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Dose-response relationships in health risk assessment of nutritional and toxicological factors in foods: development and application of novel biostatistical methods

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Abstract

Dose-response meta-analyses are of key relevance to identify causal relations between exposure and health-related endpoints based on epidemiologic evidence, thus providing a powerful tool for both investigators and risk assessors. So far, their use has been limited in food safety risk assessment and in epidemiologic studies with either experimental or non-experimental design, also since they could not be applied in studies with two levels of exposure only. However, the growing number of epidemiologic studies, the need to identify and shape relations between exposure and endpoints that are not linear, such as those L-, U- and J-shaped, and to locate possible thresholds of exposure which may characterize beneficial and adverse effects of dietary constituents, have increased the need of biostatistical tools for dose-response modelling in meta-analyses. We addressed these issues in two case-studies, the relation between cadmium exposure and breast cancer risk in non-experimental cohort studies, and between potassium exposure and blood pressure in randomized controlled trials, using a recently developed methodology for 'one-stage' dose-response modelling. This statistical methodology is based on restricted cubic spline models fit with a generalized least-squares regression, combining study-specific estimates with a restricted maximum likelihood method within a multivariable random-effects meta-analysis. Such method allows to use studies based on less than three categories of exposure, such as trials based on two arms only. The implementation of such modelling in our two case studies has shown that cadmium exposure is not generally related with breast cancer risk in cohort studies, and that potassium intake has a U-shaped relation with both systolic and diastolic blood pressure, also depending on hypertensive status. Overall, the availability and implementation of the one-stage dose-response meta-analytic approach yields a flexible and powerful tool to comprehensively summarize and model the relation between dietary constituents and health endpoints based on epidemiologic evidence, greatly favouring the implementation of the risk assessment process.

Key words: Dose-response; meta-analysis; one-stage; cadmium; potassium; breast cancer; blood pressure; randomized controlled trial; cohort study; nutrition; environment.

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Summary

The implementation of flexible dose-response meta-analyses has long been sought to provide investigators and risk assessors in the field of food safety, and more generally in any other field, with comprehensive and effective tools to perform their scientific assessments considering the overall epidemiologic evidence. The underlying reasons is the growing availability of epidemiologic studies, with both experimental and non-experimental design, and therefore the difficulty in summarizing all the available evidence from human studies. In addition, a comprehensive dose-response meta-analysis may allow to identify sources of heterogeneity in the effect observed, of major importance for the interpretations of epidemiologic studies and their inconsistencies, and to target adequately specific subgroups according to age, sex, and other characteristics (such as being pregnant or lactating women, people with high blood pressure, etc.). Finally and most importantly, only the use of flexible and comprehensive dose-response models may reliably allow to detect and shape relations between exposure and health outcome which are not linear, including those L-, U- and J-shaped, and to locate the exact threshold of exposure which may characterize both beneficial and adverse effects of foods and particularly dietary constituents.

We have addressed these issues in two case-studies, the relation between cadmium exposure and breast cancer risk (in non-experimental cohort studies), and between potassium exposure and blood pressure (in experimental cohort studies, i.e. trials), taking advantage of a recently developed methodology for one-stage dose-response modelling. This newly-available statistical methodology is based on a restricted cubic spline model fit with a generalized least-squares regression, combining study-specific estimates with a restricted maximum likelihood method within a multivariable random-effects meta-analysis. Most importantly, these methods allowed the use in the dose-response modelling of studies based on less than three categories of exposure, such as trials based on two arms only.

Our results have allowed to model flexibly the aforementioned relations, showing that cadmium exposure, as assessed through the most reliable biomarker (urinary cadmium excretion), does not appear to be related at any levels of exposure with breast cancer risk in cohort studies, with the possible exception of selected population subgroups. With reference to potassium, dietary intake of this mineral has been associated though a U-shaped relation with blood pressure, both systolic and diastolic, though the statistical precision of the effect estimates greatly differs across exposure levels, in particular after stratifying for hypertensive status.

In conclusion, the availability of the one-stage dose-response meta-analytic approach appears to yield a flexible and most useful approach to comprehensively model the relation between dietary factors and health endpoints, thus providing a useful tool to summarize large epidemiologic evidence and to carry out a risk assessment process.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

This contract/grant was awarded by EFSA to:

Contractor/Beneficiary: The consortium composed by the University of Modena and Reggio Emilia (UNIMORE) in Modena, Italy (coordinator - contact person Prof. Marco Vinceti), Karolinska Institutet (Stockholm, Sweden – contact person Prof. Nicola Orsini), the National and Kapodistrian University of Athens (NKUA, contact person Prof. Androniki Naska), and the University of Porto (UPORTO – contact person Prof. Duarte Torres).

Contract/Grant title: "Dose-response relationships in health risk assessment of nutritional and toxicological factors in foods: development and application of novel biostatistical methods"

Contract/Grant number: GP/EFSA/AFSCO/2017/01 – GA09

This report summarizes, according to the Grant Agreement section #1.7, the scientific report collaboratively carried out within the project "Dose-response relationships in health risk assessment of nutritional and toxicological factors in foods: development and application of novel biostatistical methods" and its impact assessment. This report is suitable to be published on EFSA's website, which we agreed could become publicly available.

This project has been carried out and funded by EFSA under its 2017 Partnering Grant Program: GP/EFSA/AFSCO/2017/01 – GA09, to a consortium coordinated by the University of Modena and Reggio Emilia (UNIMORE) in Modena, Italy (contact person Prof. Marco Vinceti) and also composed by Karolinska Institutet (Stockholm, Sweden – contact person Prof. Nicola Orsini,); by the National and Kapodistrian University of Athens (NKUA, contact person Prof. Androniki Naska,; and by the University of Porto (UPORTO – contact person Prof. Duarte Torres). These institutions were within the list of competent organisations adopted by EFSA Management Board according to Article 36 of European Parliament and Council Regulation (EC) No 178/2002. The project has been carried out within the time limits originally planned and authorized (February 8, 2020, plus two months for the delivery of the final report), and following all the rules which applied to this project and the related grant agreement. The financial overview and details of the project are provided in a separate report, along with the requested document. Two project meetings have been held within the project before the final telemeeting of March 25, 2020: the kick-off physical meeting held on February 9, 2018 in Parma at EFSA premises, and the interim meeting held in the form of a telemeeting on February 21, 2019.

Concerning the scientific tasks and achievements of the project, we believe that we have substantially accomplished our original aims, as hereafter explained in more details, and we have substantially met the originally planned timeline, as reported in the minutes of the kick-off meeting.

The project originally stemmed from the awareness that dose-response modelling of epidemiologic studies though a meta-analytic approach is of major importance when addressing food safety issues, with reference to both nutrients and contaminants to which humans may be exposed. However, so far this approach has not been widely utilised in nutritional and environmental epidemiology and in food safety, due to the lack of studies reporting enough information for such assessment (i.e., not reporting exposure-category specific estimates) or reporting only relative risk (RR) estimates for one category of exposure in trials, i.e. the RR in the intervention arm. In addition, the statistical routines to perform

dose-response flexible meta-analyses were unfortunately limited or even entirely missing, and therefore in most cases the published meta-analysis have focused only on the comparison between the highest versus the lowest category of exposure in term of endpoint risk/value (generally presented through a 'forest plot'), or have used a meta-regression linear approach, thus not allowing to flexibly shape the relation between exposure and health endpoint, i.e. any departure from a linear model including the identification of thresholds, U/J/L-shaped curves.

The benefit of improving our knowledge, domain, capacity to use and interpretation of dose-response meta-analysis may definitely have a key relevance in the implementation of risk assessments in food safety, and even beyond its boundaries, i.e. with reference to environmental risk assessment, drug safety, and other fields. In addition, the growing number of studies carried out on the same issues in nutritional risk assessment, and more generally in any scientific field, suggests the need to have available reliable methods to summarize in a safe and comprehensive manner all the information available, to allow both the experts and the general population to access in a transparent way the human studies underlying specific food safety issues and to assess their reliability, value, source of heterogeneity and implications.

2. Data and Methodologies

2.1. Data

2.1.1. Research question

We configured the research questions according to the PICO/PECO statement (Morgan et al. 2019; Morgan et al. 2018). This currently widely used tool turned out to be very helpful in the systematic and reliable identification of review objectives and the methods that will be implemented to answer that question. In detail, the acronym present:

- Population: the group of interest where the intervention is implemented or the population or subgroup which is exposed the exposure of interest;
- Intervention/Exposure: the compound or the chemical of interest, including the modality of administration or exposure;
- Comparator: the group to which the intervention/exposure group will be compared, e.g. placebo/control arm in experimental studies or unexposed or less-exposed group in nonexperimental studies;
- Outcome: the effect which is being expected after the administration of the intervention or the exposure.

That statement has the purpose to provide a framework for developing a key question that is being answered in a systematic review, and also to help to explicitly define inclusion and exclusion criteria in order to identify relevant studies to be included in the review. Study design: in addition to the main item mentioned above, also type of study that may be the most suitable (e.g. within experimental studies: randomized controlled trial or within nonexperimental studies: prospective studies) to answer the research question and are going to be considered.

For potassium (with blood pressure as continuous endpoint), a study was considered eligible if: (1) exposure to potassium was assessed through either use of dietary questionnaires or urinary measurements; (2) the endpoint of interest was either systolic BP (SBP) and diastolic BP (DBP); (3) an experimental design and a minimum intervention duration of four weeks had been employed; and (4) the intervention was performed using potassium containing supplement, and not through dietary modification only or by mixed intervention with other active components; and (5) measurements of urinary sodium and potassium excretion obtained before and after potassium supplementation were available.

For cadmium (with breast cancer as dichotomous outcome), a study was considered eligible if: (1) exposure to cadmium was assessed through either dietary questionnaires or urinary cadmium levels; (2) the outcome of interest was mortality or incidence of breast cancer; (3) it was a prospective cohort, case-cohort or nested case-control study with a minimum one-year follow-up; and (4) risk estimates were provided using incident rate ratio (IRR), hazard ratio (HR), risk ratio (RR), odds ratio (OR) along with the 95% confidence interval (CI), alternatively. Case-control studies with controls not recruited from the same cohort that generated the breast cancer cases, cross-sectional and animal studies were not considered.

2.1.2. Literature search

We performed the literature searches using Pubmed/Medline electronic online database. We also implemented citation chasing techniques in order to identify additional relevant studies that may be not included in the search results, e.g. error in classification in the online database, especially for older

studies or where alternatively the exposure and/or the outcome of interest is not reported in the abstract but the study has been cited in previous studies, including previous reviews on the topic. We additionally screened reference list of included studies aiming at the identification of all relevant studies and avoid the occurrence of publication bias (Begg and Mazumdar 1994).

As regards potassium and blood pressure, we used the following keywords for literature search: "potassium, dietary" or "potassium" or "potassium chloride" in association with "dietary supplements" or "supplement" for study intervention and "blood pressure" or "blood pressure determination" or "arterial pressure" or "hypertension" for study outcome. We excluded studies carried out in animals. As regard cadmium and breast cancer, we used the following keywords: "cadmium" and "breast neoplasms" or "breast cancer", with search restricted to studies carried out in humans.

We imported result of the literature searches into the Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia; www.covidence.org) for further assessment and data extraction. At least two authors from two different partners of the consortium agreement reviewed all titles and abstracts independently. In case of disagreement, the issue was discussed and agreed upon after the evaluation and a thorough discussion of a third author.

2.1.3. Risk of bias assessment

We implemented risk of bias assessment using either Risk of Bias assessment tool (RoB 2.0) for experimental studies (Higgins et al. 2011; Sterne et al. 2019) and the Risk of Bias (RoB) in Non-randomized Studies of Exposures (ROBINS-E) tool for nonexperimental studies (23). The quality of the included studies was assessed independently by all consortium partners at the Universities of Modena and Reggio Emilia, Athens and Porto. In case of disagreement for the overall evaluation of each included study, we assigned the rate which obtained the majority of the approvals after in-depth discussion between consortium partners.

For experimental studies i.e. trials, a study was allocated to an overall higher risk of bias if it was judged to be at "High risk" for at least one domain whereas we considered it as being in the "Some concerns" category when some unease existed for at least one domain. A trial was included in the review if the duration was at least four weeks, to allow for physiological adaptations over time and in line with both EFSA assessments and most systematic reviews and meta-analysis in the scientific literature. The following six risk of bias domains were considered: (1) Risk of bias arising from the randomization process; (2) Risk of bias due to deviations from the intended interventions (effect of assignment to intervention); (3) Missing outcome data; (4) Risk of bias in measurement of the outcome; (5) Risk of bias in selection of the reported result. In addition, we included an evaluation of the (6) Risk of bias for cross-over design, assessing also whether the trial implemented a wash-out period. We allocated the study to an overall higher risk of bias if it was judged to be at "High risk" for at least one domain whereas we considered the study at "Some concerns" risk of bias when some unease existed for at least one domain. Detailed guidance of RoB 2.0 assessment for experimental studies is reported in Appendix A.

For nonexperimental studies i.e. observational prospective studies, seven domains were assessed including:(1) bias due to confounding; (2) bias in selecting participants in the study; (3) bias in exposure classification; (4) bias due to departures from intended exposures; (5) bias due to missing data; (6) bias in outcome measurement; (7) bias in the selection of reported results. Each domain was characterized as low, moderate, serious or critical risk of bias. In particular, criteria implemented for risk of bias evaluation are the following: (a) Bias due to confounding: factors considered mandatory in order to judge a study at moderate risk of bias are: age, smoking habits and body mass index. Factors considered mandatory to judge a study at low risk of bias are: in addition to the aforementioned factors, use of hormone replacement therapy, alternatively energy intake for dietary intake assessment only, and creatinine adjustment for urine excretion only; (b) Bias in selecting participants in the study: selection of eligible participants must not be related to cadmium exposure; (c) Bias in exposure classification: possible exposure misclassification for studies not using a biological sample for the

assessment or exposure classification based on an assessment performed after beginning of the study; (b) Bias in departure from intended exposure: There should be no concern about departure from intended exposure due to the long term, i.e. that subjects may have changed exposure during follow-up; (e) Bias due to missing data: Definition of reasonable cutpoint of up to 10% for missing data to be considered at low risk. (f) Bias in outcome measurement: possible bias based on the modality of outcome assessment. A study was considered as of high risk if the assessment was based on self-reporting only without external validation; (g) Bias in selection of reported results: description of methods must be clearly described to be at low risk and in addition evidence that selected were presented yielded a high risk evaluation. We assigned an overall higher risk of bias to studies that were judged at high risk if at least one domain was judged at high risk of bias, and at moderate risk of bias if at least one domain was judged at moderate risk of bias. Detailed guidance of risk of assessment for nonexperimental studies is reported in Appendix B.

2.1.4. Data Extraction

Data extraction was performed by all study partners at the Universities of Modena and Reggio Emilia, Athens and Porto, with the supervision of the Karolinska Unit. For potassium, extraction was performed independently by two consortium partners in the first place (UNIMORE and NKUA), and subsequently checked and confirmed by a third partner (UPORTO). The following data were extracted: first author name, year of publication, country where the study has been carried out, duration of potassium intervention phase, number and characteristics participants, hypertensive status, use of antihypertensive drugs, design of the trial (parallel or cross-over), presence and duration of a wash-out period, modality of blood pressure measurement (type of device and position of the participants), type and quantity of the potassium supplements administrated, baseline levels of potassium excretion, achieved levels of potassium excretion at the end of the trial, sodium excretion at baseline and after the intervention, summary statistics (mean, standard deviation) of systolic blood pressure (SBP) and diastolic blood pressure (DBP) levels.

For cadmium we extracted from each eligible study: first author name, year of publication country where the study was carried out, duration of follow-up, exposure of interest (dietary intake or urinary excretion of cadmium, details about the outcome of interest (incidence or mortality), cutpoints for each category of exposure, number of cases, cohort size, variables included in most adjusted multivariable model, risk estimates with 95% confidence intervals from the most adjusted model.

2.2. Methodology

In this section we briefly describe the main features of the methodology used in this project and we refer the interested reader to the paper by Crippa et al (2019) for the details (Crippa et al. 2019). We performed dose-response meta-analyses within the general framework of mixed-effects models. The main goal of the analysis is to make inference on the average dose-response relationship based on multiple tables of empirical findings.

The response is a statistic summarizing a distribution of individual outcomes (quantitative, binary). The predictor can represent any quantitative aspect (amount, frequency, duration) of an administered drug or an exposure. The hypothesis is that a summary dose-response curve underlying a collection of similar studies addressing the same research question exists and we wish to quantify the parameters of such curve. Let's use the index $i = 1, \ldots, I$ to denote data available for the i-th study and the index $j = 1, \ldots, J_i$ to denote the allotted dose. The response μ_{ij} could represent the mean, (log) odds, or (log) rate of an individual outcome.

The simplest, yet commonly used, form of dose-response model is the following:

Model 1. Linear dose-response function

$$(\mu_{ij} - \mu_{ir}) = (\beta + b_i)(x_{ij} - x_{ir}) + \varepsilon_{ij}$$

$$b_i \sim N(0,\tau^2)$$

$$\varepsilon_{ij} \sim N(0,\Sigma)$$

The dependent variable is an estimated change in the expected outcome (mean difference, log odds ratio, log rate ratio). The independent variable is an allotted change in the dose. Where a constant change in the dose is assumed to be associated with a constant change β in the predicted outcome. Of note, both the dependent and independent variables are expressed in terms of changes relative to a common reference dose. The reference dose x_{ir} may change across studies. Each set of study-specific contrasts has a certain variances/covariances Σ reflecting the uncertainty of these contrasts. The absence of an intercept forces the study-specific dose-response line to go through the origin (0,0). The assumption of a distribution of true dose-response relationships is translated into a random-effect b_i added to the average slope β. The magnitude of variability of the true linear dose-response relationship across studies is quantified by the variance component τ^2. The published study-specific contrasts estimated within each study $(\hat{\mu}_{ij} - \hat{\mu}_{ir})$ serve as dependent variable. The corresponding inverse of the study-specific estimated variance/covariance $\hat{\Sigma}$ is used a weight. The observed dose levels being contrasted serve as the only covariate in the model. The number of contrasts J_i available within each study may vary. It should be noted that the single mixed-model estimated on the available data (or one-stage) is taking into account the structure of the data and the contribution that each study is able to provide in estimating the distribution of true dose-response relationships.

The simple, yet widely used dose-response Model 1, can be extended in different ways. In this project, we used regression splines to investigate a possible curvi-linear relationship between the dose and the predicted outcome.

Model 2. Spline dose-response function

$$(\mu_{ij} - \mu_{ir}) = (\beta_1 + b_{1i})(s_1(x)_{ij} - s_1(x_{ir})) + (\beta_2 + b_{2i})(s_2(x)_{ij} - s_2(x_{ir})) + \varepsilon_{ij}$$

$$\begin{pmatrix} b_{1i} \\ b_{2i} \end{pmatrix} \sim N\left(0, \begin{matrix} \tau_1^2 & \\ \tau_{1,2} & \tau_2^2 \end{matrix}\right)$$

$$\varepsilon_{ij} \sim N(0,\Sigma)$$

A flexible tool is represented by restricted cubic splines with three knots (k_1, k_2, k_3) spread along the range of the dose. Location of the knots are fixed percentiles (10th, 50th, 90th) of the overall dose distribution available from the I studies. A cubic spline is a variable $(x-k)_+^3 = I(x>k)(x-k)^3$ that takes value $(x_{ij}-k)^3$ when the exposure is above the knot k and 0 otherwise. The word restricted refers to the constraint of linearity before the first knot k_1 and beyond the last knot k_3. The first spline transformation of the exposure s_1 is simply the identity function $s_1(x_{ij}) = x_{ij}$. The second spline transformation of the exposure is instead a function of cubic splines and knots

$$s_2(x_{ij}) = \frac{(x_{ij}-k_1)_+^3 - \frac{(k_3-k_1)}{(k_3-k_2)}(x_{ij}-k_2)_+^3 + \frac{(k_2-k_1)}{(k_3-k_2)}(x_{ij}-k_3)_+^3}{(k_3-k_1)^2}$$

Apart from being able to describe a variety of shapes, an attractive feature of the restricted cubic spline model is that the simpler linear-response model obtained when β_2 is zero. The magnitude of the parameter β_2 quantifies the departure from the linear dose-response function (Model 1). Since the shape of the dose-response relationship is now characterized by two regression coefficients, β_1 and β_2, the random-effects are placed on both of them. The magnitude of heterogeneity in dose-response relationships across studies is captured by the 2 variances and 1 covariance components. Estimates of the unknown parameters can be obtained using likelihood methods.

Inference on the average dose-response relationship

The Wald-type test can be used for hypothesis about all or a subset of the parameters. A formal *p*-value can be obtained by comparing the Wald-type statistic to a χ^2_p distribution with *p* degrees of freedom (*p* is the number of regression coefficients). The predicted contrast in responses, $\hat{\mu}_j^* - \hat{\mu}_r^*$, together with a 95% confidence interval comparing dose x_j and x_r for the average study is given by

$$(\hat{\mu}_j^* - \hat{\mu}_r^*) = \hat{\beta}[x_j - x_r] \pm 1.96\sqrt{\text{Var}(\hat{\beta}_1)[x_j - x_r]^2}$$

when *p* =1 (Model 1) and

$$(\hat{\mu}_j^* - \hat{\mu}_r^*) = \hat{\beta}_1[s_1(x_j) - s_1(x_r)] + \hat{\beta}_2[s_2(x_j) - s_2(x_r)] \pm 1.96 \sqrt{\text{Var}(\hat{\beta}_1)[s_1(x_j) - s_1(x_r)]^2 + \text{Var}(\hat{\beta}_2)[s_2(x_j) - s_2(x_r)]^2 + 2\,\text{Cov}(\hat{\beta}_1,\hat{\beta}_2)[s_1(x_j) - s_1(x_r)][s_2(x_j) - s_2(x_r)]}$$

when *p*=2 (Model 2). The value x_j represent any dose value of interest and x_r is the chosen referent to present the summary dose-response relationship.

If the empirical estimates being modelled are on the natural log scale (i.e. log odds, log rate) then, using the equivariance property of maximum likelihood estimates, one can exponentiate the point and interval estimates given above. Estimation and visualization of the estimated dose-response models is facilitated by the statistical packages *drmeta* in Stata or *dosresmeta* in R. We next illustrate applications of the statistical model for two different scenarios.

Example 1. Tables of estimated mean differences from experimental studies

Consider tables of summarized data from 10 studies investigating the association between a quantitative dose and a quantitative outcome. The dose, randomly assigned to each individual, is positive and skewed to the right (Chi-Square with 5 degrees of freedom). Each principal investigator has categorized the quantitative dose into quantiles and conducted statistical inference on differences in population means outcomes across quantiles of the dose using a linear regression model. The 10 studies are sampled from a random effects non-linear data generating mechanism. We simulated a quadratic dose-response relationship for the average study of this form $-2(x-5) + 0.2(x^2 - 5^2)$ where the dose equal to 5 units serves as referent. Given this shape, the lowest mean outcome is expected to be at the dose of $x = -\frac{2}{2(0.2)} = 5$ units. The empirical estimates and descriptive statistics obtained by the study investigators are useful to the extent they can contribute to the understanding of the features of the distribution of dose-response relationships.

The 10 observed studies vary according to the sample size, dose categorization, and mean dose of the reference category. A total of 13,500 individuals have been involved in these studies contributing to the estimation of 19 mean outcome differences. Moving away from the bottom category of the dose (about 2 units), some studies estimated a lower mean outcome, some other studies a higher mean outcome, and some studies no substantial change (Figure 1). The visual impression is of a large variation in the dose-response association across studies. Based on the scatter plot of empirical estimates, it can be hard for the meta-analyst to imagine what kind of data generating mechanism might be underlying these tables of empirical estimates if the only knowledge available is the collected data.

Ignoring the true generating mechanism, one can assume a linear dose-response function (Model 1). Syntax and estimates obtained with Stata and R are shown in Figure 2. Every 1 unit increase of the dose is estimated to increase, on average, the mean outcome by 0.31 units (95% CI = -0.41, 1.04). The estimated standard deviation of the random-effects is 1.15, which relative to the estimated average slope of 0.31, suggests a considerable variability of the study-specific (assumed) linear trends. Data

appears to be compatible with a flat dose-response association (z=0.85, p-value=0.394). For many health researchers, a linear association with a large p-value, strong heterogeneity, and no clear visual pattern in the estimates may discourage further analysis and could be the beginning of stratified analysis, quality analysis, and wondering about possible explanations for these heterogenous observed data. Actually data may also be compatible with the hypothesis of higher mean outcomes at the dose extremes relative to the middle range of the dose (i.e. U/J-shaped). Such shape would be unlikely to be discovered forcing a linear dose-response function in all the studies.

Next, we use restricted cubic splines with 3 knots of the dose to investigate the possible curvi-linear dose-response relationship. As the linear function is a special case of the restricted cubic spline function, we can test compatibility of the data with a simpler linear function testing the hypothesis that the regression coefficient of the second spline is equal to zero (H_0:$\beta_2 = 0$). Syntax and estimates of the model are shown in the output below obtained with either Stata or R (Figure 3).

The maximized log-likelihood of a mixed-effects model using the restricted cubic spline function of the dose is, in absolute terms, about two-fifths of the maximized log-likelihood of the linear function. Even considering the higher number of estimated parameters of the spline function (2 coefficients + 3 variance/covariance of random-effects) relative the linear function (1 coefficient + 1 variance of random-effects), the AIC of the spline function is about a half the one of the linear function.

The multivariate Wald-type test rejects the null hypothesis of absence of dose-response relationship ($H_0 : \beta_1 = \beta_2 = 0$) for the average study (χ^2_2=86.21, p<0.001). Furthermore, the univariate Wald-type test (z=5.24, p-value<0.001) rejects the hypothesis a simpler linear function (H_0: $\beta_2 = 0$). Nevertheless, we still have no idea of the possible average shape relating the dose to the mean outcome. Figure 4 shows the estimated average dose-response relationship using the dose of 5 units as referent. Based on the spline model, the predicted difference in mean outcomes comparing the generic dose level x versus the reference of 5 unit is $-1.26[s_1(x_j) - s_1(x_r)] + 2.77[s_2(x_j) - s_2(x_r)$ with x ranging from 2 to 10 units. Of note, the true average dose-response relationship (blue curve) is not far from the predicted average dose-response relationship using splines (black curve). The predicted lowest mean outcome corresponds to a dose of about 5 units; which is what was expected based on the true dose-response mechanism underlying the studies.

Example 2. Tables of estimated adjusted hazard ratios from observational studies

Let's now consider tables of summarized data from 30 hypothetical prospective cohort studies investigating the association between baseline brisk walking (dose), measured in hours/week, and time until death (outcome), or end of follow-up (10 years), whichever came first. Age (confounder) is inversely associated with brisk walking and positively associated with higher mortality rates independently of brisk walking levels. Furthermore, the true summary age-adjusted mortality hazard is decreasing with higher walking levels with a threshold effect at 2 hours per week; that is $e^{-0.5(x-2)+0.5I(x>2)(x-2)}$. Principal investigators categorized brisk walking into 2/4 quantiles. Age-adjusted mortality hazard ratios comparing brisk walking categories were estimated using a Cox regression model. Each set of empirical estimates arise from a random-effects data-generating mechanism.

The scatter plot of the estimates shown in Figure 5 may suggest an overall inverse association between brisk walking and age-adjusted mortality rates. The threshold effect at 2 hours per week, however, can be hardly guessed in Figure 5. Furthermore, there are no empirical estimates between 1.5 and 2.4 hours per week, exactly in the range where the levelling-off is occurring. Once again, since the true data generating mechanism is unknown to the meta-analyst, let's assume a simple linear function (Model 1) relating walking to the age-adjusted mortality rate (Figure 6). Every increment of 1 hour/week increase in walking is estimated, in an average study, to reduce the age-adjusted mortality rate by 21% ($e^{-0.24} = 0.79$). Even if, on average, there is no effect of walking beyond 2 hours per week on mortality rate, this model would incorrectly estimate a beneficial effect of walking on age-adjusted mortality rates.

A more realistic dose-response mechanism that the meta-analyst may hypothesize is a decreasing effect of walking on mortality rates at higher levels with a plateau reached at a certain level. A restricted cubic spline model with 3 knots at fixed percentiles for walking hours per week has the possibility to capture such mechanism. The estimated parameters of the mixed model (Model 2) are presented in Figure 7. The 30 studies are compatible, on average, with a non-linear association between walking and the age-adjusted mortality rates. The multivariate Wald-type test rejects the null hypothesis of absence of dose-response relationship for the average study (χ^2_2=122, p<0.001). Furthermore, the univariate Wald-type test (z=8.7, p-value<0.001) rejects the hypothesis of a simpler linear function. Pointwise inference on the average age-adjusted mortality hazard ratios comparing any number of walking hours relative to 2 hours per week is shown in Figure 8.

The estimated spline model detects the levelling-off of the age-adjusted mortality rate somewhere between walking 2 to 3 hours per week; the true value being 2 hours per week. Below 2 hours per week, the predicted age-adjusted mortality hazard ratio is more conservative (closer to 1) compared to the true average linear trend. The fact that we described and used a cubic spline function (Model 2) it does not imply this is best option in any given scenario. According to the data generating mechanism underlying these specific studies, for example, a piecewise linear spline with a knot at 2 hours per week would be a better option.

2.2.1. Routine implementation

The methodology for dose-response model was implemented and overviewed by the KI consortium partner, in collaboration with the other partners. We used the one-stage methodology which consists of a weighted mixed effects model that allows to make inferences regarding the average dose-response relationship between the changes of the exposure and the changes in the outcome of interest. The one-stage approach also represents a major methodological advancement compared with the two-stage approach, also originally envisaged in the project. In particular, in the one-stage approach allows to included studies based on at least two levels of exposure and the pooled curve and estimates of the between-studies heterogeneity are based on the whole set of studies without any exclusion. Thus, even complex non-linear curves (splines, spike at zero exposure) defined by several parameters can be assessed and shaped. We implemented the one-stage methods within existing statistical routines of Stata software (routine *drmeta*) and for R package (routine *dosresmeta*). Details of statistical methodology are reported in web app we made publicly available (See 'Dissemination activities), while detailed illustration of routines are reported in Appendix C for *drmeta* Stata package and in Appendix D for the *dosresmeta* R package. To summarize, when continuous data are investigated, core-variables needed for the analysis are: doses for each exposure category, total number of subjects at each exposure category, mean and standard deviation at each exposure category, and mean difference (either weighted mean difference or standardized mead difference) and its standard error between higher exposure categories and the reference category. Similarly, when dichotomous data are used, core-variable for the analysis are: doses for each exposure category, number of events/cases and total number of subjects/total person-time at each exposure category, log-transformed risk estimate at each exposure category and its standard error. Data required to perform the analysis are reported in Tables 1 and 2 as example of dataset scheme.

2.2.2. Data analysis

We performed a meta-analysis using the 'metan', 'mkspline', and 'drmeta' routines within Stata statistical software (Stata Corp., College Station, TX, 2020). In particular, the drmeta command has been specifically developed for dose-response meta-analysis for Stata statistical software (Stata Corp. TX, 2019) by one consortium partner (KI), and was additionally supplemented with the one-stage approach as described in 'Routine Implementation'. For the dose-response analysis, since we did not make any specific parametric assumptions regarding the shape of the association, we used restricted cubic splines

with 3 knots at fixed percentiles (10, 50, and 90%) during the analysis (Orsini et al. 2012). For assessment of heterogeneity we used and reported I2 statistic (Higgins et al. 2003). Finally, we assessed occurrence of publication bias using graphical assessment with funnel plots for studies reporting highest versus lowest exposure.

2.2.3. Potassium

We performed a meta-analysis of SBP and DBP weighted mean differences (WMD) before and after potassium supplementation for each study and for the relevant subgroups using the 'one-stage' natural cubic spline regression model based on a random effects model (DerSimonian and Laird 1986). We used the one-stage methodology to make inferences regarding the average dose-response relationship between overall potassium excretion at the end of the trial, and changes in SBP and DBP levels. For comparison, we also included a linear function to model potassium excretion in relation to levels of BP using estimates of the parameters obtained using restricted maximum likelihood model (Crippa et al. 2019; Orsini et al. 2012).

We defined the mean difference in potassium excretion between groups of each trial as the difference between the values of potassium excretion at the end of the trial minus the ones at baseline in each arm. Similarly, we defined the mean difference in blood pressure following the intervention as the difference for SBP and DBP at the end of the trial minus the corresponding baseline value. We also carried out stratified analyses according to hypertension status. Sensitivity analysis was performed by excluding trials at high risk for bias from the main analysis.

2.2.4. Cadmium

We performed the meta-analysis based on categorical exposure to cadmium and using the one-stage meta-analytic dose-response approach (Crippa et al. 2019; Orsini et al. 2012). For each exposure category, we extracted the mean or the median or the midpoint of each exposure strata depending on the which data was available in the report. When the highest and lowest exposure categories were 'open', we used as boundary a value that was 20% higher or lower than the closest cutpoint. We excluded studies reporting effect estimates based on continuous exposure. We carried out stratified analyses according to the type of exposure assessment, i.e. dietary intake and urinary excretion.

3. Results

3.1. Potassium

- The PRISMA literature search flow-chart is presented in Figure 9 up to November 25, 2019. From the retrieved 231 records, we excluded 139 studies based on the title/abstract screening. We further excluded 60 studies full-text assessment. Reasons for exclusion are reported in Figure 1, namely studies duration of potassium supplementation was less than 4 weeks, levels of potassium and sodium excretion or of blood pressure were not reported, or studies did not implement potassium supplementation, were duplicate reports of already included studies, or eventually were studies carried out in non-adult population. Table 3 presents the main characteristics of the 32 eligible trials. Publication year ranged from 1982 to 2016. They generally included both sexes, whereas two and one trials respectively were carried out in female and male population only. Mean age of participants ranges was in the range 24-75 years. Nine trials used a parallel design, and twenty-three a cross-over design. Participants were generally hypertensives (N=27), while participants were normotensives in the remaining five trials. All studies estimated 24-hour potassium excretion both at baseline and at the end of the trial. The difference of potassium excretion at the end of the trial ranged from 17 to 131 mmol/day. In Table 4 we present result of risk of bias assessment, showing that only two of the trials were judged at high risk of bias.
- The result of the dose-response meta-analysis is presented in Figure 10 showing the mean blood pressure change between control and supplemented groups for SBP and DBP. In detail, mean SBP and DBP levels appeared to decrease in the supplemented group with increasing differences in potassium excretion, till a potassium excretion of 30 mmol/day. Further increasing levels of potassium excretion suggested a decrease in blood pressure reduction till a value of approximately 80 mmol/day. Higher levels of potassium excretion indicated a possible increase in both SBP and DBP. Mean blood pressure levels at 30, 60, 90 and 120 mmol/day cutpoint of potassium excretion resulted a SBP change of -3.27 (95% CI -4.92, -1.63), -1.95 (95% CI -3.41, -0.48), +1.08 (95% CI -2.59, +4.74), and +4.17 mmHg (95% CI -2.30, 10.63), respectively, and a DBP change of -2.27 (95% CI -3.80, -0.74), -1.31 (95% CI -2.76, +0.14), +0.86 (95% CI -2.91, +4.63) and +3.07 mmHg (95% CI -3.52, +9.66), respectively. The estimated linear function of the average predicted mean difference in blood pressure resulted in an inverse association between potassium supplementation and both SBP and DBP (Figure 10). The meta-analysis comparing blood pressure levels in the supplemented and referent groups showed a mean difference of -3.90 mmHg (95% CI -5.24, -2.56) and -2.43 mmHg (95% CI -3.76, -1.11) for systolic and diastolic BP, respectively (Figure 11).
- In Figure 12 we report the dose-response analysis in the normotensive and the hypertensive populations. It shows that mean blood pressure levels slightly decreased in association with the increase of potassium excretion up to 20-30 mmol/day in both normotensives and hypertensives, with larger effect in the latter group. When we carried out a conventional forest plot analysis, it suggested a larger blood pressure decrease due to potassium supplementation in the hypertensive population (Figure 13).
- Substantially comparable results are demonstrated after exclusion of the two trials at high risk of bias (Figures 14-15). When we assessed presence of publication bias with graphical test of funnel plots we found a slightly asymmetric distribution for SBP only, while no such evidence could be detected for DBP (Figure 16).

3.2. Cadmium

- The PRISMA flow-chart of literature search up to July 26, 2019 showed that from the retrieved 218 records, we excluded 193 studies due to title/ abstract screening (Figure 17). From the

remaining 25 studies assessed in full-text, we further excluded 14 studies since they did not have a cohort design, they were studies reporting only possible mechanism of such association, or they were review paper, or they eventually assessed cadmium exposure through environmental air cadmium levels.

- Table 5 presents characteristics of included studies we included. Six studies were carried out in the North American population, while four and one in European and Asian population, respectively. Five and six studies used dietary intake and urinary levels for cadmium exposure assessment, while eight and three studies measured breast cancer incidence and mortality, respectively. Since one study was a subsequent analysis of one of the other included study we considered in the dose-response meta-analysis the most recent report only. In Table 5 we reported risk of bias assessment: overall, none of the included studies was found to be at high risk of bias.
- The meta-analysis comparing the highest versus the lowest exposure category (Figure 18) showed substantially null association between urine levels of cadmium and risk of breast cancer risk (RR = 1.01, 95%CI 0.70-1.47), as well as for dietary intake (RR = 1.04, 95%CI 0.90-1.21). In the dose-response meta-analysis (Figure 19), there was a weak and imprecise suggestion for a decreased risk at increased urine levels (RR = 0.96, 95% CI 0.57-1.59, at 1 µg/g creatinine and 0.89, 95% CI 0.37-2.14 at 2 µg/g creatinine), while little evidence of any association was found for dietary intake (RR = 1.04, 95% CI 0.81-1.33 at 10 µg/day, and 1.12, 95% CI 0.80-1.56 at 20 µg/day). Finally, the funnel plots showed no particular evidence of publication bias, since they yielded substantially symmetric distributions (Figure 20).

Table 1: Database scheme for data required to perform analysis using mean difference.

author	id	dose	n	mean	sd	md	es_md	type
Author1 et al. year	1	0	100	148.3	4.3	0	0	4
Author1 et al. year	1	110	100	143.9	3.7	-4.4	1.41	4
Author1 et al. year	1	130	100	141.3	3.8	-7.0	1.43	4
Author2 et al. year	2	0	40	151	19.2	0	0	4
Author2 et al. year	2	90	40	144	18.1	-7.0	5.81	4

id: specifies the variable identifying study-specific contrasts.
dose: specifies the dose at each exposure level.
n: specifies the total number of subjects at each exposure level.
mean and sd: are the mean value and its standard deviation at each exposure level.
md and se_md: are the mean difference data (either weighted mean difference or standardized mead difference) and its standard error. Values must be zero for the reference dose.
type: specifies the variable indicating the type of measure used to contrast dose levels with a value of 4 for mean difference, and 5 for standardized mean difference.

Table 2: Database scheme for data required to perform analysis using risk estimates difference.

author	id	dose	cases	n	logrr	es	type
Author1 et al. year	1	11.7	677	233564	0	0	3
Author1 et al. year	1	15.0	691	228121	0.058	0.055	3
Author1 et al. year	1	17.6	744	230981	0.191	0.061	3
Author2 et al. year	2	8.4	91	1785	0	0	3
Author2 et al. year	2	12.5	93	1784	0.432	0.189	3

id: specifies the variable identifying study-specific contrasts. The reference dose is the row with a value of 0 for the standard error.
cases: specifies the number of events/cases at each exposure level.
n: specifies the total number of subjects (controls plus cases) for case-control studies (odds ratio); the total person-time for incidence rate data (hazard ratio); the total number of persons (cases plus non-cases) for cumulative incidence data (risk ratio).
logrr and es: are the log transformed risk estimate at each exposure level and its standard error. Values must be zero for the reference dose.
type: specifies the variable indicating the type of measure used to contrast dose levels with a value of 1 for odds ratio, 2 for hazard ratio, 3 for risk ratio.

Table 3: Characteristics of the 32 trials included.

Reference	Year	Country	Trial duration (a)	No. subjects (b)	Sex	Age (mean)	Age (range)	Design	Wash-out(a)	Hypertensive status	Use of anti-hyp. drugs
(Barden et al. 1986)	1986	Australia	4	44	W	32	18-55	CX	no	N	–
(Berry et al. 2010)	2010	UK	6	48	B	45	22-65	CX	≥5w	H	no
(Braschi and Naismith 2008)	2008	UK	6	56t-34c	B	35	22-65	P	–	N	–
(Bulpitt et al. 1985)	1985	UK	12	14t-19c	B	55	–	P	–	H	yes
(Chalmers et al. 1986)	1986	Australia	4	13t-11c	B	52	–	P	–	H	no
(Forrester and Grell 1988)	1988	Jamaica	4	23	B	–	>18	CX	no	H	yes
(Fotherby and Potter 1992)	1992	UK	4	18	B	75	66-79	CX	no	H	no
(Franzoni et al. 2005)	2005	Italy	4	52t-52c	B	52	–	P	–	H	no
(Gijsbers et al. 2015)	2015	The Netherlands	4	36	B	66	–	CX	no	H	no
(Graham et al. 2014)	2014	UK	6	40	B	55	40-70	CX	2-4w	H	no
(Grimm et al. 1988)	1988	US	12	148t-150c	M	58	45-68	P	–	H	yes
(Grobbee et al. 1987)	1987	The Netherlands	6	40	B	24	18-28	CX	no	H	no
(Gu et al. 2001)	2001	China	12	75t-75c	B	56	–	P	–	H	no
(He et al. 2010)	2010	UK	4	42	B	51	18-75	CX	no	H	no
(Kaplan et al. 1985)	1985	US	6	16	B	49	35-66	CX	no	H	yes
(Kawano et al. 1998)	1998	Japan	4	55	B	–	36-77	CX	no	H	yes
(MacGregor et al. 1982)	1982	UK	4	23	B	45	26-66	CX	no	H	no
(Matlou et al. 1986)	1986	South Africa	6	32	W	51	34-62	CX	no	H	no
(Matthesen et al. 2012)	2012	Denmark	4	21	B	26	18-40	CX	no	N	–
(Miller et al. 1987)	1987	US	4	64	B	42	–	CX	no	N	–

(Overlack et al. 1985)	1985	Germany	8	17	B	29	22-39	CX	no	H	no
(Overlack et al. 1991)	1991	Germany	8	12	B	37	25-59	CX	no	H	no
(Overlack et al. 1995)	1995	Germany	8	50	B	48	24-70	CX	4w	H	no
(Patki et al. 1990)	1990	India	8	37	B	50	–	CX	2w	H	no
(Richards et al. 1984)	1984	New Zealand	4	12	B	–	19-52	CX	no	H	no
(Siani et al. 1987)	1987	Italy	15	18t-19c	B	45	21-61	P	–	H	no
(Skrabal et al. 1984)	1984	Austria	4	21	B	39	21-69	CX	no	H	yes
(Smith et al. 1985)	1985	UK	4	20	B	53	30-66	CX	no	H	no
(Sundar et al. 1985)	1985	India	4	25t-25c	B	46	–	P	–	H	no
(Valdes et al. 1991)	1991	Chile	4	24	B	50	–	CX	no	H	no
(Vongpatanasin et al. 2016)	2016	US	4	30	B	54	–	CX	1w	H	no
(Whelton et al. 1995)	1995	US	4	178t-175c	B	26	30-54	P	–	N	no

–: no information available; B: both men and women; CX: cross-over; H: hypertensives; M: men; N: normotensives; P: parallel; W: women.

Table footnote a: Duration reported in weeks

Table footnote b: For parallel design, number of subjects in treated (t) and control (c) groups are reported separately.

Table 4: Overall risk of bias (RoB) assessment of included studies of potassium and blood pressure.

Reference	Domain 1[(a)]	Domain 2[(a)]	Domain 3[(a)]	Domain 4[(a)]	Domain 5[(a)]	Domani 6[(a)]	Overall RoB [(b)]
(Barden et al. 1986)	Some Conc.	Low	Low	Low	Some Conc.	Some Conc.	Some Conc.
(Berry et al. 2010)	Some Conc.	Low	Low	Low	Some Conc.	Low	Some Conc.
(Braschi and Naismith 2008)	Low	Low	Low	Low	Low	Low	Low
(Bulpitt et al. 1985)	Some Conc.	Low	Low	Low	Some Conc.	Low	Some Conc.
(Chalmers et al. 1986)	Some Conc.	Low	Low	Low	Low	Low	Some Conc.
(Forrester and Grell 1988)	High	Low	Low	Low	Some Conc.	Some Conc.	High
(Fotherby and Potter 1992)	Low	Low	Low	Low	Some Conc.	Some Conc.	Some Conc.
(Franzoni et al. 2005)	Some Conc.	Low	Low	Low	Some Conc.	Low	Some Conc.
(Gijsbers et al. 2015)	Low	Low	Low	Low	Low	Some Conc.	Some Conc.
(Graham et al. 2014)	Low	Low	Low	Low	Low	Low	Low
(Grimm et al. 1988)	Low	Low	Low	Low	Low	Low	Low
(Grobbee et al. 1987)	Low	Low	Low	Low	Some Conc.	Some Conc.	Some Conc.
(Gu et al. 2001)	Low	Low	Low	Low	Some Conc.	Low	Low
(He et al. 2010)	Low	Low	Low	Low	Some Conc.	Some Conc.	Some Conc.
(Kaplan et al. 1985)	Low	Low	Low	Low	Some Conc.	Some Conc.	Some Conc.
(Kawano et al. 1998)	Some Conc.	Low	Low	Low	Some Conc.	Some Conc.	Some Conc.
(MacGregor et al. 1982)	Low	Low	Low	Low	Some Conc.	Some Conc.	Some Conc.
(Matlou et al. 1986)	Some Conc.	Low	Low	Low	Some Conc.	Some Conc.	Some Conc.
(Matthesen et al. 2012)	Some Conc.	Low	Low	Low	Some Conc.	Low	Some Conc.
(Miller et al. 1987)	High	Low	Low	Low	Some Conc.	Low	High
(Overlack et al. 1985)	Some Conc.	Some Conc.	Low	Low	Some Conc.	Some Conc.	Some Conc.
(Overlack et al. 1991)	Some Conc.	Low	Low	Low	Some Conc.	Some Conc.	Some Conc.
(Overlack et al. 1995)	Some Conc.	Low	Low	Low	Some Conc.	Low	Some Conc.
(Patki et al. 1990)	Low	Low	Low	Low	Some Conc.	Low	Low
(Richards et al. 1984)	Some Conc.	Low	Some Conc.	Low	Some Conc.	Some Conc.	Some Conc.
(Siani et al. 1987)	Low	Low	Low	Low	Some Conc.	Low	Low
(Skrabal et al. 1984)	Some Conc.	Low	Low	Some Conc.	Some Conc.	Some Conc.	Some Conc.
(Smith et al. 1985)	Low	Low	Low	Low	Some Conc.	Some Conc.	Some Conc.
(Sundar et al. 1985)	Some Conc.	Low	Low	Low	Some Conc.	Low	Some Conc.
(Valdes et al. 1991)	Some Conc.	Low	Low	Low	Some Conc.	Some Conc.	Some Conc.
(Vongpatanasin et al. 2016)	Low	Low	Low	Low	Some Conc.	Some Conc.	Some Conc.
(Whelton et al. 1995)	Low	Low	Low	Low	Some Conc.	Low	Low

Low: low risk of bias; Some Conc.: some concerns; High: high risk of bias.

Table footnote a: The seven domains are: (1) Risk of bias arising from the randomization process; (2) Risk of bias due to deviations from the intended interventions (effect of assignment to intervention); (3) Missing outcome data; (4) Risk of bias in measurement of the outcome; (5) Risk of bias in selection of the reported result; (6) Risk of bias for cross-over design.

Table footnote b: We allocated the study to an overall higher risk of bias if it was judged to be at "High risk" for at least one domain whereas we considered the study at "Some concerns" risk of bias when some unease existed for at least one domain.

Table 5: Characteristics of the studies included for cadmium and breast cancer.

Reference	Country	Cohort acronym	Outcome	Assessment method	Cases/ population	Population (age range)	Mean Cd level[a]
(Adams et al. 2012a)	US	VITAL	Incidence	dCd	899/26801	All post-menopausal women (50-76)	10.9
(Adams et al. 2012b)	US	NHANES III	Mortality	uCd	42/8218	Pre and post menopausal women (≥17)	0.352
(Adams et al. 2014)	US	WHI	Incidence	dCd	6658/150889	All post-menopausal women (50-79)	10.9
(Adams et al. 2016)	US	WHI	Incidence	uCd	508/1050	All post-menopausal women (50-79)	0.63
(Eriksen et al. 2014)	Denmark	DCH	Incidence	dCd	1390/23815	All post-menopausal (50-65)	13.4[b]
(Eriksen et al. 2017)	Denmark	DCH	Incidence	uCd	900/23379 (898 nested)	All post-menopausal (50-65)	0.54[b]
(Garcia-Esquinas et al. 2014)	US	SHS	Mortality	uCd	25/2254	Pre and post menopausal (45-74)	0.93[b]
(Julin et al. 2012)	Sweden	SMC	Incidence	dCd	2112/55987	All post-menopausal (42-76)	15
(Lin et al. 2013)	US	NHANES III	Incidence	uCd	26/2730	All post-menopausal (≥ 50)	0.77[b]
(Sawada et al. 2012)	Japan	JPHC !-II	Incidence	dCd	402/48351	Pre and post-menopausal (45-74)	26.5
(Grioni et al. 2019)	Italy	ORDET	Incidence	dCd	481/8924	Pre and post menopausal (34-70)	7.8

dCd: dietary cadmium; uCd: urinary cadmium.
Table footnote a: values in µg/day for dietary cadmium and µg/g of creatinine for urinary cadmium.
Table footnote b: median value.

Table 6: Risk of bias assessment of studies included in the analysis of cadmium and breast cancer.

Reference	Cohort	Assessment	Domain 1[(a)]	Domain 2[(a)]	Domain 3[(a)]	Domain 4[(a)]	Domain 5[(a)]	Domain 6[(a)]	Domain 7[(a)]	Overall risk
(Adams et al. 2012a)	VITAL	dCd	Low	Low	Mod.	Low	Mod.	Low	Low	**Mod.**
(Adams et al. 2012b)	NHANES III	uCd	Mod.	Low	Low	Low	Mod.	Low	Low	**Mod.**
(Adams et al. 2014)	WHI	dCd	Low	Low	Mod.	Mod.	Mod.	Low	Low	**Mod.**
(Adams et al. 2016)	WHI	uCd	Low	Low	Low	Mod.	Low	Low	Low	**Low**
(Eriksen et al. 2014)	DCH	dCd	Mod	Low	Mod.	Low	Low	Low	Low	**Mod.**
(Eriksen et al. 2017)	DCH	uCd	Low	Low	Low	Low	Low	Low	Low	**Low**
(Garcia-Esquinas et al. 2014)	SHS	uCd	Low	Low	Low	Low	Mod.	Low	Low	**Low**
(Julin et al. 2012)	SMC	dCd	Low	Low	Mod.	Low	Low	Low	Low	**Mod.**
(Lin et al. 2013)	NHANES III	uCd	Mod	Low	Low	Low	Low	Low	Low	**Mod.**
(Sawada et al. 2012)	JPHC	dCd	Low	Low	Mod.	Low	Low	Low	Low	**Mod.**
(Grioni et al. 2019)	ORDET	dCd	Low	Low	Mod.	Low	Low	Low	Low	**Mod.**

dCd: dietary cadmium; uCd: urinary cadmium; Low: low risk of bias; Mod.: moderate risk of bias; High: serious or critical risk of bias.

Table footnote a: The seven domains are:(1) bias due to confounding; (2) bias in selecting participants in the study; (3) bias in exposure classification; (4) bias due to departures from intended exposures; (5) bias due to missing data; (6) bias in outcome measurement; (7) bias in the selection of reported results

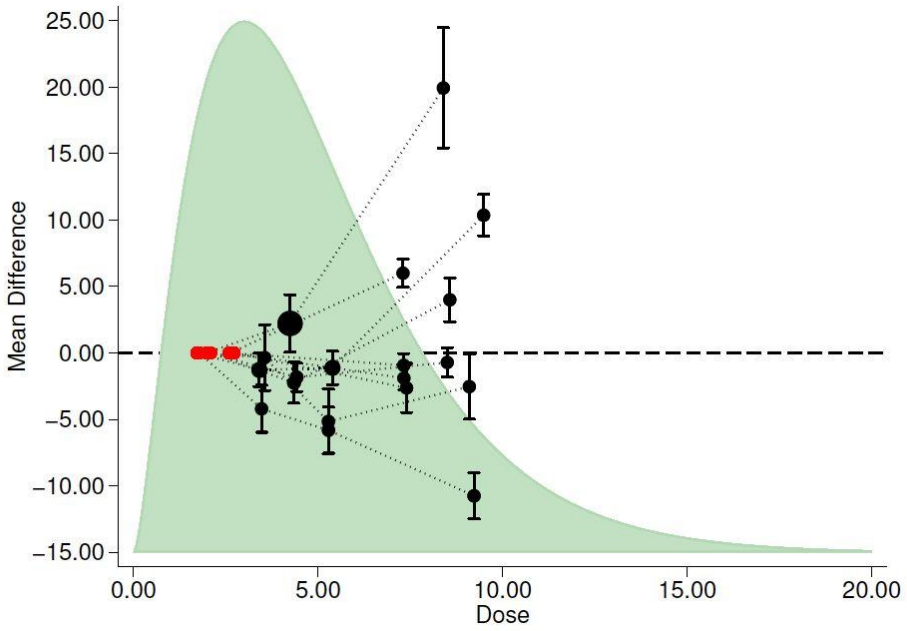

Figure 1: Graph of the study-specific estimated mean differences (95% Confidence intervals, capped spikes) arising from 10 studies of different sizes. Marker size is inversely related to its variance. Red points are the study-specific reference values. Green shaded area is the distribution of the dose in the population.

Stata

```
. drmeta md dose, se(semd) data(n sd) id(id) type(type_md) ml stddev

One-stage random-effect dose-response model      Number of studies =        10
Optimization     = ml                                Number of obs =        19
           AIC = 240.04                              Model chi2(1) =      0.73
Log likelihood = -118.02019                          Prob > chi2 =    0.3943
```

md	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
dose	.3138377	.3683861	0.85	0.394	-.4081857	1.035861

Random-effects parameters	Estimate
std(dose,dose)	1.155001

```
LR test vs. no random-effects model = 571.30704       Prob >= chi2(1) = 0.0000
```

R

```
> summary(m_1)
Call:  dosresmeta(formula = md ~ dose, id = id, n = n, sd = sd, data = md_drm,
    covariance = "md", method = "ml", proc = "1stage")

One-stage random-effects meta-analysis
Estimation method: ML
Covariance approximation: Mean Differences

Chi2 model: X2 = 0.7258 (df = 1), p-value = 0.3943

Fixed-effects coefficients
      Estimate  Std. Error       z  Pr(>|z|)  95%ci.lb  95%ci.ub
dose    0.3138      0.3684  0.8519    0.3943   -0.4082    1.0359
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Between-study random-effects (co)variance components
  Std. Dev
    1.1550

10 studies, 19 values, 1 fixed and 1 random-effects parameters
   logLik        AIC        BIC
-118.0202   240.0404   241.9293
```

Figure 2: Estimates of a mixed-effects model using a linear function based on 10 experimental studies (19 estimated mean differences) obtained with Stata and R.

Stata

```
. drmeta md doses1 doses2 , se(semd) data(n sd) id(id) type(type_md) ml stddev

One-stage random-effect dose-response model      Number of studies =        10
Optimization   = ml                                 Number of obs =        19
           AIC = 107.34                             Model chi2(2) =     86.21
Log likelihood = -48.669605                           Prob > chi2 =    0.0000
```

md	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
doses1	-1.26233	.1895313	-6.66	0.000	-1.633805	-.8908556
doses2	2.76801	.5284361	5.24	0.000	1.732295	3.803726

Random-effects parameters	Estimate
std(doses1,doses1)	.3656176
std(doses2,doses2)	1.462174
corr(doses1,doses1)	1

```
LR test vs. no random-effects model = 615.5756        Prob >= chi2(3) = 0.0000
```

R

```
> summary(m_s)
Call:  dosresmeta(formula = md ~ rcs(dose, knots), id = id, n = n, sd = sd,
    data = md_drm, covariance = "md", method = "ml", proc = "1stage")

One-stage random-effects meta-analysis
Estimation method: ML
Covariance approximation: Mean Differences

Chi2 model: X2 = 86.5004 (df = 2), p-value = 0.0000

Fixed-effects coefficients
                      Estimate  Std. Error        z
rcs(dose, knots)dose    -1.3148      0.1891  -6.9543
rcs(dose, knots)dose'    2.9633      0.5629   5.2643
                      Pr(>|z|)  95%ci.lb  95%ci.ub
rcs(dose, knots)dose     0.0000   -1.6854   -0.9443  ***
rcs(dose, knots)dose'    0.0000    1.8600    4.0666  ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Between-study random-effects (co)variance components
                      Std. Dev                  Corr
rcs(dose, knots)dose     0.3436  rcs(dose, knots)dose
rcs(dose, knots)dose'    1.5554                     1

10 studies, 19 values, 2 fixed and 3 random-effects parameters
  logLik       AIC       BIC
-48.5269  107.0537  111.7759
```

Figure 3: Estimates of a mixed-effects model using a restricted cubic spline function based on 10 experimental studies (19 estimated mean differences) obtained with Stata and R.

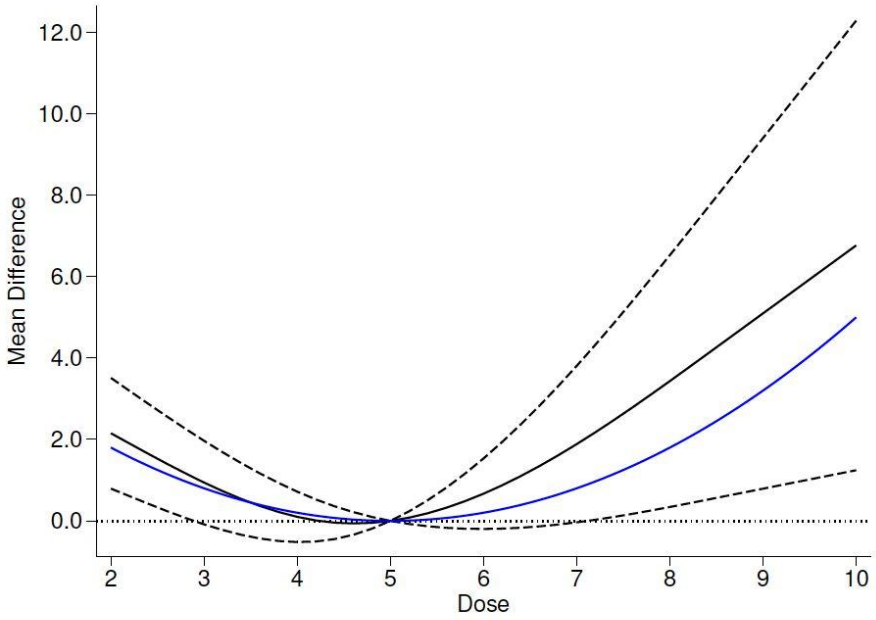

Figure 4: Estimated summary (solid line) dose-response relationship with 95% confidence intervals (dashed lines) based on 10 tables of empirical estimates. Data were fitted with a weighted mixed-effects model using restricted cubic splines for the dose with three knots located at percentiles (10th, 50th, 90th) of the overall dose distribution. The blue line is the true summary dose-response relationship. The dose value of 5 units served as referent.

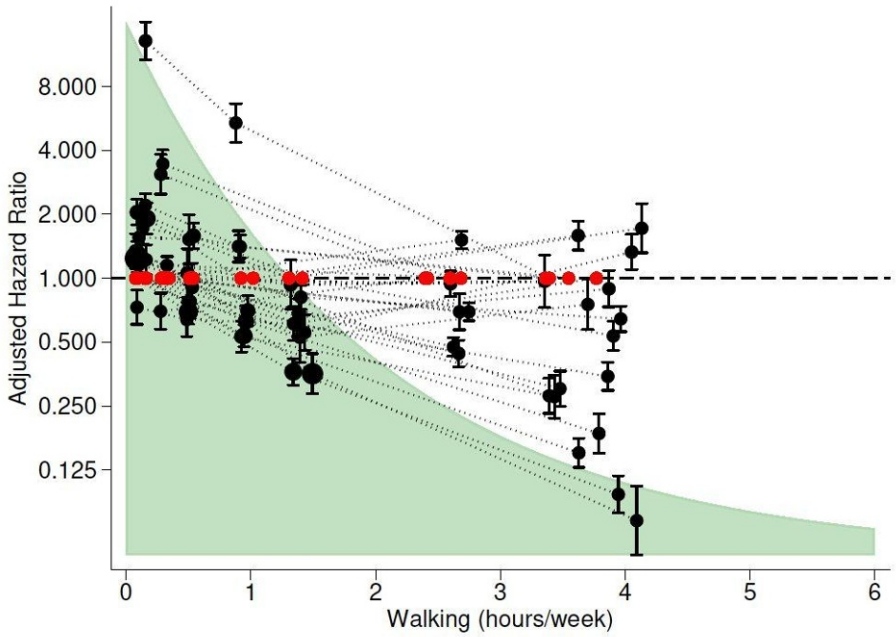

Figure 5: Graph of the study-specific estimated age-adjusted mortality hazard ratios (95% Confidence intervals, capped spikes) according to walking levels arising from 30 studies of different sizes. The red symbols indicate the study-specific reference values. Marker size is inversely related to its variance. Green shaded area is the distribution of the dose in the population.

Stata

```
. drmeta b walk   , se(seb) data(n case) type(type)  id(id) ml stddev

One-stage random-effect dose-response model      Number of studies =        30
Optimization   = ml                                  Number of obs =        61
          AIC = 294.51                               Model chi2(1) =     23.50
Log likelihood = -145.25278                            Prob > chi2 =    0.0000
```

b	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
walk	-.2413534	.0497838	-4.85	0.000	-.3389278	-.143779

Random-effects parameters	Estimate
std(walk,walk)	.2710235

```
LR test vs. no random-effects model = 2614.8966       Prob >= chi2(1) = 0.0000
```

R

```
> summary(m_1)
Call:  dosresmeta(formula = b ~ walk, id = id, type = "ir", cases = case,
    n = n, data = hr_drm, se = seb, method = "ml", proc = "1stage")

One-stage random-effects meta-analysis
Estimation method: ML
Covariance approximation: Greenland & Longnecker

Chi2 model: X2 = 23.5034 (df = 1), p-value = 0.0000

Fixed-effects coefficients
      Estimate  Std. Error        z  Pr(>|z|)  95%ci.lb  95%ci.ub
walk   -0.2414      0.0498  -4.8480    0.0000   -0.3389   -0.1438  ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Between-study random-effects (co)variance components
  Std. Dev
    0.2710

30 studies, 61 values, 1 fixed and 1 random-effects parameters
   logLik        AIC        BIC
-145.2528   294.5056   298.7274
```

Figure 6: Estimates of a mixed-effects model using a linear function based on 30 observational studies (61 estimated age-adjusted hazard ratios) obtained with Stata and R.

Stata

```
. drmeta b walks1 walks2  , se(seb) data(n case) type(type)  id(id) ml stddev

One-stage random-effect dose-response model      Number of studies =        30
Optimization     = ml                                Number of obs =        61
           AIC = 39.34                               Model chi2(2) =    122.00
Log likelihood = -14.668195                            Prob > chi2 =    0.0000
```

b	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
walks1	-.565042	.0555092	-10.18	0.000	-.6738381	-.4562459
walks2	.8556666	.0980083	8.73	0.000	.6635738	1.047759

Random-effects parameters	Estimate
std(walks1,walks1)	.2676563
std(walks2,walks2)	.3574224
corr(walks1,walks1)	-.2493728

```
LR test vs. no random-effects model = 2642.0306       Prob >= chi2(3) = 0.0000
```

R

```
> m_s <- dosresmeta(formula = b ~ rcs(walk, knots), id = id, se = seb, cases = case, n = n,
+                   method = "ml", type="ir", data = hr_drm, proc = "1stage")
> summary(m_s)
Call:  dosresmeta(formula = b ~ rcs(walk, knots), id = id, type = "ir",
    cases = case, n = n, data = hr_drm, se = seb, method = "ml",
    proc = "1stage")

One-stage random-effects meta-analysis
Estimation method: ML
Covariance approximation: Greenland & Longnecker

Chi2 model: X2 = 122.0193 (df = 2), p-value = 0.0000

Fixed-effects coefficients
                      Estimate  Std. Error         z  Pr(>|z|)  95%ci.lb  95%ci.ub
rcs(walk, knots)walk   -0.5647      0.0555  -10.1779    0.0000   -0.6735   -0.4560  ***
rcs(walk, knots)walk'   0.8539      0.0978    8.7303    0.0000    0.6622    1.0456  ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Between-study random-effects (co)variance components
                      Std. Dev                  Corr
rcs(walk, knots)walk    0.2676  rcs(walk, knots)walk
rcs(walk, knots)walk'   0.3567               -0.2487

30 studies, 61 values, 2 fixed and 3 random-effects parameters
  logLik       AIC       BIC
-14.6682   39.3363   49.8907
```

Figure 7: Estimates of a mixed-effects model using a restricted cubic spline function based on 10 experimental studies (19 estimated mean differences) obtained with Stata and R.

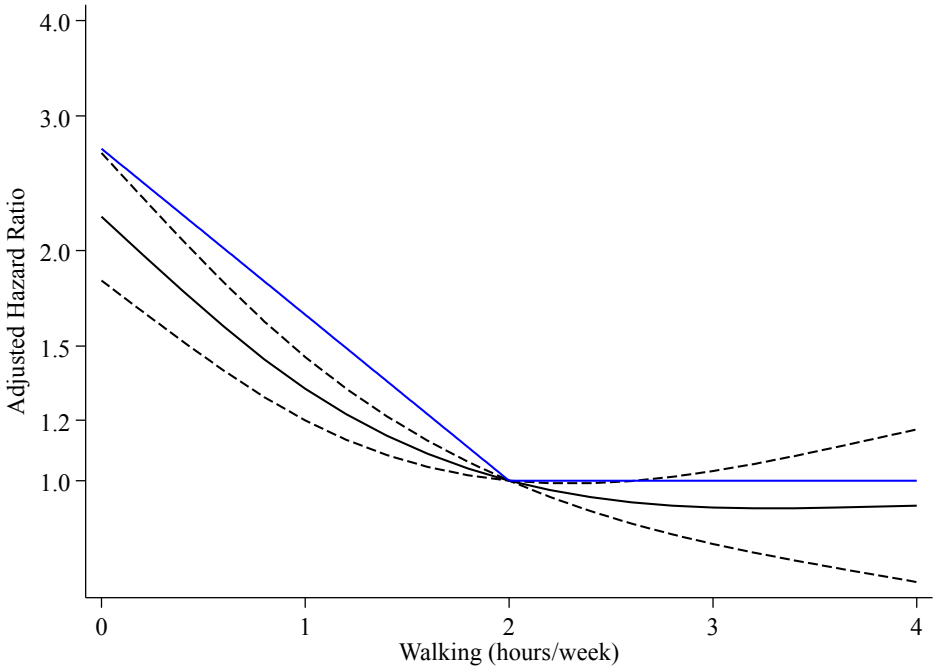

Figure 8: Estimated summary (solid line) dose-response relationship with 95% confidence intervals (dashed lines) based on 30 tables of empirical estimates. Data were fitted with a weighted mixed-effects model restricted cubic splines for brisk walking with 3 knots at fixed percentiles of its distribution. The true summary age-adjusted dose-response mechanism (blue line) is shown for comparison. The value of 2 hours/week served as referent.

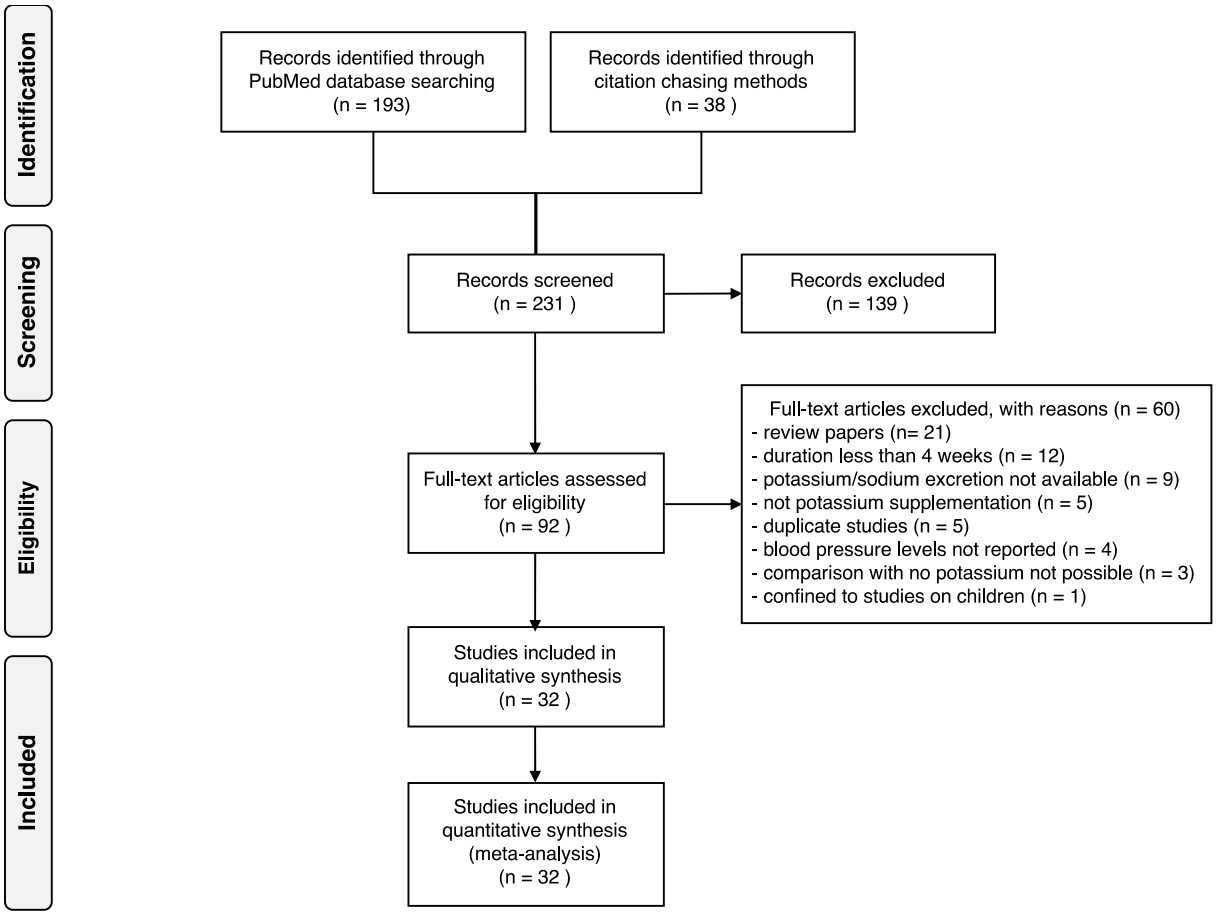

Figure 9: Flow-chart of study identification and selection for potassium and blood pressure

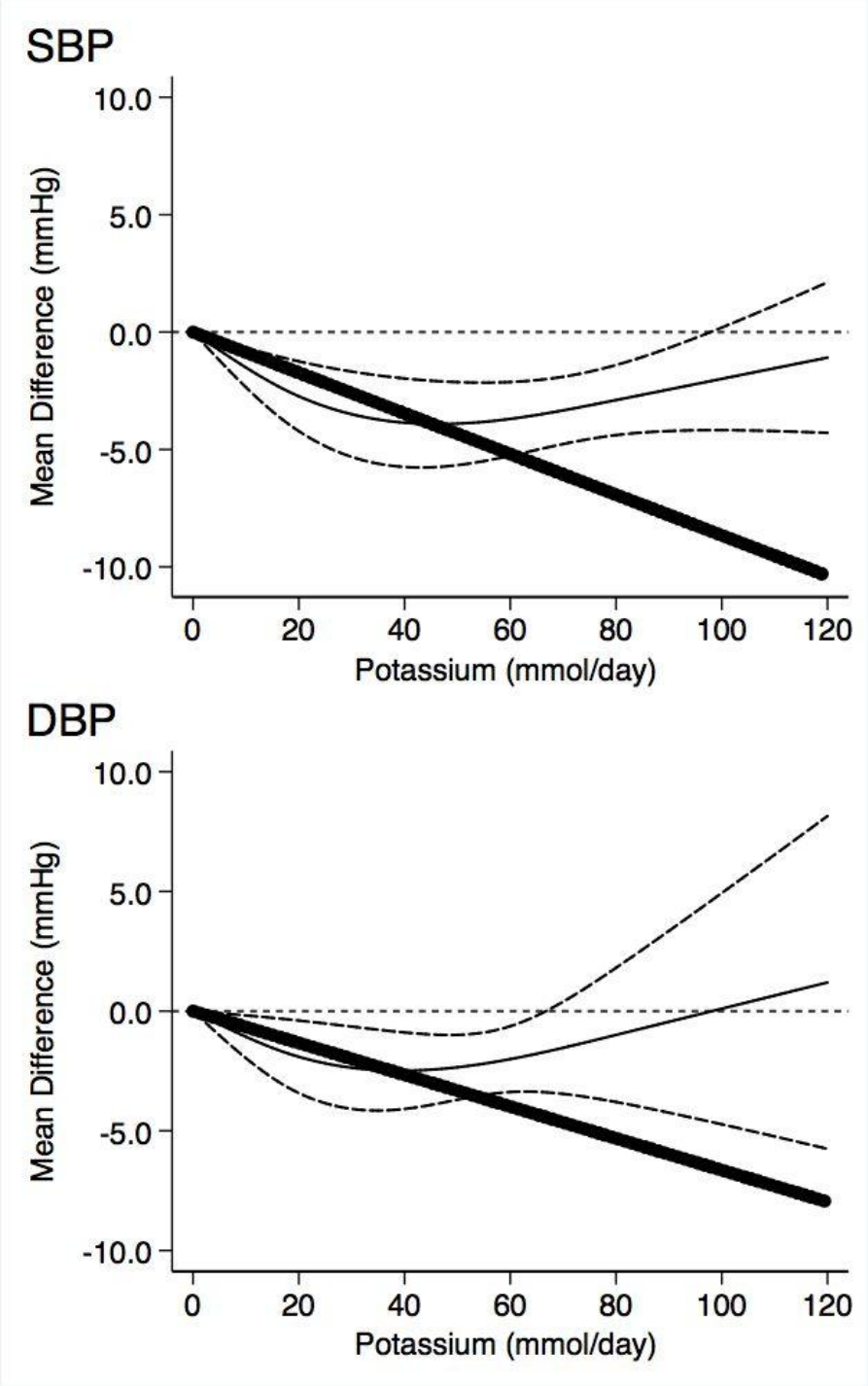

Figure 10: Dose-response meta-analysis of systolic (SBP) and diastolic (DBP) blood pressure mean changes at increasing level of difference of potassium excretion between control and supplemented groups at the end of the trial. Black thick line shows the trend of linear prediction.

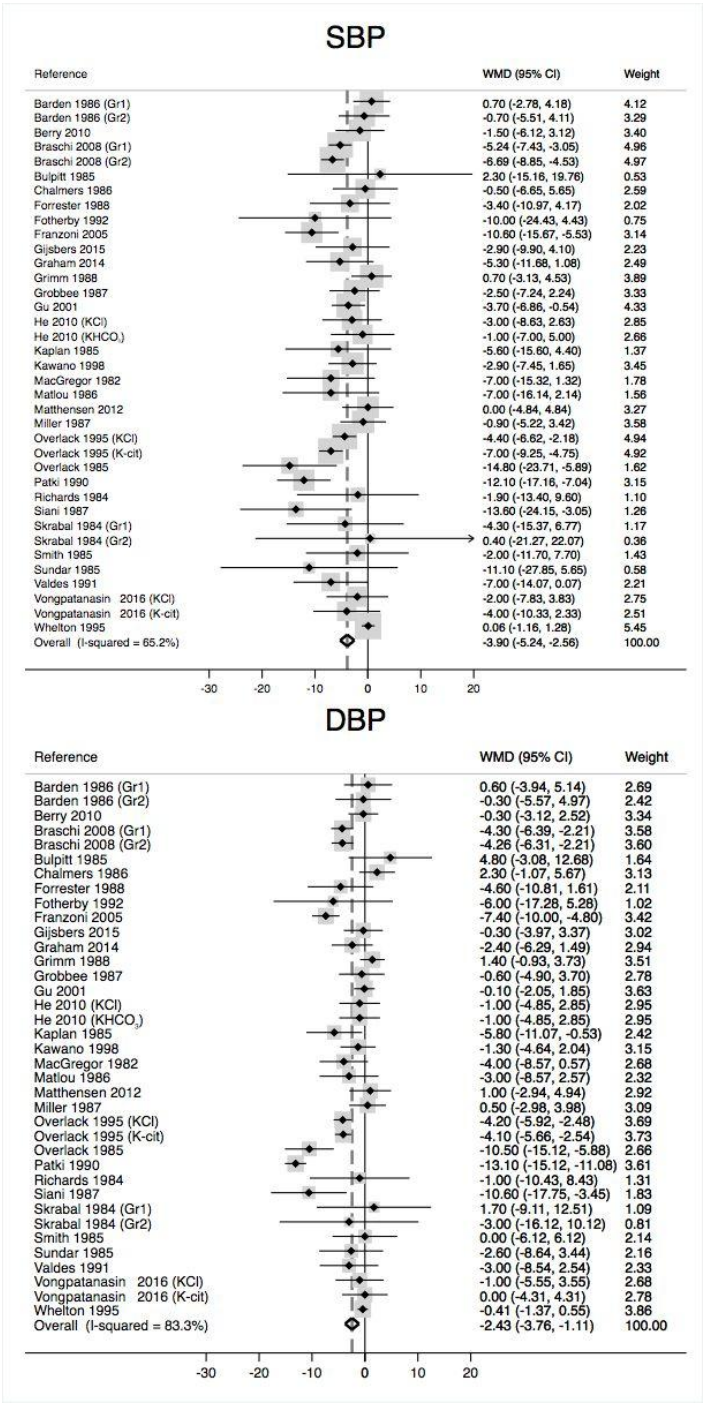

Figure 11: Meta-analysis of mean difference of systolic (SBP) and diastolic (DBP) blood pressure levels between potassium treated and non-treated groups considering overall studies.

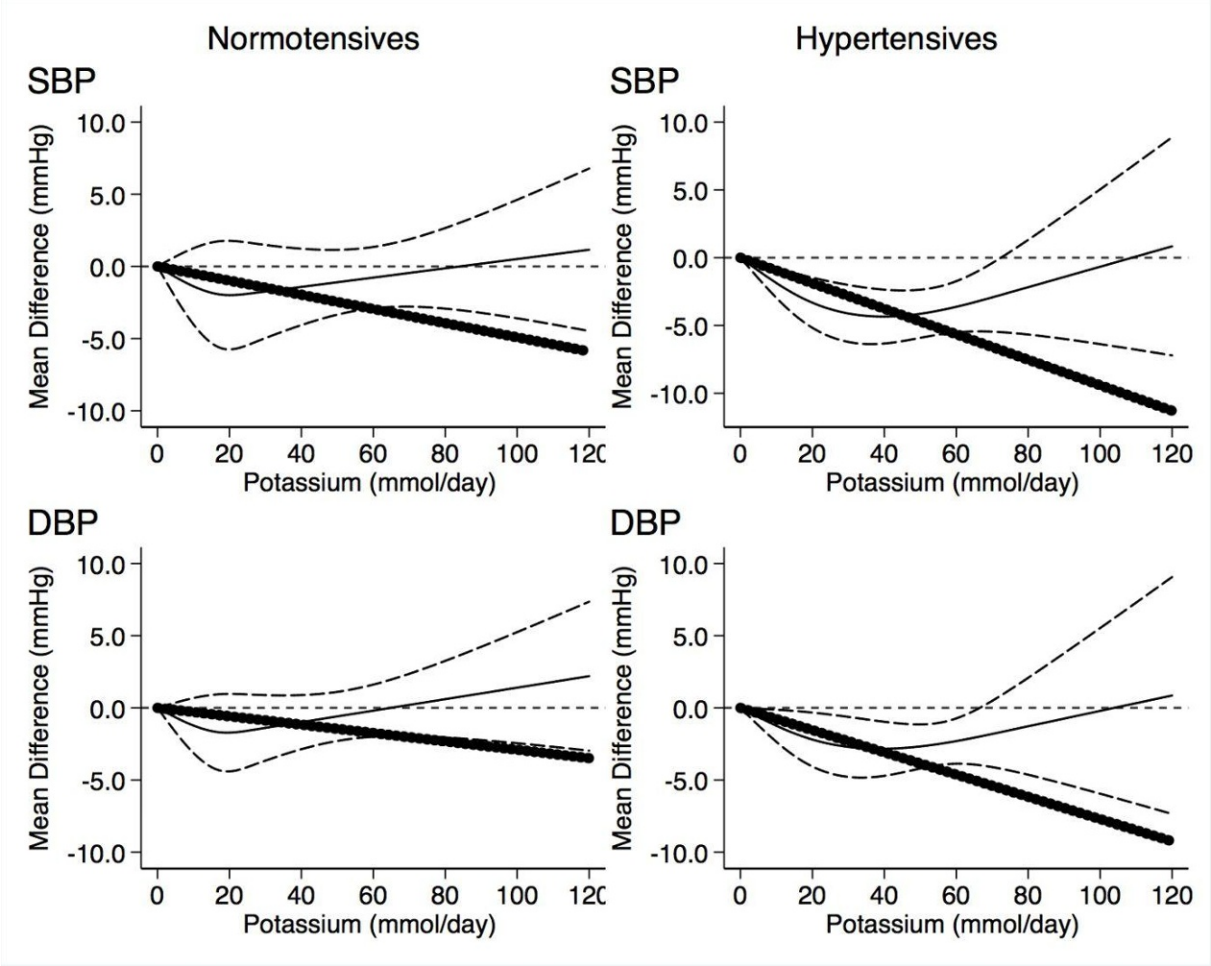

Figure 12: Dose-response meta-analysis of systolic (SBP) and diastolic (DBP) blood pressure mean changes at increasing level of the difference of potassium excretion between control and supplemented groups achieved at the end of the trial in normotensives and hypertensives. Spline curve (solid line) with 95% confidence limits (long dashed lines). Black thick line shows the trend of linear prediction.

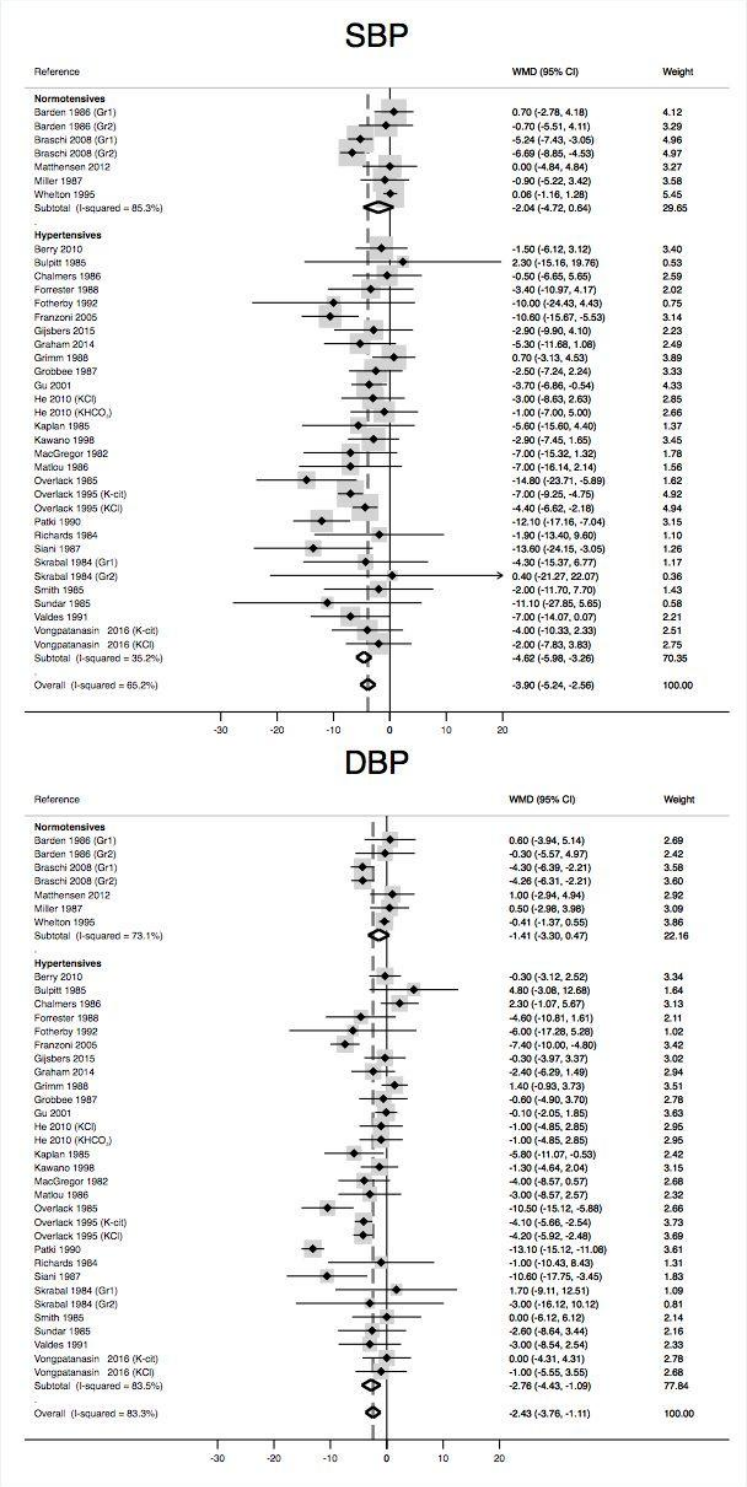

Figure 13: Meta-analysis of mean difference of systolic (SBP) and diastolic (DBP) blood pressure levels between potassium treated and non-treated groups in normotensives and hypertensives.

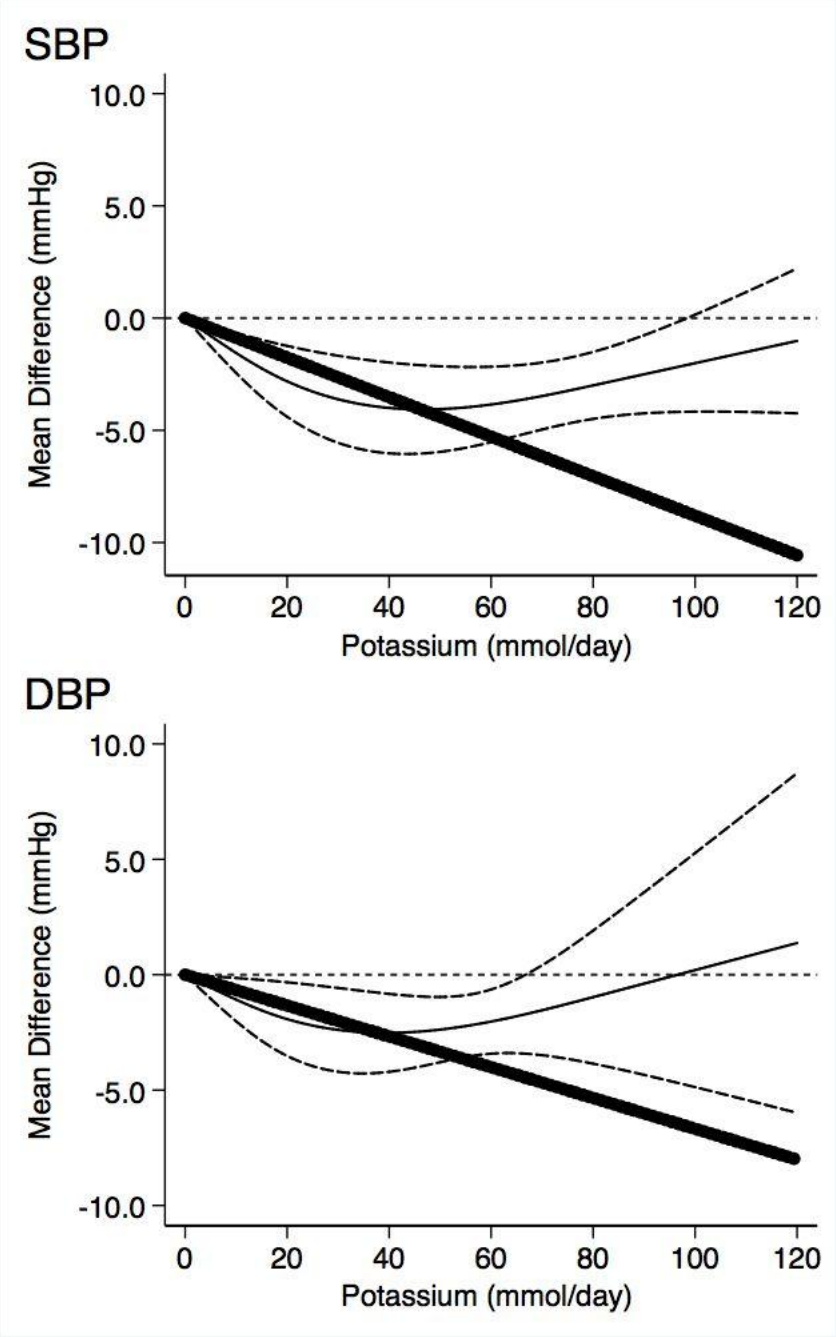

Figure 14: Dose-response meta-analysis of systolic (SBP) and diastolic (DBP) mean changes at increasing level of difference of potassium excretion between control and supplemented groups achieved at the end of the trial, after exclusion of two studies at high risk of bias. Black thick line shows the trend of linear prediction.

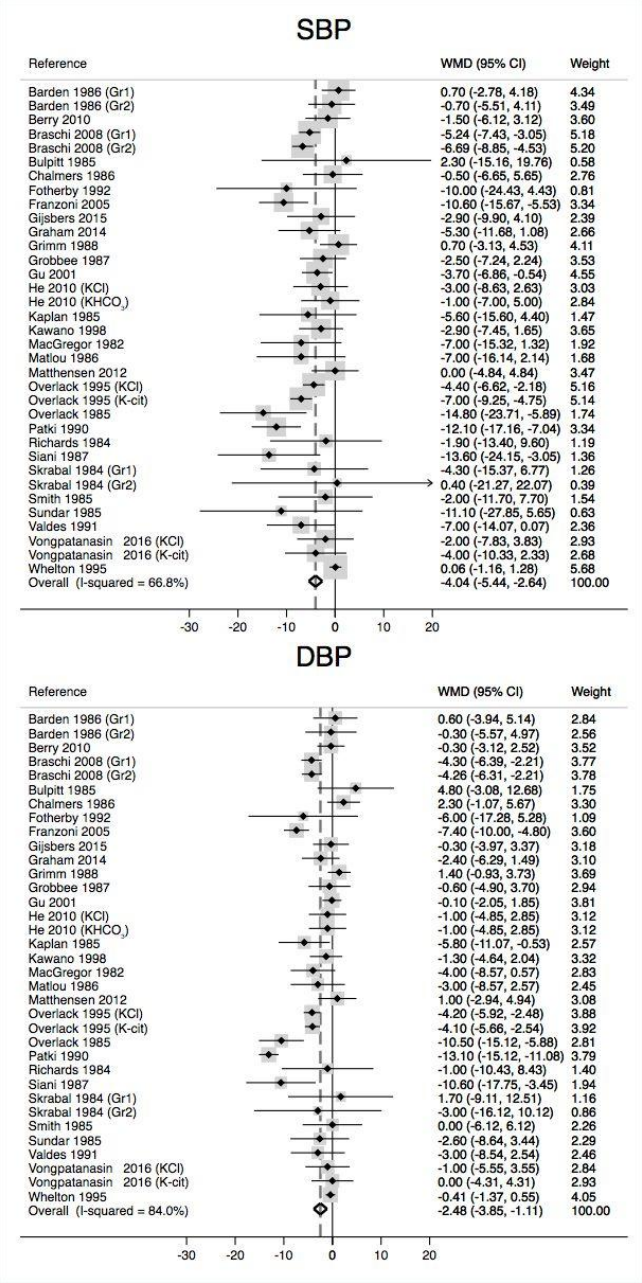

Figure 15: Meta-analysis of mean difference of systolic (SBP) and diastolic (DBP) blood pressure levels between potassium treated and non-treated groups after exclusion of two trials at high risk of bias.

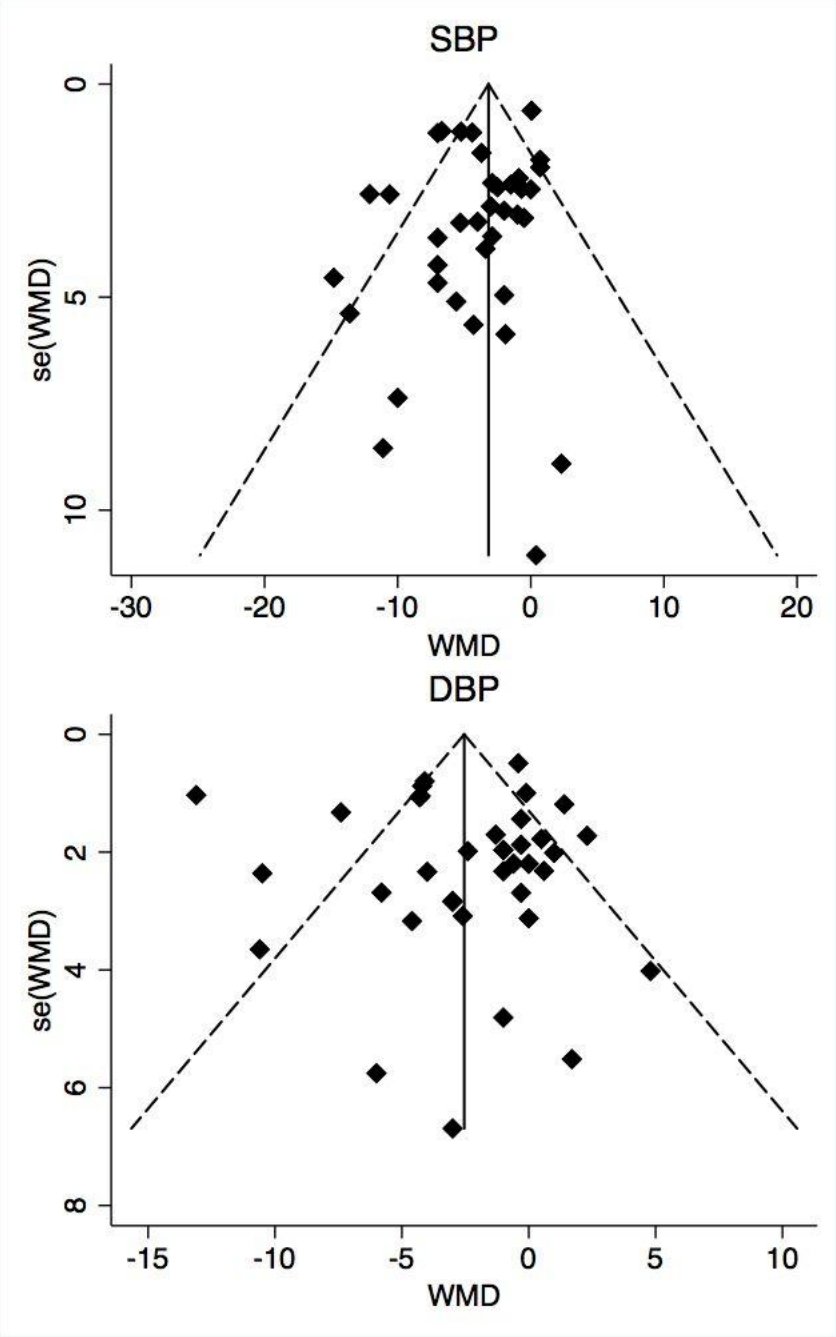

Figure 16: Funnel plots with pseudo 95% confidence limits (dash lines) for publication bias for mean difference of systolic (SBP) and diastolic (DBP) blood pressure levels (as mmHg) mean difference-WMD and its standard error-se(WMD).

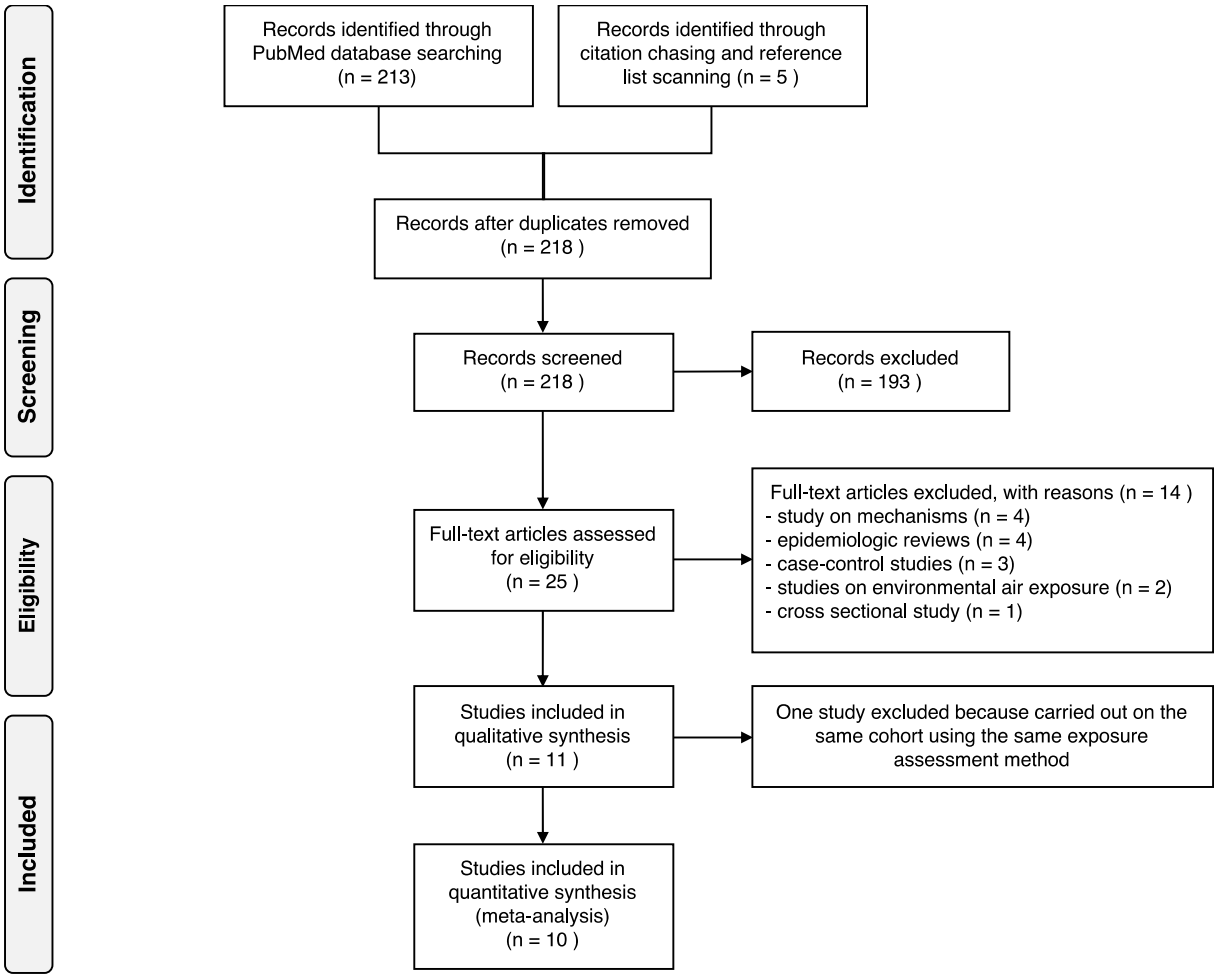

Figure 17: Flow-chart of study identification and selection for cadmium and breast cancer.

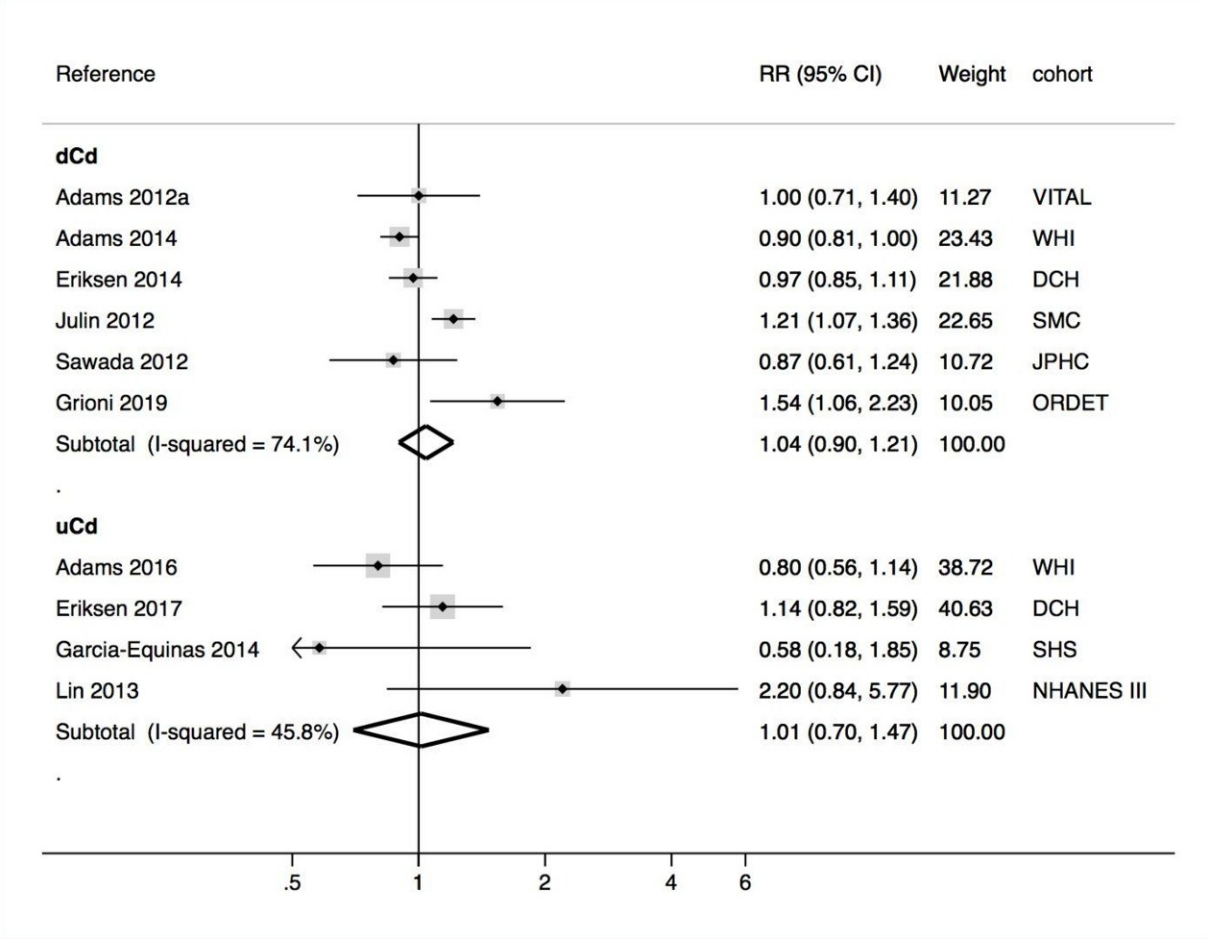

Figure 18: Risk ratio (RR) with 95% confidence interval (CI) of breast cancer risk due to cadmium exposure using dietary intake (dCd) or urinary excretion (uCd) for the assessment. The area of each grey square is proportional to the inverse of the variance of the estimated log RR. Black diamonds represent point estimates of RR and horizontal lines represent their 95% confidence intervals (CIs). The open diamonds represent the combined RR for each subgroup and the overall RR for all studies. The solid line represents RR=1. The dash line represents the point estimate of overall RR for all studies.

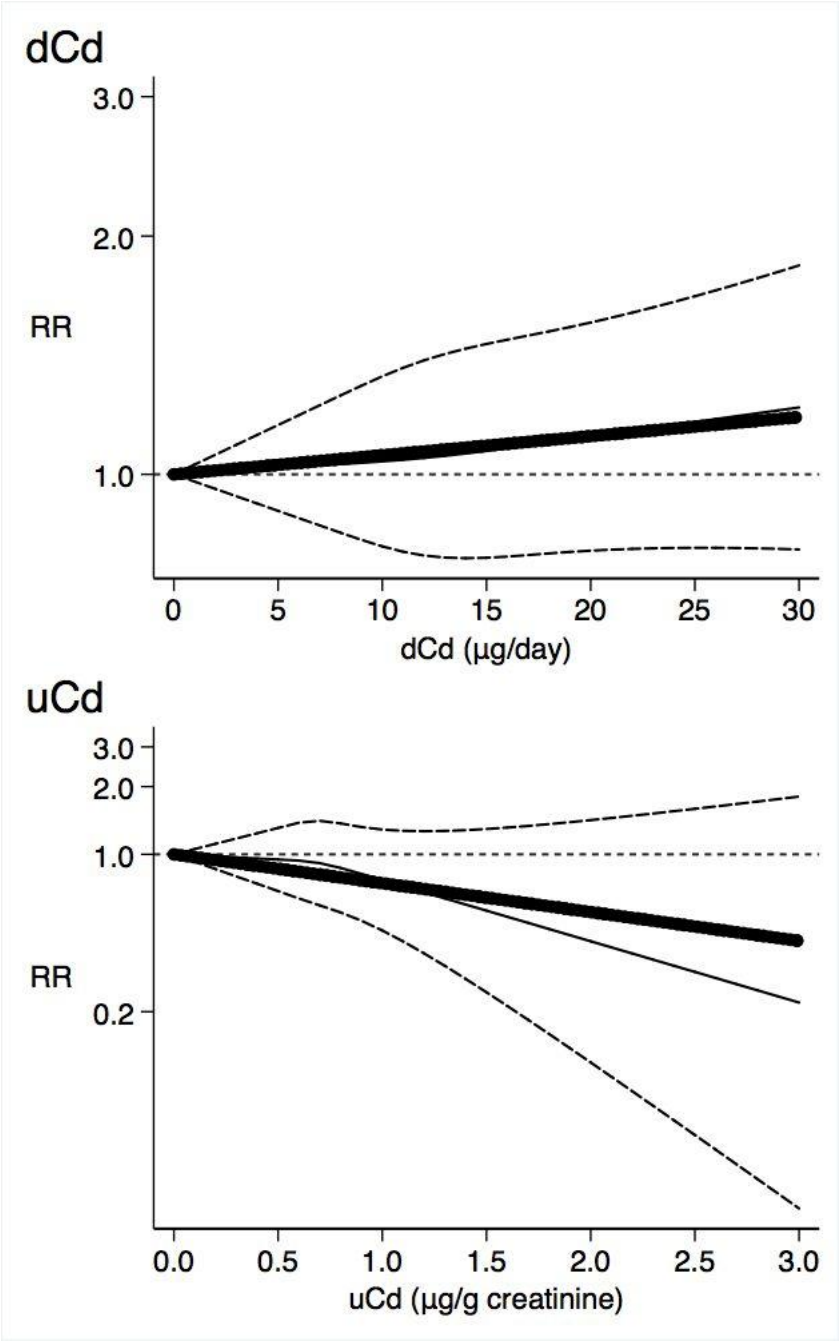

Figure 19: Dose-response meta-analysis of breast cancer risk according to cadmium exposure based on dietary intake (dCd) or urinary levels (uCd) in all post-menopausal and pre-menopausal women. Spline curve (solid line) with 95% confidence limits (long dashed lines). RR: risk ratio

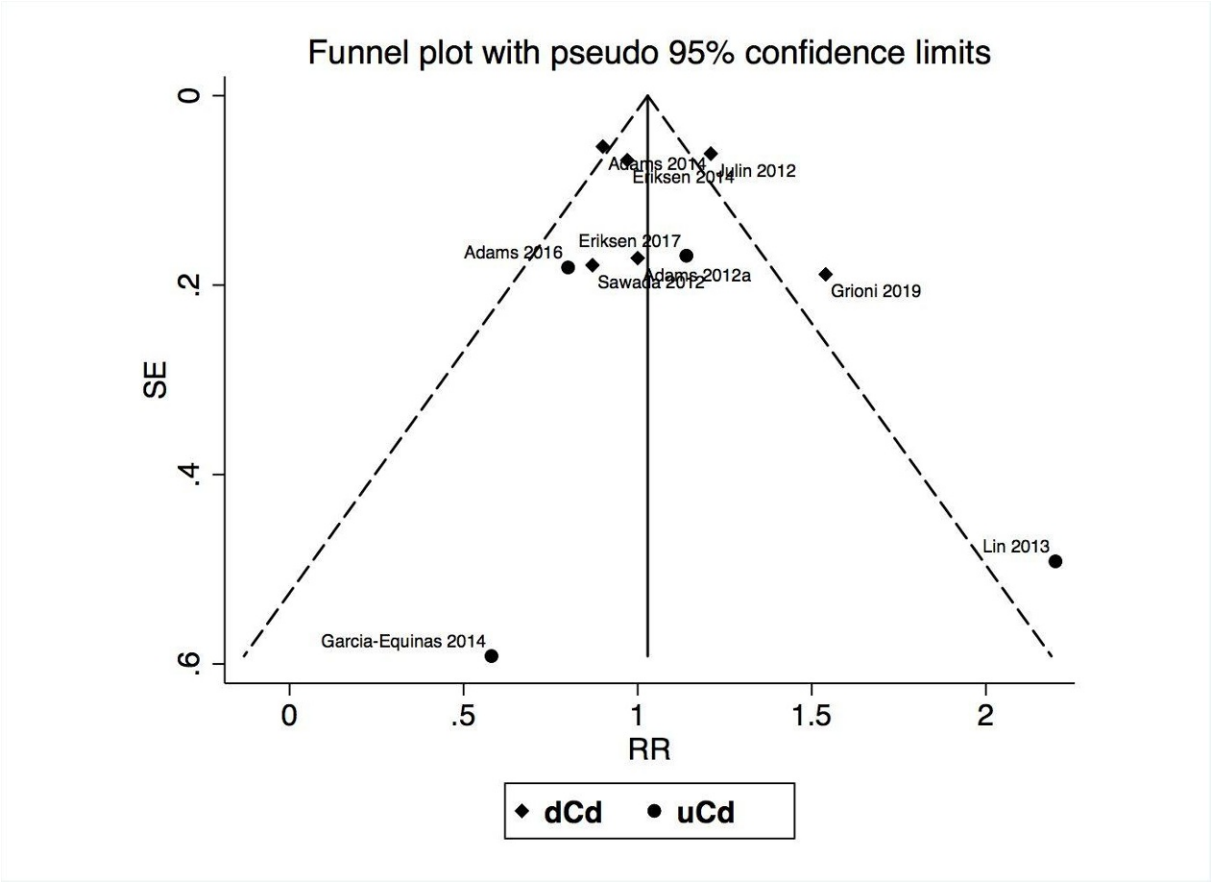

Figure 20: Funnel plots with pseudo 95% confidence limits (dash lines) for publication bias for cadmium exposure assessed using dietary intake (dCd) and urinary levels (UCd) and breast cancer risk. RR, relative risk, SE, standard error.

4. Conclusions

The herewith reported project evaluated the use of the one-stage dose-response meta-analytic, which allows modelling results of epidemiological studies even when involving two comparison groups only, something which was not possible with previously used methods. It therefore provides evidence that the one-stage dose-response meta-analytic approach is feasible for both dietary nutrients and contaminants, when assessing both experimental studies and the non-experimental epidemiological studies, addressing either continuous or dichotomous health-related endpoints. These dose-response models have allowed to carefully shape the relations between potassium and cadmium exposure and two major outcomes, blood pressure levels and breast cancer risk, respectively. Such modelling has in turn allowed to identify selected subgroups which may be more sensitive to the effect of cadmium (which overall appears to have little influence on breast cancer risk), and to characterize the shape of the potassium-blood pressure association which appears to be U-shaped, and thus unsuitable to be characterized through a traditional linear model. In addition, this project has allowed its participants to establish an exceptionally good collaboration through all its phases, such as literature search, risk of bias assessment, data extraction, data analysis, results evaluation and manuscript writing.

Based on the experience developed in this project and its results, we may conclude that the assessment of the impact of a systematic use of dose-response meta-analytic modelling in risk assessment and hazard characterization indicates the great and unique utility of this approach. Dose-response relations have long been considered as primary source of evidence for establishing causality when assessing the relation between any exposure of nutritional or toxicological interest and a health-related endpoint, also according to the Bradford-Hill criteria for causality. However, the limited availability of statistical tools to use the available data and of statistical and epidemiologic resources has long hampered the implementation of such modelling, limiting the assessment to the simple estimation of linear trends in most cases. The extensive and systematic use of the models described in this report and assessed within the project, for both risk assessment, hazard characterization and evidence synthesis, may allow to summarize the evidence in a flexible way, identifying non-linear shapes of the relations such as U-, L- and J-shaped curves, and to spot possible thresholds above or below which there are substantial changes of any association (which may become null or even opposite). We believe that the extension of such approach to any risk assessment, whenever the availability of epidemiologic data may allow this, should be systematically encouraged by agencies, scientific journals and professional societies, given its potential to improve the quality, reliability and strength of the assessment.

5. Recommendations

As project participants, we would recommend the systematic use of dose-response modelling in any risk assessment performed in food safety which may be based, even partially, on epidemiologic studies, instead of being an additional approach limited to specific instances and only in case of a large availability of studies. Such modelling should not be simply considered an extension of a traditional meta-analysis based on linear regression models or used only when conventional approaches yielded indication of an association, but should become instead the primary approach to comprehensively summarize and describe the epidemiologic evidence, entirely replacing models that assume linearity. This would allow to assess the potential changes in the shape of the relation across the entire range of exposure, as well as the possible existence of thresholds. In addition, such analysis should be performed in selected subgroups, to identify source of heterogeneity and specific susceptibilities. Given the recently availability of the one-stage approach, even a low number of studies and those based on two categories of exposure only, as several randomized controlled trials, may now be included in such dose-response modelling and contribute to the overall evidence. Finally, the possible integration of this modelling with the benchmark dose (BMD) approach, identified by EFSA as a scientifically advanced alternative to the

traditional no-observed-adverse-effect level (NOAEL) approach in food safety (EFSA Scientific Committee et al. 2017), appears to be an area definitely worth consideration and potentially fruitful for the risk assessment process.

6. Meetings and Dissemination activities

6.1. Meetings during the project

- Telemeetings: December 20, 2018; January 28, 2019; May 30, 2019
- Interim meeting: February 21, 2019: all partners
- Athens, Greece. March 17-19, 2019: UNIMORE and NKUA.
- Porto, Portugal. May 19-22, 2019: UNIMORE and UPORTO
- Venice, Italy. June 08, 2019: KI and UNIMORE
- Athens, Greece. June 19-21, 2019: UNIMORE and NKUA
- Athens, Greece. October 29-31, 2019: KI and NKUA

6.2. Dissemination activities

- Talks and seminars:
  - University of Modena and Reggio Emilia, Department of Biomedical, Metabolic and Neural Sciences. February 8, 2018. "Dose-response meta-analysis in clinical, nutritional and environmental epidemiology".
  - National and Kapodistrian University of Athens, Greece. Department of Hygiene, Epidemiology and Medical Statistics School of Medicine. October 29, 2019. Athens, Greece. "Connecting the observed data is not a dose-response meta-analysis".
  - The Royal Statistical Society International Conference. September 2-5, 2019, Belfast, Northern Ireland. "On the comparison of alternative models in dose-response meta-analysis using summarized data".
  - Nordic and Baltic Stata Users Group meeting. August 30, 2019, Stockholm, Sweden. "Model selection in dose-response meta-analysis of summarized data". https://www.stata.com/meeting/nordic-and-baltic19/slides/nordic19_orsini.pdf
- Publications:
  - Paper: Filippini T, Torres D, Lopes C, Carvalho C, Moreira P, Naska A, Kasdagli M-I, Malavolti M, Orsini N, Vinceti M. Cadmium exposure and the risk of breast cancer: a dose-response meta-analysis of cohort studies based on assessment of dietary and urinary cadmium. Environment International 2020; 142: 105879 - DOI 10.1016/j.envint.2020.105879
  - Paper: Filippini T, Naska A, Kasdagli M-I, Torres D, Lopes C, Carvalho C, Moreira P, Malavolti M, Orsini N, Vinceti M. Insights into the association of potassium intake with blood pressure: results of a dose-response meta-analysis of randomized controlled trials. Journal of the American Heart Association 2020; 9: e015719 - DOI 10.1161/JAHA.119.015719
  - Poster: Filippini T, Torres D, Lopes C, Carvalho C, Moreira P, Naska A, Kasdagli M-I, Malavolti M, Orsini N, Vinceti M. Insights into the association of potassium intake with blood pressure: results of a dose-response meta-analysis of randomized controlled trials. Poster presentation

at the 13th European Nutrition Conference, FENS 2019, Malnutrition in an Obese World: European Perspectives. Dublin, Ireland. October 15-18, 2019.

- Poster: Κάσδαγλη MI, Filippini T, Lopes C, Carvalho C, Moreira P, Malavolti M, Orsini N, Vinceti M, Νάσκα A. Σχεση δοσησ-αποκρισησ μεταξυ τησ προσληψησ καλιου και των επιπεδων αρτηριακησ πιεσησ. Αποτελεσματα μετα-αναλυσησ τυχαιοποιημενων κλινικων δοκιμων [The dose-response relationship between potassium intake and blood pressure levels. Results of meta-analysis of randomized clinical trials]. Poster presentation at the 8ο Πανελλήνιο Συνέδριο των ομαδων εργασίας [8th National (Panhellenic) Conference on Atherosclerosis, Αθήνα, Greece. November 29, 2019. Journal of Atherosclerosis Prevention and Treatment. 2019;10(Supplement 1):16. ISSN 2654-0843
- Poster: Filippini T, Kasdagli MI, Naska A, Torres D, Lopes C, Carvalho C, Moreira P, Malavolti M, Orsini N, Vinceti M. Cadmium exposure and breast cancer risk: a systematic review and dose-response meta-analysis of cohort studies. Poster presentation at the 2019 ISEE Conference, Utrecht, The Netherlands. August 25-28, 2019
- Poster: Filippini T, Torres D, Lopes C, Carvalho C, Moreira P, Naska A, Kasdagli M-I, Malavolti M, Orsini N, Vinceti M. Cadmium exposure and risk of breast cancer: a dose-response meta-analysis of cohort studies. Poster presentation at the 3rd HBM4EU Consortium Meeting. Berlin, Germany. October 8-9, 2019.

- Related software and useful links:
  - DRMETA: Stata module for dose-response meta-analysis, Statistical Software Components S458546, Boston College Department of Economics. https://ideas.repec.org/c/boc/bocode/s458546.html
  - DOSRESMETA: R Multivariate Dose-Response Meta-Analysis. CRAN. https://cran.r-project.org/web/packages/dosresmeta/index.html
  - R examples https://alecri.github.io/software/dosresmeta.html
  - Interactive Web App: http://alessiocrippa.com/shiny/dosresmeta/

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Abbreviations

Cd	cadmium
CI	confidence interval
DBP	diastolic blood pressure
dCd	dietary cadmium
EFSA	European Food Safety Authority
K	potassium
KI	Karolinska Institutet
NKUA	National and Kapodistrian University of Athens
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RR	Relative risk
SBP	systolic blood pressure
SE	standard error
uCd	urinary cadmium
UNIMORE	University of Modena and Reggio Emilia
UPORTO	University of Porto
WMD	weighted mean differences

Appendix A – Risk of bias assessment for experimental studies

Risk of bias assessment for experimental studies on potassium supplementation and blood pressure levels using RoB 2.0 tool. Responses underlined in green are potential markers for low risk of bias, and responses in red are potential markers for a risk of bias. Where questions relate only to sign posts to other questions, no formatting is used.

Domain 1: Risk of bias arising from the randomization process

Signalling questions	Comments	Response options
1.1 Was the allocation sequence random?	If the study reports a statement that it is randomized, we consider the study as randomized. Any indication of randomization is accepted as true for Y (generally 'older' trials were not used to report details of randomization process as well as allocation concealment.	Y / PY / PN / N / NI
1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?		Y / PY / PN / N / NI
1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?		Y / PY / PN / N / NI
Risk-of-bias judgement		Low / High / Some concerns

Domain 2: Risk of bias due to deviations from the intended interventions (*effect of assignment to intervention*).

Signalling questions	Comments	Response options
2.1. Were participants aware of their assigned intervention during the trial?		Y / PY / PN / N / NI
2.2. Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?		Y / PY / PN / N / NI
2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the experimental context?	According to the guidance, we can answer N or PN in case of either cessation of the intervention because of acute toxicity, or non-adherence to intervention or changes to intervention that are typical of routine case, so unrelated to the experimental context (as for example the expectations of participants that seek the experimental intervention if they feel to have been 'unlucky' (assigned to the comparator). This is an issue in clinical trial with drugs, probably it is not the case for potassium trials	NA / Y / PY / PN / N / NI
2.4. If Y/PY to 2.3: Were these deviations from intended intervention balanced between groups?		NA / Y / PY / PN / N / NI
2.5 If N/PN/NI to 2.4: Were these deviations likely to have affected the outcome?		NA / Y / PY / PN / N / NI

2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	Both intention-to-treat (ITT) analyses and modified intention to treat (mITT) analyses excluding participants with missing outcome data should be considered appropriate. (Missing outcome data are addressed in a separate domain). If not clearly stated, an ITT analysis is implemented, based on the assigned intervention at the start of the trial	Y / PY / PN / N / NI
2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?	Only to answer in case of per-protocol analysis	NA / Y / PY / PN / N / NI
Risk-of-bias judgement		Low / High / Some concerns

Domain 3: Missing outcome data

Signalling questions	Comments	Response options
3.1 Were data for this outcome available for all, or nearly all, participants randomized?	Y (all: 100%) or PY (nearly all: at least 80% of participants)	Y / PY / PN / N / NI
3.2 If N/PN/NI to 3.1: Is there evidence that the result was not biased by missing outcome data?		NA / Y / PY / PN / N
3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	Judgement of PN based mainly on two factors: if reasons of withdrawal are reported and if drop-out rate is similar between intervention and control groups	NA / Y / PY / PN / N / NI
3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?		NA / Y / PY / PN / N / NI
Risk-of-bias judgement		Low / High / Some concerns

Domain 4: Risk of bias in measurement of the outcome

Signalling questions	Comments	Response options
4.1 Was the method of measuring the outcome inappropriate?	At least two measurements to say PN/N The issue of type and modality of BP measure (supine, seated, device, cuff size) will be postponed to stratified analysis, since if such concerns are present, they are non-differential, i.e. the same at the baseline and at the end of the trial, and identical between treated and control group	Y / PY / PN / N / NI
4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	N if there is an explicit statement that was the same between intervention/placebo groups	Y / PY / PN / N / NI

4.3 If N/PN/NI to 4.1 and 4.2: Were outcome assessors aware of the intervention received by study participants?		Y / PY / PN / N / NI
4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	N for sure if an automatic device is implemented. PN if manual but trained personnel or the same person for all participants.	NA / Y / PY / PN / N / NI
4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?		NA / Y / PY / PN / N / NI
Risk-of-bias judgement		Low / High / Some concerns

Domain 5: Risk of bias in selection of the reported result

Signalling questions	Comments	Response options
5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?		Y / PY / PN / N / NI
Is the numerical result being assessed likely to have been selected, on the basis of the results, from…		
5.2. … multiple outcome measurements (e.g. scales, definitions, time points) within the outcome domain?		Y / PY / PN / N / NI
5.3 … multiple analyses of the data?		Y / PY / PN / N / NI
Risk-of-bias judgement		Low / High / Some concerns

Additional domain 6: Risk of bias for cross-over design

Signalling questions	Comments	Response options
6.1 Was a wash-out period of at least 2 weeks present?	Y if present and of at least 2 weeks. NA if parallel design	Y / N / NA
Risk-of-bias judgement	Low if Y or NA, Some Concerns if N. No high risk category in this domain	Low / Some concerns

Overall risk of bias

Risk-of-bias judgement		Low / High / Some concerns

Appendix B – Risk of bias assessment for nonexperimental studies

Risk of bias assessment for nonexperimental studies on cadmium exposure and breast cancer risk using ROBINS-E tool.

Domains	Criteria
Bias due to confounding	Factors considered mandatory in order to judge a study at moderate risk of bias are: age, smoking habits and body mass index Factors considered mandatory to judge a study at low risk of bias are: hormone replacement therapy use, energy intake (only for dietary intake), and creatinine adjustment (only for urine excretion). To be considered at low risk of bias, a study must include age, smoking body mass index and hormone therapy use, and in addition energy intake for studies using dietary cadmium exposure assessment, and creatinine adjusted for urine excretion.
Bias in selecting participants in the study	Selection of eligible participants must not be related to cadmium exposure.
Bias in exposure classification	Possible exposure misclassification for studies not using a biological sample for the assessment or based on an assessment performed after the beginning of the study.
Bias in departure from intended exposure	There should be no concern about departure from intended exposure due to the long term, i.e. that subjects may have changed exposure during follow-up. A threshold of 20 years of mean follow-up have been considered at Moderate risk for possible change in exposure from the beginning of the study to the end of follow-up.
Bias due to missing data	Definition of reasonable cutpoint for missing data. Studies with less than 10% are considered at low risk.
Bias in outcome measurement	Possible bias based on the modality of outcome assessment. High risk in case of assessment based on self-report only without external validation.
Bias in selection of reported results	Evidence that results have not been selected. Clear reporting of statistical methods.
Overall risk of bias	If at least one domain was found at risk of bias, the overall risk was considered high. If more than one domain was found at moderate risk of bias, the overall risk was considered moderate. If all domains were at low risk of bias, the overall risk was considered low.

Appendix C – Illustration of the *drmeta* Stata package

In this Appendix we report the details of *drmeta* Stata package used for illustration of Models and examples reported in the report.

```
* ssc install drmeta , replace

* Example #1. Tables of mean differences
use http://www.stats4life.se/drm/md_drm.dta, clear

* Model 1
drmeta md dose, se(semd) data(n sd) id(id) type(type_md) ml stddev

* Model 2
mkspline doses = dose, nk(3) cubic displayknots
mat knots = r(knots)
drmeta md doses1 doses2, se(semd) data(n sd) id(id) type(type_md) ml stddev

drmeta_graph , matk(knots)  dose(2(.5)10) ref(5) ///
 ytitle("Mean Difference") xtitle("Dose") list  yline(0, lp(dot) lc(black)) xlabel(2(1)10) ylabel(#7) ///
 addplot(-2*(d-5)+.2*(d^2-25))  plotopts(lc(blue) lp(l)) name(fig_md, replace)

* Example #2. Tables of hazard ratios
use http://www.stats4life.se/drm/hr_drm.dta, clear

* Model 1
drmeta b walk   , se(seb) data(n case) type(type)  id(id) ml stddev
lincom walk, eform
* Model 2
mkspline walks = walk, nknots(3) cubic displayknots
mat knots = r(knots)
drmeta b walks1 walks2  , se(seb) data(n case) type(type)  id(id) ml stddev

drmeta_graph ,  matk(knots)   dose(0(.2)4) list ref(2) ///
    addplot(-.5*(d-2)+.5*(d>2)*(d-2)) plotopts(lc(blue) lp(l))  eform  ///
        ytitle("Adjusted Hazard Ratio") xtitle("Walking (hours/week)") ///
        ylabel(1 1.2 1.5 2 3 4, angle(horiz)) name(fig_hr, replace)
```

Appendix D – Illustration of the *dosresmeta* R package

In this Appendix we report the details of *dosresmeta* R package used for illustration of Models and examples reported in the report.

```
rm()
#install.packages("devtools")
#devtools::install_github("alecri/dosresmeta")
pacman::p_load(dosresmeta, rms, tidyverse, haven, ggthemes, Epi, scales, gridExtra, ResourceSelection, epiDisplay)

# Example 1. Tables of mean differences

md_drm <- read_dta("http://www.stats4life.se/drm/md_drm.dta")

# Model 1
m_l <- dosresmeta(formula = md ~ dose, id = id,sd = sd, n = n, covariance = "md", method = "ml", data = md_drm, proc = "1stage")
summary(m_l)

# Model 2
knots <- quantile(md_drm$dose, c(.1, .5, .9), type=2)
m_s <- dosresmeta(formula = md ~ rcs(dose, knots), id = id,sd = sd, n = n, covariance = "md", method = "reml", data = md_drm, proc = "1stage")
summary(m_s)

# Tabulate predicted contrasts
newd <- data.frame(dose = seq(2, 10, 1))
pred_m_s <- round(predict(m_s, newd, expo = FALSE, xref = 5),2)
pred_m_s

# Example #2. Tables of hazard ratios
hr_drm <- read_dta("http://www.stats4life.se/drm/hr_drm.dta")

# Model 1
m_l <- dosresmeta(formula = b ~ walk, id = id, se = seb, cases = case, n = n,
                  method = "ml", type="ir", data = hr_drm, proc = "1stage")
summary(m_l)
```

```
# Model 2
knots <- quantile(hr_drm$walk, c(.1, .5, .9), type=2)
m_s <- dosresmeta(formula = b ~ rcs(walk, knots), id = id, se = seb, cases = case, n = n,
                  method = "ml", type="ir", data = hr_drm, proc = "1stage")
summary(m_s)

# Tabulate predicted contrasts
newd <- data.frame(walk = seq(0, 5, 1))
pred_m_s <- round(predict(m_s, newd, expo = TRUE, xref = 2),2)
pred_m_s
```