Etiology and Pathophysiology

Transgenerational effects of caloric restriction on appetite: a meta-analysis

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Summary
Maternal undernutrition can result in significant alterations to the post-natal offspring phenotype, including body size and behaviour. For example, maternal food restriction has been implicated in offspring hyperphagia, potentially causing increased weight gain and fat accumulation. This could result in obesity and other adverse long-term health effects in offspring. We investigated the link between maternal caloric restriction during gestation and offspring appetite by conducting the first meta-analysis on this topic using experimental data from mammalian laboratory models (i.e. rats and mice). We collected 89 effect sizes from 35 studies, together with relevant moderators. Our analysis revealed weak and statistically non-significant overall effect on offspring’s appetite. However, we found that lower protein content of restricted diets is associated with higher food intake in female offspring. Importantly, we show that a main source of variation among studies arises from whether, and how, food intake was adjusted for body mass. This probably explains many of the contradictory results in the field. Based on our results, we recommend using allometric scaling of food intake to body mass in future studies.

Keywords: Body mass, food intake, maternal diet, systematic review.

Abbreviations: CR, caloric restriction; MCMC, Markow Chain Monte Carlo; DIC, deviance information criteria; HPD, highest posterior density; P : NP, protein : non-protein macronutrient ratio; SE, standard error.

Introduction
In mammals, including humans, development of the foetus is strongly affected by maternal nutrition. Non-optimal maternal diet negatively influences the offspring’s post-natal health, including increasing the risk of obesity, insulin resistance, hypertension and endothelial dysfunction (1–4). Also, maternal nutrition can impact the development of the central nervous system at both the neuroanatomical and neurochemical levels (e.g. (5,6). It is, therefore, not surprising that non-optimal early life nutrition leads to many cognitive and behavioural changes. Such changes encompass learning, memory, activity, social and aggressive behaviour, food preference and appetite (7,8). Changes in offspring’s food intake and energy expenditure can in turn ameliorate or contribute to offspring’s obesity, diabetes and other metabolic disorders. Because of its downstream effects on suites of health problems, developmental programming of appetite via maternal nutrition deserves special attention (9–12). Yet, it remains controversial how important transgenerational nutritional effects are for appetite programming (10). Furthermore, research on rodents suggests that a range of factors can modify the effect of maternal nutrition on offspring phenotype (13).
These factors can relate either to the nutritional manipulation applied to the mothers (e.g. type, timing or duration of manipulation), or to the variables associated with the offspring (e.g. sex or age). To be able to assess the overall evidence for maternal nutritional effects, we therefore need to quantify the heterogeneity in outcomes associated with differences in experimental design, laboratory strain and the type of nutritional insult being used.

The most important factors of maternal nutritional insults include: (i) timing and duration of the nutritional manipulation (e.g. caloric vs. macronutrient restriction) and (ii) macronutrient composition of the diets used. First, timing and duration of dietary restriction can affect the extent of maternal effects on offspring phenotype. This is because the development of the foetal central nervous system has several stages (developmental time windows), during which specific brain regions or cell types are most sensitive to environmental influences (13,14). Second, severe undernutrition is most likely to have pathological effects on the offspring, such as abnormally small body size or underdeveloped brain (6). At the same time, dietary restriction can be imposed as insufficient supply of specific nutrients (most often protein) or as inadequate amount of food (calories) or both. Third, in caloric restriction (CR) experiments, composition of diet used may be critical to the outcome. For example, 50% reduction of the offered amount of low-protein chow will result in less total protein available to animals than if 50% reduction of high-protein diet was applied. There is a general agreement that protein availability is one of the key players during gestation (15).

In a similar manner to the factors related to maternal diets, there are three main offspring-related factors that one should take into account when comparing results of different experiments, namely, sex, age and offspring’s diet. First, sex differences play an important, but often understated, role in regulation of energy homeostasis (16). Behavioural data exists showing sex-specific transgenerational influences on food intake, food utilization efficiency and food preference after maternal undernutrition (17–22). Second, offspring’s age is a biological variable likely to contribute to variability in experimental results. Studies where appetite was assessed over multiple time points indicate that the differences in food intake might be more pronounced in young than in old animals (19,21,22), but the opposite pattern has also been found (18,23). Finally, the composition of the post-natal diet can also influence the degree of effects observed in the offspring. Obesogenic diets fed to the offspring before and during food intake measurements may exacerbate the effects of maternal undernutrition observed in the offspring, resulting in more pronounced hyperphagia and obesity (24,25).

Additionally, there are different ways of scaling offspring’s food intake to the offspring’s body mass. Different scaling methods (or presence/absence of such scaling) can contribute to contradictory evidence reported for the effects of maternal diet on offspring’s appetite (11). Furthermore, simply dividing food intake by body mass (assuming an isometric relationship, with a linear function and 0 intercept) may not be a physiologically realistic way of scaling (26,27). This is because the energy requirements per unit of body mass generally fall with increasing body size (28). As a consequence, using a power function could be a better option when adjusting food intake for body mass. Given that metabolic rate increases approximately with the mass to the power of 0.75 (28,29), food intake should scale to a similar power in order for animals to achieve energy homeostasis (30), at least in adult animals. Yet, we found very few examples of using power scaling of food intake in laboratory animal research (31,32).

To address these questions, we here conduct the first meta-analysis on the effect of maternal total CR on offspring appetite. We focus on the data from the CR experiments for two main reasons. First, CR experiments have an advantage of their relative simplicity in comparison to other types of dietary restriction manipulations (e.g. macro- or micronutrient modifications). For example, whether proportional protein content in a diet is reduced by substituting for carbohydrate, lipid or both, will have different effects on macronutrient balance, which might confound the protein density effect. Furthermore, animals differ in their feeding response to protein dilution, with some overeating non-protein components of the diet to stabilize protein intake (33). A given manipulation in the nutrient balance of a food might thus result in different dietary manipulations for different animals. For these reasons, CR studies seem less likely to be confounded by the details of experimental set-ups. Second, it has been shown that even mild maternal CR significantly impacts offspring’s hypothalamic-pituitary-adrenal axis (34), which suggests that CR could have strong effects on the regulation of energy metabolism, including appetite. We restrict our data to the experiments performed on rodents, where most of the relevant empirical studies have been conducted (see Methods for study selection details).

Our main aim is to quantify the magnitude of the effect of maternal CR on the offspring’s food intake. Then, using meta-regression, we investigate the aforementioned factors associated with maternal dietary manipulation (i.e. degree, timing and duration of CR, and diet macronutrient composition). We also examine the variables associated with the offspring (i.e. offspring’s sex, age and diet). We predict that these moderators may account for equivocal results from different experimental studies. Finally, we assess how scaling of the food intake to the offspring’s body mass using linear or power function changes the results of the meta-analysis.
Methods

Literature search

We followed general guidelines for systematic reviews outlined in PRISMA statement (35). We initially performed an online literature search using SCOPUS database with search terms ‘TITLE-ABS-KEY(“nutri*” OR diet* OR “feed*” OR calori* OR protein* OR food) AND (matern* OR parent* OR “gener*” OR perinatal OR prenatal OR gestat* OR fetal* OR fetus OR pregnan* OR offspring* OR progeny) AND (appetite OR “food intake” OR “phag*” OR consum* OR “prefer*” OR taste) AND NOT (human OR child* OR breast* OR infant OR baby OR patient OR women OR student OR school OR family OR postnatal* OR industry OR *toxic* OR vitamin OR salt OR leptin OR choline OR inflamm* OR phage OR insulin OR blood OR egg* OR “bird*” OR seed* OR soil OR cell OR chromosome OR chip OR energy OR industr* OR host OR habitat OR plant* OR climat* OR gene* OR mutat*)’. This search resulted in 331 hits (May 2012). From these records (and further searches based on these records), we identified 17 key reviews and an initial set of potentially relevant experimental papers. These review and experimental papers were used to perform further backward (papers cited) and forward (papers citing) searches, resulting in approximately additional 2,000 records (after removing duplicates). We also contacted key experts in the area asking for relevant published and unpublished data.

The initial eligibility screen was based on the paper’s title, keywords, abstract and occasional whole-text scan (criteria: species, timing and type of manipulation, type of behavioural outcomes measured). We identified 41 records for the second round of eligibility screening.

The final assessment of studies was based on detailed examination of full-text papers and additional information provided by the authors. We applied the following inclusion criteria: (i) the study used wild-type laboratory mammal strains (non-mutant, not having any known disorder or being selected for specific traits related to nutrition or obesity); (ii) experimental dams were subject to controlled and quantifiable CR manipulation during whole or any part of pregnancy; (iii) an appropriate control group was used; (iv) food intake of the offspring was reported and is convertible to kcal d\(^{-1}\) or to kcal d\(^{-1}\) g\(^{-1}\) body mass; (v) no additional manipulations were performed on dams or offspring (e.g. using drugs, pathogens, stress, toxins), except nutritional treatments (however, offspring can be reared in variable nutritional environments after birth, as long as these conditions are identical between the experimental and the control group); (vi) sufficient information was provided by the authors (e.g. sample sizes, experimental and statistical procedures, relevant descriptive statistics) to quantify effect sizes (if necessary, after requests for more details); and (vii) study design and reported statistics raised no concerns about good scientific practice. Additional exclusion criteria were applied: (i) species other than standard small laboratory mammals (rat, mice, guinea pig, rabbit), (ii) parenteral nutrition or any other than natural feeding of dams or offspring and (iii) studies available only as abstracts or conference proceedings.

Data extraction and coding

Two researchers (ML, SN) participated in data collection and extraction and resolved disagreements by discussion. We collected data on food intake and body mass from statistics reported in the original papers. We used GraphClick (Arizona Software, Los Angeles, CA, USA) to extract information from figures, where needed. In a few cases where mean values were reported without variance estimates, we imputed the missing values by matching the experimental variables and outcomes to the most similar available data. If a study reported outcomes at multiple data points, we pooled these data by calculating mean values over all time points.

From the means, standard deviations and sample sizes of experimental and control offspring groups, we calculated unbiased standardized mean difference – Hedge’s \(d\) (36). Hedge’s \(d\) is an effect size representing difference between the experimental group (offspring of calorically restricted dams) and the control group (offspring of control dams) in terms of standard deviation units. Positive values of the effect size imply hyperphagia (or larger body mass) in the offspring of restricted dams relative to the offspring of control dams. For each effect size, we also collected the following additional information: study’s first author name, publication year and journal, study and experiment ID, species name and strain code, start and duration of maternal CR, restriction level, maternal diet code and macronutrient composition (expressed as protein : non-protein macronutrient ratio [P : NP] by weight), offspring’s sex, offspring’s diet code and macronutrient composition (as above), timing of food intake/body mass measurements and any potentially relevant information, including comments and requests for data.

Statistical analyses

We ran statistical analyses in R v.2.15.3 (37). We performed the analyses independently for the four outcomes (response variables), which were extracted or calculated from the collected data: (i) mean body mass (g) (measured at a time closest to the food intake tests), (ii) mean food intake per day (kcal d\(^{-1}\)) (unadjusted/absolute food intake), (iii) mean food intake per day adjusted for mean body mass.
(kcal d\(^{-1}\) g\(^{-1}\)) (i.e. relative food intake with linear scaling) and (iv) mean food intake per day adjusted for mean body mass using power function (kcal d\(^{-1}\) g\(^{-0.75}\)) (i.e. relative food intake with power scaling).

We also tested our assumption that food intake scales allometrically with body mass to the power of 0.75 in laboratory rodents. For this, we fitted power function to the data on mean body mass and mean food intake from all control and experimental groups. In other words, we empirically estimated the best scaling factor for our data to see if it is close to 0.75. We used the \textit{nls} function in R for fitting non-linear functions.

To analyze collected effect sizes, we utilized Bayesian mixed-effects meta-analysis implemented in \texttt{MCMCglmm} package\cite{58,59}. We used inverse-Wishart priors (V = 1, \(nu = 0.002\)) because the model runs failed to converge with uninformative priors. For each model, we ran three Markov Chain Monte Carlo (MCMC) chains (independent runs of MCMCglmm models) and the chain with the lowest deviance information criteria (DIC) value was selected for data interpretation. Each chain was run for 4,000,000 iterations with the thinning of 4,000 after a 40,000 iterations of burn-in. We checked models for convergence and mixing by examining the Gelman–Rubin statistic among the three chains and we also checked for autocorrelation within chains\cite{60}. From the model run results, we collected information on posterior mode, mean, standard deviation and 95\% highest posterior density (HPD) intervals for meta-analytic model’s intercepts and slopes. In the results, we report posterior means as our point estimates. In a frequentist perspective, these statistics can be considered statistically significant if their 95\% HPD intervals do not include zero\cite{61}.

For each of the four outcomes, we assessed three different models. First, we constructed the null/intercept model (model 1, normal meta-analysis) in order to calculate the overall intercept as a fixed factor. Strain identity, study identity and experiment identity were included as random factors in the null model. We selected strain, rather than species, as the taxonomic variable because it better described sources of variation in the data set (there are only two species, but seven strains present). Second, the strain model (model 2), was similar to model 1, but had strain identity as a fixed effect and was used to explore differences among strains. Since the differences among strains are not among the main questions of this study, we present the results from this model in the Supporting Information. Third, our full model (model 3, meta-regression) was equivalent to model 1 with the following moderators added: maternal CR level, maternal diet P : NP, CR start and duration, offspring’s sex, offspring’s age, offspring’s diet caloric density and P : NP. We also included interactions between offspring’s sex and maternal CR levels, and between offspring’s sex and maternal diet P : NP. The construction of this full model was driven by biological hypotheses outlined in the introduction. All continuous moderators were log-transformed and scaled prior to the analyses.

From the full model results, we extracted separate overall mean values for each sex. Negative mean values indicate that offspring of calorically restricted dams were generally smaller or eating less than offspring of control dams, while keeping all the other moderators constant at their mean values. Positive mean values indicate larger mean body size or hyperphagia in experimental offspring, accordingly. For continuous moderators, negative effect size values can be interpreted as increased likelihood of positive effects (i.e. larger body mass, hyperphagia in experimental offspring) with decreasing levels of continuous moderator (e.g. less calories available to experimental mothers, less protein in maternal diet, shorter restriction duration, earlier onset of restriction). More specifically, regression coefficients of scaled continuous moderators should be treated as the amount of change in effect size when the moderator value changes by one standard deviation, while all other moderators are fixed at their average values\cite{61}. We calculated regression slopes for each sex group, separately for maternal CR levels and for maternal diets macronutrient composition.

We quantified heterogeneity using a modified \(I^2\) statistics, following the same approach as has been detailed elsewhere\cite{62,63,64}. Overall heterogeneity for each model is the percentage of total variance explained by all random factors and residuals (i.e. total variance excluding sampling error variance divided by total variance). \(I^2\) indicates whether the model adequately explains the variance present in the data. \(I^2\) values of the random effects represent percentage contribution of a given random effect to the overall heterogeneity. Values of \(I^2\) around 25\%, 50\% and 75\% can be considered low, moderate and high levels of heterogeneity, respectively\cite{65}.

**Publication bias**

Publication bias arises when statistically non-significant results are less likely to be published than results showing large effects, misleading the conclusions of a meta-analysis. Non-random absence of effect sizes from the data set can also result in a distortion of the data distribution in the so-called funnel plots, where effect sizes are plotted against their precision\cite{66}. Therefore, we explored the possibility of publication bias by visual inspection of funnel plots for the presence of data distribution asymmetry. We also performed Egger’s regression on data points consisting of the residuals and sampling errors from the full models\cite{67}. The intercept of Egger’s regression will be close to zero if there exists little evidence for publication bias in the data.
Subset analysis

In rodents, appetite regulatory centres continue to develop after birth until third post-natal week (47). Therefore, it can be expected that dietary restriction extending into the lactation period can mask the effects of dietary restriction during gestation. In order to reveal such potentially confounding influence of experimental set-up, we reduced our dataset to the studies where maternal CR was applied only in the prenatal period. We repeated our statistical analyses on this data subset. The potential differences in the results between the full dataset and the subset could indicate the effects of post-natal maternal dietary restriction.

Results

Literature search and the final dataset

The results of our search for experimental data are summarized in Supporting Information Fig. S1. We contacted five authors for additional information on four papers (Supporting Information Table S1). One author provided us with additional details on their study. We received no reply from the other contacted authors and we had to exclude their studies from our dataset due to insufficient information. We also excluded two papers that were based on the datasets presented in other publications. Finally, we extracted 89 effect sizes from 35 experimental studies reporting food intake of laboratory mammals (Table 1). The majority (85) of the data points originated from experiments on rats and the remaining four data points were from mouse studies. In total, seven laboratory strains of rodents were used in the included research.

Characteristics of the included data points

Almost all included studies reported some measures of offspring food intake and body mass, allowing us to calculate absolute and relative food intakes as alternative outcomes of each experiment. Forty-three data points were originally reported as absolute food intake, 44 as food intake per unit of body mass and two data points as both. Notably, another four data points were also reported as food intake adjusted for body mass using a power function (Table 1).

Maternal CR levels in included experiments ranged from 5% to 70%, with the most common being reduction by 50% or 70% relative to the control group. At the same time, the maternal diets differed in their macronutrient composition among the experiments. This was evident in the calculated P : NP varying between 0.184 and 0.613 (Fig. 1a). This range represents protein content in the diet of approximately 20–30% by weight. In all cases, except three, litter sizes were standardized to the same pup number (usually eight) for all dams in an experiment. After weaning, in approximately one-third of the cases, the offspring were kept on different diets than their mothers (Fig. 1b). The offspring’s age at the time of food intake tests ranged from 21 to 510 d.

Scaling food intake relative to body mass in control animals

Figure 2 shows that the power function has a better fit to the data than the linear function. The estimated exponent of the power function fitting offspring’s food intake relative to offspring’s body mass was 0.740 (standard error [SE] = 0.005). This estimate thus confirms our expectation that scaling of food intake to body mass to the power of 0.75 is a good approximation for the allometry of the food intake in rodents (Fig. 2, for estimates based on the data split by sex and experimental group, see Supporting Information Fig. S2).

Overall effects of maternal CR

The intercept-only (null) model for body mass showed that offspring of calorically restricted dams generally tend to be smaller than the offspring of control dams (Bayesian mixed-effects meta-analysis: \( \beta_{\text{meta-analytic mean}} = -0.642, 95\% \text{ HPD} = -1.420 \text{ to 0.026} \); Supporting Information Table S3). Also, there was only weak and non-significant overall effect of maternal CR on offspring’s absolute food intake (\( \beta_{\text{meta-analytic mean}} = -0.041, 95\% \text{ HPD} = -0.264 \text{ to 0.150} \); Supporting Information Table S5), food intake per unit of body mass (\( \beta_{\text{meta-analytic mean}} = 0.212, 95\% \text{ HPD} = -0.180 \text{ to 0.601} \); Supporting Information Table S7) and food intake per unit of body mass to the power of 0.75 (\( \beta_{\text{meta-analytic mean}} = 0.170, 95\% \text{ HPD} = -0.019 \text{ to 0.323} \); Supporting Information Table S9).

In the null models for body mass, for absolute food intake and for intake per unit of body mass, we observed high overall heterogeneity, with \( F \) value ranging from 52.1% to 95.2% (Supporting Information Tables S2, S4, S6). Such high heterogeneity called for inclusion of moderators (such as sex and experimental variables) in the models. The \( F \) value of the null model for the food intake adjusted using power function of body mass was dramatically smaller (22.4%; Supporting Information Table S8) than in the other three null models. This result indicates significant decrease of heterogeneity in the data when power function was used to adjust offspring’s food intake for the body mass. We also noted that, in the null models, between 5.0% and 23.9% of the variation in the data was attributed to the strain effects (Supporting Information Tables S2, S4, S6, S8). The results of analyses of differences among strains showed that some strains responded more
strongly to maternal CR than others (see Supplementary Materials for details).

**Moderator effects**

The inclusion of offspring sex and continuous moderators in the full models revealed substantial complexity of the effects of maternal CR on offspring food intake and body mass (Fig. 3). Consistent with the overall effect, both female- and male-specific overall means were not different from zero for body mass, unadjusted food intake and food intake adjusted for body mass using the power function (Supporting Information Tables S3, S5, S9). In contrast, female- and male-specific food intake adjusted using linear function in the experimental offspring was higher than in the control offspring ($\beta_{\text{Overall mean for females}} = 0.583$; $95\%$ HPD = 0.051 to 1.052; $\beta_{\text{Overall mean for males}} = 0.522$, $95\%$ HPD = 0.056 to 0.988; Supporting Information Table S7), indicating that the overall conclusions may depend on the way the appetite was expressed, after exclusion of the potentially confounding mixed-sex group. We note that, when looking at the sex effects, we decided not to focus on the results from the mixed-sex group because this group consisted only of six data points. Moreover, visual inspection of the raw effect plots (Supporting Information Figs S3–S6) suggested that some of the significant results obtained for this group could be driven by a single data point.

**Table 1** List of studies included in our meta-analysis: species and strain name; N, number of effect sizes (control group vs. treatment group value comparisons) extracted from each study; reference information for the study and details of the source of data used to calculate effect sizes (figures and tables in the original publications, raw data from the authors); offspring’s intake originally reported as AI, absolute food intake; RI, relative food intake (AI/body mass); $\text{RI}^\wedge$, relative food intake calculated using power function on body mass

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Reference</th>
<th>Source of data for effect size</th>
<th>Reported offsprings’ food intake</th>
<th>N_{ES}</th>
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<td>Rat</td>
<td>McCollum</td>
<td>Hsueh et al. (1973) (31)</td>
<td>Table 1</td>
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<td>Lee and Chow (1965) (84)</td>
<td>Tables 1 and 2</td>
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<td>Halas et al. (1977) (85)</td>
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<td>AI</td>
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<td>ICR</td>
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<td>Fig. 1, Table 1</td>
<td>AI, RI</td>
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<td>C57BL/6J</td>
<td>Yura et al. (2005) (88)</td>
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<td>AI</td>
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effect was observed in offspring’s allometrically adjusted food intake: female offspring of calorically restricted dams were more likely to exhibit hyperphagia if their mothers were fed low-protein diets during pregnancy ($\beta_{[\text{Dam dietP:N P– slope for females}]} = -0.229$, 95% HPD = −0.445 to −0.002; Supporting Information Table S9). Similar result was obtained for linearly adjusted food intake ($\beta_{[\text{Dam dietP:N P– slope for females}]} = -0.450$, 95% HPD = −0.865 to −0.050; Supporting Information Table S7), but not for body mass and absolute food intake (Supporting Information Tables S3, S5).

We have included several other biologically relevant moderators in our full models to test the effects of differences in the experimental protocols used in the included studies. These moderators were CR level, starting day of maternal CR, duration of maternal CR, offspring age at the time food intake was measured, nutritional value of the offspring’s diet and protein level in the offspring’s diet. Of these moderators, duration of CR seems to influence offspring’s body mass, unadjusted food intake and linearly adjusted food intake. Longer durations of maternal CR contributed to the smaller body mass in the experimental offspring relative to the control offspring ($\beta_{[\text{Dam CR duration}]} = -0.672$, 95% HPD = −1.160 to −0.082; Supporting Information Table S3) and to the lower total food consumption ($\beta_{[\text{Dam CR duration}]} = -0.168$, 95% HPD = −0.363 to −0.008; Supporting Information Table S5). In contrast, longer durations of maternal CR was associated with higher food intake per unit of body mass ($\beta_{[\text{Dam CR duration}]} = 0.324$, 95% HPD = 0.083 to 0.624; Supporting Information Table S7).

![Figure 1](image1.png)  
**Figure 1** Diets of control and experimental dams: (a) protein: non-protein macronutrient ratio (P : NP) vs. caloric restriction level, expressed as percentage reduction of the calories available to the experimental dams relative to the control dams; (b) transgenerational differences in P : NP and caloric density of food fed to dams and their offspring. The arrows connect pairs of compared control and experimental dam groups in panel a, and mother–offspring diet change within experiments in panel b.

![Figure 2](image2.png)  
**Figure 2** Offspring’s food intake plotted against body mass. Each dot stands for average values from one group of animals. Dotted line represents a linear function commonly used for adjusting food intake for body mass. Continuous line shows a power function with an exponential of 0.75 used as an alternative way for the food intake adjustment in our meta-analysis. Dashed line indicates a power function estimated from the data (exponential = 0.740). Only data from rats was used.
CR starting later in pregnancy was more likely to result in reduced total food intake in the experimental offspring \((\beta_{\text{Dam CR start}} = -0.350, 95\% \text{ HPD} = -0.680 \text{ to } -0.061; \text{Supporting Information Table S5})\). Post-natal diet affected the differences in body mass between experimental and control offspring; offspring of calorically restricted dams were more likely to be larger than the control offspring if they were fed calorie-rich diet after weaning \((\beta_{\text{Offspring diet E}} = 0.501, 95\% \text{ HPD} = 0.012 \text{ to } 0.997; \text{Supporting Information Table S3})\).

**Publication bias**

Visual inspection of funnel plots revealed data distribution asymmetry for body mass and food intake per unit of body mass (Fig. 4). The intercepts of Egger’s regressions performed on the model residuals and measurement errors were not significantly different from zero, except for the food intake per unit of body mass \((\beta_{\text{Intercept}} = 1.034, 95\% \text{ HPD} = 0.032 \text{ to } 2.074)\).

**Subset analysis**

We repeated our statistical analyses on the subset of the data, containing only effect sizes from experiments where maternal dietary restriction did not extend into post-natal period. The results of these analyses are generally similar to the results from the full dataset: the null (overall intercept) models showed no overall effect of gestational CR on offspring’s body mass and the three measures of appetite (Supporting Information Tables S10–S17). However, in the full models, we observed several different patterns for the offspring’s body mass data (Supporting Information Fig. S7). The relationships between the maternal CR duration, the caloric value of offspring’s diet, and the magnitude of the effect size for body mass became statistically non-significant after the studies where dams were fed calorie-rich diet after weaning \((\beta_{\text{Offspring diet E}} = 0.501, 95\% \text{ HPD} = 0.012 \text{ to } 0.997; \text{Supporting Information Table S3})\).

The results of these analyses are generally similar to the results from the full dataset: the null (overall intercept) models showed no overall effect of gestational CR on offspring’s body mass and the three measures of appetite (Supporting Information Tables S10–S17). However, in the full models, we observed several different patterns for the offspring’s body mass data (Supporting Information Fig. S7). The relationships between the maternal CR duration, the caloric value of offspring’s diet, and the magnitude of the effect size for body mass became statistically non-significant after the studies where dams were fed calorie-rich diet after weaning \((\beta_{\text{Offspring diet E}} = 0.501, 95\% \text{ HPD} = 0.012 \text{ to } 0.997; \text{Supporting Information Table S3})\).

When the food intake was adjusted for body mass using power function, female offspring of calorically restricted dams were more likely to exhibit hyperphagia if their mothers were fed low-protein diets during pregnancy \((\beta_{\text{Dam diet P:N P– slope for females}} = -0.538, 95\% \text{ HPD} = -1.019 \text{ to } -0.066; \text{Supporting Information Table S15})\), as in the full dataset. Female and male-specific overall food intake per unit of body mass in the experimental offspring was no longer statistically different from the control offspring in the data subset (Supporting Information Table S15). Also, male experimental offspring ate less food than control males, per unit of body mass, after severe maternal CR \(\beta_{\text{Dam diet CR – slope for males}} = -0.726, 95\% \text{ HPD} = -1.372 \text{ to } -0.121; \text{Supporting Information Table S15})\).

In the full model for offspring’s unadjusted (absolute) food intake, we found the same sex-specific effect of maternal protein on appetite \((\beta_{\text{Dam diet P:N P– slope for males}} = -0.267, 95\% \text{ HPD} = -0.481 \text{ to } -0.053; \text{Supporting Information Table S17})\), as in the original results for the full dataset and similarly to the results for linear food intake. In addition, we now noted an effect of maternal CR level on male offspring appetite \(\beta_{\text{Dam diet CR – slope for males}} = -0.293, 95\% \text{ HPD} = -0.588 \text{ to } -0.025; \text{Supporting Information Table S17})\), similar to that observed for linear food intake in the data subset. Data distributions in the funnel plots seemed asymmetrical for body mass and food intake per unit of body mass (Supporting Information Fig. S8). However, the Egger’s tests showed little evidence for publication bias in the subset data.

**Discussion**

In this study, using published rodent experimental data, we have explored three main issues associated with the transgenerational effects of maternal CR on offspring’s appetite: (i) the overall effect and consistencies across studies, (ii) the variables affecting experimental outcomes and (iii) whether the results are affected by different ways of scaling food intake to body mass. We found generally weak and non-significant effects of gestational undernutrition on offspring’s food intake later in life, although the amount of statistical heterogeneity varied among different analyses. We identified two main factors contributing to variation in experimental results, namely, offspring sex and the amount of protein in the maternal diet during pregnancy. Most importantly, we
demonstrated that different ways of adjusting for body mass when reporting food intake do not affect the overall meta-analytic means. However, they have a large influence on the heterogeneity observed in the data, and also the outcomes of meta-regression analyses. This suggests that a major contribution to the apparent inconsistency among studies is due to the method used by researchers to adjust for body mass-dependent food intake, rather than differences in experimental design or in the true effect size. Additionally, the subset analysis indicated that the key results are robust to the exclusion of studies where maternal CR was extended into the lactation period. We discuss our main findings in detail below, starting from the assessment of methods for adjusting food intake to body mass.

Scaling of food intake by body mass

One of the most important findings of our study is that appropriate accounting for body mass differences is of paramount importance when comparing food intake of laboratory animals from the same species and strain. Although discussed at length elsewhere (26,27), this caveat was mentioned by only a handful of review authors (11,48) in our survey. The current practice in research is to report either unadjusted food intake or food intake per unit of body mass, which could lead to contradictory results, even for the same data (48). Using data gleaned from the experimental papers, we were able to show that food intake of rats scales with body mass to the power of approximately 0.75. This fits very well with how metabolism scales with body mass (26,27), and clearly demonstrates that using allometric adjustment for body mass is a more biologically meaningful way for reporting appetite of laboratory rodents than using absolute or linearly adjusted intakes. Accordingly, the results of our meta-regression analyses differed when different scaling methods were used. Notably, both lack of scaling and linear scaling resulted in much higher statistical heterogeneities in the data than when scaling with power function. This result shows that the former two measurements of appetite can produce inconsistent and distorted results. Consequently, we argue that adjustment for body mass using the appropriate power function produces a more accurate picture of the factors influencing offspring appetite. Our conclusions are therefore focused on the results from the allometrically adjusted appetite measures.

Effects of maternal CR on offspring food intake

Our results indicate that maternal CR has little effect on offspring’s appetite in laboratory rodents, which is in agreement with a recent narrative review (11). Such an overall weak effect of pre-birth undernutrition on food intake regulation can be explained from the developmental point of view. In rodents, neuronal connections involved in regulating food intake mature in the early post-natal life (9), which implies that the critical time window for altering long-term feeding behaviour might be located outside gestational period. This is supported by the differences in overall effect size between the full dataset and the subset of data restricted to gestational CR. Moreover, although changes to offspring appetite may not be a general rule, as implied by the lack of overall effect in our data, such changes might still be possible under special circumstances. Specifically, the inclusion of additional biological and experimental variables in our statistical models (i.e. meta-regression models) revealed that the offspring’s sex and the proportional protein content in the maternal diets significantly contribute to the variation in the included data (Fig. 3d). The results suggest that female offspring are likely to exhibit hyperphagia if their mothers were fed low-protein diet during gestational CR. The implications of this finding are twofold. First, it confirms the importance of protein deficiency for foetal development and its long-term
consequences for energy intake and metabolism (15). Second, this finding highlights that sensitivity to maternal protein deprivation is likely to be sex specific, as has been implied for other physiological traits (16). Interestingly, the effects of differences in protein levels among experiments were not the focus of most of the original studies and therefore not standardized. Despite this, the inter-study macronutrient variation in the maternal diets contributed to the magnitude of changes in the offspring’s feeding behaviour, by increasing or decreasing the chances of developing hyperphagia. This suggests that macronutrient limitation is more important than energy intake reduction per se for maternal effects on offspring appetite. This fits well with the observation that exposure to diet-derived molecules during embryonic development can have lasting consequences for offspring food preferences in mammals (49).

It also mirrors recent results implicating macronutrient balance, rather than CR per se, in the effects of moderate food deprivation on longevity (50,51).

### Effects of maternal CR on offspring body mass

The lack of overall effect on body mass matches the observation of Remmers and Delemarre-van de Waal (11) and is not surprising given high heterogeneity in the available data. We were able to attribute much of the variation among the reported outcomes to the following factors: strain differences, duration of maternal CR and offspring diet. Similar factors have been implicated in changes in offspring’s body composition after gestational CR (11). Such similarities may indicate common mechanisms for developmental programming of body mass and body composition. The subset analysis revealed that gestational maternal CR in combination with maternal low-protein diet can be sufficient to produce decrease in offspring body mass. It should be borne in mind, however, that body mass was measured at different time points after weaning, and therefore, we did not assess the effects on offspring’s birth mass, but rather, we evaluated the long-term programming of adult body size. The smaller adult body size of experimental offspring is not due to decreased food intake; in fact, female offspring from mothers with low P:NP are likely to exhibit hyperphagia. These results thus possibly demonstrate an effect of low maternal protein intake on programming of energy metabolism or changes in allocation of nutrients. The latter should be evident as differences in body composition, as suggested by findings from several studies (52,53). In turn, the differences in body composition may influence the food intake of animals. Specifically, the amount of fat tissue affects energetic requirements of mammals (54,55). Unfortunately, body fat data was not available for most studies included in our analyses, so that the effect of adiposity could not be accounted for. Also, changes in metabolic rates, and possibly food requirements, with age (56,57) might have contributed some bias to our results, despite controlling for offspring’s age. Furthermore, this meta-analytic study used data from laboratory rodents and, thus, our conclusions are not necessarily transferable to other mammals that differ in their life-history traits, such as humans. Further quantitative synthesis based on data from other species, including humans, is needed to test the wider generality of our findings.

Unfortunately, extrapolating results from experimental studies to humans is especially challenging because the existing human evidence is mainly based on limited epidemiological data. In such data, foetal undernutrition may result from causes other than direct undernutrition, such as famine, maternal eating disorders or low socioeconomic status. In such cases, CR and nutrient restriction cannot be separated, which does not allow for specific testing of caloric vs. nutrient effects. However, several human-based studies have shown that offspring of undernourished mothers can exhibit altered feeding behaviour and growth patterns (58,59), change in food preference without change in total food intake (60) and increased adiposity in female offspring (61).

We acknowledge that multiple additional confounding factors – including maternal age, dietary macronutrient supplementation, offspring cross-fostering, litter size adjustment, housing conditions, duration of the food intake measurement period, etc. – could not be included in our statistical models due to the limited sample size and missing information in the original papers. Although the overall effect on offspring food intake was weak, different traits, especially physiological parameters, might still be
Hedge's $d$

Precision (1/SE)

Egger's regressed effect sizes

(a) Body mass (g)

(b) Body mass (g)

(c) Absolute intake (kcal d$^{-1}$)

(d) Absolute intake (kcal d$^{-1}$)

(e) Linear relative intake (kcal d$^{-1}$ g$^{-1}$)

(f) Linear relative intake (kcal d$^{-1}$ g$^{-1}$)

(g) Non-linear relative intake (kcal d$^{-1}$ g$^{-0.75}$)

(h) Non-linear relative intake (kcal d$^{-1}$ g$^{-0.75}$)
significantly affected in the offspring (e.g. 1–4). Finally, we note that we found little evidence for publication bias in our dataset and, therefore, our conclusions are likely to be statistically robust.

Conclusions and future directions
To our knowledge, our work represents the first attempt to quantitatively synthesize the available data on effects of maternal undernutrition on offspring’s appetite in animal models. Our meta-analytic results indicate that there is likely to be no or only small transgenerational effects of maternal CR during gestation on offspring’s appetite. However, we have shown that the reduction of the amount of available protein in maternal diets is likely to influence the appetite of female offspring, although such reductions were experimental ‘by-products’ rather than the target of the experiments. This finding suggests that nutrient availability, rather than calories per se, may play a pivotal role in programming of the offspring’s appetite.

Despite being limited to rodent data, our study leads to three important recommendations. First, given the small effect of gestational CR on appetite, shifting our focus to the effects of undernutrition during lactation might be more fruitful. Second, experiments where protein deficiency is imposed on mothers are more likely to cause substantial changes in offspring’s body size and behaviour. Hence, a focus on macro- and micronutrient composition of maternal diet may lead to novel insights into the developmental origins of long-term maternal effects. Third, following other authors, we suggest that care should be taken when comparing results from experimental studies that apply different ways of scaling of the physiological parameters associated with body mass. In particular, we propose examining the data to ascertain the appropriate scaling function, or for laboratory rodents using power function, as a simple and universal way to account for differences in body mass in relation to food intake.

Conflict of interest statement
No conflict of interest was declared.

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Supporting information
Additional Supporting Information may be found in the online version of this article, http://dx.doi.org/10.1111/obr.12138

Figure S1. PRISMA diagram.
Figure S2. Food intake against body mass.
Figure S3. Effects on body mass.
Figure S4. Effects on absolute food intake.
Figure S5. Effects on linear food intake.
Figure S6. Effects on allometric food intake.
Figure S7. Forest plots for subset analysis.
Figure S8. Funnel plots for subset analysis.
Table S1. List of excluded full-text publications, with reasons.
Table S2. Body mass: models summary.
Table S3. Body mass: results summary.
Table S4. Absolute food intake: models summary.
Table S5. Absolute food intake: results summary.
Table S6. Linear food intake: models summary.
Table S7. Linear food intake: results summary.
Table S8. Allometric food intake: models summary.
Table S9. Allometric food intake: results summary.
Table S10. Subset analysis – body mass: models summary.
Table S11. Subset analysis – body mass: results summary.
Table S12. Subset analysis – absolute food intake: models summary.
Table S13. Subset analysis – absolute food intake: results summary.
Table S14. Subset analysis – linear food intake: models summary.
Table S15. Subset analysis – linear food intake: results summary.
Table S16. Subset analysis – allometric food intake: models summary.
Table S17. Subset analysis – allometric food intake: results summary.
Appendix S1. Supplementary methods, analyses and results.
Appendix S2. Supplementary data.

References
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