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REVIEW

Little appetite for obesity: meta-analysis of the effects of maternal obesogenic diets on offspring food intake and body mass in rodents

M Lagisz[1,2,3], H Blair[4], P Kenyon[4], T Uller[5], D Raubenheimer[6,7] and S Nakagawa[1,2,3]

BACKGROUND: There is increasing recognition that maternal effects contribute to variation in individual food intake and metabolism. For example, many experimental studies on model animals have reported the effect of a maternal obesogenic diet during pregnancy on the appetite of offspring. However, the consistency of effects and the causes of variation among studies remain poorly understood.
METHODS: After a systematic search for relevant publications, we selected 53 studies on rats and mice for a meta-analysis. We extracted and analysed data on the differences in food intake and body weight between offspring of dams fed obesogenic diets and dams fed standard diets during gestation. We used meta-regression to study predictors of the strength and direction of the effect sizes.
RESULTS: We found that experimental offspring tended to eat more than control offspring but this difference was small and not statistically significant (0.198, 95% highest posterior density (HPD) = – 0.118–0.627). However, offspring from dams on obesogenic diets were significantly heavier than offspring of control dams (0.591, 95% HPD = 0.052–1.056). Meta-regression analysis revealed no significant influences of tested predictor variables (for example, use of choice vs no-choice maternal diet, offspring sex) on differences in offspring appetite. Dietary manipulations that extended into lactation had the largest effect on body weight. Subgroup analysis revealed that high protein to non-protein ratio of the maternal diet may promote increased body weight in experimental offspring in comparison with control offspring; low protein content in the maternal chow can have opposite effect.
CONCLUSIONS: Exposure to maternal obesogenic diets in early life is not likely to result in a substantial change in offspring appetite. Nevertheless, we found an effect on offspring body weight, consistent with permanent alterations of offspring metabolism in response to maternal diet. Additionally, it appears that protein content of the obesogenic diet and timing of manipulation modulate the effects on offspring body weight in later life.

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INTRODUCTION

The maternal phenotype can alter fetal development and, thus, offspring phenotype.[1,2] The long-lasting influences of early life conditions on an individual's physiology, behaviour and morphology are often referred to as developmental programming. Developmental programming is the core of the concept of developmental origins of health and disease. For example, developmental programming via maternal nutritional stress (underfeeding, overfeeding or nutritional imbalance) during gestation has been implicated as one of the factors potentially contributing to obesity epidemics in humans.[3]

An increasing number of women at reproductive age are overweight.[4] This raises the question of whether a maternal obesogenic diet can predispose offspring for increased weight gain in adult life.[5] In human epidemiological data, the shared post-natal environment between offspring and their mothers makes it difficult to assess the contribution of maternal effects. In contrast, animal models allow the separation of the effects of the maternal diet during gestation from the effects of post-natal environment. Indeed, a recent review of experimental data on laboratory animals, such as rats and mice, concluded that maternal high-fat diet likely promotes weight gain in the next generation.[6] Yet, we still know very little about the mechanisms of the altered body weight regulation in the offspring of mothers fed obesogenic diets.[7,8]

Offspring behavioural alterations could offer one of the simplest mechanisms for maternal effects on offspring body weight. For example, offspring of obese mothers may be less active and/or consume more food than offspring of control mothers. There is limited evidence to support reduced activity levels in offspring of dams subject to obesogenic treatments,[9] but several experimental studies have revealed hyperphagia in offspring of obese dams (reviewed in Rooney and Ozanne[5] and Parlee and MacDougald[10]). However, there are also examples of studies where hyperphagia was not observed (for example, Dunn and Bale,[11] White *et al.*[12] and Zhang *et al.*[13]).

[1]Evolution and Ecology Research Centre, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, New South Wales, Australia ; [2]Division of Diabetes and Metabolism, Garvan Institute of Medical Research, Sydney, New South Wales, Australia; [3]Department of Zoology, University of Otago, Dunedin, New Zealand; [4]Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand; [5]Department of Zoology, University of Oxford, Oxford, UK; [6]Charles Perkins Centre, Faculty of Veterinary Sciences, and School of Biological Sciences, University of Sydney, Sydney, New South Wales, Australia and [7]Institute of Natural Sciences, Massey University, Auckland, New Zealand. Correspondence: Dr M Lagisz, School of Biological, Earth and Environmental Sciences, University of New South Wales, Biological Sciences Building, Sydney, New South Wales 2052, Australia.
E-mail: losialagisz@yahoo.com
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Such mixed experimental results could be partly explained by the different ways in which food intake can be quantified (typically as total food intake per day or as food intake per gram of body weight per day). Different quantification methods could lead to different study conclusions.[14] There are also many other likely sources of heterogeneity among the results, including the diversity of experimental designs (for example, severity and timing of dietary manipulation), and a range of biological factors (for example, species, offspring sex or age).

In studies on laboratory rodents, obesogenic diet treatment is imposed on dams in two main ways: (1) by feeding with obesogenic chow (usually with high fat content and/or energy density; hereafter, no-choice diet) and (2) by allowing dams to choose among various obesogenic food items, such as cookies, pastry and sweetened condensed milk (often termed 'cafeteria diet' or 'junk food diet'; hereafter, choice diet). No-choice (chow) diets have fixed ratios of nutrients and the animals can only regulate how much chow is consumed (food is usually offered *ad libitum*). In contrast, choice diets may allow animals to not only actively adjust energy intake, but also to balance nutrient ratios (depending on compositional range of food stuffs used). Thus, choice diets could potentially allow the animals to stay closer to their nutritional optimum compared with no-choice chow diets.[15] For this reason, the effects of maternal exposure to obesogenic diets on the offspring might differ between experiments using choice and no-choice diets. Surprisingly, such differences have not been quantified.

As indicated above, it is not only the quantity of calories consumed, but also the composition of the food eaten. Food composition is critically important for almost every aspect of an organism's functioning. For example, protein restriction has been shown to be a more influential mediator of life-extending effects than caloric restriction.[16] Similarly, in mice fed *ad libitum*, metabolic health and longevity are affected by protein content of the food.[17] It remains to be tested whether variation in diet macronutrient composition can explain differences in outcomes among studies using different obesogenic diets.[18] Such analyses can be reliably performed only for no-choice (chow) diets, where it is easy to quantify the exact ratios of macronutrients eaten by the animals. This, however, is not the case for choice diets.

Timing of the maternal nutritional manipulation is another important factor reflecting sensitive time windows for the development of parts of the fetal brain that control food intake.[19,20] Timing is also critical for the ability of the dam to supply the foetus with the right levels of energy and nutrients. The dam's requirement for energy does not change much during gestation, but the demand for protein increases as foetuses grow and remains high during lactation.[21,22] It is, therefore, possible that late gestational or lactational protein limitation (or both) resulting from inability to consume more of calorically dense food is responsible for developmental programming of offspring traits. Other factors potentially contributing to variation in experimental outcomes include: timing and duration of maternal nutritional treatment, offspring diet composition, offspring sex and age at measurement. Therefore, whenever possible, these factors should be also taken into account when comparing experimental outcomes of different studies.

In this paper, we conduct a systematic review and meta-analysis of the vast experimental data available on rodent models of nutritional developmental programming of offspring's appetite and body weight. Our study has four main aims: (1) to quantify the overall effect of maternal obesogenic diet on developmental programming of offspring's food intake and body weight; (2) to compare the effects of chow (no-choice) vs choice (junk food) maternal obesogenic diets; (3) to investigate the effects of macronutrient composition, particularly the ratio between protein and non-protein macronutrients in the obesogenic no-choice diets and (4) to assess the influence of additional experimental and biological factors, namely, timing of maternal dietary manipulation, offspring sex and diet characteristics.

MATERIALS AND METHODS

Literature search and study selection

To locate relevant experimental data for meta-analysis, we performed systematic literature searches following guidelines outlined in PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement.[23] We started from an online literature search using SCOPUS and ISI Web of Science databases for articles published up to March 2014 (search terms and parameters are listed in Supplementary Information). Additional records were identified via reviews, forward (papers citing) and backward (papers cited) searches, author-based searches and directly contacting leading researchers in the field.

Retrieved titles and abstracts were screened to identify experimental studies on wild-type laboratory rodents, where dams were subjected to an obesogenic dietary treatment around gestation time and offspring phenotypes were measured after birth. Next, for the resultant records, we screened full-text articles using the following inclusion criteria: (1) the study was performed on wild-type laboratory strains (non-mutant, healthy and unselected for body weight or metabolic parameters); (2) experimental dams were fed obesogenic diet before and/or during whole or any part of pregnancy and a control group was available where dams were fed standard/non-obesogenic diet, all other experimental conditions being equal (comparisons of groups fed diets differing only in the source of macronutrients, for example, fat, were excluded); (3) food intake of the offspring was reported in a form convertible to kcal/day or to kcal/day/g body mass, and the body mass of the offspring around the time when food intake was measured; (4) offspring were fed a no-choice (chow) diet during food intake measurements (if food preference was affected by the treatment, opportunity to choose between different food items would influence total food intake and confound the results); (5) dams and offspring were only subjected to nutritional treatments (no surgery, drugs, pathogens, and so on used); (6) the authors provided descriptions of experimental and statistical procedures, relevant descriptive statistics or raw data, allowing us to quantify effect sizes and moderator values (requests for more data or details were made where necessary), and the study/data raised no quality concerns. We allowed for inclusion of studies published in languages other than English and for unpublished studies.

Data extraction and coding

Two researchers (ML and SN) extracted data from the included papers and, if needed, resolved disagreements by discussion. From the original papers, or the raw data provided by the authors, we extracted mean, variance and sample sizes of food intake and body mass measurements for offspring of experimental dams and for offspring of control dams. When data were available in the form of figures only, we used GraphClick (Arizona Software, Switzerland) to extract information from the figures. In studies with multiple experimental and control groups, we only extracted data for the pairs of experimental and control groups that matched our inclusion criteria.

Body weights were collected alongside the food intake data to control for potential differences in body sizes between the offspring from the two groups (birth weights were not available in majority of the studies and thus were not collected). We applied allometric scaling of food consumption to body mass for each group of animals within included experiments, by dividing mean total daily food intake by the mean body mass raised to the power of 0.75, following Lagisz *et al.*[14]

We used Hedges' *g*, that is, unbiased standardized mean difference between two groups, as a measure of effect size.[24] Positive values of the effect size imply hyperphagia in the offspring of experimental dams relative to the offspring of control dams. Additionally, from the same data, we calculated effect sizes for the offspring body mass measures, to quantify effects of maternal treatments on offspring body size. Positive values imply higher mass for offspring from dams fed an obesogenic diet.

From the papers, we also collected information on the study's first author name, publication year and journal, species (mouse/rat) and strain of the animals used, start and duration of maternal nutritional manipulation, maternal diet type (no-choice/choice), code and macronutrient composition (expressed as protein to non-protein ratio by calories), offspring sex, offspring's diet code and macronutrient composition (as above), timing of food intake/body mass measurements and any other potentially relevant information (full list of variables available in Supplementary Information).

Statistical analyses

All analyses were run in R v. 3.1.1 statistical language and environment,[25] using the same procedures for the data on offspring food intake and body mass. The effect sizes were calculated using *compute.es* package[26] and analysed in the Bayesian mixed-effect models framework implemented in *MCMCglmm* package.[27,28] For each model, we ran three MCMC chains (independent runs of MCMCglmm models) for 1 100 000 iterations with the thinning of 1000 after 100 000 iterations of burn-in, resulting in 1000 samples from the chain. We used inverse-Gamma priors ($V = 1$, $nu = 0.002$), because the model runs failed to converge with uninformative priors ($V = 1$, $nu = 0$). We checked model chains for convergence and mixing by examining the Gelman-Rubin statistic among the three chains and we also checked for autocorrelation within chains.[29] From the chain with the lowest DIC value, we extracted posterior mode, mean, standard deviation and 95% highest posterior density (HPD) intervals for estimating model intercepts and slopes. Posterior means, which we used as our point estimates, can be considered statistically significant if their 95% HPD intervals do not include zero.

Strain identity, study identity and effect size identity were included as random factors in the models run (except for the strain model, where strain identity was set as a fixed factor). Because there are only two species (rat and mouse), but eight strains (three rat and five mice) present in our data set, we used strain, rather than species, as the taxonomic variable. Four models were assessed for the full data set (Supplementary Table S2). First, we ran an intercept-only model (null model), to estimate the overall intercept as a fixed factor. In the second model (strain model), we estimated intercepts for each strain, to explore differences among the strains (we do not report results from this model in the main text, because we did not have any specific hypothesis for the pattern of differences among strains). In the third model (full model), we used the following moderators as fixed factors in the model: offspring's sex, dam diet type (no-choice/choice), dam diet start day, dam diet end day, offspring's diet caloric density, offspring's diet protein to non-protein ratio and offspring's age. The fourth model (alternative full model) differed from the third model by replacing start and end days of dam's nutritional manipulation with a single categorical variable coding whether nutritional manipulation extended into lactation. Although all the models are multilevel models, the first model can be seen as meta-analysis (addressing the overall effect), and the second to fourth models as meta-regression models (cf. Nakagawa and Santos[30]). Models three and four address the influences of dam diet presentation (no-choice/choice), dam diet timing and offspring-related predictors (moderators). All continuous moderators were *z*-transformed before the analyses, so they had a mean of 0 and a standard deviation of 1. For continuous moderators, positive estimates from the full models can be interpreted as increased likelihood of hyperphagia (or larger body mass) in experimental offspring with increasing levels of the continuous moderator, while all other moderators are fixed at their average values.[16]

We quantified overall heterogeneity (that is, total variance excluding sampling error variance divided by total variance) for the intercept-only (null) models using modified I^2 statistics.[30] Values of I^2 around 25, 50 and 75% are considered as low, moderate and high levels of heterogeneity, respectively.[31] High I^2 values suggest that most of the variability across studies is due to heterogeneity rather than chance and warrant investigation of the potential sources of heterogeneity.

Within some studies, the control group was shared among multiple treatment groups. We statistically controlled for such inter-dependence among effect sizes, by calculating variance and covariance values adjusted for the presence of a shared control group using equations 19.18 and 19.19 from Gleser and Olkin.[32] We included this variance-covariance matrix in the statistical models using the *MCMCglmm* function.

Subset analysis

As above, we aimed to investigate how the properties of the obesogenic diets fed to the dams may affect the food intake and body mass of the offspring. Thus, we created a data subset including only the experiments where chow-based diets were fed to the dams (no-choice data subset), where we had reliable information on diet caloric density and macronutrient ratios. For this data subset, we ran analyses analogous to these performed on the full data set. In the models with moderators, we replaced moderators representing offspring diet characteristics (used for the full data set) with caloric density and protein to non-protein ratio of dams' obsogenic no-choice diets.

Publication bias

Publication bias can potentially influence the results of meta-analysis, if results with small and statistically non-significant results are less likely to be published than results showing large and statistically significant effects. We tested for publication bias in our data by visually inspecting funnel plots (effect sizes plotted against their precision) for the presence of data distribution asymmetry. Additionally, we ran Egger's regression on data points consisting of the residuals and sampling errors from the full models. If there is publication bias in the data, the intercept of Egger's regression will be significantly different from zero.[33] The total number of statistical tests performed in our study was 102, although the majority of these tests were not independent.

RESULTS

Our literature search is summarised in the PRISMA diagram presented in Figure 1. We sent requests for data or experimental details to 15 authors about 19 studies. We received raw data for three studies and additional details for another two studies (Table 1). Finally, from 53 studies we extracted 116 effect sizes (data points, i.e. experimental – control group comparisons), which met all our inclusion criteria. The excluded studies with the reasons for their exclusion are listed in Supplementary Table S1.

The final data set comprises 89 effect sizes from rat studies and 27 from mouse studies, representing 8 laboratory rodent strains in total. The data were based on 2468 unique individuals for food intake measurements, and on 2671 unique individuals for body weight measurements. Dam nutritional manipulation extended into lactation for 93 effect sizes/data points; in the remaining 23 cases obesogenic diet was ceased at birth. For 97 data points, litter

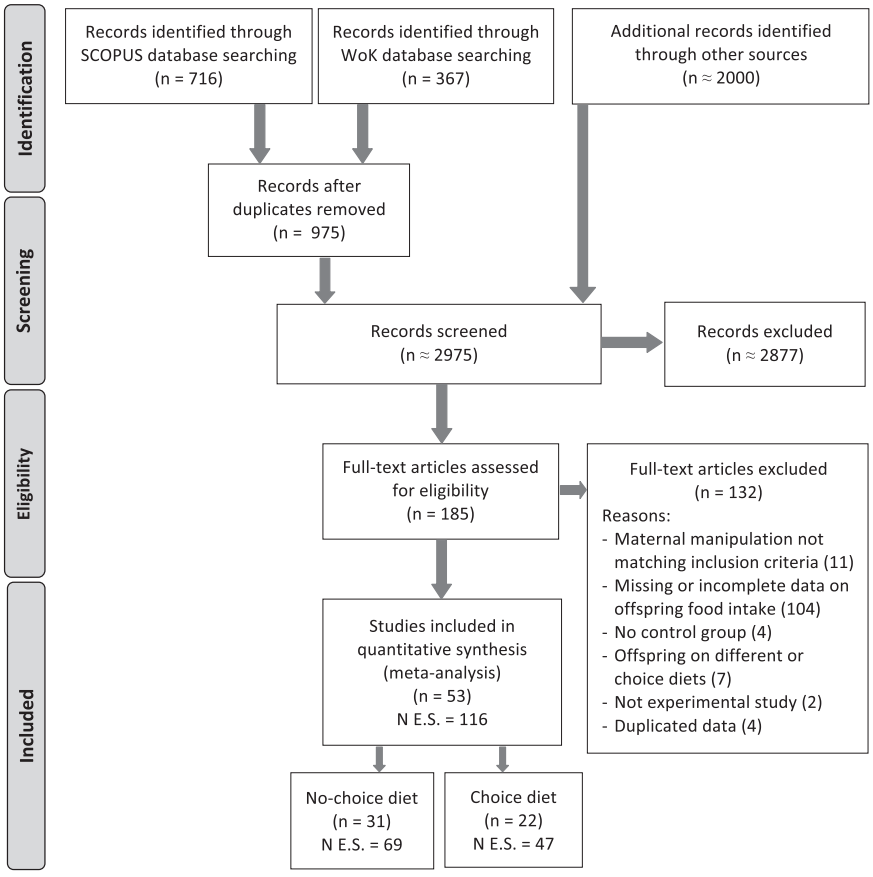

Figure 1. PPRISMA flow diagram: literature search and study selection process.

sizes were standardised to the same pup number (usually 8); in the remaining 19 cases information was either not available or litters were not standardised. Age of the offspring during food intake measurements was 125.4 ± 62.4 (mean ± s.d., median = 114.5) days post conception, indicating that most of the measurements were taken on adult individuals. The majority of the collected data was for male offspring (69 data points); a few data points were reported for mixed-sex groups.

Chow-based no-choice obesogenic diets were fed to the experimental dams in 31 studies providing 69 effect sizes. For these data points we could determine composition of the experimental diets and compare them with control group diets and to diets fed to the offspring. As expected, obesogenic diets usually contained more fat (approx. 40% more energy from fat) and less carbohydrates than control diets (Supplementary Figure S1A). This resulted in high caloric density of experimental diets. Protein to non-protein macronutrient ratio was also reduced in many cases (Supplementary Figure S1B). The majority of offspring were reared on the same or similar diet to the one fed to control dams (Supplementary Data File).

The effect sizes for food intake allometrically scaled to body mass were not correlated with effect sizes for body mass (Spearman's $r = -0.036$, $t = -0.390$, df = 114, $P = 0.698$). In contrast, when food intake was not scaled to body mass, differences between experimental and control offspring in total food intake were linked to differences in body weights (Spearman's $r = 0.595$, $t = 7.913$, df = 114, $P < 0.001$), indicating that adjusting for differences in body weight between groups is necessary.

Effects on offspring appetite and body mass

Our main meta-analysis (the intercept-only/null model) revealed a small and statistically non-significant difference in allometrically adjusted food intake between offspring of dams fed obesogenic diets and offspring of control dams (Bayesian mixed-effects meta-analysis: $\beta_{[\text{meta-analytic mean}]} = 0.198$, 95% HPD = −0.118–0.627; Figure 2; Supplementary Table S3). In the null model, we observed low to moderate overall heterogeneity, with mean I^2 value of 41.6% (Supplementary Table S2), suggesting that a large proportion of the variability across studies (*ca.* 60%) was due to sampling error rather than heterogeneity.

The intercept-only model for body mass showed that offspring of experimental dams generally are heavier than offspring of control dams ($\beta_{[\text{meta-analytic mean}]} = 0.591$, 95% HPD = 0.052–1.056; Figure 2; Supplementary Table S3). In the null model for body mass, we observed high overall heterogeneity, with I^2 value of 88.1% (Supplementary Table S2).

Additionally, we examined how alternative representations of food intake data could influence the results of meta-analysis. We ran the intercept-only model on the food intake data not scaled to body mass and on the food intake data scaled linearly to body mass. The average daily total food intake (unscaled) was higher in experimental offspring than in control offspring ($\beta_{[\text{meta-analytic mean}]} = 0.495$, 95% HPD = 0.161–0.833), reflecting higher body mass of experimental offspring. Also, the overall heterogeneity was higher, with mean I^2 value of 76.5%. When average daily total food intake was linearly scaled to body mass, the difference in food intake per gram of body mass was small and statistically non-significant ($\beta_{[\text{meta-analytic mean}]} = 0.160$, 95% HPD = −0.265–0.629), as for the allometrically scaled data. However, the overall heterogeneity was higher, with mean I^2 value of 67.1%, implying that although the overall conclusion was similar, the different scaling method resulted in more variation in effect sizes among studies. Taken together, this shows that not accounting for differences in body mass between groups of animals can lead to

Table 1. List of studies included in our meta-analysis

Nr	*Species*	*Strain*	*Dam diet type*	*NES*	*Reference*	*Data source*
1	Mouse	C57BL/6	No-choice	2	Dunn and Bale[11]	SFig1, Fig2
2	Mouse	C57BL/6	No-choice	3	Gregorio *et al.*[43]	Tab2, text p762
3	Mouse	C57BL/6 J	No-choice	2	King *et al.*[44]	text p2517, Fig2
4	Mouse	C57BL/6 J	No-choice	1	Magliano *et al.*[45]	Tab4
5	Mouse	C57BL/6 J	Choice	1	Oben *et al.*[46]	Fig3ab
6	Mouse	C57BL/6 J	Choice	2	Ornellas *et al.*[47]	Tab3, Fig3
7	Mouse	C57BL/6 J	Choice	2	Samuelsson *et al.*[48]	Fig2
8	Mouse	C57BL/6 J	Choice	2	Samuelsson *et al.*[49]	Fig2B
9	Mouse	C57BL/6 J	No-choice	2	Tuohetimulati *et al.*[50]	text p220, Fig1b
10	Mouse	FVB	No-choice	2	Turdi *et al.*[51]	Tab2
11	Mouse	ICR	No-choice	4	Masuyama *et al.*[52]	Fig2ab
12	Mouse	ICR	No-choice	2	Platt *et al.*[53]	raw_data
13	Mouse	NMRI	No-choice	2	Dahlhoff *et al.*[54]	Tab2, Tab3
14	Rat	Long-Evans	No-choice	2	Kozak *et al.*[55]	Fig2
15	Rat	Long-Evans	No-choice	2	Kozak *et al.*[56]	text p2890
16	Rat	Long-Evans	No-choice	2	White *et al.*[12]	Fig5B,A
17	Rat	Sprague-Dawley	Choice	1	Bahari *et al.*[57]	Fig2
18	Rat	Sprague-Dawley	Choice	1	Caruso *et al.*[58]	Tab2
19	Rat	Sprague-Dawley	No-choice	2	Chang *et al.*[59]	Fig1
20	Rat	Sprague-Dawley	Choice	4	Chen *et al.*[60]	Tab3
21	Rat	Sprague-Dawley	Choice	1	Chen and Morris[61]	text p1357
22	Rat	Sprague-Dawley	Choice	1	Chen *et al.*[62]	Tab1
23	Rat	Sprague-Dawley	No-choice	1	Chen *et al.*[63]	Tab1
24	Rat	Sprague-Dawley	No-choice	4	Desai *et al.*[64]	Fig5, Fig4
25	Rat	Sprague-Dawley	Choice	1	Flynn *et al.*[65]	Tab1, Fig1
26	Rat	Sprague-Dawley	No-choice	1	Jackson *et al.*[66]	Tab2, Fig1a
27	Rat	Sprague-Dawley	No-choice	2	Khan *et al.*[67]	Fig1c
28	Rat	Sprague-Dawley	No-choice	2	Khan *et al.*[68]	Fig4
29	Rat	Sprague-Dawley	No-choice	4	Khan *et al.*[69]	Fig2
30	Rat	Sprague-Dawley	No-choice	2	Kirk *et al.*[70]	Fig2c
31	Rat	Sprague-Dawley	No-choice	2	Page *et al.*[71]	Tab2
32	Rat	Sprague-Dawley	Choice	2	Rajia *et al.*[72]	Tab3
33	Rat	Sprague-Dawley	Choice	2	Rajia[73]	Fig5.5b, Fig5.1
34	Rat	Sprague-Dawley	Choice	1	Rajia *et al.*[74]	Tab1
35	Rat	Sprague-Dawley	No-choice	1	Sun *et al.*[75]	Fig2ab
36	Rat	Sprague-Dawley	No-choice	1	Walker *et al.*[76]	Fig4a
37	Rat	Sprague-Dawley	No-choice	3	Wu *et al.*[77]	Tab4
38	Rat	Sprague-Dawley	No-choice	1	Zhang *et al.*[78]	SFig1
39	Rat	Sprague-Dawley	Choice	4	Zhang *et al.*[13]	raw_data
40	Rat	Sprague-Dawley	Choice	2	Zhang *et al.*[79]	raw_data
41	Rat	Wistar	Choice	10	Akyol *et al.*[80]	Fig5, Fig3
42	Rat	Wistar	Choice	2	Bayol *et al.*[81]	raw data
43	Rat	Wistar	No-choice	2	Beltrand *et al.*[82]	Fig5, Fig4
44	Rat	Wistar	Choice	1	Bouanane *et al.*[83]	Tab2
45	Rat	Wistar	No-choice	6	Couvreur *et al.*[84]	Tab5
46	Rat	Wistar	No-choice	4	Ferezou-Viala *et al.*[85]	Tab2
47	Rat	Wistar	Choice	2	Gugusheff *et al.*[86]	text p351, Fig2
48	Rat	Wistar	Choice	3	Jacobs *et al.*[87]	Tab3
49	Rat	Wistar	No-choice	2	Mitra *et al.*[88]	Fig4,6
50	Rat	Wistar	Choice	1	Mucellini *et al.*[89]	Fig2,3
51	Rat	Wistar	No-choice	2	Nivoit *et al.*[90]	Fig3c
52	Rat	Wistar	No-choice	1	Oliveira *et al.*[91]	Fig1
53	Rat	Wistar	Choice	1	Shalev *et al.*[92]	text p497, Tab1

Species and strain name; dam diet type—whether choice or no-choice obesogenic diet was used for experimental dam group; NES—number of effect sizes (control group vs treatment group value comparisons) extracted from each study; reference information for the original study; data source—data used to calculate effect sizes (figures and tables in the original publications, raw data from the authors).

spurious conclusions and that allometric scaling ameliorates variation in food intake resulting from differences in body mass between groups.

Moderator analysis

Meta-regression (full) models for allometrically scaled offspring food intake did not reveal any factors significantly contributing to the heterogeneity of the data (Figure 2). In contrast, the effects on body mass appeared to be influenced by the timing of dams' nutritional manipulation. Experimental offspring were more likely to be heavier than control offspring when the dams were fed obesogenic diet not only during gestation but also during lactation (as continuous predictor $\beta_{[\text{Dam diet end time}]} = 0.315$, 95% HPD = 0.148–0.485; Figure 2; as categorical predictor $\beta_{[\text{Dam diet during lactation}]} = 0.448$, 95% HPD = 0.075–0.840; Supplementary Table S3; Figure 3). In none of the full models did we observe effects of maternal diet type (no-choice/choice), offspring diet characteristics (caloric density, protein ratio) and offspring sex or age.

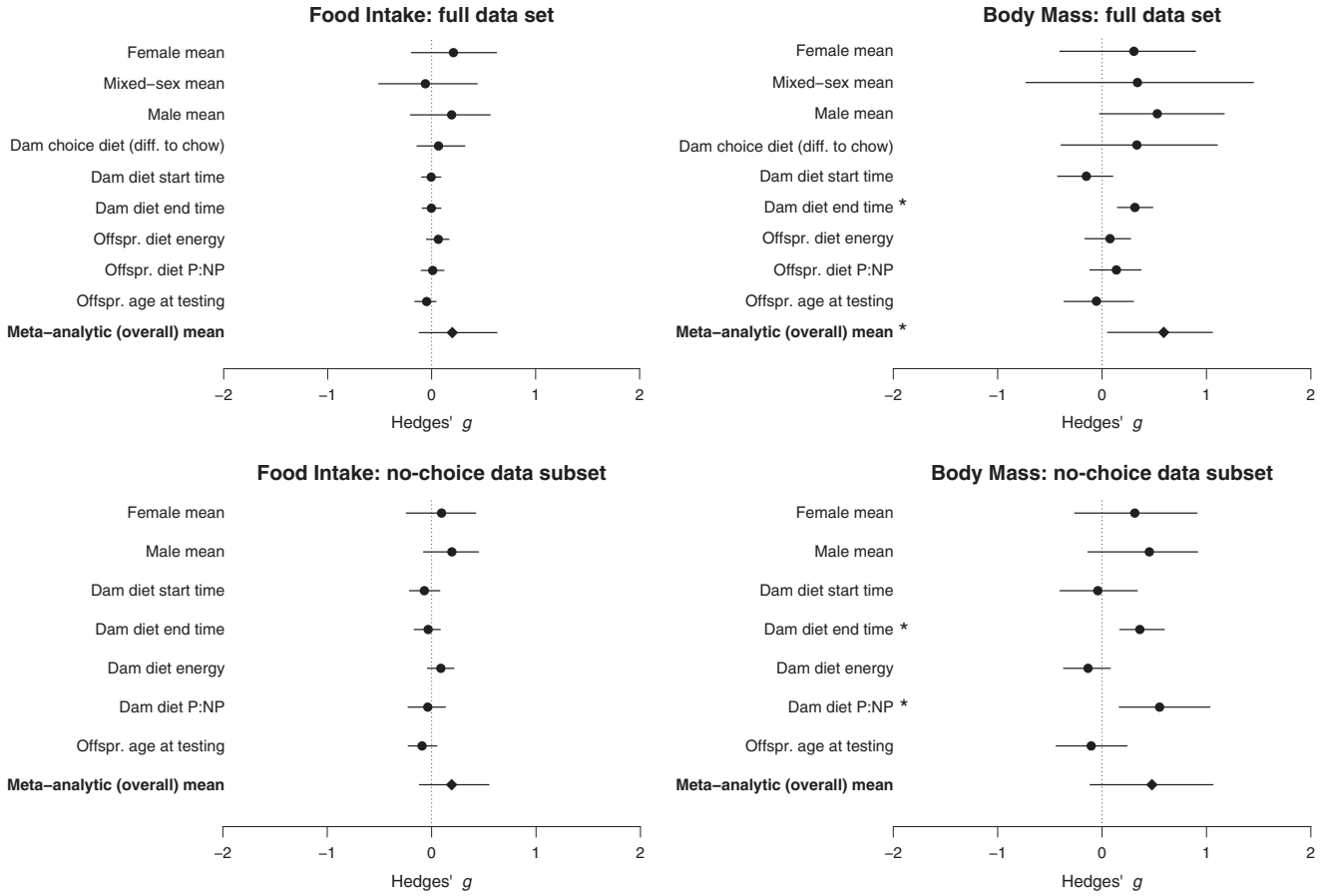

Figure 2. Forest plots of effect size (Hedges' *g*) estimates from the full models for offspring food intake and body mass. For the intercepts, positive effect sizes indicate that offspring of dams fed obesogenic diets were overall eating more or were bigger than offspring of dams fed control diets (while keeping all the other moderators at mean value). The effects are considered statistically significant when their 95% HPD intervals (horizontal error bars in the plot) do not cross zero (marked with stars next to the effect labels). For continuous moderators, positive values indicate positive relationship between moderator value and effect size (for example, larger body size of experimental offspring with increasing protein content in experimental diet). Included moderators for the full data set: offspring sex, dam offered no-choice/choice diet, dam diet: start and end day, offspring diet total energy (caloric density) and protein to non-protein ratio, offspring mean age when outcome measurements were taken. Included moderators for the no-choice diet data subset: offspring sex, dam diet: start and end day, dam diet total energy (caloric density) and protein to non-protein ratio, offspring mean age when outcome measurements were taken. Continuous moderators were scaled, and thus the intercepts lay at the average value of each continuous variable.

Subset analysis

We repeated our statistical analyses on the no-choice subset of the data, containing only effect sizes from experiments where dams were fed obesogenic chow diet. In the subset analysis, we obtained results similar to those from the full data set. There were small and statistically non-significant differences in offspring food intake ($\beta_{[\text{meta-analytic mean}]} = 0.192$, 95% HPD = −0.116–0.548; Figure 2; Supplementary Table S3). The overall effect on offspring body mass was moderate and in the same direction as in the full data set, but became statistically non-significant in the data subset ($\beta_{[\text{meta-analytic mean}]} = 0.478$, 95% HPD = −0.114–1.061; Figure 2; Supplementary Table S3). However, the effect of the timing of nutritional manipulation on the changes in offspring weight remained significant (fitted as continuous predictor $\beta_{[\text{Dam diet end time}]} = 0.362$, 95% HPD = 0.171–0.595; Figure 2; as categorical predictor $\beta_{[\text{Dam diet during lactation}]} = 0.656$, 95% HPD = 0.055–1.258; Supplementary Table S3). Additionally, we found that when experimental dams were given chow with less than average protein to non-protein ratio, their offspring were smaller than control offspring. However, when obesogenic chow contained protein at levels greater than the average, then experimental offspring were heavier than control ones ($\beta_{[\text{meta-analytic mean}]} = 0.551$, 95% HPD = 0.165–1.032; Supplementary Table S3; Figure 4).

Publication bias

Visual inspection of funnel plots revealed no data distribution asymmetry for food intake effect sizes (Supplementary Figure S2). Accordingly, the intercepts of Egger's regressions performed on the model residuals including measurement errors were not significantly different from zero (full data set: $\beta_{[\text{Intercept}]} = 0.256$, 95% HPD = −0.032–0.538; no-choice subset: $\beta_{[\text{Intercept}]} = -0.244$, 95% HPD = −0.694–0.179; Supplementary Figure S3). In contrast, distributions of effect sizes for body mass data in the funnel plots appeared asymmetrical (Supplementary Figure S2). Accordingly, Egger's tests showed evidence for publication bias in the body mass data (full data set: $\beta_{[\text{Intercept}]} = 3.178$, 95% HPD = 2.183–4.216; no-choice subset: $\beta_{[\text{Intercept}]} = 3.498$, 95% HPD = 2.058–4.942; Supplementary Figure S3).

DISCUSSION

The meta-analysis of the experimental data on developmental programming of offspring's appetite in laboratory rodents indicated that a maternal obesogenic nutritional treatment during gestation usually has only a small effect on offspring's appetite. However, there appears to be a substantial positive effect on

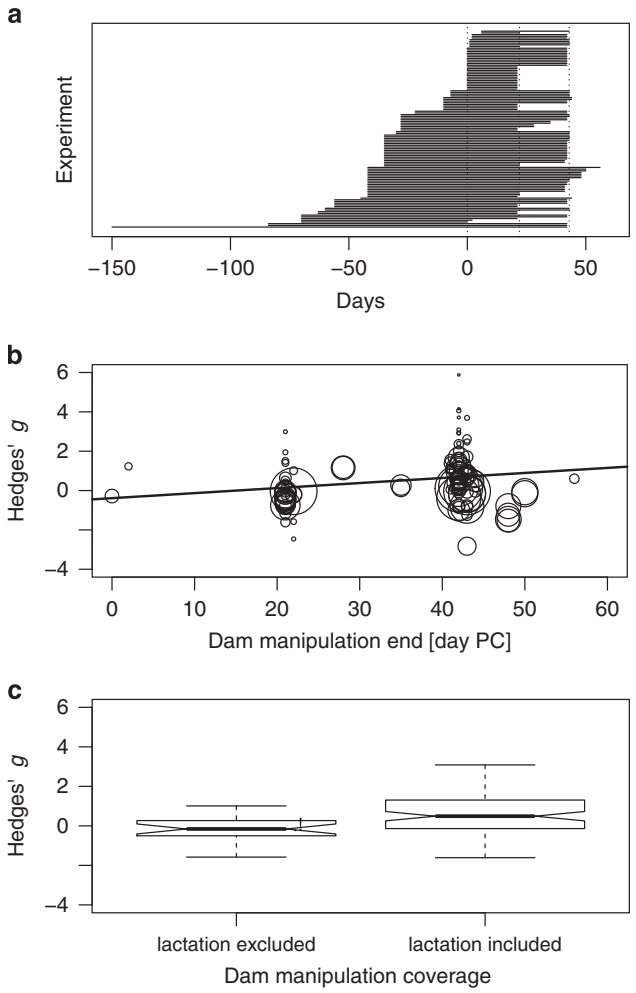

Figure 3. Plots of the timings of maternal dietary manipulations in the full data set and the effects of ending time of maternal treatment on difference in body weights between offspring of experimental and control dams (Hedges' *g*). Distribution of timings of experimental dam manipulations: vertical dashed lines indicate conception, birth and end and weaning of the offspring (**a**); relationship between end day of dam treatment and effect size: regression line fitted to the raw data is shown (**b**); and effect sizes split by whether dam treatment extended into lactation period: the horizontal bar inside the box represents the median, notches represent confidence bounds for the median, edges of the box represent the lower and upper quartiles, and whiskers represent the range of the data (**c**).

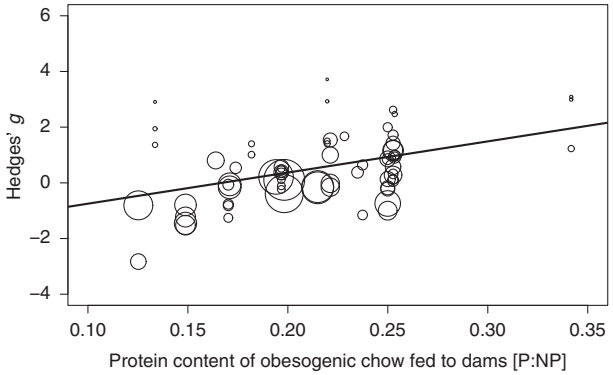

Figure 4. Effect sizes (Hedges' *g*) representing differences in offspring body weight between offspring of experimental and control dams plotted against protein to non-protein macronutrient ratio of experimental dam diets. Data come from the no-choice dam diet subset, i.e., studies where obesogenic diet was given in the form of chow food, so that diet macronutrient composition was constant and quantifiable. The size of bubbles represents relative precision of each effect size estimate, i.e., the smallest bubbles are least precise and the largest ones are most precise and influence the results of analyses most. Negative effect sizes indicate that offspring of overfed dams were smaller than offspring of control dams, and vice versa for positive effect sizes.

offspring weight in adult life. This effect seems to not be linked with the effect on appetite.

Our results suggest that offspring appetite is not highly modified by a maternal obesogenic diet. This finding is in contrast with opinions expressed in many narrative reviews (for example, Rooney and Ozanne[5] and Parlee and MacDougald[10]). It is possible that the programming effect on appetite could be larger in early life and that it may get reversed if offspring are fed non-obesogenic diet in later stages of development and growth (as is the case in most studies included in our analyses). However, in our analyses offspring age and diet characteristics did not explain much variation in the data, nor did the other moderators. Furthermore, we found no signs of publication bias in the food intake data set. Therefore, our estimate of the meta-analytic mean for the effect on appetite, for the included experiments, is likely to be robust. Moreover, we observed little heterogeneity in the data after food intake was allometrically scaled to body mass, and we noted larger heterogeneity if food intake was not scaled or scaled linearly. This suggests that contradictory conclusions of separate experimental studies often stem from the fact that researchers do not account for the allometric relationship between food intake and body mass, given differences in body size between experimental and control groups, and report either unscaled food intake or linearly-scaled food intake.[14]

Our additional aim was to confirm and quantify the effect of maternal diet on offspring body weight after weaning. We also tested the influence of several predictor variables (moderators) on collected effect sizes. Most moderators did not appear to influence differences in offspring body mass. Particularly, we expected that non-choice diet would be more detrimental to the offspring than choice diet, because allowing diet selection might have allowed animals to better balance their macronutrient and energy intake when they could choose among food items of different composition (Raubenheimer and Simpson,[15] but see Lefcheck *et al.*[34]). The absence of a differential effect between choice and no-choice diets in our data set may be attributed to the obesogenic food items typically being high in energy and low in protein (as well as being high in other potentially detrimental ingredients, for example, salt), making it difficult for animals to balance their macronutrient intake. Also, we did not observe an effect of offspring diet composition, which could be due to the majority of our data coming from studies where offspring were fed non-obesogenic/standard chow.

Experimental offspring were, nonetheless, heavier than control offspring. Given that the overall effect on body weight is larger than the overall effect on appetite, and that effect sizes coming from the same experiments are not correlated, differences in food intake are unlikely to be the sole explanation of differences in body weight. Taken together, this implies that changes at the metabolic, rather than the behavioural level, are largely responsible for increased body weight of offspring from obese mothers. It remains to be quantified how the change in adult body mass relates to birthweight, adiposity and health of the offspring, which suggests future meta-analyses are warranted.

Interestingly, the caloric density of the maternal obesogenic diet did not significantly affect offspring weight. Instead, protein content of the chow used is likely to be a key player. Maternal protein availability during gestation has been implicated in

determining offspring phenotype[1,2,35] and hence it has been proposed that the rise in obesity and metabolic disorders is linked to the relatively low protein content of modern diets.[36] Notably, the experimental evidence comes mainly from studies where dietary protein availability was directly manipulated between control and experimental groups within a study. In such studies, proteins are usually replaced with carbohydrates, while fat content (and caloric density) is kept constant. In contrast, in research on the effects of obesogenic maternal diets, experimental diets typically have high fat content and also reduced protein to non-protein macronutrient ratio, in comparison with control diets. Our results indicate that differences across studies in protein levels available to the dams could potentially explain some of the contradictory experimental results. According to the Protein-Leverage Hypothesis, protein intake is prioritised over fat and carbohydrate intake in many animals, including humans,[37] rats[15] and mice.[38] Therefore, when restricted to diets low in protein, dams tend to ingest excessive energy and become obese. Nevertheless, when fed energy-dense low-protein diets, dams may not be able to meet their protein intake target and subsequently their offspring develop under protein-restricted conditions. Maternal protein restriction experiments provide no evidence for programming of increased offspring body weight.[39] This observation is in line with our finding that decreased offspring body weight was more likely when maternal obesogenic diet contained low ratios of protein. Overall, we speculate that maternal protein limitation via exposure to obesogenic chow diet modifies the extent of the developmental programming effects in the offspring, at least for body weight.

Our study also provides review-generated evidence (following Cooper[40]) for the importance of the timing of diet manipulation. Exposure to a maternal obesogenic diet that extended into the suckling period was more influential for programming of the offspring's adult body mass than was exposure during gestation only. This result is consistent with conclusions from cross-fostering experiments and highlights the importance of the lactation period for developmental programming of health in rodents.[10,18,41,42]

Finally, we showed high heterogeneity in the existing experimental data on offspring adult body mass. This heterogeneity can be partly attributable to some of the moderators included in our study. Unaccounted heterogeneity, rather than publication bias, could contribute to the evidence for funnel asymmetry in the data set, and warrants further investigation of the factors influencing offspring body mass in later life. We do not expect publication bias *sensu stricto* to exist in this data set, because measuring offspring body mass was seldom an aim or focus of the included articles.

Conclusions and future directions

Our meta-analysis revealed that the increased body weight of experimental offspring is not associated with increased food intake when it is scaled allometrically to the body mass. Therefore, future work should focus at the alterations at the physiological level. In rodents, lactation might be a critical period for programming offspring body mass in later life. Our findings also highlight the importance of protein deficiency for fetal development and its long-term consequences, even when protein restriction was not directly imposed on the dams. Therefore, optimising macronutrient balance in the maternal diet might be more important than reducing the calories for ameliorating developmental programming of increased offspring body weight. In conclusion, although there may be a minor effect of maternal diet on offspring appetite, this appears to be overstated in the current literature, at least for laboratory rodents.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed to conceiving and designing the work, collecting and analysing the data, interpreting the results and writing the manuscript. All authors approved the final version of the manuscript.

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