47	24,9	4,45	3,33	1,37	1,09
Heiss B2015	0	0	60, SD: 8,94	26,5	5,35	4,06	1,39	1,33
Hermansen2005	47 (100 % post menopause)	39	59,54, SD: 3,89	26,075	6,73	4,41	1,41	MD
Hoch2009	100 (none post menopause)	0	24, SD: 3,21	22,1	MD	MD	MD	MD
Khan2014	32,98	84,625	52,38, SD: 9,67	28,8	5,2	MD	MD	MD
Lee2015	0	0	22, SD: 3,74	23,4	MD	MD	MD	MD
Lorenzo Ghiadoni2018	0	0	43, SD: MD	25	MD	MD	MD	MD
Olthof2006	42,5	MD	59, SD: 5	25,5	5,8	MD	MD	MD

Rammos2014	36	MD	63, SD: 6	24	6,15	MD	MD	MD
Salden2016	57,5	0	53,5, SD: 14,5	28,95	MD	MD	MD	MD
Sansone2015	47,5	MD	44,5, SD: 8,5	25,5	4,8	3,05	1,55	1,2
Siasos2008	70	100	24,3, SD: 2,31	MD	MD	MD	MD	MD
Siasos2009	58	100	24,4, SD: MD	22,31	4,59	MD	1,43	0,75
Simons2000	100 (100 % post menopause)	0	59, SD: MD	26,8	5,86	3,94	1,42	1,12
van der Made2017	44	0	61, SD: 7	28,3	MD	MD	MD	MD
van Mierlo2010	0	MD	31,4, SD: 9	23,2	4,8	3,2	1,4	1
Wang- Polagruto2006	100 (100 % post menopause)	0	56,62, SD: 5,76	25,088 2353	6,13	3,86	1,78	1,06
West2014	49,9 (100 % post menopause)	0	51,7, SD: 6,57	27,8	5,09	3,18	1,37	1,2
Wilmink2000	50	20	23, SD: 3,4	22,8	MD	MD	MD	MD
Wong2013	57 (100 % post menopause)	0	61, SD: 6,88	33,3	MD	MD	MD	MD
Woo 1999	11,7	11,7	54, SD: 10	MD	5,7	MD	MD	MD
Woodman2004	30,7	0	49, SD: 10,2	28,1	5,3	3,2	1,39	1,27

Table 5: meta-regression on acute FMD

Variable	Rate of available variable	beta	95 CI	P value
Women proportion	26	-0.0027	-0.0263 0.0209	0.8223
<b>Smoker proportion</b>	<b>20</b>	<b>0.0155</b>	<b>0.0010 0.0300</b>	<b>0.0357</b>
Age	27	-0.0009	-0.0372 0.0353	0.9594
BMI	24	0.0347	-0.0622 0.1316	0.4827
Cholesterol	14	-0.2947	-1.7042 1.1149	0.6820
LDL	9	0.3678	-0.6050 1.3405	0.4587
HDL	12	0.3763	-4.4218 5.1744	0.8778
Triglyceride	12	-1.0934	-3.2645 1.0778	0.3236
FMD before intervention	19	0.0227	-0.5083 0.5536	0.9332
Funding	40	-0.2338	-1.1557 0.6882	0.6192

Table 6: meta-regression on chronic FMD

Variable	Rate of available variable	beta	95 CI	P value
Women proportion	40	0.0057	-0.0076 0.019	0.40
Smoker proportion	33	-0.0002	-0.0230 0.0227	0.9884
Age	40	0.0015	-0.0309 0.0338	0.9294
BMI	33	-0.0513	-0.1422 0.0396	0.2686
Cholesterol	25	0.5471	-0.1057 1.1998	0.1005
LDL	22	0.5204	-0.0707 1.1115	0.0844
HDL	23	4.3288	-0.4387 9.0963	0.0751
Triglyceride	22	0.2048	-1.4024 1.8120	0.8028
FMD before intervention	30	-0.1015	-0.4085 0.2055	0.5169
<b>Duration of supplementation</b>	<b>40</b>	<b>0.0110</b>	<b>0.0006 0.0225</b>	<b>0.0381</b>
<b>Funding</b>	<b>40</b>	<b>-1.3358</b>	<b>-2.2440 -0.4277</b>	<b>0.0039</b>

Figure 20: acute effect of NO donors on NMD

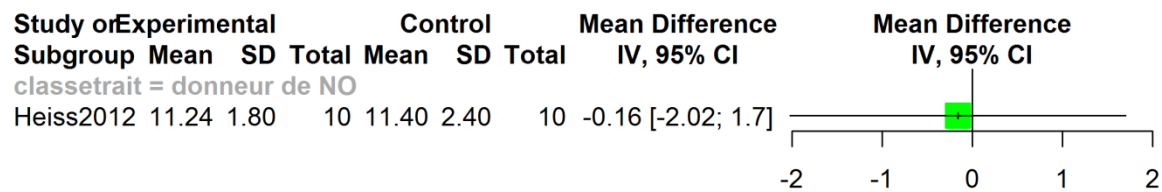


Figure 21: acute effect of NOS substrates on NMD

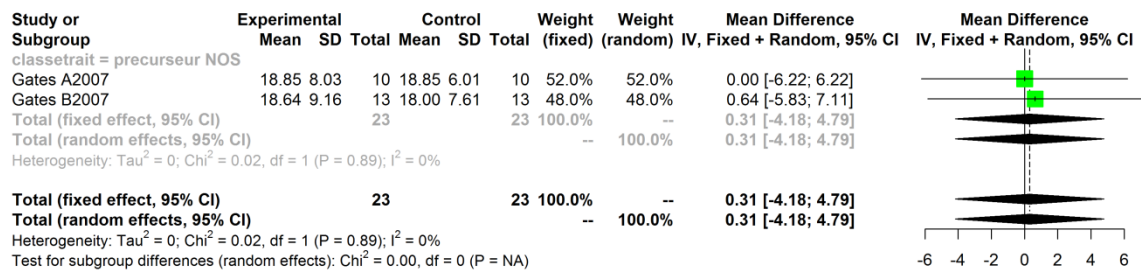


Figure 22: acute effect of polyphenol on NMD

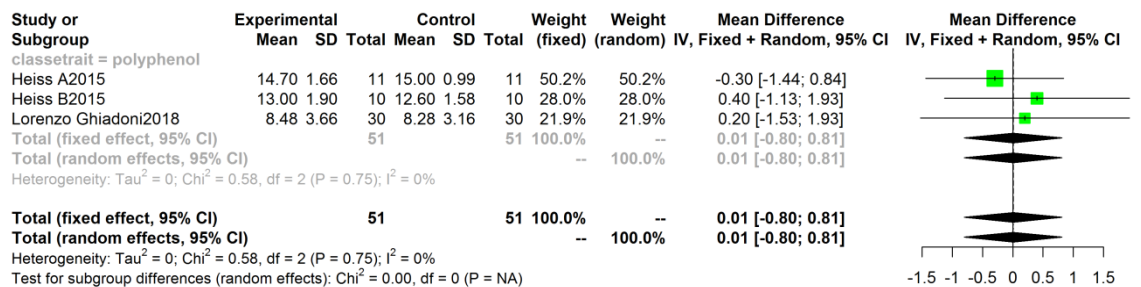


Figure 23: chronic effect of NOS substrates on NMD

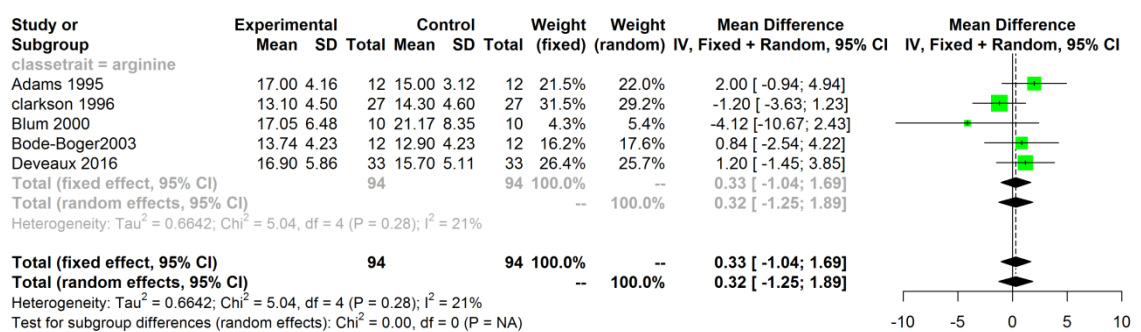


Figure 24: chronic effect of folic acids on NMD

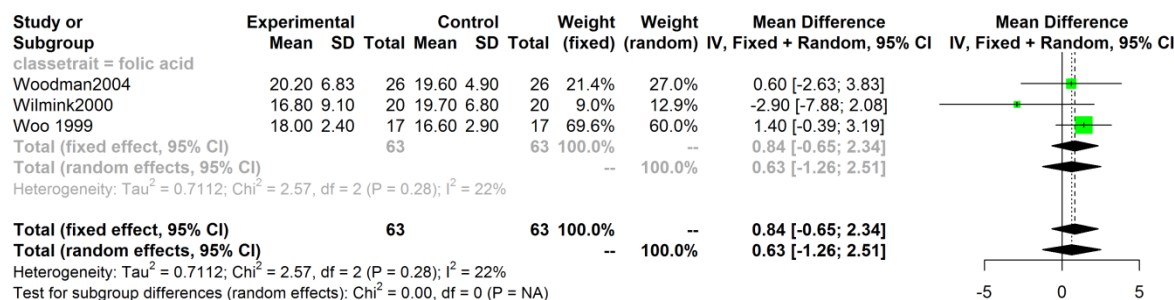


Figure 25: chronic effect of polyphenols on NMD

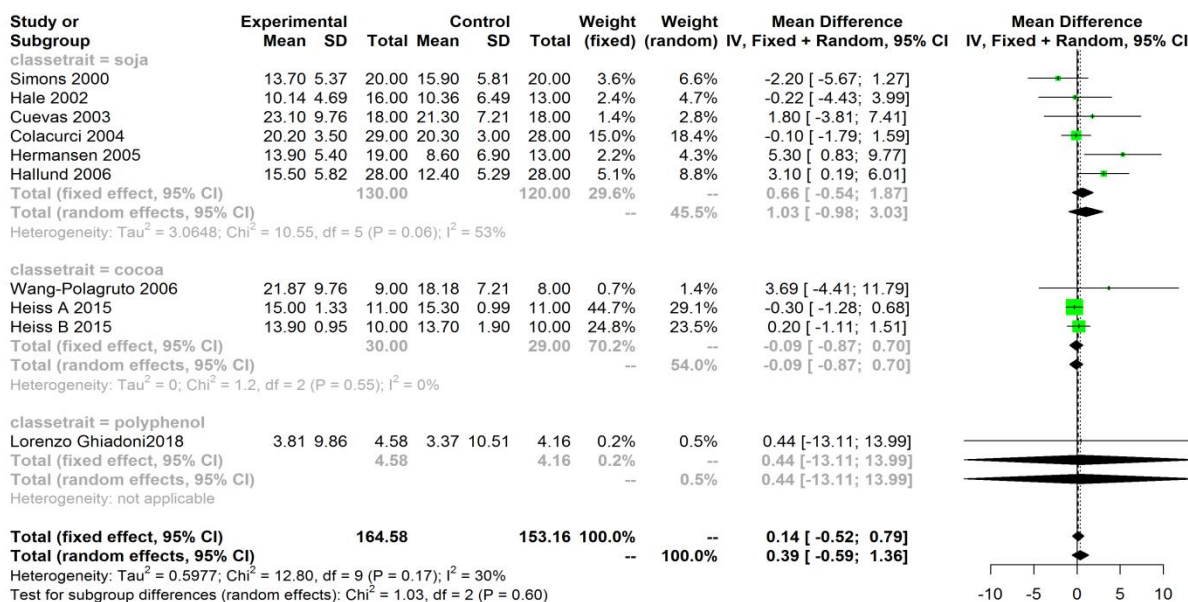


Figure 26: placebo effect on acute supplementation

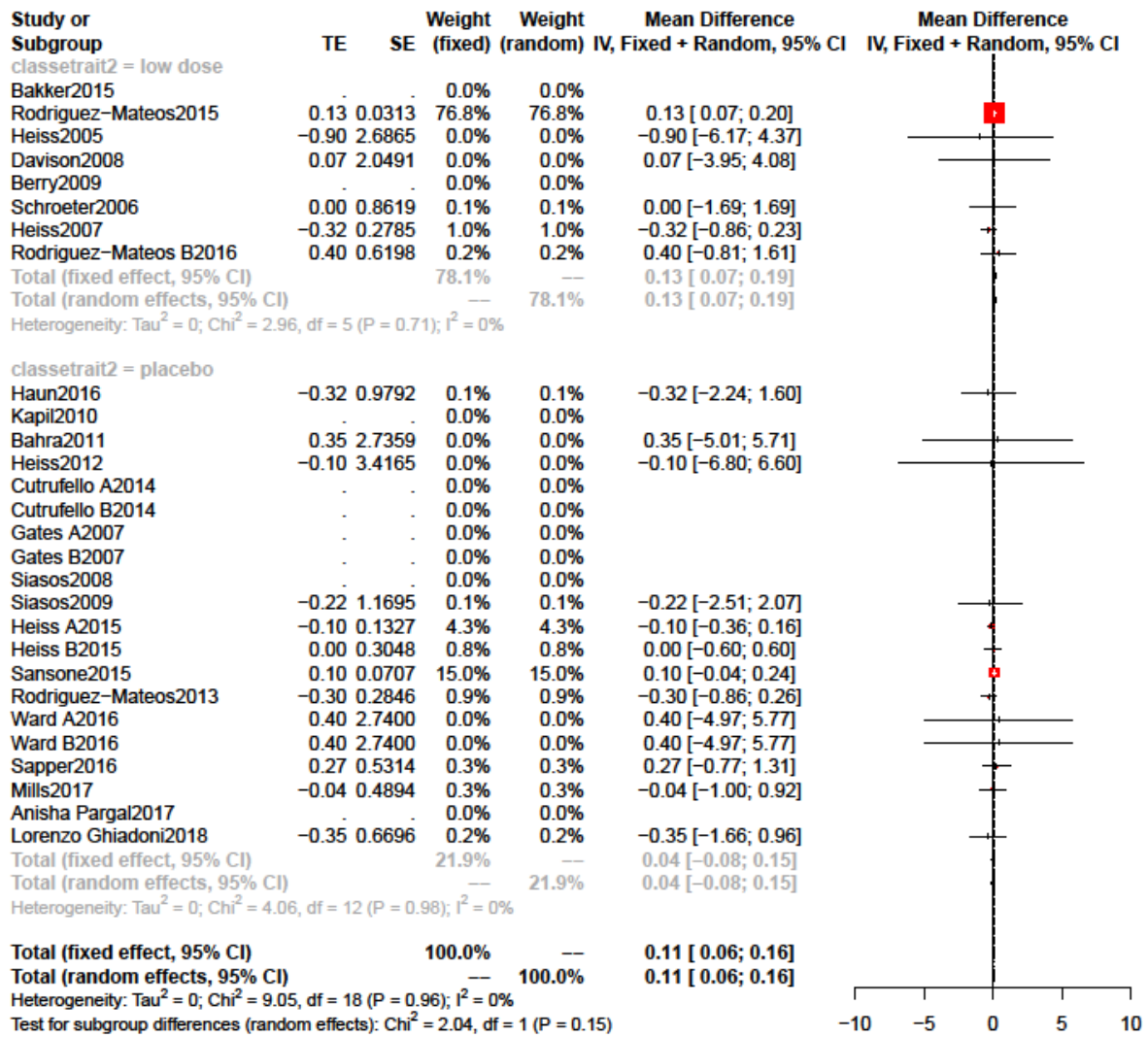


Figure 27: placebo effect on chronic supplementation

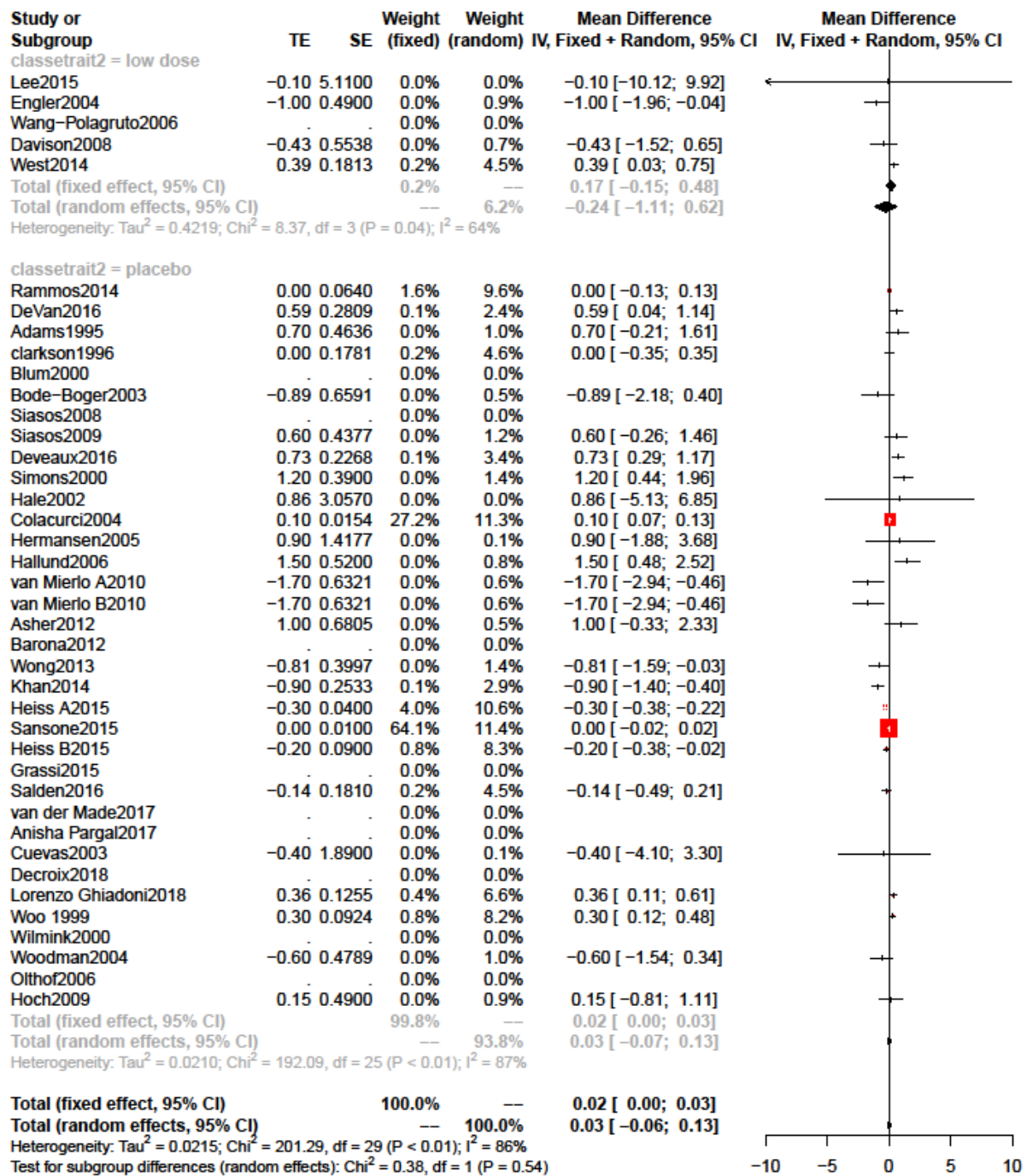




Tableau 1: GRADE assesement in acute supplementation

	Number of studies	Number of volunteers	Heterogeneity	MD (95%CI)	Sensitivity analysis MD (95%CI)	High quality studies (%)	Risk of bias	Publication bias	Inconsistency	Imprecision	Indirectness	Overall quality of evidence
Overall ACUTE	24	503										
NO donors	6	65	47%	1.07 (0.68; 1.45)	1.0685 (0.0118; 2.1252)	0	serious limitations no high quality study Sensitivity analysis coherent	Undetected 1 industrial funded study	Moderate heterogeneity	Required information size not reach (174) but Z-curve favor to an effect of NO donors	Applicability: FMD between 3.6 and 12.6 %	3 ⊗⊗⊗○
NOS substrates	4	67	0%	0.29 (-0.63; 1.2)	0.442 (-0.5530; 1.4370)	0	Serious limitations Sensitivity analysis coherent	Undetected	Low heterogeneity	Rate down Required information size not reach (3288) and CI superior to 2	Applicability: FMD between 3.83 and 12.9%	2 ⊗⊗○○
polyphenols	14	371	81%	1.45 (0.84; 2.05)	0.8709 (0.4421; 1.3197)	3	No serious limitation (21% high quality), sensitivity analysis reaches a significant positive result	Suspected on funnel plot, Egger test negative, 8 industrial funded study	Rate down of one Substantial heterogeneity	Required information size reach (203) and Z-curve favor to an effect of polyphenols	Applicability: FMD between 3.25 and 10.25%	2 ⊗⊗○○

Tableau 2: GRADE assessment in chronic supplementation

	Number of studies	Number of volunteers	Heterogeneity	MD (95% CI)	Sensitivity analysis MD (95% CI)	High quality studies (%)	Risk of bias	Publication bias	Imprecision	Inconsistency	Indirectness	Overall quality of evidence
Overall CHRONIC	38	1076										
NO donors	3	56	47%	0.78 (0.16; 1.40)	2.0406 (0.5965; 3.4848)	0	Serious limitations Sensitivity analysis reach a significant more positive result	Undetected	Rate down Required information size not reach (433) and Z-curve not significant	Moderate heterogeneity	Applicability: FMD between 3.73 and 17.8 % Rate down for one	1 ⊗○○○
NOS substrates	7	116	85%	1.22 (-0.64; 3.7)	1.5212 (-0.5027; 3.5452)	0	Serious limitations Sensitivity analysis coherent	Undetected, 1 industrial funded study	Rate down Required information size not reach (4115) and Z-curve not significant and Ci superior to 2	Rate down of one Substantial heterogeneity	Applicability: FMD between -0.25 and 7.4	1 ⊗○○○
polyphenols	23	789	77%	0.83 (0.36; 1.30)	0.8854 (0.3152; 1.4555)	3	No serious limitations (13% high quality studies) Sensitivity analysis reach a significant positive result	Undetected, Egger test negative, 14 industrial funded study	Required information size reach (788) and Z-curve favor to an effect of polyphenols	Rate down of one Substantial heterogeneity	Applicability: FMD between 0.56 and 14.1% Rate down for one	2 ⊗⊗○○
Folic acids	5	115	58%	1.1 (-0.10; 2.91)	0.7238 (-0.8863; 2.3340)	0	Serious limitations Sensitivity analysis coherent	Undetected, 1 industrial funded study	Rate down Required information size not reach (765) and Z-curve not significant and CI superior to 2	Rate down of one Moderate heterogeneity	Applicability: FMD between 2.8 and 10.6%	1 ⊗○○○



# **Serment de Galien**



**« Je jure en présence des Maîtres de la Faculté, des Conseillers de l'Ordre des Pharmaciens et de mes condisciples :**

**D'honorer ceux qui m'ont instruit(e) dans les préceptes de mon art et de leur témoigner ma reconnaissance en restant fidèle à leur enseignement.**

**D'exercer, dans l'intérêt de la santé publique, ma profession avec conscience et de respecter non seulement la législation en vigueur, mais aussi les règles de l'honneur, de la probité et du désintéressement.**

**De ne jamais oublier ma responsabilité et mes devoirs envers le malade et sa dignité humaine ; en aucun cas, je ne consentirai à utiliser mes connaissances et mon état pour corrompre les mœurs et favoriser des actes criminels.**

**Que les hommes m'accordent leur estime si je suis fidèle à mes promesses. Que je sois couvert(e) d'opprobre et méprisé(e) de mes confrères si j'y manque ».**

## Myrtille BEAU GUILLAUMOT

### COMPARAISON DE STRATEGIES NUTRITIONNELLES CIBLANT LA VOIE DU MONOXYDE D'AZOTE AFIN D'AMELIORER LA FONCTION VASCULAIRE : REVUE SYSTEMATIQUE ET META-ANALYSE

**Résumé** : Dans les cellules endothéliales, la production de monoxyde d'azote (NO) par la NO synthase (NOS) permet la vasodilatation. De nombreuses pathologies avec dysfonctions vasculaires (diabète, maladie cardiovasculaires...) affectent cette voie.

Le critère de jugement principal est la vasodilatation dépendante du flux (FMD) : un critère de jugement intermédiaire de la fonction vasculaire reflétant la libération endothéliale de NO. Nous avons étudié des sujets sains pour éviter des confusions avec les pathologies. Notre protocole est prédéfini selon l'énoncé PRISMA et enregistré sur PROSPERO (CRD42018090965). Nous avons recherché des essais cliniques contrôlés, randomisés (RCTs) en double aveugle dans Medline et clinicaltrial.gov jusqu' 4 juillet 2018, portant sur les substrats de la NOS : L-arginine et L-citrulline, les acides foliques, les nitrates inorganiques et les polyphénols (aliment ou complément nutritionnel). Le critère de jugement secondaire est la vasodilatation dépendante de nitrate (NMD).

55 études sont inclues, 24 en aigu et 38 en chronique. L'efficacité des nitrates inorganique et des polyphénols (principalement le cacao) est significative en aigu comme en chronique. Aucun résultat significatif n'a été trouvé pour les substrats de la NOS, les acides foliques, la NMD et l'effet placebo. Les méta-régressions significatives portent sur le financement, la durée et le taux de fumeur. Nos résultats sont cohérents les analyses de sensibilités excluant les études de faible qualité.

### COMPARAISON OF NUTRITIONNAL STRATEGIES TARGETING THE NITRIC OXYDE PATHWAY TO IMPROVE MACROVASCULAR FUNCTION: A SYSTEMATIC REVIEW AND A META ANALYSIS

**ABSTRACT**: In endothelial cells, production of Nitric Oxide (NO) by NO synthase (NOS) lead to vasodilatation. In many pathologies associated with vascular dysfunction (diabetes, cardiovascular diseases, scleroderma...) this pathway is impacted.

Our main outcome was flow mediated dilatation FMD, used as a surrogate of vascular efficiency and reflecting the endothelial NO release. We studied healthy participants to avoid confounding induced by pathological states. Predefined protocol was registered on PROSPERO (CRD42018090965) and followed PRISMA statement. We searched for double-blind randomized controlled trials (RCTs) in Medline and clinicaltrial.gov up to July 4th, 2018. Evaluated strategies were NOS substrates: L-arginine and L-citrulline, folic acids, inorganic nitrates and polyphenol, supplied by dietary ingredient or nutrient supplement. The secondary outcome was nitrate mediated dilatation (NMD).

55 studies were included, 24 in acute and 38 in chronic supplementation. Acute and chronic efficiency of inorganic nitrates and polyphenols were significant on FMD. Among polyphenols, cocoa displayed the larger effect size. No significant result was found for NOS substrates, folic acids, for all on NMD and on placebo effect. Significant meta regression were on funding, duration of supplementation and smoking. The results were consistent in sensitivity analysis excluding low quality studies.

**MOTS CLÉS** : méta-analyse, monoxyde d'azote, donneurs d'oxyde nitrique, polyphénols, acide folique, L-arginine, l-citrulline, vasodilatation dépendante de l'endothélium/ effets des médicaments et des substances chimiques

FILIÈRE : Pharmacie hospitalière Pratique et Recherche