Transgenerational effects of obesogenic diets in rodents: a meta-analysis

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ABBREVIATIONS:

F0 –parental generation, i.e., first generation exposed to an obesogenic diet (and an appropriate non-exposed control group)

F1 – first generation offspring, i.e., descendants of F0 animals first exposed to an obesogenic diet (and an appropriate non-exposed control group)

F2 –second generation of descendants of F0 animals first exposed to an obesogenic diet (and an appropriate non-exposed control group)

F3 –third generation of descendants of F0 animals first exposed to an obesogenic diet (and an appropriate non-exposed control group)

lnRR – log-transformed response ratio, i.e., an effect size expressing the ratio of trait means between control and treatment grand-offspring groups

lnCVR – log-transformed coefficient of variation ratio, i.e., an effect size expressing the ratio of trait variabilities between control and treatment grand-offspring groups, controlling for a potential mean-variance relationship

PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analyses

CI – Confidence Interval

PI – Prediction Interval

Abstract

Obesity is a major health condition that affects millions worldwide. There is an increased interest in understanding the adverse outcomes associated with obesogenic diets. A multitude of studies have investigated the transgenerational impacts of maternal and parental obesogenic diets on subsequent generations of offspring, but results have largely been mixed. We conducted a systematic review and meta-analysis on rodent studies to elucidate how obesogenic diets impact the mean and variance of grand-offspring traits. Our study focused on transgenerational effects (i.e., F2 and F3 generations) in one-off and multigenerational exposure studies. From 33 included articles, we obtained 407 effect sizes representing pairwise comparisons of control and treatment grand-offspring groups pertaining to measures of body weight, adiposity, glucose, insulin, leptin, and triglycerides. We found evidence that male and female grand-offspring descended from grandparents exposed to an obesogenic diet displayed phenotypes consistent with metabolic syndrome, especially in cases where the obesogenic diet was continued across generations. Further, we found stronger evidence for the effects of grand-maternal than grand-paternal exposure on grand-offspring traits. A high-fat diet in one-off exposure studies did not seem to impact phenotypic variation, whereas in multigenerational exposure studies it reduced variation in several traits.

Introduction

Obesity – the excess accumulation of adipose tissue – is a major health condition that leads to impaired physical and psychosocial health and well-being [1,2]. Obesity affects millions of people worldwide, and alarmingly, the World Health Organization estimates that global rates of obesity have tripled since 1975 [3] and continue to rise. Increased food supply and its higher caloric content are major driving factors of this epidemic [4]. An increasing number of animal studies incorporate obesogenic diets to evaluate adverse outcomes of obesity and explore potential remedies [5].

Obesity has a strong genetic component, but an individual's lifestyle and environment also play a large role in the development of obesity and associated metabolic disorders [6,7]. However, early life nutrition is thought to be one the most critical factors [8]. In mammals, an individual's phenotype can be influenced by the in-utero environment, determined by maternal condition and nutrition – a form of "developmental programming" [9]. Developmental programming describes the phenomenon whereby conditions present during early development render an individual susceptible to metabolic disease later in life [10]. For example, there is accumulating evidence that maternal obesity during pregnancy has adverse effects on offspring health [11], including the increased likelihood of obesity [12], impaired leptin signalling [13], hypertension [14], hyperglycemia [15], reduced insulin tolerance [16], and type 2 diabetes [17]. While the effects of maternal nutrition are well-recognised, and supported by extensive empirical research, there is also emerging evidence for strong effects of paternal nutrition [18–22]. Further, both maternal and paternal effects can be carried beyond the F1 (offspring) generation and this may involve epigenetic mechanisms, which work to alter the phenotype through changes in gene expression rather than eliciting changes to the DNA sequence itself [23–27]. Epigenetic effects transferred across multiple generations, from the

87 parents (F0) to grand-offspring (F2 and beyond), are termed “transgenerational effects” [28]
88 (for “truly transgenerational effects”, see [29–31]).

89 There are two main manipulations used in empirical studies of transgenerational
90 effects: one-off exposure and multigenerational exposure (Figure 1a). One-off exposure
91 involves exposing only the F0 generation to the experimental treatment (i.e., obesogenic
92 diet). All generations that follow are devoid of any dietary manipulation (i.e., they are kept on
93 a control diet). In contrast, multigenerational exposure involves exposing not only the F0
94 animals but also the generations that follow. Such study designs allow researchers to
95 disentangle mechanisms associated with indirect (i.e., epigenetic) and direct (i.e.,
96 developmental programming) influences of obesogenic diets. Specifically, one-off exposure
97 experiments expose subtle transgenerational epigenetic changes, while multigenerational
98 exposure experiments show cumulative effects of continuous exposure across generations so
99 that epigenetic influences can interact with direct effects of obesogenic diets on development.
100 When testing the effects of high-fat diets across generations, one must use an appropriate
101 animal model. Laboratory rodents, such as mice and rats, have been used for over three
102 decades to study various aspects of metabolic syndrome [32] because the experimental
103 conditions can be manipulated with ease to observe treatment effects across generations
104 within a relatively short period [33]. Many studies use rodents maintained on a high-fat diet to
105 determine health effects [34]. The composition of high-fat diets used can vary widely among
106 studies [35]. Reported direct effects on offspring include increased body weight; hyperphagia;
107 adiposity; insulin and leptin resistance [13,36–38]; impaired glucose tolerance [39,40]; hypertension
108 [41]; and raised plasma lipids [42]. Further, there is evidence that these effects can impact
109 offspring in a sex-specific manner [43–45].

110 Despite overwhelming evidence suggesting detrimental impacts associated with
111 obesogenic diets, there is very little work synthesizing studies on transgenerational effects.

112 Such syntheses are critical for understanding the effects of obesogenic diets across
113 generations. Systematic reviews and meta-analyses are standard tools for summarizing
114 empirical studies in many fields. Typically, meta-analytic studies on nutrition and
115 development almost exclusively focus on comparing means between experimental and
116 control groups [46]. Consequently, inter-subject variability is rarely explored. However,
117 phenotypic variability in obesity has been shown to have an inherited component [47,48]. Thus,
118 understanding the effects of a given treatment on the variability of an outcome is just as
119 important as understanding its effects on the mean, as variation in biological systems forms
120 the basis of ecological and evolutionary processes (i.e., natural selection). As such, the
121 importance of trait variability has been increasingly argued for in ecology, evolutionary
122 biology [46,49], and medical sciences [50,51]. Recently, a robust method was developed for the
123 analysis of variance in meta-analytic models [52]. Using this method, as well as an established
124 framework for analyses of the effects on the mean trait values, we conducted a systematic
125 review and meta-analysis of rodent studies (rats and mice strains) in one-off and
126 multigenerational dietary exposure experiments.

127 Our study aims to address four main questions: (1) What are the overall magnitudes of
128 effects of a one-off grandparental (i.e., F0) and a multigenerational exposure to obesogenic
129 diets on grand-offspring (i.e., F2 and F3)? (2) How do the effects of an obesogenic diet on
130 grand-offspring compare between grand-maternal and grand-paternal exposure? (3) Do the
131 effects of obesogenic diets impact female and male grand-offspring differently? and (4) What
132 traits are most strongly impacted in grand-offspring because of grand-parental obesogenic
133 diet exposure? For each question, we examine the effects on grand-offspring trait mean and
134 variability, comparing control (normal food conditions) and treatment (obesogenic diets)
135 groups.

Methods

We followed The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [53] in reporting our systematic review and meta-analysis. We provide full details of searches and screening in Supporting Information. Before searches, we registered a protocol of all our methods on the Open Science Framework (https://osf.io/sg6wj/register/565fb3678c5e4a66b5582f67). We present amendments to this protocol, with justifications, in the Supporting Information.

Literature search

We performed a comprehensive systematic review of the academic literature using four online databases (Scopus, Medline and the core collection in ISI Web of Knowledge, Embase) and other sources (Google Scholar, snowballing). Our initial search took place in April 2018. We also explored grey literature across three online platforms: Trove, OpenGrey, and ProQuest. We performed an update of our searches in May 2020, following the same procedures.

Screening and study selection

We screened titles and abstracts of downloaded bibliometric records using Rayyan QCRI [54]. We aimed to identify experimental studies on wild-type laboratory rodents (excluding strains that were genetically modified or selected for metabolism-related traits), where founders (F0) were exposed to an obesogenic dietary treatment and grand-offspring (F2, F3) phenotypes were reported. Two researchers (HA and AA, and then HA and ML for the search update) independently screened all records to locate potentially relevant studies. We then used the following criteria to screen full-texts of studies that passed the first stage of screening: (1) study had to be empirical work using laboratory rodent models (rats, mice); (2) the rodents used must be from wild-type strains (not mutant, knockdown, or selected for metabolism-

160 related traits, e.g., lean or obese strains); (3) F0-generation rodents of any sex must have been
161 exposed to an obesogenic diet (e.g., high-fat, high-energy, Western diet) alongside a control
162 (not-exposed) group; (4) animals were bred to produce subsequent generations up to a
163 minimum of F2 generation (grand-offspring), and these subsequent generations were kept on
164 a control diet (one-off exposure), or were kept on the diet matching that of their parents
165 (multigenerational exposure); (5) study reported morphological or physiological traits
166 associated with obesity or metabolic syndrome (body weight, adiposity, blood glucose,
167 insulin, leptin and triglyceride levels) for F2 and / or subsequent generations; (6) study
168 presented relevant data and statistics (mean, standard error / standard deviation, sample sizes)
169 allowing calculation of effect sizes (we attempted contacting authors for missing data and
170 information from recent studies where possible).

171 *Data extraction and coding*

172 Our initial extraction and coding captured paper-level information, such as author, title,
173 publication year, and place (see Supporting Information for the full list of variables and
174 descriptions). We then coded experiment-level information (some studies included more than
175 one experiment or multiple exposure lines), such as rodent species, strain, exposure type
176 (one-off or multigenerational), the composition of obesogenic diets, timing of exposure of F0
177 generation to obesogenic diet, and sex of exposed animals in the F0 generation. We also
178 coded grand-offspring generation (as F2 or F3), grand-offspring sex, diet, and age at
179 measurement. We collected quantitative data for the traits of interest in the grand-offspring:
180 body weight, adiposity, blood glucose (fasting glucose and glucose tolerance), leptin, and
181 triglyceride levels. For each measurement, we extracted the mean, standard deviation (or
182 standard error, as reported), and sample size for the treatment group (offspring of
183 grandparents exposed to obesogenic diets) and appropriate control group (Figure 1a). We
184 used the *R* package *metaDigitise* v.1.0.0 [55] to retrieve quantitative data from figures.

We categorized glucose data into measurements of fasting blood glucose (representing baseline glucose levels), and glucose tolerance tests (representing the organism's response to a sudden increase of glucose in the blood), both used as indicators of diabetes. Tolerance test data were usually presented as tolerance test curves displaying glucose levels over a test period (usually 2 hours), with initial measurements taken as a baseline on fasted animals before glucose injection. We amalgamated tolerance test curve data into a single AUC (i.e., total area under the curve) estimate for each group of animals; we also used initial measurement from each curve as a fasting glucose estimate. Insulin data extractions and processing followed the same procedure, with measurements categorized as plasma / serum fasting insulin or insulin tolerance tests.

We categorised adiposity data according to the body location of adipose tissue (e.g., visceral fat, total fat). Methods to quantify adiposity mainly involved weighing dissected fat pads (10 studies), with some studies employing x-rays (2 studies) and high-resolution imaging (1 study). Values presented as a proportion of body weight were recalculated into grams using associated body weight data (see Supporting Information). For triglyceride and leptin data, we noted the type of blood extraction (plasma / serum). Additionally, we collected body weight data for F0 founders (where reported) to compare grandparents' body mass around the end of the obesogenic diet treatment to the body mass of their grand-offspring at a similar age. Further details of data extraction and calculations are described in Supporting Information.

Calculating effect sizes

We used the ln-transformed response ratio (*lnRR*) – commonly used in meta-analyses in the biological and medical sciences [56,57]. It expresses the ratio of trait means between control and treatment grand-offspring groups. To compare variances between control and treatment grand-offspring groups, we used ln-transformed coefficient of variation ratio (*lnCVR*) to

control for a potential mean-variance relationship [52,58]. We calculated these effect sizes and their associated measurement error variances (s^2) following equations (5), (6), (11), and (12) in Nakagawa et al. [52]. Positive values of estimated effect sizes can be interpreted as trait mean value or variability being greater in the grand-offspring of parents exposed to obesogenic diets, relative to the grand-offspring of parents not exposed to obesogenic diets.

Meta-analysis and meta-regression models

We ran analyses in RStudio v.1.2.1335 [59] using R v.3.6.0 [60]. Statistical models were run using the R package *metafor* v.2.0 [61]. We ran multilevel meta-analytic and meta-regression models, which are extensions of standard random-effects models [62]. Our dataset had cases where the different sets of treatment groups were compared against the same group of control animals [63]. To account for this non-independence, we calculated a variance-covariance matrix (equations (19.18) and (19.19) in Olkin and Glesser [64]) and used it as a variance component in meta-analytic and meta-regression models. In all meta-analytic and meta-regression models, we used Paper ID, Rodent Strain ID, Effect Size ID, and Trait as our random factors (except the models where Trait was used as a fixed factor). We calculated overall heterogeneity for meta-analytic models using the multilevel versions of the I^2 statistic [62]. We conducted all analyses for both *lnRR* and *lnCVR* effect sizes. We performed analyses separately for one-off and multigenerational experiment data because they represent different biological processes and questions. We first estimated overall means for these two datasets using multilevel meta-analytic models (i.e., intercept-only models). We then merged these two datasets to formally compare the magnitudes of estimated average effects of one-off and multigenerational exposures using meta-regression with Exposure Type as a moderator (fixed factor). We ran all subsequent meta-regression analyses separately on one-off and multigenerational datasets. Our main meta-regression models (with one fixed factor at a time) included three moderators: sex of the exposed grandparent (an additional model examined

differences between grandparents exposed before mating), sex of grand-offspring and measured trait category. We estimated marginal R^2 values for each meta-regression model following Nakagawa and Schielzeth (2013), to determine the contribution of these moderators to explaining variation across studies. We created forest-like plots using the *orchaRd* package [66] to visualise distributions of the raw effect sizes and their mean estimates, together with associated Confidence Intervals (CI) and Prediction Intervals (PI). In the text, we converted point estimates into percentage change, for easier interpretation.

Full model and model selection analysis

The full meta-regression model included all three fixed effects of interest (with no interactions): sex of exposed grandparents, sex of measured grand-offspring, and measured trait type. Based on this multivariate model, we performed model selection using the *MuMIn* package [67] to determine the most influential moderator and moderator combinations, for both data sets and both effect size types.

Publication bias analyses

We assessed publication bias in the one-off and multigenerational datasets by visually inspecting funnel plots for asymmetry [68] in the distribution of the residuals of effect sizes (*sensu* [62]) from a full multivariate model with all three fixed effects. In addition, we performed a modified multilevel-model version of Egger's regression [69] by including sampling variance in a full meta-regression model. Finally, we investigated whether there are time trends in the distribution of effect sizes (time lag effect [70]), by using a meta-regression model with publication year as a continuous moderator.

Additional analyses

We also ran three additional univariate meta-regression models on both one-off and multigenerational datasets. To explore the effects of the severity of the obesogenic diet on the

F0 generation, we analysed total diet energy (kcal / g), diet protein to non-protein ratio (by weight), and duration of exposure to obesogenic diet (in days). We also tested whether average effects on F2 grand-offspring differ from effects on F3 great grand-offspring. Further, we used body weight data for F0 grandparents around the end of their obesogenic diet treatment (where reported) to compare the effects of obesogenic diets on the body mass of F0 grandparents with the effects in grand-offspring. As a supplement, we also explored the effects of rodent type and period of exposure in females. We conducted all additional analyses for both *lnRR* and *lnCVR* effect sizes (except for F0 grandparent's body weight, in which analyses were only conducted with *lnRR*). All R code and datasets are available at https://github.com/Apex619/meta-analysis.

Results

Dataset description

Results of our literature search are summarized in the PRISMA diagram in Figure S1 and Supplementary Methods, Figures S2 – S3, Tables S1 – S3, in the Supplementary Information. From the 33 included articles (Table 1), we obtained 407 effect sizes representing pairwise comparisons of control and treatment groups pertaining to body weight, adiposity, glucose, insulin, leptin, and triglycerides in grand-offspring generations F2 and F3. Individual articles contributed between 2 to 57 effect sizes. The measurements were taken from 1164 and 1090 unique grand-offspring from treatment and control groups, respectively.

We found 23 articles with one-off exposure type (272 effect sizes) and 15 articles with multigenerational exposure experiments (135 effect sizes; some articles include both types of exposure; see Figure 1). A few articles presented data from the same or very similar experiments, and we categorised these as representing the same study – in total this yielded 19 one-off exposure and 12 multigenerational exposure studies in our data set.

In terms of rodent species used, the dataset comprised of 242 effect sizes from mice (from 18 articles), and 165 from rats (from 15 articles), representing 6 laboratory strains in total. Mice and rats were almost equally represented in one-off and multigenerational exposure studies (Table 1). The dataset was dominated by experiments where F0 females were exposed (F0 exposed females: 297 effect sizes from 26 studies, F0 exposed males: 98 effect sizes from 7 studies; we had only one study where both females and males were exposed and then bred together to yield F1. Transgenerational data came mainly from F2 grand-offspring (325 effect sizes from 30 studies), as opposed to F3 great grand-offspring (82 effect sizes from 10 studies). Grand-offspring measurements were distributed evenly between the sexes (females: 197 effect sizes from 23 studies, males: 193 effect sizes from 24 studies, mixed-sex groups: 17 effect sizes from 5 studies).

Body weight was the best-represented offspring trait (173 effect sizes from all 31 studies), followed by adiposity (73 effect sizes from 13 studies). We had fewer data points for triglycerides (42 effect sizes from 14 studies), glucose tolerance tests (35 effect sizes from 13 studies), fasting glucose (17 effect sizes from 8 studies), fasting insulin (29 effect sizes from 10 studies), insulin tolerance test (21 effect sizes from 7 studies), and leptin (17 effect sizes from 8 studies) (it is important to note that studies were mixed regarding the method of measurement, with only a few confirming unstressed measures). Figure 1 presents a summary of numbers of effect sizes in one-off and multigenerational datasets by the exposed grandparent sex (b), grand-offspring sex (c), and grand-offspring trait type (d).

The included studies varied in terms of the type and timing of the obesogenic diet treatments. Energetic value ranged from 4.1 to 5.7 kcal / g (mean 4.9 kcal / g, SD 0.4 kcal / g) in the obesogenic diets and 3.1 to 4.1 kcal / g (mean 3.7 kcal / g, SD 0.3 kcal / g) in the control diets. Protein content ranged from 16 to 30% (mean 23%, SD 3%) by weight in obesogenic diets, and 14 to 27% (mean 20%, SD 3%) in control diets, with protein to non-

protein ratio (by weight) ranging from 0.16 to 0.46 (mean 0.32, SD 0.08) in obesogenic diets, and 0.18 to 0.49 (mean 0.30, SD 0.07) in control diets. These diet parameters were similarly distributed among the data points included in one-off and multigenerational datasets (Figure S4). The duration of grandparental exposure to obesogenic diets ranged from 21 to 140 days (mean 83 days, SD 30 days), for males finishing at mating (day 0) and for females often extending into gestation (31% of studies) and / or even lactation (42% of studies). The distributions of the timing of exposures were generally similar between one-off and multigenerational datasets (Figure S5).

316 *Effects of exposure type*

317 Our overall analyses examined the effects of grandparental exposure to an obesogenic diet on
318 grand-offspring separately for one-off and multigenerational exposure data (for both males
319 and females). Although statistically non-significant, grand-offspring descended from exposed
320 grandparents in one-off exposure studies tended to have overall mean trait values 9% higher
321 than their control counterparts (*lnRR* = 0.085, CI = -0.076 to 0.247, $p = 0.301$; Figure 2a;
322 Table S4). Grand-offspring descended from exposed grandparents in multigenerational
323 exposure experiments, however, had mean trait values 43% higher than control counterparts.
324 This effect was statistically significant (*lnRR* = 0.358, CI = 0.096 to 0.620, $p = 0.007$; Table
325 S4). The difference between average effects of two exposure types was statistically non-
326 significant (meta-regression model on merged data sets with exposure type as moderator:
327 $lnRR_{\text{difference}} = -0.187$, CI = -0.256 to -0.118; Table S5). Total heterogeneity among effect
328 sizes was high for both one-off and multigenerational datasets ($I^2 = 95.7\%$ and 99.2%,
329 respectively; Table S4), warranting analyses of moderators to explain the variation in the
330 effect sizes for effects on mean trait values.

331 The average effects on trait variability for both types of exposure were small and
332 statistically non-significant (one-off data: increase of 3%, *lnCVR* = 0.033, CI = -0.134 to
333 0.200, $p = 0.698$; multigenerational data: decrease of 7%, *lnCVR* = -0.074, CI = -0.282 to
334 0.134, $p = 0.486$; Figure 2b; Table S4; $lnCVR_{\text{difference}} = -0.024$, CI = -0.173 to 0.124, $p =$
335 0.750, $R^2 = 0.001$; Table S5). Total data heterogeneity was also moderately high for both one-
336 off and multigenerational data ($I^2 = 63.0\%$ and 71.6%, respectively; Table S4).

337

338 *Effects of exposed grandparents sex (F0)*

339 In one-off exposure experiments, we found no clear effect of the sex of exposed grandparents
340 on mean trait values of grand-offspring ($lnRR_{\text{difference}} = -0.017$, CI = -0.065 to 0.032, $p =$

341 0.508, R^2 = 0.001; Table S6) and the meta-regression model explained less than 1% of the
342 variation among effect sizes (Figure 3a; Table S6). In addition, there was also no clear effect
343 of the sex of grandparents exposed only before mating (Table S20). Similarly, effects on
344 variability of grand-offspring traits (*lnCVR*) were indistinguishable between grand-maternal
345 and grand-paternal one-off exposure lines (R^2 = 0.006; Figure 3c; Table S6). This was also
346 the case for grandparents exposed only before mating (Table S20).
347 In multigenerational exposure experiments, grand-maternal exposure to an obesogenic
348 diet had a large and statistically significant effect on mean trait values of grand-offspring
349 (grandmothers: increase of 51%, *lnRR* = 0.412, CI = 0.140 to 0.683, *p* = 0.003; Figure 3b;
350 Table S6). The same was true for grand-maternal exposure before mating (Table S20). In
351 contrast, grand-paternal exposed lines were associated with a small and statistically non-
352 significant effect on mean trait values of grand-offspring (grandfathers: increase of 16%,
353 *lnRR* = 0.146, CI = -0.183 to 0.475, *p* = 0.384; Figure 3b) compared to more marked effects
354 from maternal exposure lines ($lnRR_{\text{difference}}$ = -0.266, CI = -0.517 to -0.015, *p* = 0.038). This
355 meta-regression model explained 7% of variation among effect sizes. Grand-paternal
356 exposure before mating, however, was associated with a statistically significant effect on
357 grand-offspring, albeit smaller than grand-maternal exposure before mating (Table S20). The
358 effects on variability of grand-offspring traits were indistinguishable from zero for both
359 grand-maternal and grand-paternal exposures, with no statistically significant difference
360 between them ($lnRR_{\text{difference}}$ = -0.132, CI = -0.420 to 0.157; R^2 = 0.017; Figure 3d; Table S6).
361
362 *Grand offspring sex effects*
363 For one-off exposures, the effects on both granddaughter and grandson mean trait values
364 were small and statistically not different from zero, or each other (granddaughters: 10%, *lnRR*
365 = 0.091, CI = -0.056 to 0.238, *p* = 0.226; grandsons: 11%, *lnRR* = 0.103, CI = -0.044 to

366 0.250, $p = 0.170$; Figure 4a; Table S7). The meta-regression model explained only 0.3% of
367 variation among effect sizes. There was also no effect of grand-offspring sex on trait
368 variability (Figure 4c; Table S7).

369 For multigenerational exposures, the effects on granddaughter and grandson mean
370 trait values were large and statistically different from zero (granddaughters: 45%, *lnRR* =
371 0.373, CI = 0.087 to 0.658, $p = 0.010$; grandsons: 39%, *lnRR* = 0.331, CI = 0.044 to 0.618, p
372 = 0.024; Figure 4b; Table S7). On average, there was no statistically significant difference
373 between effects on granddaughters and grandsons ($lnRR_{\text{difference}} = -0.042$, CI = -0.157 to
374 0.074, $R^2 = 0.002$). There was also no apparent difference between granddaughters and
375 grandsons in the average effect on variability of the traits ($lnRR_{\text{difference}} = -0.133$, CI = -0.381
376 to 0.116; $R^2 = 0.002$; Figure 4d; Table S7).

377 *Effects of offspring trait category*

378 For one-off exposures, grand-offspring of grandparents exposed to obesogenic diets were on
379 average more obese than offspring from unexposed lines, although the effect was small (20%,
380 *lnRR* = 0.182, CI = 0.025 to 0.338, $p = 0.023$; $R^2 = 0.093$; Figure 5a; Table S8). While also
381 small, the average concentration of leptin was increased in grand-offspring of grandparents
382 fed obesogenic diets (20%, *lnRR* = 0.205, CI = 0.029 to 0.381, $p = 0.022$). Average effects on
383 adiposity and leptin levels were significantly larger than in the remaining trait categories
384 (Table S8). The effects on trait variability (*lnCVR*) were usually small and not statistically
385 different from zero for all trait categories ($R^2 = 0.064$; Figure 5c, Table S8).

386 Similar to what was observed in one-off exposures, after multigenerational exposure
387 to obesogenic diets, grand-offspring had significantly, and remarkably higher levels of
388 adiposity (121%, *lnRR* = 0.794, CI = 0.578 to 1.010, $p < 0.001$; Figure 5b; Table S8). They
389 also had higher levels of fasting insulin (58%, *lnRR* = 0.457, CI = 0.138 to 0.776, $p = 0.005$),
390 leptin (139%, *lnRR* = 0.872, CI = 0.577 to 1.168, $p < 0.001$) and triglycerides (41%, *lnRR* =

391 0.340, CI = 0.088 to 0.592, $p = 0.008$). The average effects on adiposity and leptin levels
392 were significantly larger than in the remaining traits ($R^2 = 0.370$; Table S8). Surprisingly, the
393 effect on average body mass was small and not statistically significant (11%, *lnRR* = 0.100,
394 CI = -0.062 to 0.262, $p = 0.225$). The effects of multigenerational exposure on grand-
395 offspring trait variability were small and statistically non-significant for all traits except one
396 (Figure 5d, Table S8). Namely, grand-offspring of parents exposed to obesogenic diets across
397 at least two generations had less variability in levels of blood triglycerides than their non-
398 exposed counterparts (*lnCVR* = -0.388, CI = -0.756 to -0.020, $p = 0.039$; $R^2 = 0.166$).
399
400 *Full model and model selection analysis*

401 The full meta-regression model included sex of exposed grandparents, sex of measured
402 grand-offspring, and measured trait type (Table S9). Although there was still a great deal of
403 model uncertainty, these three moderators jointly explained 13% of the variation in the
404 effects on mean trait values in one-off exposure data, 38% in multigenerational data, and
405 7.9% of the effects on trait variability, in both datasets. Model selection analysis indicated
406 that trait type was the most influential moderator of average effect sizes on the trait means in
407 one-off data, and for both trait means and variabilities in multigenerational data (Table S10).

408 *Publication bias analyses*

409 Visual inspection of enhanced-contour funnel plots of residuals did not show clear data
410 distribution skewness indicative of publication bias (Figure S6). A variant of Egger's
411 regression test using full multilevel meta-regression models indicated significant funnel plot
412 asymmetry only for the multigenerational *lnRR* dataset (Table S11). Finally, the slope of the
413 linear regression between publication year and the effect size was not significantly different
414 from zero for all data sets (Table S12).

Additional analyses

We performed three additional analyses: 1) to examine the effects of diet composition and exposure time; 2) compare average effects between F2 and F3 offspring and; 3) compare F0 and grand-offspring body mass. Results of additional models examining effects of rodent type and period of exposure in females can be found in Tables S19 and S21, respectively.

Diets with higher total energy content in multigenerational experiments had larger effects on mean grand-offspring trait values ($lnRR_{slope}$ = 0.188, CI = 0.062 to 0.313, p = 0.003; R^2 = 0.153; Table S13). Neither relative protein content of the obesogenic diet, nor the duration of the exposure of grandparents to the obesogenic diet, appeared to significantly influence the magnitude of effect sizes in one-off and multigenerational datasets and effect measures (Table S14 and Table S15).

Moreover, we found no difference between the average magnitudes of the effects between grand-offspring from F2 and F3 generations (Table S16).

Exposed grandparents from both one-off and multigenerational datasets were, on average, 14.9% heavier than their non-exposed counterparts ($lnRR$ = 0.139, CI = 0.062 to 0.216; Table S17, Table S18). For comparison, predicted differences between their grand-offspring at similar age (around 100 days old) would be around 7% in one-off experiments, and around 16.9% after multigenerational experiments (but note very wide confidence and prediction intervals; Table S17, Table S18).

Discussion

We addressed four main questions relating to the effects of obesogenic diets on morphological and physiological traits of grand-offspring from F2 and F3 generations. First, we have shown that grand-offspring in multigenerational exposure lines had mean trait values 43% larger than control grand-offspring. We have also shown an analogous, although weaker, trend in one-off exposure experiments. Second, we show that grand-maternal effects were larger than grand-paternal effects in multigenerational experiments, but not in one-off exposure experiments (9% larger). Third, the effects on grandsons and granddaughters were similar in both multigenerational and one-off exposure experiments. Fourth, we found that adiposity was the most affected grand-offspring trait in both types of exposure experiments. In multigenerational experiments, leptin, fasting insulin, and triglyceride levels were also elevated by the obesogenic diet treatment, with weaker effects on body weight. Also, effects on grand-offspring trait variability were usually small and statistically non-significant, apart from triglyceride levels in multigenerational experiments, where inter-subject variation was lower in treatment grand-offspring in comparison to control counterparts. Notably, leptin, fasting insulin, and fasting glucose exhibited a similar trend that inter-subject variability appears lower in the treatment group. We discuss these findings, and additional insights, in detail below.

One-off vs. Multigenerational exposure

Grand-offspring traits of treatment animals from One-off experiments tended to be 9% higher than control grand-offspring, although this overall effect was statistically non-significant. This finding is unsurprising, as it has been previously shown that subtle effects of obesogenic diets can persist beyond the F0 generation even without further diet manipulation[71–74]. Quantifying this effect for the first time across rodent studies supports the importance of F0

nutrition in shaping the health of grand-offspring, with the caveat that the effect is usually small, thus requiring large sample sizes to be reliably detected in empirical studies.

In contrast, grand-offspring traits of treatment animals from Multigenerational experiments were 43% higher than their control counterparts. This result matched our expectations that effect would be exacerbated when obesogenic diets are applied across generations, because observed changes in grand-offspring traits result from the cumulative influence (or interaction) of trans-generational, inter-generational, and direct nutritional effects. Notably, three-quarters of the animals in multigenerational studies were fed a high-fat diet around the time of trait measurements. As such, direct effects of grand-offspring diet could explain, in part, the large differences in trait means between experimental and control grand-offspring, while potentially masking more subtle purely transgenerational effects.

Grand-parental (F0) sex effects

For one-off exposure experiments, impacts on grand offspring traits did not depend on the sex of exposed grandparents (i.e., there was no effect of F0 sex, regardless of exposure period). The lack of a statistically significant grandparental sex effect in one-off exposure experiments is in line with the fact that effect sizes are both small and highly heterogeneous, and there are relatively few studies with grand-paternal exposures (5 out of 18 articles with one-off exposure). This lack of bias towards either sex has been shown before in a diet-induced obesity study (intergenerational) which noted equal strength of epigenetic inheritance from both male and female gametes[75]. Certainly, the same may be happening on a transgenerational scale, especially if offspring in question are not exposed to an obesogenic diet. In contrast, for multigenerational experiments, grand-offspring from exposed grandmothers had mean trait values 23% higher than treatment grand-offspring from exposed grandfathers. This result reflects more limited opportunities for transmitting nutritional insults by males at each generation. In mammals, mothers can easily pass on nutritional

484 insults to their offspring during gestation and lactation. Fathers can influence their offspring
485 only via sperm and seminal fluid during mating. Because of the latter, paternal one-off and
486 transgenerational studies would hint more clearly towards epigenetic mechanisms, which
487 modify the epigenome of sperm [76].

488 ### *Grand-offspring (F2 and F3) sex effects*

489 The average magnitudes of the dietary exposure effects were similar between female and
490 male grand-offspring for both one-off and multigenerational exposure studies. This is
491 surprising because previous studies examining the transgenerational effects of obesogenic
492 diets have shown sex-specific effects [27,77,78]. Although mechanisms underlying sex-specific
493 effects remain poorly understood, it has been suggested that one sex may be more sensitive
494 than the other due to the role of sex chromosomes [79] and factors acting during development,
495 such as ontogenetic changes in gene expression [80,81]. Our work shows that, on average, there
496 is no consistent pattern of sex-dependent offspring vulnerability to obesogenic diets, at least
497 in the assessed offspring traits and dietary conditions. Further work is needed to clarify this
498 given that sex-dependent responses can depend on the type of diet [82,83].

499 ### *Grand-offspring (F2 and F3) trait type effects*

500 Our analyses yielded three interesting findings for the effects in different categories of grand-
501 offspring traits. Firstly, we found adiposity to be the most affected trait. We expected that
502 treatment offspring would display abnormally high levels of adiposity in response to
503 developmental programming by obesogenic diets, especially in multigenerational
504 experiments. In line with this prediction, treatment grand-offspring had on average 20% more
505 fat than control offspring in one-off exposure experiments and 121% more fat in
506 multigenerational experiments. Unexpectedly, the increase in body fat was not accompanied
507 by an equivalent increase in body weight. Treatment grand-offspring tended to be only 4%

heavier than control grand-offspring in one-off experiments, and 11% heavier in multigenerational experiments. This discrepancy can be partially attributed to the fact that only 3 studies, out of 12 studies with adiposity data, reported total fat measurements using imaging techniques. The remaining studies reported adipose mass measurements for fat pads, which could be differentially affected by nutrition.

Secondly, physiological traits that are functionally linked to adipose tissue showed similar patterns of effects as adiposity measurements. As adipose tissue stores triglycerides [84], it is unsurprising that we found a parallel increase of triglyceride levels in treatment grand-offspring in multigenerational experiments. Treatment grand-offspring in multigenerational experiments also had significantly greater levels of leptin (139% higher). Leptin is one of the major players involved in energy homeostasis. It is produced by adipocytes and has a central role in the regulation of food intake and body weight [85]. However, individuals with obesity not only have elevated leptin levels but also develop 'leptin resistance', a complex phenomenon, where increased circulating leptin does not reduce appetite and body mass [86, 87]. We showed that treatment grand-offspring in multigenerational experiments also had significantly greater levels of fasting insulin (58% higher). Increased insulin is required to maintain normal glucose tolerance, also known as compensatory hyperinsulinemia [88]. This potentially explains why we did not observe abnormal glucose levels in multigenerational exposure experiments. In one-off exposure experiments, the overall pattern of effects was similar, but all effects were small and statistically non-significant. Finally, taken together, the above results suggest weak transgenerational inheritance of the metabolic syndrome, a cluster of conditions that increase the risk of heart disease and diabetes [89]. Large effects observed in multigenerational exposure experiments, likely arise predominantly due to maternal effects and direct offspring diet effects. The surprisingly small effect on body weights coupled with

large effects on physiology in multigenerational experiments is in line with the concept of 'normal weight obesity', which highlights the need to stratify risk based on underlying metabolic factors, such as adiposity, and metabolic changes, rather than body weight alone [90]. Moreover, since many of the included papers reported body weights of grandparents, we were able to show that when controlling for age, the magnitude of the effect of obesogenic diet in multigenerational exposure experiments was similar for grandparents and grand-offspring (15% and 17%, respectively). This result suggests a lack of strong cumulative effects of multigenerational exposure, at least on body weight.

Thirdly, our study also examined differences in variation between control and treatment grand-offspring. Effects on grand-offspring trait variability were small and not statistically significant, apart from triglyceride levels in multigenerational experiments. While work in flies showed that high-fat diets increase phenotypic variation [91], we found that triglyceride levels varied significantly less in treatment than in the control grand-offspring (32% difference). While non-significant, it is noteworthy that leptin, fasting insulin, and fasting glucose also generally followed this pattern. One possible explanation is that the effects of a high-fat diet are impacted by a ceiling effect whereby levels are elevated to their physiological capacity, effectively lowering variation and masking potential differences between individuals. Such a scenario was previously proposed to explain the lack of increase in glucose concentrations of rodents under a high-fat diet [92].

Additional findings

We investigated whether obesogenic diet composition and duration of grand-parental exposure to such a diet can moderate the effects on grand-offspring traits in both one-off and multigenerational datasets. The minimum protein to support adequate growth and reproduction in mice is ~16% in rats and ~14%, by weight [93]. Studies included in our meta-

analysis had adequate, but not particularly high protein content, with protein by weight % ranging from 16% to 30%. We found no effect of protein content in the obesogenic diets on grand-offspring trait values. This finding is surprising given the expectation that low protein intake may decrease thermogenesis and satiety, as well as lead to an increase in subsequent energy intake [94]. However, our result is consistent with a study showing that protein content is not a major factor in regulating energy intake and causing an obesogenic response (i.e., adiposity), rather dietary fat is [95]. In line with this, we revealed that experimental diets with higher total energy content (usually containing more fat) had a significantly larger impact on mean trait values of grand-offspring in multigenerational experiments. Foods high in energy have been shown to have a robust and significant effect on satiety and satiation, thereby facilitating overconsumption of fat, leading to obese phenotypes [96]. It has also been established in previous studies that longer exposure to obesogenic diet results in larger effects on morphological and physiological traits in directly exposed animals [97–100]. However, we found no statistically significant influence of duration of grand-parental diet exposure on the magnitude of effect sizes in grand-offspring, indicating that this effect may get diluted as it is passed to subsequent generations. We also found no difference between F2- and F3-generation grand-offspring in the average magnitude of effects (for both one-off and multigenerational studies), indicating weak, but durable, transgenerational transfer of dietary effects.

Limitations and future directions

Our meta-analysis has several limitations. Firstly, we worked with unevenly distributed numbers of available effect sizes when attempting to answer our questions regarding exposure type (66% of the data for one-off exposure studies), F0 sex effects (74% of the data from grand-maternal exposure), and grand-offspring trait effects (besides body weight, all other traits did not exceed 17% of effect sizes). All studies (except one) reported body

583 weights, but not necessarily other traits, depending on the study's focus. Further, most studies
584 did not report outcomes pertaining to the F0 generation itself (i.e., adiposity). This made it
585 difficult to answer other questions concerning the effects of grandparental exposure to an
586 obesogenic diet (i.e., separating the effects of dietary exposures and the resulting changes in
587 the bodies of parents).
588
589 Secondly, given the subtlety of effects in certain cases (i.e., one-off exposure studies had an
590 average effect of 9%), large sample sizes are required to detect changes due to experimental
591 manipulations in empirical studies. In our data sets, numbers of measured grand-offspring in
592 treatment groups ranged from 3 to 34, with a mean of 13 and a median of 10, thus limiting
593 power to detect small effects. The magnitudes of effect sizes from our meta-analysis can be
594 used in power calculations to guide the appropriate sample sizes required in future empirical
595 studies [101].
596
597 Thirdly, truly transgenerational effects are those that are found in generations that were not
598 exposed to the factor that triggered the change in phenotype [29]. In other words, the effects can
599 be considered truly transgenerational only if grand-offspring have no direct contact with the
600 grand-parental environment. In mammals, this definition is important when comparing grand-
601 maternal and grand-paternal effects. When an F0 mother is exposed to an obesogenic diet, the
602 developing offspring (F1) is directly affected in utero and during lactation, as well as F2 germ
603 cells inside these developing F1 offspring [30]. As such, the F2 generation may already be at an
604 adverse risk of developing metabolic disease, and a minimum F3 generation is required to
605 detect true transgenerational effects. When an F0 father is exposed, their germline (future F1)
606 is exposed, but not F2 germ cells. Thus, in grand-paternal exposure studies, F2 is sufficient
607 for detecting true transgenerational effects [29,31]. In our dataset, approximately 80% of effect

608 sizes were from F2 grand-offspring. The remaining 20% of effect sizes is from F3 grand-
609 offspring: 82 effect sizes overall, including 58 from one-off exposure experiments (only 44
610 from great-grand-maternal exposure). Given that our dataset is dominated by grand-maternal
611 exposures and F2 grand-offspring data, it is hard to completely disentangle truly
612 transgenerational mechanisms from developmental programming effects. However, our
613 analyses also show that the average magnitude of observed effects did not significantly differ
614 between F2 and F3 grand-offspring, indicating that the difference between transgenerational
615 effects in F2 and "truly transgenerational effects" in F3 is also subtle.
616
617 Fourthly, the pre-planned moderators we used in our meta-regression analyses did not
618 effectively account for the high level of variation present in effect sizes across studies, with
619 R^2 values ranging from 0 to 0.37. Improved reporting, as well as a consistent framework for
620 study designs, may help limit heterogeneity, and facilitate more detailed analyses. As such,
621 we highly recommend following updated guidelines for reporting animal research [102].
622
623 Lastly, our publication bias tests indicated some potential funnel plot asymmetry, but only in
624 the multigenerational *lnRR* (mean trait values) dataset. Funnel plot asymmetry may stem
625 from true unexplained biological heterogeneity, not publication bias [69]. In any case, quality
626 reporting including all results, regardless of their outcome, is recommended for the primary
627 studies and care should be taken when interpreting meta-analytical results [103].
628
629 To our knowledge, our meta-analysis is the first to examine differences in variance between
630 obesogenic and control group diets. As most meta-analyses focus on mean differences, we
631 hope to open another avenue of meta-analytical exploration, as the difference in variance are

not only medically relevant [104], but also evolutionary and ecologically relevant [52], as natural selection processes act on variation.

Conclusions

This meta-analysis on rodent studies aimed to address how an obesogenic diet impacts the mean and variance of metabolic traits across generations. Overall, we have demonstrated that the grand-offspring of exposed grandparents display phenotypes consistent with the metabolic syndrome, especially if the effect has been exacerbated by multigenerational exposure to obesogenic diets. Maternal factors have the strongest influence via developmental programming, and both male and female offspring are equally susceptible. Furthermore, the caloric density of the diet plays a significant role in promoting an obesogenic phenotype, and certain metabolic traits, such as adiposity, are more reliable as indicators of metabolic syndrome transfer across generations. A high-fat diet may also result in a ceiling effect, reducing the amount of phenotypic variation in grand-offspring in multigenerational exposure groups. Further empirical and meta-analytical research is needed to elucidate mechanisms underlying true transgenerational inheritance of effects of obesogenic diets, particularly for maternal exposure, as most included studies fail to extend beyond the F2 generation.

CONFLICTS OF INTERESTS: none

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Tables

Table 1)

Full list of included studies with species and strain used as well as information on dietary fat for both control and obesogenic diets used for F0 (percent of fat by weight).

Author (Year)	Title	Species/Strain	Diet (% fat by weight: Control, Obesogenic)
Adedeji et al. (2019) [105]	Multigenerational effects of dietary macronutrient intake on the metabolic phenotype of male Wistar rats	Rat/Wistar	C = 10.3% O = 60.0%
Andreas et al. (2019) [106]	The effect of maternal high-fat/high-sugar diet on offspring oocytes and early embryo development	Mouse/C57BL/6J	C = 5.0% O = 36.0%
Armitage et al. (2007) [107]	Programmed aortic dysfunction and reduced Na+, K+ - ATPase activity	Rat/Sprague Dawley	C = 5.3% O = 25.7%

Author (Year)	Title	Species/Strain	Diet (% fat by weight: Control, Obesogenic)
	present in first generation offspring of lard-fed rats does not persist to the second generation		
Barbosa et al. (2020) [108]	Maternal high-fat diet triggers metabolic syndrome disorders that are transferred to first and second offspring generations	Rat/Sprague Dawley	C =4.0% O = 39.5%
CastroBarbosa et al. (2016) [109]	High-fat diet reprograms the epigenome of rat spermatozoa and transgenerationally affects metabolism of the offspring	Rat/Fischer	C = 4.2% O = 21.2%
CastroBarbosa et al.	Paternal high-fat diet transgenerationally	Rat/Sprague Dawley	C = 4.2% O = 21.2%

Author (Year)	Title	Species/Strain	Diet (% fat by weight: Control, Obesogenic)
(2019) [110]	impacts hepatic immunometabolism		
de Assis et al. (2012) [111]	High-fat or ethinyl-oestradiol intake during pregnancy increases mammary cancer risk in several generations of offspring	Rat/Sprague Dawley	C = 7.0% O = 23.0%
Ding et al. (2014) [112]	DNA hypomethylation of inflammation-associated genes in adipose tissue of female mice after multigenerational high fat diet feeding	Mouse/C57BL/6	C = not reported O = 34.9%
Dunn and Bale	Maternal high-fat diet promotes body length	Mouse/C57BL/6	C = 5.8% O = 24.0%

Author (Year)	Title	Species/Strain	Diet (% fat by weight: Control, Obesogenic)
(2009) [113]	increases and insulin insensitivity in second-generation mice		
Dunn and Bale (2011) [71]	Maternal high-fat diet effects on third-generation female body size via the paternal lineage	Mouse/C57BL/6	C = 6.0% O = 24.0%
Fullston et al. (2012) [114]	Diet-induced paternal obesity in the absence of diabetes diminishes the reproductive health of two subsequent generations of mice	Mouse/C57BL/6	C = 6.0% O = 21.0%
Giraudo et al. (2010) [115]	Maternal high fat feeding and gestational dietary restriction: effects on	Mouse/C57BL/6	C = 11.0% O = 24.0%

Author (Year)	Title	Species/Strain	Diet (% fat by weight: Control, Obesogenic)
	offspring body weight, food intake and hypothalamic gene expression over three generations in mice		
Gniuli et al. (2008) [17]	Effects of high-fat diet exposure during fetal life on type 2 diabetes development in the progeny	Mouse/Swiss	C = 3.6% O = 34.0%
Graus-Nunes et al. (2015) [116]	Pregestational maternal obesity impairs endocrine pancreas in male F1 and F2 progeny	Mouse/C57BL/6	C = 7.0% O = 27.0%
Hanafi et al. (2016) [72]	Transgenerational effects of obesity and malnourishment on diabetes risk in F2 generation	Rat/Wistar	C = 4.3% O = 26.5%

Author (Year)	Title	Species/Strain	Diet (% fat by weight: Control, Obesogenic)
Harasymowicz et al. (2020) [117]	Intergenerational transmission of diet-induced obesity, metabolic imbalance, and osteoarthritis in mice	Mouse/C57BL/6J	C = 4.3% O = 34.9%
Hoile et al. (2015) [118]	Fat and carbohydrate intake over three generations modify growth, metabolism and cardiovascular phenotype in female mice in an age-related manner	Mouse/C57BL/6	C = 5.0% O = 21.0%
Huang et al. (2017) [119]	Maternal high-fat diet impairs glucose metabolism, beta-cell function and proliferation in the	Rat/Sprague Dawley	C = 5.0% O = 24.0%

Author (Year)	Title	Species/Strain	Diet (% fat by weight: Control, Obesogenic)
	second generation of offspring rats		
King et al. (2013) [120]	Maternal obesity has little effect on the immediate offspring but impacts on the next generation	Mouse/C57BL/6	C = 4.8% O = 35.8%
Lannes et al. (2015) [73]	Both hepatic lipogenesis and beta-oxidation are altered in offspring of mothers fed a high-fat diet in the first two generations (F1 and F2)	Mouse/C57BL/6	C = 7.5% O = 27.0%
Li et al. (2012) [121]	Accumulation of endoplasmic reticulum stress and lipogenesis in the liver	Mouse/C57BL/6	C = not reported O = 34.9%

Author (Year)	Title	Species/Strain	Diet (% fat by weight: Control, Obesogenic)
	through generational effects of high fat diets.		
Martins Terra et al. (2019) [122]	Multigenerational effects of chronic maternal exposure to a high sugar/fat diet and physical training	Rat/Wistar	C = 4.0% O = 45.1%
Masuyama et al. (2015) [123]	The effects of high-fat diet exposure in utero on the obesogenic and diabetogenic traits through epigenetic changes in adiponectin and leptin gene expression for multiple generations in female mice	Mouse/ICR	C = 4.2% O = 35.0%
Masuyama et al.	The effects of paternal high-fat diet exposure	Mouse/ICR	C = 4.4% O = 35.0%

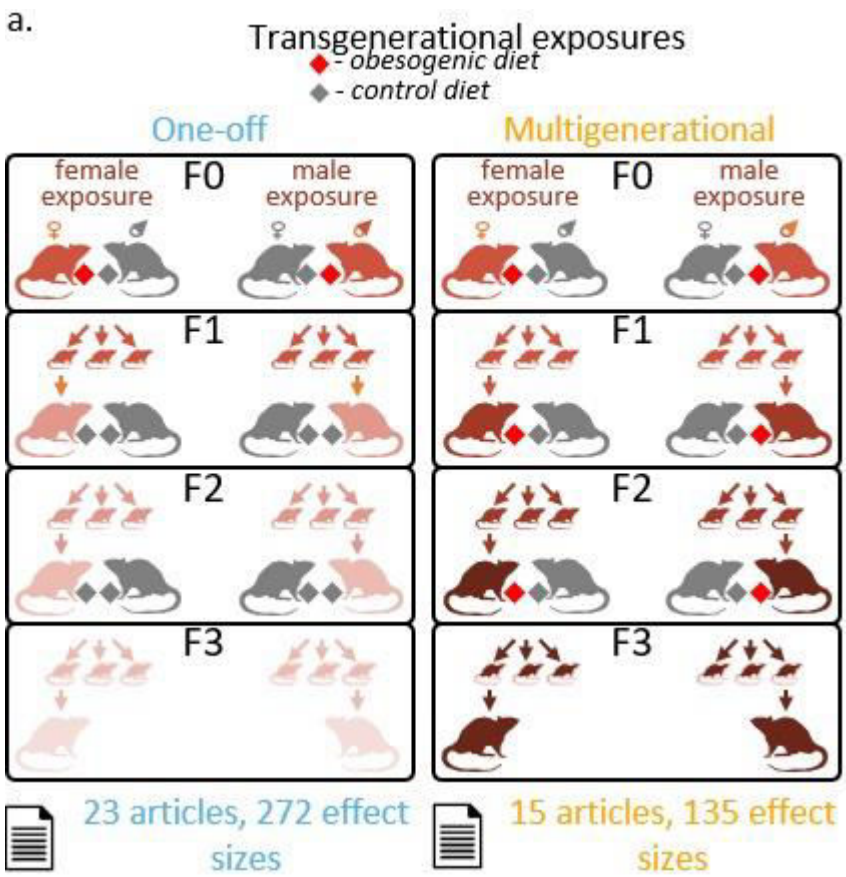
Author (Year)	Title	Species/Strain	Diet (% fat by weight: Control, Obesogenic)
(2016) [124]	on offspring metabolism with epigenetic changes in the mouse adiponectin and leptin gene promoters		
Nasu et al. (2007) [125]	Effect of a high-fat diet on diabetic mother rats and their offspring through three generations	Rat/Wistar	C = 4.6% O = 32.0%
Oshio et al. (2020)[126]	A paternal hypercaloric diet affects the metabolism and fertility of F1 and F2 Wistar rat generations	Rat/Wistar	
Park et al. (2018) [127]	Diet-induced obesity leads to metabolic dysregulation in	Mouse/C57BL/6	C = 7.0% O = 27.0%

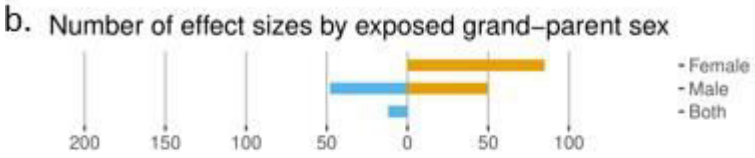
Author (Year)	Title	Species/Strain	Diet (% fat by weight: Control, Obesogenic)
	offspring via endoplasmic reticulum stress in a sex-specific manner		
Sarker et al. (2018) [128]	Transgenerational transmission of hedonic behaviors and metabolic phenotypes induced by maternal overnutrition	Mouse/C57BL/6	C = 4.5% O = 35.0%
Schellong et al. (2020) [129]	Maternal but not paternal high-fat diet (HFD) exposure at conception predisposes for 'diabesity' in offspring generations	Rat/Wistar	C = 3.3% O = 15.5%

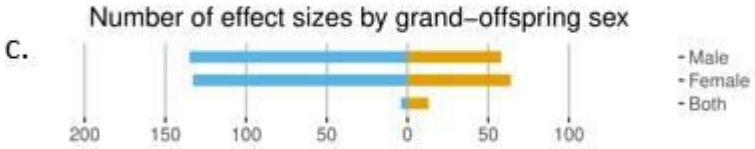
Author (Year)	Title	Species/Strain	Diet (% fat by weight: Control, Obesogenic)
Tait et al. (2015) [130]	Successive generations in a rat model respond differently to a constant obesogenic environment	Rat/Wistar	C = 5.0% O = 24.0%
Thompson et al. (2019) [131]	Transgenerational impact of maternal obesogenic diet on offspring bile acid homeostasis and non-alcoholic fatty liver disease	Mouse/C57BL/6J	C = 5.0% O = 36.0%
Winther et al. (2019) [132]	Grandmaternal high-fat diet primed anxiety-like behaviour in the second-generation female offspring	Rat/Sprague Dawley	C = 4.2% O = 34.9%

Author (Year)	Title	Species/Strain	Diet (% fat by weight: Control, Obesogenic)
Zhang et al. (2019) [133]	Paternal programming of liver function and lipid profile induced by a paternal pre-conceptional unhealthy diet: potential association with altered gut microbiome composition	Rat/Sprague Dawley	C = 7.0% O = 20.7%

Figures


a.
Transgenerational exposures
- obesogenic diet
- control diet
One-off
Multigenerational
female exposure
F0
male exposure
F1
F2
F3
23 articles, 272 effect sizes
15 articles, 135 effect sizes


b. Number of effect sizes by exposed grand–parent sex
- Female
- Male
- Both
200
150
100
50
0
50
100


c.
Number of effect sizes by grand–offspring sex
- Male
- Female
- Both
200
150
100
50
0
50
100

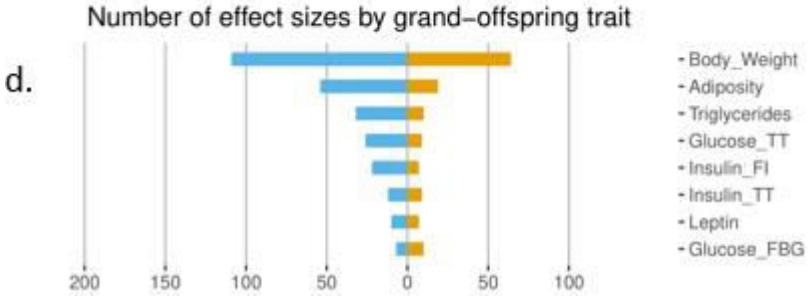

d.
Number of effect sizes by grand–offspring trait
- Body_Weight
- Adiposity
- Triglycerides
- Glucose_TT
- Insulin_FI
- Insulin_TT
- Leptin
- Glucose_FBG
200
150
100
50
0
50
100

Figure 1

a) Conceptual diagram of two main types of transgenerational experiments included in the meta-analysis. In one-off experiments, only F0 generation adults are exposed to obesogenic diet before and / or during breeding. Then, all animals are kept on control / standard diets. As a result, in subsequent generations the effects of exposure to obesogenic diet is expected to become progressively weaker. In multigenerational experiments, F0 and subsequent generations are exposed to obesogenic diets before and / or during breeding. As result, in subsequent generations, the effects of exposure to obesogenic diets are compounded. b – d) Summaries of the counts of effect sizes for the main analysed factors in one-off and multigenerational datasets used for meta-analysis.

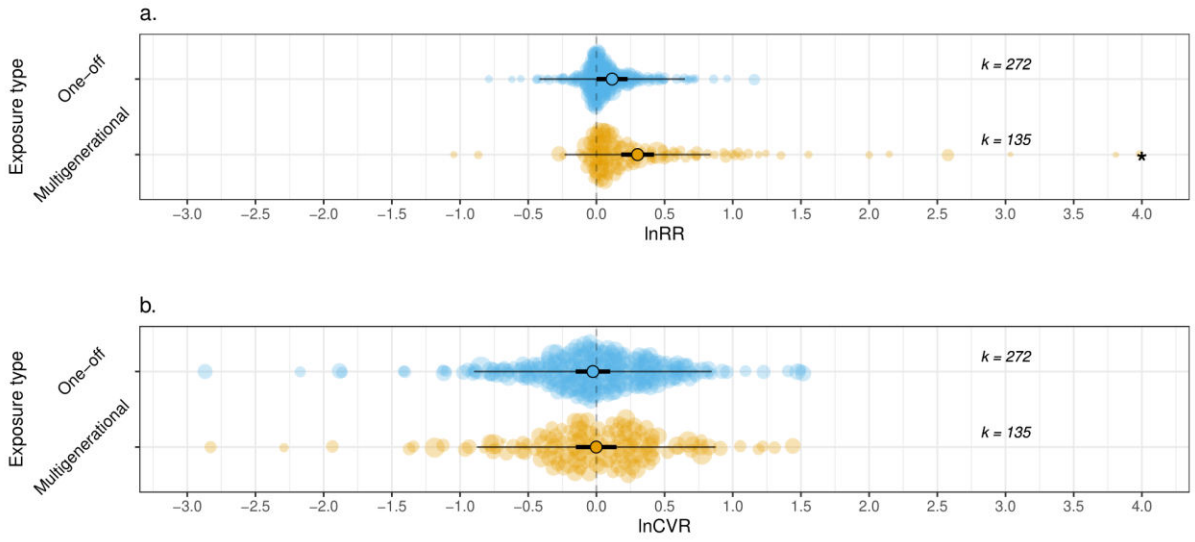


Figure 2

Forest-like (orchard) plots of effect size estimates from meta-regression model with experiment type (one-off or multigenerational) as a moderator: a) effects on the mean values of grand-offspring traits (*lnRR*), and b) effects on the variances of grand-offspring traits (*lnCVR*). Thick horizontal lines indicate 95% confidence intervals (*CI*), thin horizontal lines indicate 95% prediction intervals (*PI*), with mean estimates at the centre; *k* are the numbers of

effect sizes. Pale blue and orange circles represent individual effect sizes, with circle sizes scaled accordingly to precision (weights). Statistically significant effect sizes (CI not crossing zero) are marked with *.

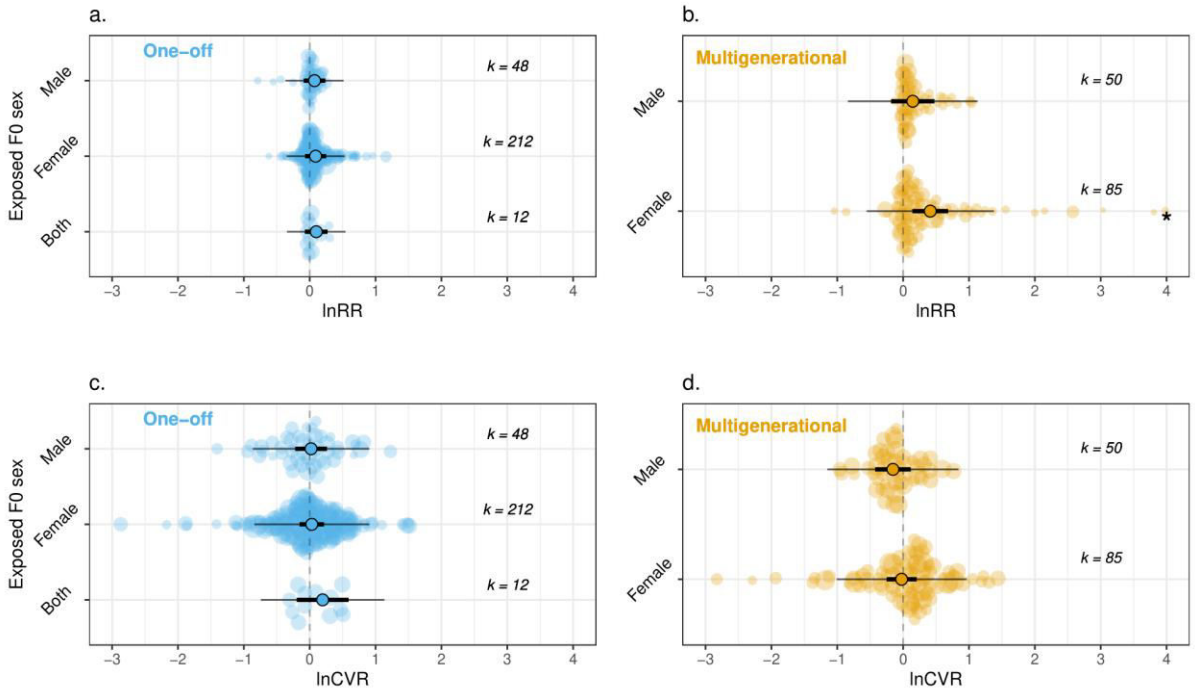


Figure 3

Forest-like (orchard) plots of effect size estimates from meta-regression models with the sex of exposed grandparents as a moderator, for one-off and multigenerational datasets: a, b) effects on the mean values of grand-offspring traits (*lnRR*), and c, d) effects on the variances of grand-offspring traits (*lnCVR*). Thick horizontal lines indicate 95% confidence intervals (*CI*), thin horizontal lines indicate 95% prediction intervals (*PI*), with mean estimates at the centre; k are the numbers of effect sizes. Pale blue and orange circles represent individual effect sizes, with circle sizes scaled accordingly to precision (weights). Statistically significant effect sizes (CI not crossing zero) are marked with *.

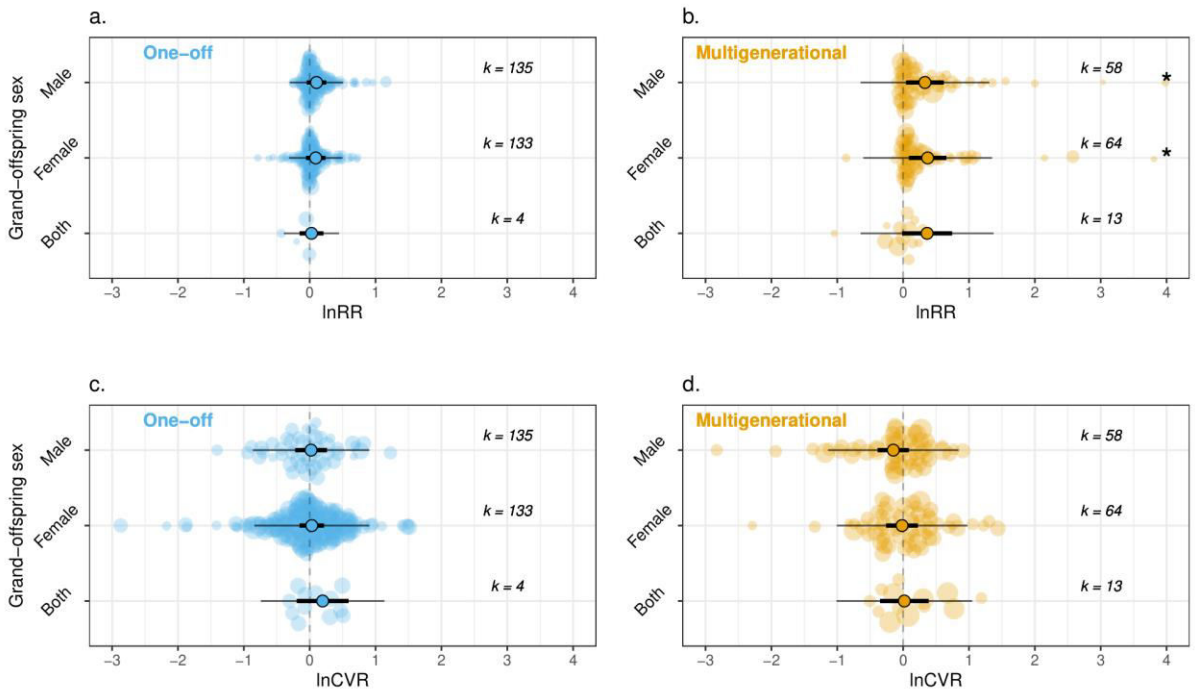


Figure 4

Forest-like (orchard) plots of effect size estimates from meta-regression models with the sex of measured grand-offspring as a moderator, for one-off and multigenerational datasets: a, b) effects on the mean values of grand-offspring traits (*lnRR*), and c, d) effects on the variances of grand-offspring traits (*lnCVR*). Thick horizontal lines indicate 95% confidence intervals (*CI*), thin horizontal lines indicate 95% prediction intervals (*PI*), with mean estimates at the centre; *k* are the numbers of effect sizes. Pale blue and orange circles represent individual effect sizes, with circle sizes scaled accordingly to precision (weights). Statistically significant effect sizes (CI not crossing zero) are marked with *.

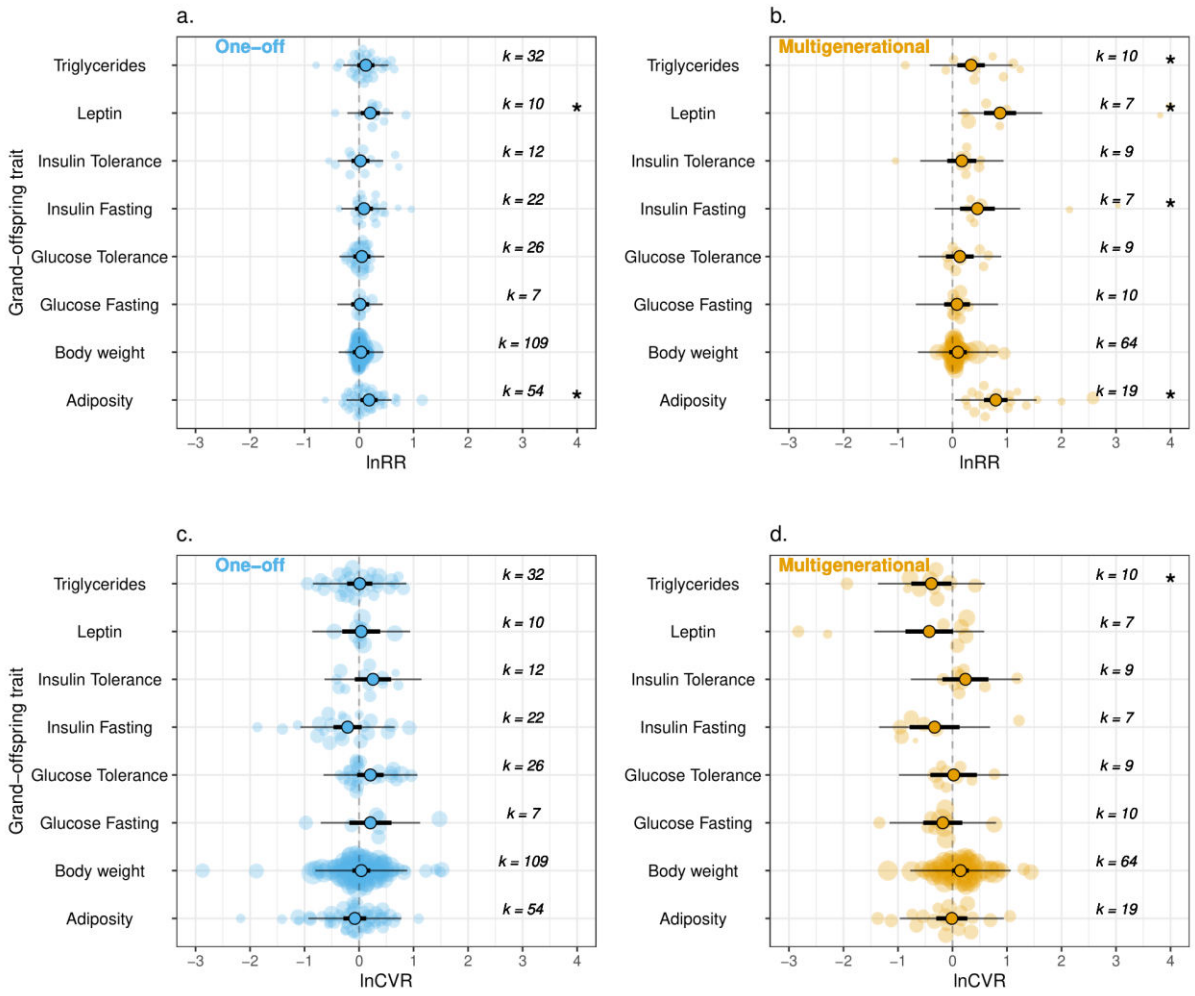


Figure 5

Forest-like (orchard) plots of effect size estimates from meta-regression models with the grand-offspring trait type as a moderator, for one-off and multigenerational datasets: a, b) effects on the mean values of grand-offspring traits (*lnRR*), and c, d) effects on the variances of grand-offspring traits (*lnCVR*). Thick horizontal lines indicate 95% confidence intervals (*CI*), thin horizontal lines indicate 95% prediction intervals (*PI*), with mean estimates at the centre; *k* are the numbers of effect sizes. Pale blue and orange circles represent individual effect sizes, with circle sizes scaled accordingly to their precision (weights). Statistically significant effect sizes (CI not crossing zero) are marked with *.

Supporting Information

Transgenerational effects of obesogenic diets in rodents:

a meta-analysis

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KEYWORDS: Systematic review, obesity, grand-parents, grand-offspring

RUNNING TITLE: Meta-analysis of transgenerational effects

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# equal contribution as senior authors

31 May 2020

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Supplementary Methods

Supplementary information for the literature search

Literature search and study selection

We performed a comprehensive systematic review of the academic literature, as presented in **Figure S1** (PRISMA diagram). We used pre-piloted keyword strings to search four online databases: Scopus, ISI Web of Knowledge, Medline and Embase (search strings in **Table S1**). We ran the main database searches in April 2018 and updated our search in 2020. We merged references from these databases and removed duplicate copies before exporting a single .RIS file for title and abstract screening in Rayyan QCRI (Ouzzani et al., 2016). Two researchers (HA, AA) independently screened all records. After independent screens, each record with a decision conflict was crosschecked and discussed, and a final decision to include or exclude was made via consensus. **Figure S2** presents a decision tree representing our inclusion criteria used in screening titles and abstracts of the bibliometric records.

We supplemented the above literature database searches by snowballing (forward and backward citation searches) from studies deemed to have matched our inclusion criteria after full-text assessment. Snowballing involved screening titles and abstracts of references cited by each paper (backward) as well as references it had been cited by (forward) at the time of screening. We considered grey literature and developed search strings using three databases: Trove, OpenGrey and ProQuest. Additional searches were also conducted using Google Scholar using combinations of relevant keywords (i.e., rodent, high fat, obesogenic, transgenerational, multigenerational).

Papers that passed abstract and title screening were downloaded as PDF files along with supplementary files for full-text screening. Our criteria for full-text studies to be included for quantitative synthesis are presented as a decision tree in **Figure S3**. Studies that were excluded during this stage were recorded along with reasons for exclusions (see **Table S2**). We repeated searches and screening processes in April 2020 to update our dataset.

The search of two main databases in 2018 yielded a total of 761 records, and the 2020 update of these searches 237 records. Searchers from other sources yielded almost 3000 additional records for screening. Following title and abstract screening of all found records, we screened full texts of 59 studies in 2018 and another 15 in 2020. We excluded 34 articles and 4 articles, respectively, mainly due to them not being transgenerational studies, not using an obesogenic diet treatments or using mutant rodents. After removing 5 duplicated articles, we included 31 unique articles for analyses. After suggestions from reviewers to also seach the databases Medline and Embase, we found a further 2 articles suitable for our analyses (resulting in the total of 33 articles).

Data extraction and coding

General data extractions

We created a coding system to standardize commonly reported data in the included papers. The full list of the main extracted variables (meta-data) is provided in **Table S3**. Data were extracted from the text, tables or figures, as available. If needed, we contacted authors for missing information or clarifications regarding papers published within the last 5 years. Where complex experiments were performed in the original papers, we only extracted exposure lines matching our two main types of exposure (one-off or multigenerational), alongside with appropriate control groups. A few of the articles presented data from the same or very similar experiments, and thus we categorised the data points from these as representing the same study (Dunn and Bale 2009, 2011; Masuyama et al. 2015, 2016; Castro-Barbosa et al. 2016, 2019). To take this into account, we created a Study_ID variable, which was used instead of Paper_ID in the analyses.

Data extractions from figures

In figures, where symbols used for the mean overlapped error bars, we took a conservative estimate by selecting the middle of the symbol for the mean, and the edge of the symbol for the error. In some cases, we deemed authors to have reported the incorrect statistics name (e.g., SE instead of SD). As such, we back calculated the values based on what we inferred was used. We used the *R* package *metaDigitise* v.1.0.0 (Pick et al., 2018) to extract quantitative data from figures.

Body weights and adiposity data extractions

We extracted body weight data from the offspring from generations F2 and F3 (we found no papers reporting data for further generations). All body weight data were standardized to grams. When the body weight growth curves were presented in the included papers, we extracted data from the available time points closest to birth (0 days), weaning (21 days) and adult life stage (100-200 days) and the last reported time point. Additionally, we also extracted body weight data for F0 parents (grandparents), where reported. Extractions followed the same procedure as for the grand-offspring data (e.g., corresponding age ranges and units). For adiposity data presented as proportion of body weight, we recalculated grams / milligrams of fat using associated body weight data for the same cohort of animals at the time point closest to the adiposity measurement.

Glucose and insulin data extractions

We amalgamated tolerance test data extracted from the response curves to obtain an AUC (area under curve) estimate. We calculated AUC by estimating area of rectangular columns under the curve (area = width × height), with the width equating to the length of the time interval between midpoints of glucose measurements, and the height equating to the value of the glucose measurement. Analogous calculations were performed for associated standard deviation values. Obtaining halfway points was necessary to include time 0 measurements. The sum of all areas for time points and standard deviation provided us our new mean AUC and its SD value. We then calculated standard error of AUC from standard deviation.

Obesogenic diet data extractions

We extracted the following information about obesogenic diets used in the experiments: total energy content (kcal / g), protein, carbohydrate and fat percentage by weight and by energy. From these values we calculated relative protein content (protein to non-protein ratio by weight). For data extraction, we first looked at information about diet composition provided in the included articles. When this information was insufficient, we looked at other publications from the same research group published around the same time and potentially using same diets, but providing more detailed diet descriptions. We also consulted data on the respective diets provided by commercial rodent chow producers. We had to calculate some of the values from the other available information, e.g., kJ/kg into kcal/g,

macronutrient percentage by energy to proportion by weight, or vice versa. From the included papers, we also collected data on the timing of the F0 generation exposures to obesogenic diets, with day 0 set as the day of F0 animal mating. Negative values of exposure start indicate pre-mating exposure, and positive values of exposure end indicate that exposure was continued into gestation and/or lactation (for F0 females). We also calculated total duration of exposure of F0 generation to obesogenic diets (in days). For statistical analyses, we considered total diet energy (kcal / g), percentage of energy from fat as the key indicators of the obesogenic potential of the used diets. We scaled these variables (and also exposure duration), when we used them as moderators in meta-regression models (i.e., these fixed effects were *Z*-transformed, so that their mean is at 0 and SD is 1 in the models).

Comparing body weights of grandparents and grand-offspring

We collected F0 body weight around the end of exposure to obesogenic diets from 17 studies that reported this information. We obtained 27 effect sizes comparing body weight of exposed to non-exposed grandparents of the same sex. The ages were centered around 100 days of age, when most of the exposures finished (at or after F0 mating). We fitted a meta-regression model with age at body weight measurement as a moderator and effect sizes for body weight as a response (random effects: Rodent Strain, Study ID and Effect Size ID, variance-covariance matrix used to control for non-independence of some of the comparisons). An analogous meta-regression was run on the body weight data of the grand-offspring, separately for One-off and Multigenerational datasets. We then used these meta-regression models to predict the magnitudes of effect sizes for effects on mean body weights of grandparents and grand-offspring at around 100 days of age. We expressed the results as percent difference between average body sizes of control and treatment groups of animals.

Protocol amendments

We registered a protocol of all our methods on the Open Science Framework (https://osf.io/sg6wj/register/565fb3678c5e4a66b5582f67). During the course of the project we had to make the following deviations from that protocol:

1. Search: we performed an update of the literature search one year after the original search, to keep our data set up to date with new publications.
2. Data collection categories: in the protocol we mention “serum glucose” as a trait category to be extracted. During data collection we realized that many studies measured glucose in blood, so we broadened this category to serum or blood glucose measurements. We included measurements taken after fasting and also after glucose injections (glucose tolerance tests).
3. Data collection across generations: since most of the included studies did not report measurements taken on F0 or F1 generations, we decided not to include data on these generations. We made an exception for F0 body weight measurements in multigenerational exposure experiments. Thus, the only cross-generations model we run was for comparing effects of multigenerational exposure on body weights between generations F0 and F2/F3.
4. Data transformations: for some of the trait categories it was not possible to bring all the measurements to the same units, e.g., when authors reported results in “arbitrary units”. In such cases, we kept original units. The effect sizes we used are standardized, so they are unitless and should not be affected by original measurement units.
5. Data coding: we ultimately decided not to code each treatment group of animals within each generation as “dietary”, “gestational”, “gametic” (plus combination of these three, as applicable) or “none”, because combination of already coded data on the experiment time, sex and generation was usually sufficient to infer exposure type. Also we did not code transmission lines (and breeding design) as “maternal” or “paternal”, because this information was already coded as “F0 exposed sex” variable. Additionally, we had a new Lineage_HFD variable, which represents both breeding designs and exposure transmission line, but was not used during analyses due to the unbalanced distribution of data points across levels of this factor.

Supplementary Tables

Table S1.

Search keywords and strings for the database searches.

Database	Search String
SCOPUS *(Search results: 661)*	TITLE-ABS- KEY ("rats" OR "rat" OR "mice" OR "mouse" OR "rodent*") AND (" DIO" OR "diet-induced-obesity" OR "diet-induced obesity" OR "diet induced obesity" OR "overfe*" OR "TWD" OR "HFHSD" OR "high-fat-high-sucrose" OR "high-sugar diet" OR "high sugar diet" OR "obesogenic diet" OR "HFD" OR "high-fat diet" OR "high fat diet" OR "western diet" OR "cafeteria diet" OR "dietary fat" OR "lipid diet") AND ("transgenerational effects" OR "trans-generational effects" OR "multiple generations" OR "across generations" OR "grand offspring" OR "grand-offspring" OR "F2" OR "F3" OR "F4" OR "intergenerational effects" OR "inter-generational effects" OR "2 generations" OR "3 generations") AND NOT (bovine OR sheep OR pig* OR drosophila OR cattle OR bull O R vitro OR cow OR fish) AND NOT TITLE (women OR men OR patient* OR human* OR child*) A ND (LIMIT-TO (DOCTYPE , "ar "))
ISI Web of Science Core Collection *(Search results: 100)*	(TS = (("rats" OR "rat" OR "mice" OR "mouse" OR "rodent*") AND ("DIO" OR "diet-induced-obesity" OR "diet-induced obesity" OR "diet induced obesity" OR “overfe*” OR "TWD" OR "HFHSD" OR "high-fat-high-sucrose" OR "high-sugar diet" OR "high sugar diet" OR "obesogenic diet" OR "HFD" OR "high-fat diet" OR "high fat diet" OR "western diet") AND ("transgenerational effects" OR "trans-generational effects" OR "multiple generations" OR "across generations" OR "grand offspring" OR "grand-offspring" OR "F2" OR "intergenerational effects" OR "inter-generational effects") NOT ("bovine" OR "sheep" OR "pig*" OR "drosophila" OR "cattle" OR "bull" OR “vitro” OR “cow” OR “fish”)) NOT TI= (women OR men OR patient* OR human* OR child*)) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article)
Trove *(Search results: 169)*	("rats" OR "rat" OR "mice" OR "mouse" OR "rodent*") AND ("DIO" OR "diet-induced-obesity" OR "diet-induced obesity" OR "diet induced obesity" OR “overfe*” OR "TWD" OR "HFHSD" OR "high-fat-high-sucrose" OR "high-sugar diet" OR "high sugar diet" OR "obesogenic diet" OR "HFD" OR "high-fat diet" OR "high fat diet" OR "western diet")
OpenGrey *(Search results: 36)*	("rats" OR "rat" OR "mice" OR "mouse" OR "rodent*") AND ("DIO" OR "diet-induced-obesity" OR "diet-induced obesity" OR "diet induced obesity" OR “overfe*” OR "TWD" OR "HFHSD" OR "high-fat-high-sucrose" OR "high-sugar diet" OR "high sugar diet" OR "obesogenic diet" OR "HFD" OR "high-fat diet" OR "high fat diet" OR "western diet")
ProQuest	(noft("rats") OR noft("rat") OR noft("mice") OR noft("mouse") OR

Database	Search String
(Search results: 4)	noft("rodent*")) AND (noft("DIO") OR noft("diet-induced-obesity") OR noft("diet-induced obesity") OR noft("diet induced obesity") OR noft(“overfe*”) OR noft("TWD") OR noft("HFHSD") OR noft("high-fat-high-sucrose") OR noft("high-sugar diet") OR noft("high sugar diet") OR noft("obesogenic diet") OR noft("HFD") OR noft("high-fat diet") OR noft("high fat diet") OR noft("western diet")) AND (noft("transgenerational effects") OR noft("trans-generational effects") OR noft("multiple generations") OR noft("across generations") OR noft("grand offspring") OR noft("grand-offspring") OR noft("F2") OR noft("intergenerational effects") OR noft("inter-generational effects"))
Medline (up to 2020) *(Search results: 116)*	(TS = (("rats" OR "rat" OR "mice" OR "mouse" OR "rodent*") AND ("DIO" OR "diet-induced-obesity" OR "diet-induced obesity" OR "diet induced obesity" OR “overfe*” OR "TWD" OR "HFHSD" OR "high-fat-high-sucrose" OR "high-sugar diet" OR "high sugar diet" OR "obesogenic diet" OR "HFD" OR "high-fat diet" OR "high fat diet" OR "western diet") AND ("transgenerational effects" OR "trans-generational effects" OR "multiple generations" OR "across generations" OR "grand offspring" OR "grand-offspring" OR "F2" OR "intergenerational effects" OR "inter-generational effects") NOT ("bovine" OR "sheep" OR "pig*" OR "drosophila" OR "cattle" OR "bull" OR “vitro” OR “cow” OR “fish”)) NOT TI= (women OR men OR patient* OR human* OR child*)) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article)
Embase (up to 2020) *(Search results: 78)*	((rat* OR mice OR mouse OR rodent*) AND (DIO OR diet-induced-obesity OR diet-induced obesity OR (diet induced adj3 obesity) OR overfe* OR TWD OR HFHSD OR high-fat-high-sucrose OR (high-sugar adj3 diet) OR (high sugar adj3 diet) OR (obesogenic adj3 diet) OR HFD OR (high-fat adj3 diet) OR (high fat adj3 diet) OR (western adj3 diet)) AND ((transgenerational adj3 effects) OR (trans-generational adj3 effects) OR (multiple adj3 generations) OR (across adj3 generations) OR (grand adj3 offspring) OR grand-offspring OR F2 OR (intergenerational adj3 effects) OR (inter-generational adj3 effects)) NOT (bovine OR sheep OR pig* OR drosophila OR cattle OR bull OR vitro OR cow OR fish women OR men OR patient* OR human* OR child*)).ti,ab.

Table S2.
List of studies excluded at full-text screening, with main reasons for exclusion.

Short reference	Paper Title	Main reason for exclusion
(Adams, Coon and Poling, 1974)	Insecticides in the Tissues of Four Generations of Rats Fed Different Dietary Fats Containing a Mixture of Chlorinated Hydrocarbon Insecticides	Irrelevant traits/data

Short reference	Paper Title	Main reason for exclusion
(Adedeji et al. 2019)	Dietary intake of parents affects antioxidant activity and inflammatory status in F2 offspring	Irrelevant traits/data
(Alm et al., 2017)	Grandpaternal-induced transgenerational dietary reprogramming of the unfolded protein response in skeletal muscle	Duplicated data
(Almind and Kahn, 2004)	Genetic determinants of energy expenditure and insulin resistance in diet-induced obesity in mice	Not an obesogenic diet treatment
(Benyshek, Johnston and Martin, 2004)	Post-natal diet determines insulin resistance in fetally malnourished, low birthweight rats (F1) but diet does not modify the insulin resistance of their offspring (F2).	No appropriate control group
(Billah et al., 2019)	A Novel Micronutrient Supplement to Mitigate the Transgenerational Effects of Paternal Obesity on Body Composition of Male Offspring (P11-138-19)	Poster with not enough data
(Burdge et al., 2011)	Progressive, Transgenerational Changes in Offspring Phenotype and Epigenotype following Nutritional Transition	Irrelevant traits/data
(Cai et al., 2012)	Oral advanced glycation endproducts (AGEs) promote insulin resistance and diabetes by depleting the antioxidant defenses AGE receptor-1 and sirtuin 1	Not an obesogenic diet treatment
(Chambers et al., 2015)	Does grandparents' diet affect weight and risk of hypogonadism in subsequent generations?	Poster without extractable data
(Chambers et al., 2016)	High-fat diet disrupts metabolism in two generations of rats in a parent-of-origin specific manner	Not wild-type lab rodents
(Cropley et al., 2016)	Male-lineage transmission of an acquired metabolic phenotype induced by grand-paternal obesity	Not wild-type lab rodents
(Diaz and Taylor, 1998)	Abnormally high nourishment during sensitive periods results in body weight changes across generations	Not an obesogenic diet treatment
(Dunn, 2012)	Transgenerational epigenetic effects of parental high fat diet exposure	Duplicated data
(Eaton et al., 2018)	Maternal obesity heritably perturbs offspring metabolism for three generations without serial programming	Not wild-type lab rodents
(Fullston et al., 2013)	Paternal obesity initiates metabolic disturbances in two generations of mice with incomplete penetrance to the F2 generation and alters the transcriptional profile of testis and sperm microRNA content	Duplicated data
(Gallou-Kabani et al., 2007)	Resistance to high-fat diet in the female progeny of obese mice fed a control diet during the periconceptual, gestation, and lactation periods	Not an obesogenic diet treatment
(Han et al., 2017)	Transgenerational Effects of Branched Chain Amino Acids Supplement Combined with High Fat Diet in Male Mice	No appropriate control group
(Hiramatsu et	Maternal exposure to Western diet affects adult body	Not a

Short reference	Paper Title	Main reason for exclusion
al., 2017)	composition and voluntary wheel running in a genotype-specific manner in mice	transgenerational study
(Kumazawa et al., 2007)	Searching for genetic factors of fatty liver in SMXA-5 mice by quantitative trait loci analysis under a high-fat diet	Not wild-type lab rodents
(Le et al., 2017)	Binge-like sucrose self-administration experience inhibits cocaine and sucrose seeking behavior in offspring	Not an obesogenic diet treatment
(Levin et al., 2003)	A new obesity-prone, glucose-intolerant rat strain (F.DIO)	Not wild-type lab rodents
(Marissal-Arvy et al., 2014)	QTLs influencing carbohydrate and fat choice in a LOU/CxFischer 344 F2 rat population	Not an obesogenic diet treatment
(Massiera et al., 2010)	A Western-like fat diet is sufficient to induce a gradual enhancement in fat mass over generations	No appropriate control group
(Miranda et al., 2017)	Cross-fostering reduces obesity induced by early exposure to monosodium glutamate in male rats	Not an obesogenic diet treatment
(Nguyen et al., 2017)	Maternal intake of high n-6 polyunsaturated fatty acid diet during pregnancy causes transgenerational increase in mammary cancer risk in mice	Not wild-type lab rodents
(Ogassawara et al., 2018)	Food deprivation in F0 generation and hypercaloric diet in F1 generation reduce F2 generation astrogliosis in several brain areas after immune challenge	Not a transgenerational study
(Pentinat et al., 2010)	Transgenerational inheritance of glucose intolerance in a mouse model of neonatal overnutrition	Not an obesogenic diet treatment
(Phatak et al., 2016)	Multi-Generational Effect of Western Diet on Colorectal Cancer and Impact of Green Tea on Cancer Prevention	Unpublished/Insufficient data
(Phatak et al., 2019)	Impact of the Total Western Diet for Rodents on Colon Mucosal Gene Expression in a Multigenerational Murine Model of Colitis-associated Colorectal Cancer (OR04-03-19)	Poster with not enough data
(Poutahidis et al., 2015)	Dietary microbes modulate transgenerational cancer risk	Not a transgenerational study
(Ruegsegger et al., 2017)	Maternal Western diet age-specifically alters female offspring voluntary physical activity and dopamine- and leptin-related gene expression	Rodents subjected to exercise
(Saben et al., 2016)	Maternal Metabolic Syndrome Programs Mitochondrial Dysfunction via Germline Changes across Three Generations	Irrelevant traits/data
(Sarker et al. 2019)	Maternal overnutrition programs hedonic and metabolic phenotypes across generations through sperm tsRNAs	No appropriate control group
(Skolnikova et al. 2020)	Grandmother's diet matters: Early life programming with sucrose influences metabolic and lipid parameters in second generation of rats	Not willd-type lab rodents
(Steffensen,	Obesity and Intestinal Tumorigenesis in Adult Min/ plus	Not wild-type lab

Short reference	Paper Title	Main reason for exclusion
2016)	Mice from Early-life High-fat Diet Exposure Were Not Inherited Transgenerationally	rodents
(Takasaki et al., 2012)	Continuous intake of a high-fat diet beyond one generation promotes lipid accumulation in liver and white adipose tissue of female mice	No appropriate control group
(Thakali et al., 2015)	Maternal High-Fat Diet Programs Sex-Specific Intergenerational Effects in Male and Female F1 Mouse Progeny	Unpublished/Insuffi cient data
(Uddin et al., 2016)	Head to Head Comparison of Short-Term Treatment with the NAD+Precursor Nicotinamide Mononucleotide (NMN) and 6 Weeks of Exercise in Obese Female Mice	Not a transgenerational study
(Wu, 1999)	The effects of high-fat diet feeding over generations on body fat accumulation associated with lipoprotein lipase and leptin in rat adipose tissues	Not a transgenerational study
(York, Lei and West, 1997)	Inherited non-autosomal effects on body fat in F2 mice derived from an AKR/J _ SWR/J cross	Not a transgenerational study
(Zhou et al. 2018)	Diet-Induced Paternal Obesity Impairs Cognitive Function in Offspring by Mediating Epigenetic Modifications in Spermatozoa	Irrelevant traits/data
(Zuberi et al., 2008)	Increased adiposity on normal diet, but decreased susceptibility to diet-induced obesity in mu-opioid receptor-deficient mice	Not wild-type lab rodents

Table S3

List of the main variables extracted from included studies, with descriptions.

Column	Description
Paper_ID	Unique ID assigned to each paper (first author surname combined with year of publication, e.g., Johnson2018)
Study_ID	Unique ID assigned to each lab group common to papers where major authors overlap and experiments likely overlap (coded first/corresponding/last author surnames combined)
Cohort_ID	Unique ID assigned to each cohort of Treatment offspring animals examined in generation F2 or further. The ID was formed by combining the Paper ID with the lineage code and exposure type code
Control_ID_Control	Unique ID's to identify same control animals used in different experiments
Shared_Control_Code	Unique code assigned to every control group used as comparison against treatment groups within each experiment

Column	Description
Title	Title of the original publication
Journal	Name of the journal of the original publication
Year	Publication year of the original publication
Rodent_Type	Common name of animals used in an experiment (Rat, Mouse)
Rodent_Strain	Strain of rodent species used in an experiment
Exposure_Type	One-off (only F0) or multigenerational (F0 and subsequent generations) exposure to obesogenic diet in Treatment group
F0_Parent_Exposed	Sex of F0 parent(s) exposed to an obesogenic diet
Treatment_Diet_Code	Unique ID assigned to an obesogenic rodent diet used for F0 Treatment group (manufacturer codes used, if relevant)
Treatment_Diet_Notes	Additional notes on the obesogenic diet used for F0 Treatment group, including sources of information on the composition
Treatment_Diet_Prot_pww	Percent by weight of protein in the obesogenic diet used for F0 Treatment group
Treatment_Diet_Carb_pww	Percent by weight of carbohydrates in the obesogenic diet used for F0 Treatment group
Treatment_Diet_Fat_pww	Percent by weight of fat in the obesogenic diet used for F0 Treatment group
Treatment_Diet_Energy_kcal_g	Total energy content of the obesogenic diet used for F0 Treatment group
Treatment_Diet_Prot_pE	Percent of energy from protein in the obesogenic diet used for F0 Treatment group
Treatment_Diet_Carb_pE	Percent of energy from protein in the obesogenic diet used for F0 Treatment group
Treatment_Diet_Fat_pE	Percent of energy from fat in the obesogenic diet used for F0 Treatment group
Treatment_Diet_PC_ratio	Ratio of Protein to Carbohydrate by weight in the obesogenic diet used for F0 Treatment group
Treatment_Diet_PNP_ratio	Ratio of Protein to Non-Protein (Carbohydrate and Fat) by weight in the obesogenic diet used for F0 Treatment group
Treatment_Start_F0	Start of exposure to the obesogenic diet of F0 Treatment group (in days, 0 is the day of mating)
Treatment_End_F0	End of exposure to the obesogenic diet of F0 Treatment group (in days, 0 is the day of mating)
Treatment_Duration_F0	Duration of exposure to the obesogenic diet of F0 Treatment group (in days)

Column	Description
Treatment_Duration_F0_Notes	Noytes on duration of exposure to the obesogenic diet of F0 Treatment group
Offspring_Generation	Generation of animals being examined (F2, F3)
Offspring_Sex	Sex of animals examined in generation F2 or further
Lineage_HFD	Sex lineage of the obesogenic treatment in each generation (e.g., f-f-fm, indicates female F0 parent exposed, female F1 offspring bred, and male/female F2 offspring measurements reported together as a single group/cohort)
Age_at_Measurement_days	Age at which offspring were measured (in days since birth)
Diet_at_Measurement	Type of diet being fed to measured offspring ariund the time of measurement (either HFD or Standard)
Trait	Trait category of the measured trait
Trait_Info	Details of the measured trait
Unit_of_Measurement	Units of trait measurements
Mean_Control	Mean trait value for the Control group
SD_Control	Standard deviation for the mean trait value for the Control group
SEM_Control	Standard error of the mean trait value for the Control group
Sample_Size_n_Control	Sample size for the treat measurement for the Control group
Estimated_or_Exact_SampleCcontrol	Sample size detail for the control group (whether exact sample size was reported or estimate was used based on other reported values, such as range of sample sizes)
Mean_Treatment	Mean trait value for the Treatment group
SD_Treatment	Standard deviation for the mean trait value for the Treatment group
SEM_Treatment	Standard error of the mean trait value for the Treatment group
Sample_Size_n_Treatment	Sample size for the treat measurement for the Treatment group
Estimated_or_Exact_Sample_Treatment	Sample size detail for the treatment group (whether exact sample size was reported or estimate was used based on other reported values, such as range of sample sizes)
Data_Source	Source of the extracted values for the trait measurement (figure, table or page number in the original paper)
Group_Label_Paper	Names of Treatment and Control groups, as reported in the original paper

Column	Description
Notes	Any other relevant notes and comments about paper or data extraction

Table S4

Meta-analysis models for two exposure types (One-off and Multigenerational) and two effect size types (*lnRR*, *lnCVR*). For fixed effects, we show mean intercept estimates from intercept-only models, with 95% Confidence Intervals (*CI*) and *p*-values. For random effects, we show variance components, heterogeneity (I^2) estimates and numbers of levels (N). Bold font indicates estimates with *CI* not crossing zero.

Data		Fixed effects				Random effects		
		Mean	CI.lb	CI.ub	*p*		I^2	N levels
One-off lnRR	Intercept	0.085	-0.076	0.247	0.301	Total	95.7	272
						Rodent Strain	74	6
						Study	0.3	21
						Trait	8.0	8
						Unit	13.4	272
One-off lnCVR	Intercept	0.033	-0.134	0.200	0.698	Total	63.0	272
						Rodent Strain	4.8	6
						Study	1.9	21
						Trait	3.1	8
						Unit	53.2	272
Multigenerational lnRR	**Intercept**	**0.358**	**0.096**	**0.620**	**0.007**	Total	99.2	135
						Rodent Strain	0	5
						Study	26.6	12
						Trait	38.9	8
						Unit	33.8	135
Multigenerational lnCVR	Intercept	-0.074	-0.282	0.134	0.486	Total	71.6	135
						Rodent Strain	2.7	5
						Study	0.0	12
						Trait	8.3	8
						Unit	60.6	135

Table S5

Univariate meta-regression models with exposure type as a moderator. We combined One-off and Multigenerational data and run separate models for two effect size types (*lnRR*, *lnCVR*). For fixed effects, we show mean intercept estimates and a difference between these intercepts, with 95% Confidence Intervals (*CI*) and *p*-values. We show numbers of effect sizes at each factor level (*k*) and proportion of variance explained (R^2). Bold font indicates estimates with *CI* not crossing zero.

Data	Exposure type	Mean	CI.lb	CI.ub	*k*	R^2
lnRR						0.099
	One-off	**0.115**	**0.001**	**0.229**	272	
	Multigenerational	**0.302**	**0.181**	**0.422**	135	
	One-off − Multigenerational	-0.187	-0.256	-0.118		
lnCVR						0.001
	One-off	-0.026	-0.152	0.101	272	
	Multigenerational	-0.001	-0.151	0.148	135	
	One-off − Multigenerational	-0.024	-0.173	0.124		

Table S6

Univariate meta-regression models with sex of exposed grandparents (F0) as a moderator. We run models separately for combinations of One-off and Multigenerational datasets and two effect size types (*lnRR*, *lnCVR*). For fixed effects, we show mean intercept estimates and a difference between these intercepts, with 95% Confidence Intervals (*CI*) and *p*-values. We show numbers of effect sizes at each factor level (*k*) and proportion of variance explained (R^2). Bold font indicates estimates with *CI* not crossing zero.

Data	Sex of exposed grandparents	Mean	CI.lb	CI.ub	*k*	R^2
One-off lnRR						0.001
	Females	0.090	-0.070	0.250	212	
	Males	0.073	-0.091	0.237	48	
	Both sexes	0.099	-0.074	0.272	12	
	Females – Males	-0.017	-0.065	0.032		
	Females – Both	0.009	-0.061	0.080		
	Males – Both	0.026	-0.052	0.104		
One-off lnCVR						0.006
	Females	0.031	-0.155	0.217	212	
	Males	0.020	-0.222	0.262	48	
	Both sexes	0.196	-0.198	0.590	12	
	Females – Males	-0.011	-0.234	0.212		
	Females – Both	0.165	-0.209	0.538		
	Males – Both	0.176	-0.229	0.580		
Multigenerational lnRR						0.069
	Females	**0.412**	**0.140**	**0.683**	85	
	Males	0.146	-0.183	0.475	50	
	Females – Males	**-0.266**	**-0.517**	**-0.015**		
Multigenerational lnCVR						0.017
	Females	-0.023	-0.252	0.206	85	
	Males	-0.155	-0.425	0.116	50	
	Females – Males	-0.132	-0.420	0.157		

Table S7

Univariate meta-regression models with sex of measured grand-offspring (F2, F3) as a moderator. We run models separately for combinations of One-off and Multigenerational datasets and two effect size types (*lnRR*, *lnCVR*). For fixed effects, we show mean intercept estimates and a difference between these intercepts, with 95% Confidence Intervals (*CI*) and *p*-values. We show numbers of effect sizes at each factor level (*k*) and proportion of variance explained (R^2). Bold font indicates estimates with CI not crossing zero.

Data	Sex of measured grand-offspring	Mean	CI.lb	CI.ub	*k*	R^2
One-off lnRR						0.003
	Females	0.091	-0.056	0.238	133	
	Males	0.103	-0.044	0.250	135	
	Both sexes	0.027	-0.155	0.208	4	
	Females – Males	0.012	-0.022	0.046		
	Females – Both	-0.064	-0.185	0.057		
	Males – Both	-0.076	-0.197	0.044		
One-off lnCVR						0.005
	Females	0.018	-0.173	0.208	133	
	Males	0.060	-0.128	0.247	135	
	Both sexes	-0.145	-0.726	0.436	4	
	Females – Males	0.042	-0.131	0.215		
	Females – Both	-0.162	-0.746	0.421		
	Males – Both	-0.204	-0.786	0.378		
Multigenerational lnRR						0.002
	Females	**0.373**	**0.087**	**0.658**	64	
	Males	**0.331**	**0.044**	**0.618**	58	
	Both sexes	0.364	-0.016	0.744	13	
	Females – Males	-0.042	-0.157	0.074		
	Females – Both	-0.009	-0.366	0.348		
	Males – Both	0.033	-0.328	0.394		
Multigenerational lnCVR						0.020
	Females	-0.017	-0.259	0.225	64	
	Males	-0.149	-0.388	0.089	58	
	Both sexes	0.018	-0.351	0.388	13	
	Females – Males	-0.133	-0.381	0.116		
	Females – Both	0.035	-0.337	0.407		
	Males – Both	0.168	-0.205	0.541		

Table S8

Univariate meta-regression models with trait type of measured grand-offspring (F2, F3) as a moderator. We run models separately for combinations of One-off and Multigenerational datasets and two effect size types (*lnRR*, *lnCVR*). For fixed effects, we show mean intercept estimates and a difference between these intercepts, with 95% Confidence Intervals (*CI*) and *p*-values. We show numbers of effect sizes at each factor level (*k*) and proportion of variance explained (R^2). Bold font indicates estimates with *CI* not crossing zero.

Data	Grand-offspring trait type	Mean	CI.lb	CI.ub	*k*	R^2
One-off lnRR						0.093
	Adiposity	**0.182**	**0.025**	**0.338**	54	
	Body weight	0.037	-0.114	0.187	109	
	Glucose fasting	0.019	-0.145	0.184	7	
	Glucose tolerance	0.051	-0.104	0.206	26	
	Insulin fasting	0.091	-0.072	0.254	22	
	Insulin tolerance	0.025	-0.139	0.190	12	
	Leptin	**0.205**	**0.029**	**0.381**	10	
	Triglycerides	0.124	-0.032	0.208	32	
	Adiposity – Body weight	**-0.145**	**-0.198**	**-0.092**		
	Adiposity – Glucose fasting	**-0.163**	**-0.246**	**-0.079**		
	Adiposity – Glucose tolerance	**-0.131**	**-0.192**	**-0.070**		
	Adiposity – Insulin fasting	**-0.091**	**-0.171**	**-0.011**		
	Adiposity – Insulin tolerance	**-0.156**	**-0.245**	**-0.068**		
	Adiposity – Leptin	0.023	-0.080	0.127		
	Adiposity – Triglycerides	-0.058	-0.117	0.001		
	Body weight – Glucose fasting	-0.017	-0.093	0.059		
	Body weight – Glucose tolerance	0.014	-0.031	0.060		
	Body weight – Insulin fasting	0.054	-0.014	0.123		
	Body weight – Insulin tolerance	-0.011	-0.089	0.067		
	Body weight – Leptin	**0.169**	**0.072**	**0.265**		
	Body weight – Triglycerides	**0.087**	**0.037**	**0.138**		
	Glucose fasting – Glucose tolerance	0.032	-0.049	0.113		
	Glucose fasting – Insulin fasting	0.072	-0.023	0.166		
	Glucose fasting – Insulin tolerance	0.006	-0.100	0.112		
	Glucose fasting – Leptin	**0.186**	**0.071**	**0.301**		
	Glucose fasting – Triglycerides	**0.105**	**0.020**	**0.190**		
	Glucose tolerance – Insulin fasting	0.040	-0.037	0.117		
	Glucose tolerance – Insulin tolerance	-0.025	-0.110	0.059		

Data	Grand-offspring trait type	Mean	CI.lb	CI.ub	*k*	R^2
	Glucose tolerance – Leptin	**0.154**	**0.053**	**0.256**		
	Glucose tolerance – Triglycerides	**0.073**	**0.013**	**0.133**		
	Insulin fasting – Insulin tolerance	-0.066	-0.165	0.034		
	Insulin fasting – Leptin	0.114	0.003	0.226		
	Insulin fasting – Triglycerides	0.033	-0.046	0.112		
	Insulin tolerance – Leptin	**0.180**	**0.061**	**0.299**		
	Insulin tolerance – Triglycerides	**0.099**	**0.011**	**0.186**		
	Leptin – Triglycerides	-0.081	-0.184	0.021		
One-off lnCVR						0.064
	Adiposity	-0.079	-0.288	0.130	54	
	Body weight	0.041	-0.123	0.204	109	
	Glucose fasting	0.208	-0.178	0.594	7	
	Glucose tolerance	0.208	-0.035	0.451	26	
	Insulin fasting	-0.210	-0.470	0.049	22	
	Insulin tolerance	0.255	-0.078	0.589	12	
	Leptin	0.039	-0.309	0.387	10	
	Triglycerides	0.012	-0.219	0.242	32	
	Adiposity – Body weight	0.119	-0.061	0.300		
	Adiposity – Glucose fasting	0.287	-0.107	0.680		
	Adiposity – Glucose tolerance	**0.287**	**0.041**	**0.533**		
	Adiposity – Insulin fasting	-0.132	-0.404	0.141		
	Adiposity – Insulin tolerance	0.334	-0.003	0.671		
	Adiposity – Leptin	0.118	-0.243	0.478		
	Adiposity – Triglycerides	0.091	-0.133	0.314		
	Body weight – Glucose fasting	0.167	-0.204	0.539		
	Body weight – Glucose tolerance	0.167	-0.048	0.383		
	Body weight – Insulin fasting	**-0.251**	**-0.487**	**-0.014**		
	Body weight – Insulin tolerance	0.215	-0.102	0.532		
	Body weight – Leptin	-0.002	-0.343	0.340		
	Body weight – Triglycerides	-0.029	-0.232	0.175		
	Glucose fasting – Glucose tolerance	0.000	-0.406	0.406		
	Glucose fasting – Insulin fasting	-0.418	-0.836	0.000		
	Glucose fasting – Insulin tolerance	0.047	-0.426	0.521		
	Glucose fasting – Leptin	-0.169	-0.654	0.316		
	Glucose fasting – Triglycerides	-0.196	-0.602	0.209		
	Glucose tolerance – Insulin fasting	**-0.418**	**-0.716**	**-0.121**		

Data	Grand-offspring trait type	Mean	CI.lb	CI.ub	*k*	R^2
	Glucose tolerance – Insulin tolerance	0.047	-0.313	0.408		
	Glucose tolerance – Leptin	-0.169	-0.552	0.213		
	Glucose tolerance – Triglycerides	-0.196	-0.460	0.068		
	Insulin fasting – Insulin tolerance	**0.466**	**0.092**	**0.84**		
	Insulin fasting – Leptin	0.249	-0.142	0.641		
	Insulin fasting – Triglycerides	0.222	-0.063	0.508		
	Insulin tolerance – Leptin	-0.216	-0.659	0.226		
	Insulin tolerance – Triglycerides	-0.244	-0.596	0.109		
	Leptin – Triglycerides	-0.027	-0.398	0.344		
Multigenerational lnRR						0.370
	Adiposity	**0.794**	**0.578**	**1.010**	19	
	Body weight	0.100	-0.062	0.262	64	
	Glucose fasting	0.082	-0.154	0.319	10	
	Glucose tolerance	0.134	-0.120	0.389	9	
	Insulin fasting	**0.457**	**0.138**	**0.776**	7	
	Insulin tolerance	0.171	-0.095	0.438	9	
	Leptin	**0.872**	**0.577**	**1.168**	7	
	Triglycerides	**0.340**	**0.088**	**0.592**	10	
	Adiposity – Body weight	**-0.694**	**-0.873**	**-0.514**		
	Adiposity – Glucose fasting	**-0.711**	**-0.951**	**-0.472**		
	Adiposity – Glucose tolerance	**-0.659**	**-0.923**	**-0.396**		
	Adiposity – Insulin fasting	**-0.337**	**-0.657**	**-0.017**		
	Adiposity – Insulin tolerance	**-0.623**	**-0.901**	**-0.345**		
	Adiposity – Leptin	0.078	-0.209	0.366		
	Adiposity – Triglycerides	**-0.454**	**-0.701**	**-0.207**		
	Body weight – Glucose fasting	-0.018	-0.226	0.190		
	Body weight – Glucose tolerance	0.034	-0.182	0.250		
	Body weight – Insulin fasting	**0.357**	**0.064**	**0.649**		
	Body weight – Insulin tolerance	0.071	-0.161	0.303		
	Body weight – Leptin	**0.772**	**0.501**	**1.043**		
	Body weight – Triglycerides	**0.240**	**0.019**	**0.461**		
	Glucose fasting – Glucose tolerance	0.052	-0.235	0.339		
	Glucose fasting – Insulin fasting	**0.374**	**0.028**	**0.721**		
	Glucose fasting – Insulin tolerance	0.089	-0.211	0.388		

Data	Grand-offspring trait type	Mean	CI.lb	CI.ub	*k*	R^2
	Glucose fasting – Leptin	**0.790**	**0.473**	**1.106**		
	Glucose fasting – Triglycerides	0.258	-0.013	0.529		
	Glucose tolerance – Insulin fasting	0.322	-0.029	0.674		
	Glucose tolerance – Insulin tolerance	0.037	-0.238	0.311		
	Glucose tolerance – Leptin	**0.738**	**0.410**	**1.066**		
	Glucose tolerance – Triglycerides	0.206	-0.086	0.497		
	Insulin fasting – Insulin tolerance	-0.286	-0.650	0.078		
	Insulin fasting – Leptin	**0.415**	**0.050**	**0.781**		
	Insulin fasting – Triglycerides	-0.117	-0.464	0.23		
	Insulin tolerance – Leptin	**0.701**	**0.360**	**1.042**		
	Insulin tolerance – Triglycerides	0.169	-0.136	0.474		
	Leptin – Triglycerides	**-0.532**	**-0.847**	**-0.217**		
Multigenerational lnCVR						0.166
	Adiposity intercept	-0.013	-0.299	0.273	19	
	Body weight intercept	0.147	-0.007	0.300	64	
	Glucose fasting	-0.179	-0.537	0.180	10	
	Glucose tolerance	0.021	-0.406	0.448	9	
	Insulin fasting	-0.329	-0.788	0.129	7	
	Insulin tolerance	0.237	-0.185	0.660	9	
	Leptin	-0.427	-0.863	0.009	7	
	Triglycerides	**-0.388**	**-0.756**	**-0.020**	10	
	Adiposity – Body weight	0.159	-0.149	0.467		
	Adiposity – Glucose fasting	-0.166	-0.608	0.276		
	Adiposity – Glucose tolerance	0.034	-0.464	0.532		
	Adiposity – Insulin fasting	-0.317	-0.835	0.202		
	Adiposity – Insulin tolerance	0.250	-0.247	0.747		
	Adiposity – Leptin	-0.415	-0.906	0.077		
	Adiposity – Triglycerides	-0.375	-0.814	0.064		
	Body weight – Glucose fasting	-0.325	-0.702	0.051		
	Body weight – Glucose tolerance	-0.125	-0.556	0.305		
	Body weight – Insulin fasting	**-0.476**	**-0.938**	**-0.014**		
	Body weight – Insulin tolerance	0.091	-0.336	0.517		
	Body weight – Leptin	**-0.574**	**-1.023**	**-0.125**		
	Body weight – Triglycerides	**-0.534**	**-0.914**	**-0.155**		
	Glucose fasting – Glucose tolerance	0.200	-0.351	0.750		

Data	Grand-offspring trait type	Mean	CI.lb	CI.ub	*k*	R^2
	Glucose fasting – Insulin fasting	-0.151	-0.722	0.421		
	Glucose fasting – Insulin tolerance	0.416	-0.132	0.964		
	Glucose fasting – Leptin	-0.249	-0.794	0.297		
	Glucose fasting – Triglycerides	-0.209	-0.701	0.282		
	Glucose tolerance – Insulin fasting	-0.350	-0.961	0.260		
	Glucose tolerance – Insulin tolerance	0.216	-0.323	0.755		
	Glucose tolerance – Leptin	-0.448	-1.035	0.138		
	Glucose tolerance – Triglycerides	-0.409	-0.949	0.131		
	Insulin fasting – Insulin tolerance	0.567	-0.042	1.176		
	Insulin fasting – Leptin	-0.098	-0.706	0.510		
	Insulin fasting – Triglycerides	-0.059	-0.629	0.512		
	Insulin tolerance – Leptin	**-0.665**	**-1.253**	**-0.076**		
	Insulin tolerance – Triglycerides	**-0.625**	**-1.168**	**-0.082**		
	Leptin – Triglycerides	0.039	-0.500	0.579		

Table S9

Multivariate meta-regression (full) models with sex of exposed grandparents, sex of measured grand-offspring and trait type of measured grand-offspring as moderators. We run models separately for combinations of One-off and Multigenerational datasets and two effect size types (*lnRR*, *lnCVR*). For mean fixed effects estimates, we show 95% Confidence Intervals (*CI*) and *p*-values. We show numbers of effect sizes in dataset (*k*) and proportion of variance explained by the model (R^2). Bold font indicates estimates with *CI* not crossing zero.

Data	Fixed effects	Mean	CI.lb	CI.ub	*k*	R^2
One-off lnRR					272	0.099
	Grandparents both sexes exposed, Grand-offspring both sexes measured, Adiposity (intercept)	0.129	-0.065	0.324		
	Sex of exposed grandparents: Both - Female	-0.007	-0.081	0.067		
	Sex of exposed grandparents: Both - Male	-0.032	-0.113	0.050		
	Sex of grand-offsprings: Both - Female	0.076	-0.053	0.205		
	Sex of grand-offsprings: Both - Male	0.087	-0.041	0.215		
	Trait: Adiposity – Body weight	**-0.147**	**-0.200**	**-0.093**		
	Trait: Adiposity – Glucose fasting	**-0.165**	**-0.251**	**-0.078**		
	Trait: Adiposity – Glucose tolerance	**-0.134**	**-0.196**	**-0.072**		
	Trait: Adiposity – Insulin fasting	**-0.093**	**-0.174**	**-0.011**		
	Trait: Adiposity – Insulin tolerance	**-0.161**	**-0.251**	**-0.071**		
	Trait: Adiposity – Leptin	0.016	-0.089	0.121		
	Trait: Adiposity – Triglycerides	-0.060	-0.119	0.000		
One-off lnCVR					272	0.001
	Grandparents both sexes exposed, Grand-offspring both sexes measured, Adiposity (intercept)	-0.105	-0.828	0.618		
	Sex of exposed grandparents: Both – Female	-0.174	-0.555	0.208		
	Sex of exposed grandparents: Both – Male	-0.178	-0.589	0.233		
	Sex of grand-offsprings: Both – Female	0.175	-0.417	0.768		
	Sex of grand-offsprings: Both – Male	0.224	-0.368	0.816		
	Trait: Adiposity – Body weight	0.119	-0.063	0.301		
	Trait: Adiposity – Glucose fasting	0.289	-0.107	0.684		
	Trait: Adiposity – Glucose tolerance	**0.291**	**0.044**	**0.537**		
	Trait: Adiposity – Insulin fasting	-0.140	-0.414	0.134		
	Trait: Adiposity – Insulin tolerance	**0.350**	**0.009**	**0.690**		
	Trait: Adiposity – Leptin	0.118	-0.244	0.481		
	Trait: Adiposity – Triglycerides	0.094	-0.130	0.318		

Data	Fixed effects	Mean	CI.lb	CI.ub	*k*	R^2
Multigenerational lnRR					135	0.096
	Grandparents female sex exposed, Grand-offspring both sexes measured, Adiposity (intercept)	**0.800**	**0.443**	**1.157**		
	Sex of exposed grandparents: Female - Male	-0.253	-0.517	0.010		
	Sex of grand-offsprings: Both - Female	0.070	-0.299	0.438		
	Sex of grand-offsprings: Both - Male	0.044	-0.333	0.421		
	Trait: Adiposity – Body weight	**-0.673**	**-0.854**	**-0.492**		
	Trait: Adiposity – Glucose fasting	**-0.697**	**-0.939**	**-0.456**		
	Trait: Adiposity – Glucose tolerance	**-0.667**	**-0.930**	**-0.405**		
	Trait: Adiposity – Insulin fasting	-0.289	-0.611	0.033		
	Trait: Adiposity – Insulin tolerance	**-0.617**	**-0.894**	**-0.340**		
	Trait: Adiposity – Leptin	0.047	-0.240	0.335		
	Trait: Adiposity – Triglycerides	**-0.473**	**-0.720**	**-0.225**		
Multigenerational lnCVR					135	0.001
	Grandparents female sex exposed, Grand-offspring both sexes measured, Adiposity (intercept)	0.086	-0.366	0.538		
	Sex of exposed grandparents: Female - Male	-0.076	-0.366	0.215		
	Sex of grand-offsprings: Both - Female	0.003	-0.392	0.398		
	Sex of grand-offsprings: Both - Male	-0.116	-0.523	0.291		
	Trait: Adiposity – Body weight	0.130	-0.184	0.445		
	Trait: Adiposity – Glucose fasting	-0.180	-0.636	0.276		
	Trait: Adiposity – Glucose tolerance	-0.001	-0.505	0.504		
	Trait: Adiposity – Insulin fasting	-0.322	-0.844	0.200		
	Trait: Adiposity – Insulin tolerance	0.216	-0.289	0.720		
	Trait: Adiposity – Leptin	-0.429	-0.924	0.066		
	Trait: Adiposity – Triglycerides	-0.380	-0.821	0.061		

Table S10

Model selection analyses for multivariate meta-regression models with sex of exposed grandparents, sex of measured grand-offspring and trait type of measured grand-offspring as moderators. *K* is the number of parameters in the model including the intercept and the residual error estimates, LogLik is Log Likelihood, AICc is Akaike Information Criteria with correction for small sample sizes; ΔAIC is the difference between model, *i*, and the top model; weight stands for model weights.

Data	Model: Fixed effects	*K*	logLik	AICc	ΔAIC	weight
One-off lnRR						
	Trait	11	150.42	-277.83	0.00	0.69
	Trait + Offspring Sex	13	151.33	-275.25	2.58	0.19
	Trait + F0 Parent Exposed	13	150.66	-273.91	3.92	0.10
	Trait + Offspring Sex + F0 Parent Exposed	15	151.68	-271.48	6.35	0.03
	Intercept-only (no moderators)	4	129.82	-251.50	26.33	0
	Offspring Sex	6	131.32	-250.33	27.50	0
	F0 Parent Exposed	6	129.84	-247.36	30.47	0
	F0 Parent Exposed + Offspring Sex	8	131.39	-246.23	31.60	0
One-off lnCVR						
	Intercept-only (no moderators)	4	-209.35	426.84	0.00	0.53
	Trait	11	-202.89	428.8	1.95	0.20
	F0 Parent Exposed	6	-209.05	430.42	3.57	0.09
	Offspring Sex	6	-209.09	430.5	3.65	0.09
	Trait + Offspring Sex	13	-202.4	432.22	5.37	0.04
	Trait + F0 Parent Exposed	13	-202.42	432.25	5.40	0.04
	F0 Parent Exposed + Offspring Sex	8	-208.82	434.18	7.34	0.01
	Trait + Offspring Sex + F0 Parent Exposed	15	-202.00	435.87	9.03	0.01
Multigenerational lnRR						
	Trait + F0 Parent Exposed	12	-53.44	133.43	0.00	0.61
	Trait	11	-55.38	134.92	1.48	0.29
	Trait + Offspring Sex + F0 Parent Exposed	14	-53.18	137.87	4.43	0.07
	Trait + Offspring Sex	13	-55.10	139.21	5.78	0.03
	F0 Parent Exposed	5	-86.21	182.89	49.46	0
	F0 Parent Exposed + Offspring Sex	7	-85.45	185.79	52.35	0
	Intercept-only (no moderators)	4	-89.44	187.18	53.75	0
	Offspring Sex	6	-88.96	190.58	57.14	0
Multigenerational lnCVR						
	Trait	11	-111.92	247.99	0.00	0.30

Data	Model: Fixed effects	*K*	logLik	AICc	ΔAIC	weight
	Intercept-only (no moderators)	4	-120.12	248.55	0.57	0.23
	F0 Parent Exposed	5	-119.33	249.13	1.14	0.17
	Trait + F0 Parent Exposed	12	-111.57	249.70	1.71	0.13
	Offspring Sex	6	-119.19	251.04	3.06	0.07
	Trait + Offspring Sex	13	-111.25	251.51	3.52	0.05
	F0 Parent Exposed + Offspring Sex	7	-118.69	252.27	4.28	0.04
	Trait + Offspring Sex + F0 Parent Exposed	14	-111.08	253.67	5.68	0.02

Table S11

Multilevel-model version of Egger's regression with sampling variance (sqrt(VlnRR)) included in a full meta-regression model. We run models separately for combinations of One-off and Multigenerational datasets and two effect size types (*lnRR*, *lnCVR*). For fixed effects, we show mean intercept estimates and a difference between these intercepts, with 95% Confidence Intervals (*CI*) and *p*-values. We show proportion of variance explained (R^2) by each model. Bold font indicates estimates with *CI* not crossing zero.

Data	Fixed effects	Mean	CI.lb	CI.ub	*p*	R^2
One-off lnRR						0.126
	Grandparents both sexes exposed, Grand-offspring both sexes measured, Adiposity (intercept)	0.136	-0.064	0.337	0.181	
	sqrt(VlnRR)	-0.062	-0.451	0.327	0.754	
	Sex of exposed grandparents: Both - Female	-0.006	-0.081	0.068	0.865	
	Sex of exposed grandparents: Both - Male	-0.032	-0.114	0.051	0.448	
	Sex of grand-offspring: Both - Female	0.077	-0.053	0.207	0.244	
	Sex of grand-offspring: Both - Male	0.088	-0.041	0.218	0.181	
	Trait: Adiposity – Body weight	**-0.152**	**-0.214**	**-0.089**	**0.000**	
	Trait: Adiposity – Glucose fasting	**-0.169**	**-0.261**	**-0.077**	**0.000**	
	Trait: Adiposity – Glucose tolerance	**-0.137**	**-0.203**	**-0.071**	**0.000**	
	Trait: Adiposity – Insulin fasting	**-0.093**	**-0.175**	**-0.011**	**0.026**	
	Trait: Adiposity – Insulin tolerance	**-0.164**	**-0.255**	**-0.072**	**0.001**	
	Trait: Adiposity – Leptin	0.016	-0.090	0.122	0.772	
	Trait: Adiposity – Triglycerides	-0.061	-0.122	0.000	0.050	
One-off lnCVR						0.103
	Grandparents both sexes exposed, Grand-offspring both sexes measured, Adiposity (intercept)	-0.430	-1.242	0.382	0.298	
	sqrt(VlnRR)	0.834	-0.059	1.727	0.067	
	Sex of exposed grandparents: Both - Female	-0.192	-0.572	0.189	0.322	
	Sex of exposed grandparents: Both - Male	-0.189	-0.599	0.221	0.365	

Data	Fixed effects	Mean	CI.lb	CI.ub	*p*	R^2
	Sex of grand-offspring: Both - Female	0.167	-0.426	0.760	0.579	
	Sex of grand-offspring: Both - Male	0.230	-0.363	0.823	0.446	
	Trait: Adiposity – Body weight	**0.225**	**0.012**	**0.437**	**0.038**	
	Trait: Adiposity – Glucose fasting	0.326	-0.070	0.722	0.106	
	Trait: Adiposity – Glucose tolerance	**0.328**	**0.080**	**0.577**	**0.010**	
	Trait: Adiposity – Insulin fasting	-0.118	-0.392	0.156	0.399	
	Trait: Adiposity – Insulin tolerance	**0.380**	**0.039**	**0.721**	**0.029**	
	Trait: Adiposity – Leptin	0.173	-0.193	0.539	0.352	
	Trait: Adiposity – Triglycerides	0.12	-0.105	0.345	0.295	
Multigenerational lnRR						0.420
	Grandparents female sex exposed, Grand-offspring both sexes measured, Adiposity (intercept)	**0.517**	**0.084**	**0.951**	**0.020**	
	sqrt(VlnRR)	**1.605**	**0.510**	**2.700**	**0.004**	
	Sex of exposed grandparents: Female - Male	-0.269	-0.546	0.008	0.057	
	Sex of grand-offspring: Both - Female	0.104	-0.295	0.502	0.608	
	Sex of grand-offspring: Both - Male	0.092	-0.317	0.501	0.657	
	Trait: Adiposity – Body weight	**-0.513**	**-0.727**	**-0.299**	**0.000**	
	Trait: Adiposity – Glucose fasting	**-0.554**	**-0.819**	**-0.288**	**0.000**	
	Trait: Adiposity – Glucose tolerance	**-0.579**	**-0.854**	**-0.304**	**0.000**	
	Trait: Adiposity – Insulin fasting	**-0.353**	**-0.685**	**-0.022**	**0.037**	
	Trait: Adiposity – Insulin tolerance	**-0.602**	**-0.885**	**-0.318**	**0.000**	
	Trait: Adiposity – Leptin	0.023	-0.270	0.317	0.876	
	Trait: Adiposity – Triglycerides	**-0.488**	**-0.741**	**-0.236**	**0.000**	
Multigenerational lnCVR						0.209

Data	Fixed effects	Mean	CI.lb	CI.ub	*p*	R^2
	Grandparents female sex exposed, Grand-offspring both sexes measured, Adiposity (intercept)	0.530	-0.157	1.217	0.129	
	sqrt(VlnRR)	-1.175	-2.513	0.163	0.085	
	Sex of exposed grandparents: Female - Male	-0.117	-0.465	0.230	0.505	
	Sex of grand-offspring: Both - Female	0.092	-0.320	0.504	0.658	
	Sex of grand-offspring: Both - Male	-0.083	-0.498	0.331	0.692	
	Trait: Adiposity – Body weight	-0.021	-0.376	0.333	0.905	
	Trait: Adiposity – Glucose fasting	-0.287	-0.760	0.186	0.232	
	Trait: Adiposity – Glucose tolerance	0.030	-0.484	0.544	0.909	
	Trait: Adiposity – Insulin fasting	-0.346	-0.875	0.182	0.197	
	Trait: Adiposity – Insulin tolerance	0.227	-0.286	0.740	0.382	
	Trait: Adiposity – Leptin	-0.483	-0.985	0.018	0.059	
	Trait: Adiposity – Triglycerides	-0.438	-0.884	0.009	0.055	

Table S12

Univariate meta-regression models with (scaled) year of publication as a moderator. We run models separately for combinations of One-off and Multigenerational datasets and two effect size types (*lnRR*, *lnCVR*). We show mean intercept estimates for the model intercept and slope for publication year, with 95% Confidence Intervals (*CI*) and *p*-values. We show proportion of variance explained (R^2) by each model. Bold font indicates estimates with *CI* not crossing zero.

Data	Publication year	Mean	CI.lb	CI.ub	*p*	R^2
One-off lnRR						0.001
	Intercept	0.087	-0.067	0.240	0.268	
	Slope	0.007	-0.010	0.025	0.406	
One-off lnCVR						0.005
	Intercept	0.040	-0.133	0.213	0.652	
	Slope	-0.032	-0.125	0.062	0.507	
Multigenerational lnRR						0.059
	Intercept	**0.354**	**0.073**	**0.636**	**0.014**	
	Slope	0.118	-0.028	0.263	0.113	
Multigenerational lnCVR						0.049
	Intercept	-0.071	-0.251	0.109	0.440	
	Slope	-0.109	-0.232	0.014	0.084	

Table S13

Univariate meta-regression models with (scaled) energy content of obesogenic diets as a moderator. We run models separately for combinations of One-off and Multigenerational datasets and two effect size types (*lnRR*, *lnCVR*). We show mean intercept estimates for the model intercept and slope for the diet energy, with 95% Confidence Intervals (*CI*) and *p*-values. We show proportion of variance explained (R^2) by each model. Bold font indicates estimates with *CI* not crossing zero.

Data	Obesogenic diet total energy	Mean	CI.lb	CI.ub	*p*	R^2
One-off lnRR						0.000
	Intercept	0.082	-0.083	0.247	0.331	
	Slope	-0.004	-0.027	0.019	0.732	
One-off lnCVR						0.001
	Intercept	0.027	-0.144	0.198	0.759	
	Slope	-0.014	-0.119	0.092	0.800	
Multigenerational lnRR						0.153
	Intercept	**0.347**	**0.088**	**0.606**	**0.009**	
	Slope	**0.188**	**0.062**	**0.313**	**0.003**	
Multigenerational lnCVR						0.018
	Intercept	-0.072	-0.294	0.150	0.527	
	Slope	-0.068	-0.224	0.087	0.389	

Table S14

Univariate meta-regression models with (scaled) relative protein content (protein to non-protein ratio - by weight) of obesogenic diets as a moderator. We run models separately for combinations of One-off and Multigenerational datasets and two effect size types (*lnRR*, *lnCVR*). We show mean intercept estimates for the model intercept and slope for the diet protein, with 95% Confidence Intervals (*CI*) and *p*-values. We show proportion of variance explained (R^2) by each model. Bold font indicates estimates with *CI* not crossing zero.

Data	Obesogenic diet relative protein content	Mean	CI.lb	CI.ub	*p*	R^2
One-off lnRR						0.001
	Intercept	0.085	-0.074	0.244	0.295	
	Slope	0.006	-0.017	0.028	0.629	
One-off lnCVR						0.000
	Intercept	0.036	-0.138	0.210	0.684	
	Slope	-0.008	-0.123	0.108	0.897	
Multigenerational lnRR						0.020
	Intercept	**0.367**	**0.102**	**0.632**	**0.007**	
	Slope	0.068	-0.077	0.213	0.361	
Multigenerational lnCVR						0.006
	Intercept	-0.073	-0.292	0.147	0.517	
	Slope	-0.040	-0.184	0.104	0.585	

Table S15

Univariate meta-regression models with (scaled) grandparental exposure duration to obesogenic diets as a moderator. We run models separately for combinations of One-off and Multigenerational datasets and two effect size types (*lnRR*, *lnCVR*). We show mean intercept estimates for the model intercept and slope for the exposure duration, with 95% Confidence Intervals (*CI*) and *p*-values. We show proportion of variance explained (R^2) for each model. Bold font indicates estimates with *CI* not crossing zero.

Data	Obesogenic diet relative protein content	Mean	CI.lb	CI.ub	*p*	R^2
One-off lnRR						0.002
	Intercept	0.083	-0.075	0.241	0.301	
	Slope	-0.008	-0.029	0.012	0.413	
One-off lnCVR						0.015
	Intercept	0.015	-0.145	0.174	0.856	
	Slope	-0.053	-0.162	0.055	0.336	
Multigenerational lnRR						0.017
	Intercept	**0.354**	**0.085**	**0.623**	**0.010**	
	Slope	0.063	-0.069	0.194	0.351	
Multigenerational lnCVR						0.000
	Intercept	-0.073	-0.288	0.143	0.508	
	Slope	-0.003	-0.127	0.121	0.964	

Table S16

Univariate meta-regression models with grand-offspring generation as a moderator. We run models separately for combinations of One-off and Multigenerational datasets and two effect size types (*lnRR*, *lnCVR*). For categorical moderator, we show mean intercept estimates and a difference between these intercepts, with 95% Confidence Intervals (*CI*). We show numbers of effect sizes at each factor level (*k*) and proportion of variance explained (R^2) for each model. Bold font indicates estimates with *CI* not crossing zero.

Data	Grand-offspring generation	Mean	CI.lb	CI.ub	*k*	R^2
One-off lnRR						0.004
	F2	0.091	-0.072	0.254	82	
	F3	0.059	-0.110	0.227	325	
	F2 – F3	-0.032	-0.082	0.017		
One-off lnCVR						0.020
	F2	0.060	-0.102	0.223	82	
	F3	-0.087	-0.323	0.149	325	
	F2 – F3	-0.147	-0.374	0.079		
Multigenerational lnRR						0.009
	F2	0.378	0.115	0.640	82	
	F3	0.263	-0.038	0.565	325	
	F2 – F3	-0.114	-0.299	0.07		
Multigenerational lnCVR						0.002
	F2	-0.084	-0.304	0.136	82	
	F3	-0.025	-0.350	0.301	325	
	F2 – F3	0.059	-0.249	0.368		

Table S17

Univariate meta-regression models with (scaled) age at measurement as a moderator for body weights of grandparents and gran-offspring. We run models separately for grandparents, and grand-offspring form One-off and Multigenerational datasets. We only investigated effects on mean body weight values (*lnRR*). We show mean intercept estimates and a slope for the effect of age, with 95% Confidence Intervals (*CI*), *p*-values and proportion of variance explained (R^2) for each model. Bold font indicates estimates with *CI* not crossing zero.

Data	Age at measurement	Mean	CI.lb	CI.ub	*p*	R^2
Grandparents body weight lnRR						0.220
	Intercept	0.009	-0.093	0.110	0.870	
	Slope	**0.001**	**0.001**	**0.002**	**0.000**	
OF grand-offspring body weight lnRR						0.006
	Intercept	0.056	-0.006	0.118	0.076	
	Slope	0.000	0.000	0.000	0.272	
MG grand-offspring body weight lnRR						0.062
	Intercept	0.060	-0.080	0.201	0.401	
	Slope	**0.001**	**0.000**	**0.001**	**0.000**	

Table S18

Predicted differences (*lnRR*) in body weights of grandparents and grand-offspring from One-off and Multigenerational datasets, at 100 days age. We show mean estimates, with Standard Errors (SE), 95% Confidence Intervals (*CI*), and 95% Prediction/Credibility Intervals (PI).

Data	Mean	SE	CI.lb	CI.ub	PI.lb	PI.ub
lnRR for predicted grandparents body weights	0.139	0.040	0.062	0.216	-0.105	0.383
lnRR for One-off grand-offspring predicted body weights	0.067	0.030	0.008	0.126	-0.089	0.223
lnRR for Multigenerational grand-offspring predicted body weights	0.156	0.070	0.020	0.293	-0.331	0.644

Table S19

Univariate meta-regression models with rodent type (mouse, rat) as a moderator. We run models separately for combinations of One-off and Multigenerational datasets and two effect size types (*lnRR*, *lnCVR*). For fixed effects, we show mean intercept estimates and a difference between these intercepts, with 95% Confidence Intervals (*CI*). We show numbers of effect sizes at each factor level (*k*) and proportion of variance explained (R^2). Bold font indicates estimates with CI not crossing zero.

Data	Rodent type	Mean	CI.lb	CI.ub	*k*	R^2
One-off lnRR						0.138
	Mouse	-0.015	-0.258	0.228	177	
	Rat	0.170	-0.060	0.398	95	
	Mouse - Rat	0.184	-0.144	0.512		
One-off lnCVR						0.094
	Mouse	-0.116	-0.253	0.020	177	
	Rat	**0.157**	**0.003**	**0.311**	95	
	Mouse – Rat	**0.273**	**0.097**	**0.449**		
Multigenerational lnRR						0.011
	Mouse	**0.411**	**0.953**	**0.726**	65	
	Rat	**0.311**	**0.007**	**0.614**	70	
	Rat - Mouse	-0.100	-0.421	0.221		
Multigenerational lnCVR						0.048
	Mouse	0.047	-0.189	0.282	65	
	Rat	-0.170	-0.384	0.046	70	
	Rat - Mouse	-0.216	-0.464	0.032		

Table S20

Univariate meta-regression models with sex of exposed grandparents (F0) as a moderator. We run models separately for combinations of One-off and Multigenerational datasets and two effect size types (*lnRR*, *lnCVR*). Data is subsetted for F0 animals that were only exposed to an obesogenic diet ***before*** mating / pregnancy. For fixed effects, we show mean intercept estimates and a difference between these intercepts, with 95% Confidence Intervals (*CI*). We show numbers of effect sizes at each factor level (*k*) and proportion of variance explained (R^2). Bold font indicates estimates with *CI* not crossing zero. Note: for multigenerational data, *k*=8 for females, from one study; and *k*=50 for males, from only 4 studies; for one-off data, *k*=34 for females from 3 studies; and *k*=43 for males from 5 studies

Data	Sex of exposed grandparents	Mean	CI.lb	CI.ub	*k*	R^2
One-off lnRR						0.000
	Female	0.068	-0.007	0.142	34	
	Male	0.065	-0.002	0.131	43	
	Female – Male	-0.003	-0.058	0.051		
One-off lnCVR						0.031
	Female	0.069	-0.204	0.343	34	
	Male	-0.049	-0.267	0.168	43	
	Female - Male	-0.119	-0.409	0.171		
Multigenerational lnRR						0.078
	Female	**0.517**	**0.291**	**0.742**	8	
	Male	**0.317**	**0.142**	**0.492**	50	
	Female – Male	**-0.200**	**-0.390**	**-0.009**		
Multigenerational lnCVR						0.010
	Females	-0.259	-0.880	0.363	8	
	Males	-0.143	-0.423	0.139	50	
	Females – Males	0.116	-0.494	0.725		

Table S21

Univariate meta-regression models with period during which females were exposed to the obesogenic diet treatment as a moderator. We run models separately for combinations of One-off and Multigenerational datasets and two effect size types (*lnRR*, *lnCVR*). Data is subsetted to include only F0 females. For fixed effects, we show mean intercept estimates and a difference between these intercepts, with 95% Confidence Intervals (*CI*). We show numbers of effect sizes at each factor level (*k*) and proportion of variance explained (R^2). Bold font indicates estimates with *CI* not crossing zero.

Data	Exposure period	Mean	CI.lb	CI.ub	*k*	R^2
One-off lnRR						0.000
	Before mating (BP)	0.093	-0.089	0.274	34	
	Before, during and after mating (BDA)	0.106	-0.062	0.273	175	
	During and after mating (DA)	0.106	-0.062	0.274	20	
	BP – BDA	0.013	-0.067	0.093		
	BP - DA	0.014	-0.067	0.094		
	BDA – DA	0.001	-0.012	0.014		
One-off lnCVR						0.020
	Before mating	0.200	-0.116	0.516	34	
	Before, during and after mating	0.015	-0.179	0.210	175	
	During and after mating	0.039	-0.195	0.273	20	
	BP - BDA	-0.185	-0.501	0.131		
	BP - DA	-0.161	-0.502	0.179		
	BDA - DA	0.023	-0.132	0.179		
Multigenerational lnRR						0.000
	Before mating	0.459	-0.322	1.241	8	
	Before, during and after mating	**0.426**	**0.032**	**0.820**	77	
	During and after mating	**0.426**	**0.032**	**0.820**	9	
	BP - BDA	-0.033	-0.322	1.241		
	BP - DA	-0.034	-0.810	0.742		
	BDA - DA	-0.000	-0.020	0.020		
Multigenerational lnCVR						0.055
	Before mating	-0.507	-1.213	0.200	8	
	Before, during and after mating	0.012	-0.257	0.280	77	
	During and after mating	0.019	-0.275	0.313	9	
	BP - BDA	0.518	-0.204	1.240		
	BP - DA	0.526	-0.207	1.258		
	BDA- DA	0.008	-0.123	0.138		

Supplementary Figures

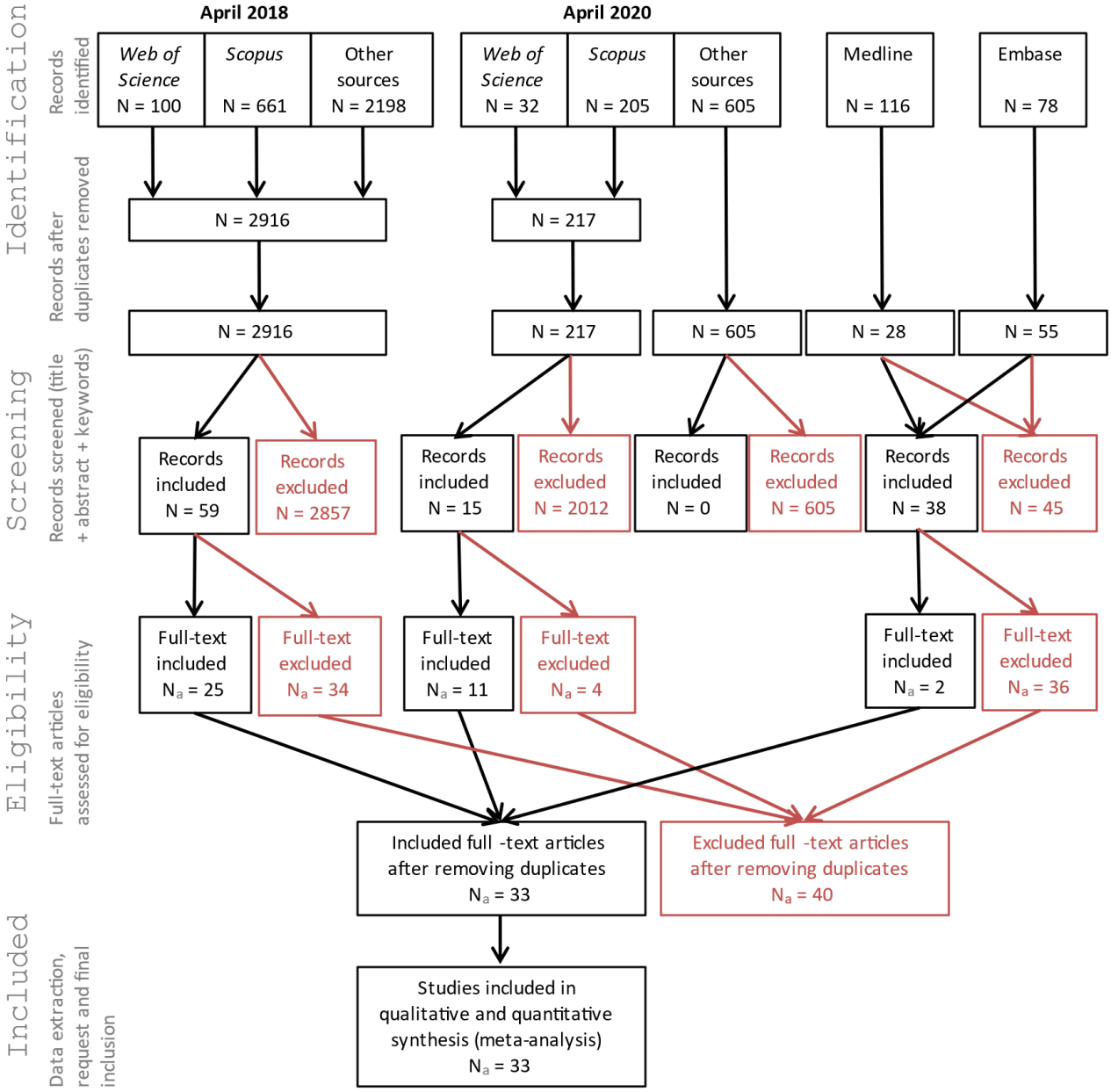

Figure S1

PRISMA flow diagram of literature search and screening process. N = number of references, N_a = number of full-text articles.

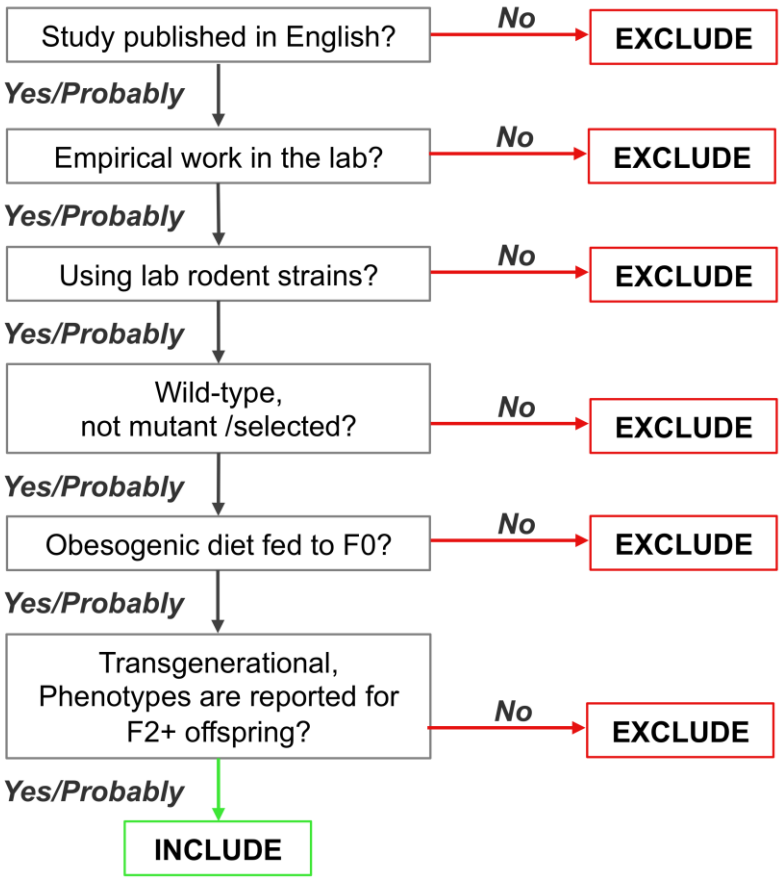

Figure S2

Decision tree used to screen titles and abstracts from bibliometric records of retrieved publications.

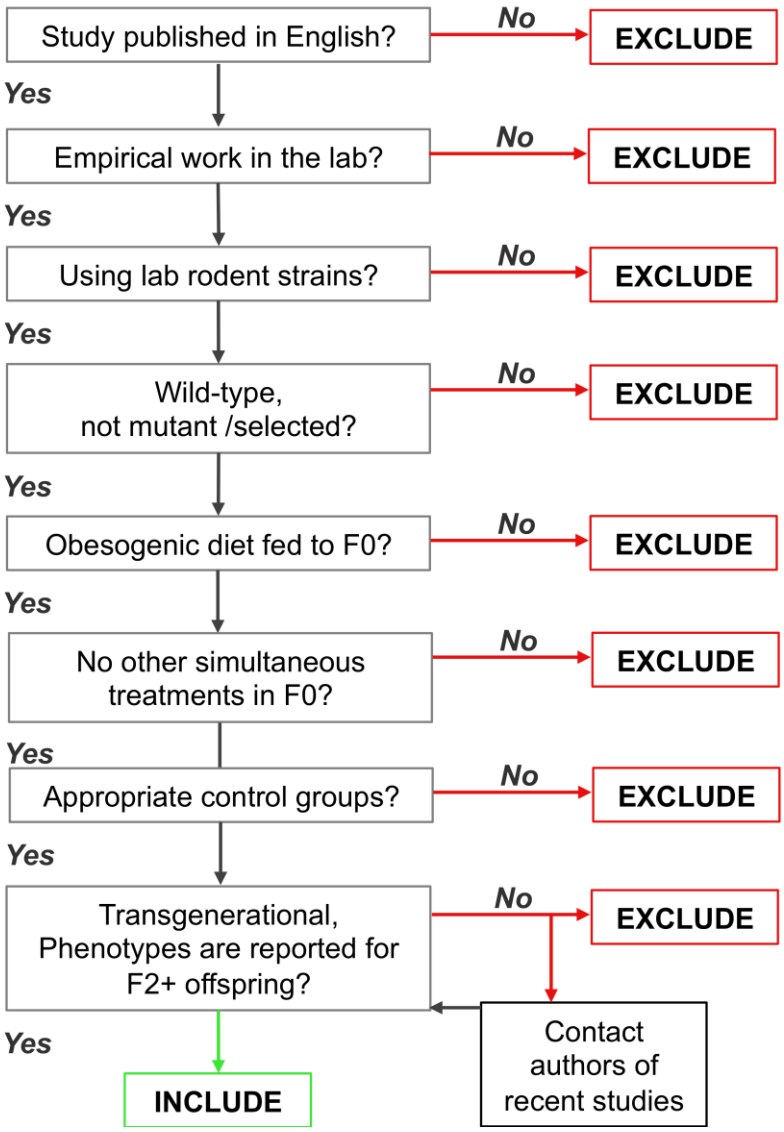

Figure S3.

Decision tree used to screen retrieved full-text publications.

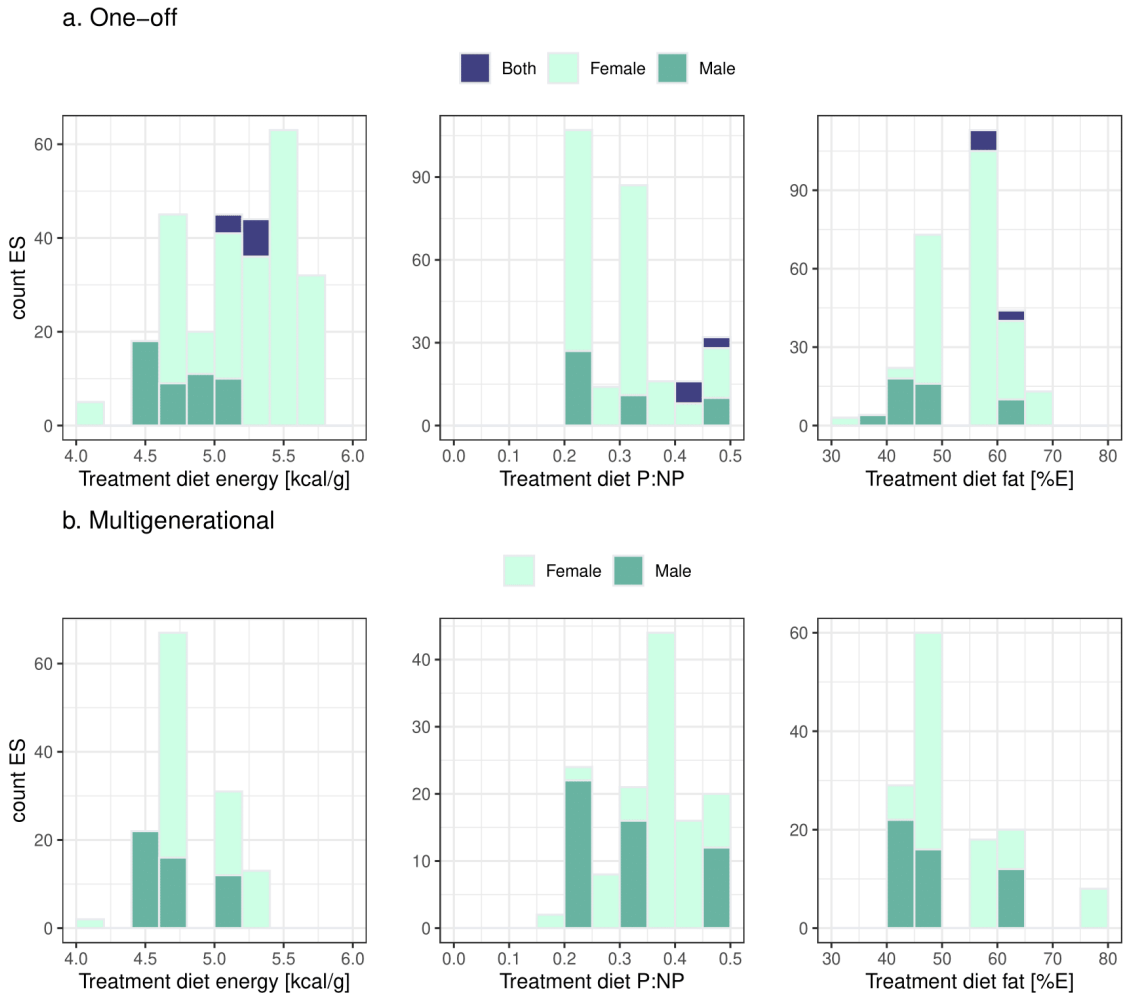

Figure S4

Key properties of the obesogenic (treatment) diets used in the studies included in the meta-analysis: total energy content of the obesogenic diets [kcal/g], ratio of protein to non-protein components of the diet (P:NP, by weight), and percent of diet energy from fat. The plots are split by treatment type: a) One-off exposures, where only F0 (grandparental) generation was exposed to obesogenic diets, and b) Multigenerational exposures, where F0 and subsequent generations were exposed to obesogenic diets. Shades of green and purple indicate sex of the animals exposed in the F0 generation.

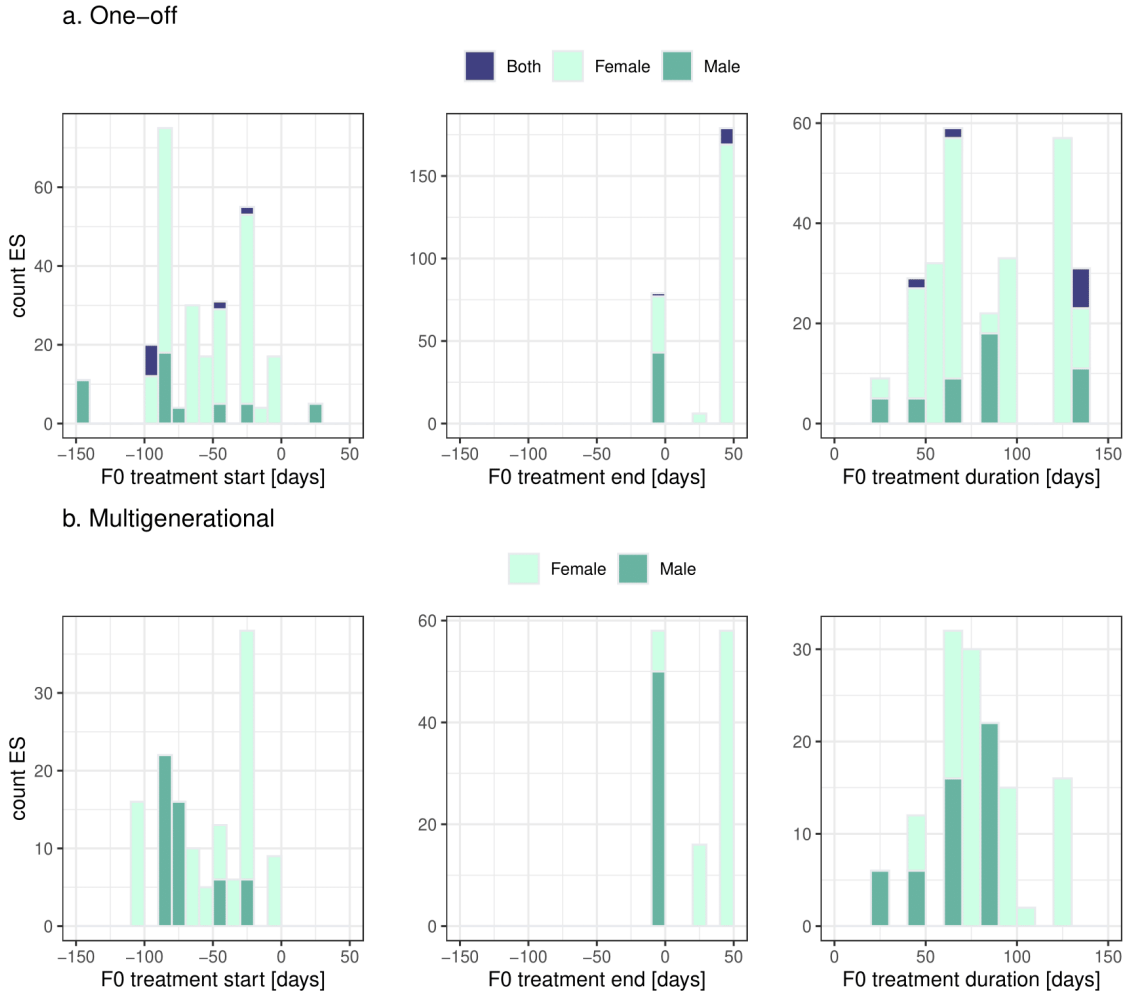

Figure S5

Timing of the obesogenic diet (treatment) at F0 (grandparental) generation: start, end and total duration of exposure [days]. For start and end of exposure, day 0 signifies day of mating of F0 animals. The plots are split by treatment type: a) One-off exposures, where only F0 generation was exposed to obesogenic diets, and b) Multigenerational exposures, where F0 and subsequent generations were exposed to obesogenic diets. Shades of green and purple indicate sex of the animals exposed in the F0 generation.

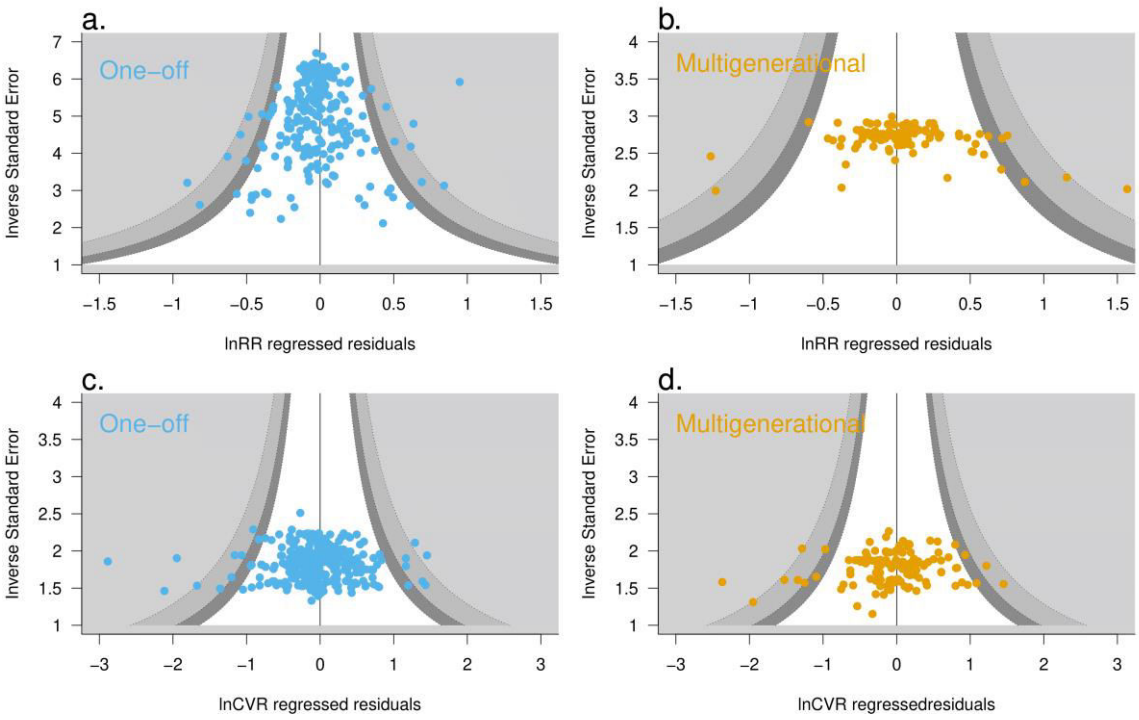

Figure S6

Residual funnel plots from the multivariate meta-regression models with sex of exposed grandparent, sex of measured grand-offspring and grand-offspring trait as moderators. a) *lnRR* effect sizes for One-off exposures, where only F0 generation was exposed to obesogenic diets, and b) *lnRR* effect sizes for Multigenerational exposures, where F0 and subsequent generations were exposed to obesogenic diets. c) *lnCVR* effect sizes for One-off exposures, and d) *lnCVR* effect sizes for Multigenerational exposures. Inverse Standard Error is equivalent to precision 1/sqrt(V).

Supplementary references

Ouzzani, M., Hammady, H., Fedorowicz, Z., & Elmagarmid, A. (2016). Rayyan---a web and mobile app for systematic reviews. *Systematic Reviews*, *5*(1), 210. https://doi.org/10.1186/s13643-016-0384-4

Pick, J. L., Nakagawa, S., & Noble, D. W. A. (2018). Reproducible, flexible and high throughput data extraction from primary literature: The metaDigitise R package. *BioRxiv*, 247775. https://doi.org/10.1101/247775