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Schipper, L; Harvey, L; van der Beek, E M; van Dijk, G

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Obesity/Experimental Models

Home alone: a systematic review and meta-analysis on the effects of individual housing on body weight, food intake and visceral fat mass in rodents

L. Schipper^{1,2}, L. Harvey³, E. M. van der Beek^{2,4} and G. van Dijk¹

¹Groningen Institute for Evolutionary Life Sciences (GELIFES), Neurobiology Cluster, Department Behavioural Neurosciences, University of Groningen, Groningen, The Netherlands, ²Nutricia Research, Utrecht, The Netherlands, ³Nutricia Research, Singapore, and ⁴Department of Paediatrics, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands

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Address for correspondence: G van Dijk, GELIFES-Neurobiology, Dep. Behavioural Neurosciences, University of Groningen, Nijenborg 7, 9747AG Groningen, The Netherlands.
Email: gertjan.van.dijk@rug.nl

Summary

Rats and mice are widely used to study environmental effects on psychological and metabolic health. Study designs differ widely and are often characterized by varying (social) housing conditions. In itself, housing has a profound influence on physiology and behaviour of rodents, affecting energy balance and sustainable metabolic health. However, evidence for potential long-term consequences of individual versus social housing on body weight and metabolic phenotype is inconsistent. We conducted a systematic literature review and meta-analyses assessing effects of individual versus social housing of rats and mice, living under well-accepted laboratory conditions, on measures of metabolic health, including body weight, food intake and visceral adipose tissue mass. Seventy-one studies were included in this review; 59 were included in the meta-analysis. Whilst housing did not affect body weight, both food intake and visceral adipose tissue mass were significantly higher in individually compared with socially housed animals. A combination of emotional stress and lack of social thermoregulation likely contributed to these effects. Increased awareness of consequences and improved specifications of housing conditions are necessary to accurately evaluate efficacy of drugs, diets or other interventions on metabolic and other health outcomes because housing conditions are rarely considered as possible moderators of reported outcomes.

Keywords: Energy balance, housing condition, metabolic phenotype, rodents.

Abbreviations: BAT, brown adipose tissue; BMR, basal metabolic rate; HPA, hypothalamic-pituitary-adrenal; SNS, sympathetic nervous system; UCP-1, uncoupling protein 1.

Introduction

Mice and rats are widely used to study the effects of physical and psychological influences on physiological, metabolic and mental health outcomes. These rodents are social species; living in a group benefits the survival of the individual by lowering predation risk and reducing energy costs via social thermoregulation (1,2). Guidelines for housing and husbandry for laboratory rodents are based on the natural living

conditions of the species, and general recommendations suggest social housing before and after weaning (3). However, these recommendations often collide with reality of experimental conditions. Assessment of many parameters, especially when automated, can be difficult in group-housed animals as it is challenging to differentiate between individuals. Thus, assessment of individual behavioural, metabolic and physiological parameters may require (temporary or otherwise) isolation of an animal from its cage mates.

Consequently, mice and rats are often housed individually, in specifically designed cages, to enable detection of activity patterns, energy intake/expenditure or collection of faeces/urine. They may also be housed individually to avoid aggression and fighting with conspecifics or to avoid damage of surgically implanted materials, such as indwelling cannulae and exteriorized electrodes. In addition, several common models of neuropsychiatric diseases purposely apply social isolation to induce a certain phenotype, e.g. isolation rearing (4), social defeat (5) and chronic mild stress (6). The consequence is that single housing affects the animal's intrinsic behavioural, metabolic and/or physiological parameters.

Solitary versus social housing has previously been shown to affect energy balance regulation and fuel homeostasis (7,8), which may affect metabolic phenotype and modulate an animal's response to other interventions or environmental influences. It has been suggested that social isolation is a stimulus-poor condition that may lead to boredom and increased reward-sensitivity, which, in the case of *ad libitum* access to food, may provoke increased food intake. In addition, deprivation of social cues related to food intake regulation (e.g. social facilitation, feeding competition (9) and influence of social status (10–12)) is likely to change both energy use and availability. Whereas the absence of some social behaviours may save energy, the absence of social thermoregulation would increase loss of energy at temperatures below thermoneutrality (1,2). Sustained deprivation of social behaviours and inability to exchange the animal homologues of empathy, altruism and compassion may also provoke chronic emotional stress (13,14). Chronic emotional stress is known to affect the activity of several neuroendocrine, autonomic and/or immune axes, which in turn can influence fuel homeostasis and energy balance regulation (15,16), leading to alterations in body weight and fat deposition (14,17,18).

The impact and consequences of individual housing, especially longer term, are also likely to be affected by the age at which individual housing commences. Execution of social behaviours, such as play behaviour, during adolescence (i.e. the period from weaning to about 8 weeks of age in mice and rats) is important for neurodevelopment, and social deprivation during this stage affects cognitive and affective functioning in adulthood (19–24). Importantly, behavioural deficits induced by social isolation during adolescence differ from those induced by isolation during adulthood (25). An increased vulnerability during adolescence to the effects of individual housing may also apply to metabolic health. During adolescence, rats and mice grow fast, with a sharp increase in lean body mass (26,27), increasing energy requirements by up to threefold–fourfold compared with adulthood maintenance requirements (28). Differences in food intake and changes in the availability of energy during this period will directly affect growth rate and body composition development due

to changes in energy partitioning. Specifically, visceral adipose tissue depots are a closely related marker for future metabolic health (29).

The effects of housing conditions on body weight and metabolic health in rats and mice have been investigated in several studies, with varying results. Studies have reported higher (30,31), lower (32,33) or equal (34,35) body weight and related indices following individual housing. These inconsistencies may depend on differences in experimental design, age or other characteristics of the rodents used. Notably, few studies have evaluated both juvenile and adult stages in rats/mice to understand the consequences of individual housing during distinct life stages. Literature reviews on the effect of individual versus social housing on body weight and metabolic health indices of rats and mice are lacking. Understanding whether, when and under what conditions individual housing affects body weight and metabolic health is an often overlooked component of the study design. This is true not only for studies that focus on body weight, obesity and metabolic health outcomes, but also relevant for virtually all other experimental outcomes that could be confounded by isolation-induced changes in body weight and metabolic health.

Thus, the aims of this study were to (i) quantify the overall effect of individual housing on body weight, food intake and visceral fat mass; (ii) compare the effects of the life stage when individual housing is applied (starting during adolescence or during adulthood) on adolescent and adult body weight, food intake and visceral fat mass; and (iii) assess the influence of species, sex and diet of the animal, the number of animals housed per cage in the social groups and total duration of the individual housing on our parameters of interest. To this end, we used a systematic approach to search the literature for studies that compared body weight and metabolic health outcomes of rats and mice housed individually versus those housed in social conditions, and performed meta-analyses and meta-regressions where possible. In addition, we describe the potential underlying mechanisms by which individual housing may influence metabolic health in rodents.

Methods

Search strategy

Using ProQuest, we performed a search of the following electronic databases: CAB abstracts, Embase and MEDLINE. The search was performed on 21 March 2016 using the following search terms:

(social* OR communal OR individual* OR singl* OR isolat* OR solit* OR group* OR colony OR colonies OR pair* OR trio OR triad OR separate*) N/1 (rear* OR hous* OR environment* OR condition* OR enrich* OR depriv* OR confin*) OR ('isolation stress' OR

'separation stress' OR 'social segregation' OR 'housing density' OR 'cage density' OR 'social isolation' OR 'socially isolated') AND (metaboli* OR calorimetr* OR thermoregula* OR diabetes OR diabetic* OR adipo* OR obes* OR body weight OR 'weight loss' OR 'weight gain' OR hyperphag* OR hypophag* OR overconsumption OR fat OR WAT OR BAT OR adipokin* OR satiety OR glucose OR insulin OR leptin OR glucose OR 'plasma lipid*' OR 'diet-induced' OR 'high-fat' OR 'physical activity' OR locomot*) OR (body) N/1 (weight OR mass OR temp* OR composit*) OR (diet* OR energy OR satiety OR food OR feeding OR reward OR meal OR nutrient* OR eating OR nutrition* OR sucrose OR calori* OR lipid OR homeostatic OR hedonic) N/1 (control OR regulation OR expenditure OR balance OR homeostasis OR signaling OR intake OR consum* OR behav* OR regulat* OR pattern* OR motivat* OR preference* OR addiction OR choice OR induced OR system OR sensitivity) AND (*Mice OR mus OR *mouse OR murine OR murinae OR rat OR rats OR cottonrat OR cottonrats).

There were no language restrictions in the search. Additional records were identified via screening of reference lists of articles that met the inclusion criteria and via manual search in Google.

Inclusion and exclusion criteria

Articles were initially screened on title and abstract using the following inclusion criteria: (i) experiment featured at least two different post-weaning (social) housing conditions, namely individual and social housing (pair or group); (ii) social housing conditions were applied continuously (24 h/d) and for at least 48 h; (iii) experiments were conducted in mice (*Mus musculus*) and/or rats (*Rattus norvegicus*); (iv) title and/or abstract mentioned body weight and/or outcomes relevant to metabolic health, i.e. food intake, adipose tissue, energy expenditure or body temperature; (v) title appeared relevant to the inclusion criteria stated earlier, but no abstract was available; (vi) publication was in English; (vii) publication was peer reviewed; and (viii) publication was a research report.

Full texts were then obtained for the articles that passed title/abstract screening and screened according to the earlier stated criteria, as well as the following additional inclusion criteria: (i) study reported body weight of rodents during/directly after the social housing intervention; (ii) animals in the experimental groups did not differ from each other (within and between groups) in characteristics (e.g. strain, age, sex, social history and predetermined social status), treatment(s) prior to and during the experiment and housing situations (cage enrichment or other factors that increase cage complexity; differences between groups in cage size and volume of bedding were allowed); (iii) animals in the socially housed experimental group were housed with

cage mates that had equal characteristics (e.g. strain, age, sex, social history and predetermined social status); (iv) the individual and social experimental groups were derived from the same study; (v) the two experimental groups compared were composed of different subjects (animals were not used as own control); (vi) the experimental groups were *ad libitum* fed and watered prior to and throughout the study, and experimental animals were not derived from dams exposed to food restriction; (vii) animals were not individually housed prior to the start of the experiment; (viii) the experiment was performed on non-mutant animals, which were not subjected to invasive procedures such as surgery that may have required anaesthesia and/or fasting, or additional drug, stress, alcohol, exercise or other possible confounding interventions prior to and/or during the experiment. Exceptions were animals that were exposed to an obesogenic or palatable diet (high fat diet/high sucrose diet) with the purpose of inducing obesity or metabolic derangements. Injections with saline, handling procedures, inanimate cage enrichment, as long as both groups were exposed, and the use of wild type controls were allowed; (ix) animals were not exposed to previous social interventions; (x) the individual housing started after weaning (>postnatal day 21); (xi) the study used a minimum of five cases (i.e. animals) per experimental group; (xii) the ambient temperature did not fall outside the range of 20–26°C. This temperature range represents the general recommendations for environmental temperature for laboratory rodents (3) and is slightly larger than the regular range (between 20 and 22°C) used by several laboratories (36). Studies using ambient temperature outside this range (<20 and >26°C) were considered to be specifically targeting ambient temperature as an experimental factor.

For the articles that passed full text screening, we extracted data only from relevant matched groups of individual and socially housed experimental animals that met the criteria listed earlier, excluding experimental groups from the same article that did not meet the inclusion criteria. In some articles, additional changes in social housing situation or additional interventions or treatments commenced after the start of the housing paradigm. In these cases, data were extracted only from the period before additional/subsequent treatment. When multiple relevant matched groups were described within the same study, we extracted all relevant matched groups separately. This included cases where one experimental group was used for more than one relevant comparison between individually and socially housed animals (for instance, isolated animals were compared with both pair and group housed animals).

Data extraction

From the included articles, we extracted information related to study design, including species, strain, sex, age, exposure

period, number of animals per cage and diet. For each matched group, the body weight of individually housed animals was extracted in a categorical manner as 'higher', 'lower' or 'equal' compared with body weight of the socially housed animals, according to the interpretation of the articles' authors and independent of the unit that was reported. For the meta-analyses, we extracted quantitative data of the experimental groups, where reported. When data were only presented graphically, the outcome was deducted as precisely as possible. Studies that reported results as mean and standard deviation or standard error per experimental group and number of animals per group were included for meta-analyses. When effects of individual housing on body weight were described at multiple time points after initiation of the housing paradigm, we selected the latest time point for data extraction. Where reported, information about food intake and visceral white adipose tissue was extracted. Care was taken to only include articles that reported on visceral adipose tissue mass rather than total body fat or subcutaneous adipose tissue to ensure the use of similar outcome parameters across the articles, as visceral and subcutaneous adipose depots may be different in their function and their response to external factors (37). There was variation in the specific visceral adipose depot(s) described in the articles, ranging from one (38,39) to three different adipose tissue depots (40) or total abdominal fat (41,42). We excluded data from studies in which socially housed animals were temporarily isolated in order to obtain food intake data. We contacted 12 authors of 13 studies published in or after the year 2000 to request information regarding missing experimental details or data and received information for 10 studies (35,43–51).

Quality assessment

The quality and risk of bias of body weight data in the included studies were independently assessed by two reviewers (L. S., L. H.), using a modified version of the Systematic Review Center for Laboratory Animal Experimentation Risk of Bias Tool for animal intervention studies (52). The tool contains 10 questions to investigate potential sources of bias including errors in randomization, blinding and selective outcome reporting. The risk of selection bias by method of allocation concealment was not assessed, as it is impossible to physically conceal the allocation of the groups when comparing different housing conditions. The risk of reporting bias by selective outcome reporting was not assessed, as body weight monitoring was not always described as part of the study protocol, however, it is a standard activity related to animal welfare. Articles that did not report body weight in their results section were excluded from the review. Two questions from the tool were split. Risk of selection bias via unequal baseline characteristics was assessed as (i) whether body weight was balanced

between groups at the start of the housing intervention and (ii) if the author used an analysis or reporting method to adjust for unequal distribution of baseline body weights. For risk of other bias from other sources, potential risk of bias from (i) conflict of interest and (ii) unit analysis errors were assessed as separate questions. Disagreements between the reviewers were resolved through discussion until consensus was reached.

Effect size calculation

Effect sizes (ESs) for body weight, food intake and visceral fat mass were calculated for each independently matched comparison of individually and socially housed animals. When multiple experimental groups within one study shared the same control group, a synthetic mean was computed by combining these experimental groups, which was used to compute the ES (53). As quantitative data were presented as either absolute or relative data and using different units (e.g. kilogram, fold change, Table 1), we used Hedges' *g* unbiased standardized mean difference as a measure of ES (105). Positive ESs represent higher body weight, food intake or visceral fat mass as a result of individual housing compared with social housing. Based on Cohen's *d*, ESs of 0.2, 0.5 and 0.8 indicate small, medium and large effects, respectively (106).

Statistical analysis

The meta-analysis was conducted using Comprehensive Meta Analysis software (Version 2.2.064, BioStat, Englewood, NJ, USA). We computed Hedges' *g*, 95% confidence intervals (95% CI) and associated *p* values for each individual matched group. Summary estimates were calculated using random effects models; separate estimates were obtained for body weight, food intake and visceral fat mass. Heterogeneity among studies was assessed using I^2 and the chi-squared statistic (*Q*) (105). Measures of heterogeneity of 25%, 50% and 75% were considered low, moderate and high, respectively (107). Moderator analyses were conducted to examine the effects of species (rats and mice), sex (male and female), diet (normal and obesogenic) and life stage (adult–adult, young–adult, young–young). We classified animals as young if they were ≤ 8 weeks of age and as adult if they were > 8 weeks of age (end of adolescence). The life stage moderator comprised both the timing of the start of the isolation and the timing of data collection (i.e. young–adult represents animals who were young when the isolation began, but adult when data were collected). The influence of the total duration of individual housing (in weeks) and the number of animals per cage in the social groups were analysed (as continuous variables) using meta-regression models on body weight, food intake and visceral fat mass. Stratified analyses included calculation

Table 1 Overview of characteristics of all included studies.

First author, year of publication (subgroup)	Species (strain)	Sex	Age at start of isolation (weeks)	Duration of isolation (weeks)	#animals in social group	Obesogenic diet?	Measure of body weight	Isolated animals' body weight compared with socially housed?	Isolated animals' food intake compared with socially housed?	Isolated animals' white adipose tissue compared with socially housed?
Abou-Ismaïl, 2014(54)	Rats (Wistar)	M	3	5	3	Normal	Absolute (g) change (g)	Equal		
Abramov, 2004(50)	Mice (WT 129sv/ C57BL6)	M	13	3	8	Normal	Change (g)	Lower		
Abramov, 2004(50)	Mice (WT 129sv/ C57BL6)	F	13	3	8	Normal	Change (g)	Equal		
Ader, 1963(55)	Rats (SD)	M	4	8	2	Normal	Descriptive only	Equal		
Ali, 2012(51)	Mice (Swiss albino)	M	8	4	5	Normal	Absolute (g)	Equal		
Amir, 1979(56)	Rats (Wistar)	M	8	5	2	Normal	Absolute (g)	Higher		
Arcego, 2014(57)	Rats (Wistar)	M	3	1	4	Normal	Change (g)	Equal	Equal ⁴	
Arcego, 2014(57)	Rats (Wistar)	M	3	1	4	HFD	Change (g)	Equal	Higher ⁴	
Arcego, 2014(57)	Rats (Wistar)	M	3	1	4	HSD	Change (g)	Equal	Equal ⁴	
Baker, 2007(58)	Rats (SD)	F	10	3	2	Normal	Absolute (g) change (g and %)	Higher		
Barnhart, 1982(59)	Mice (Ha: ICR)	M	29	14	4	Normal	Absolute (g)	Equal		
Barnhart, 1982(59)	Mice (Ha: ICR)	M	4	37	4	Normal	Absolute (g)	Equal		
Barnhart, 1982(59)	Mice (Ha: ICR)	F	4	37	4	Normal	Absolute (g)	Equal		
Bartolomucci, 2009a(44)	Mice (Swiss CD-1)	M	12	1	3	Normal	Fold change from basal	Lower	Lower ³	
Bartolomucci, 2009a(44)	Mice (Swiss CD-1)	M	12	4	3	HFD	Fold change from basal	Higher	Higher ⁶	Higher ⁷
Bartolomucci, 2009a(44)	Mice (Swiss CD-1)	M	12	4	3	Normal	Fold change from basal	Lower	Lower ³	Lower ⁷
Bartolomucci, 2009b (non-siblings)(60)	Mice (Swiss CD-1)	F	10	1	3	Normal	Change (g)	Equal ⁹		
Bartolomucci, 2009b (siblings)(60)	Mice (Swiss CD-1)	F	10	1	3	Normal	Change (g)	Equal ⁹		
Ben Menachem-Zidon, 2008(46)	Mice (WT C57BL/6 X CBA)	M	10	4	9	Normal	Absolute (g)	Lower		
Benite-Ribeiro, 2013(41)	Rats (Wistar)	F	7	2	2	HFD		Equal	Equal	
Benite-Ribeiro, 2013(41)	Rats (Wistar)	F	7	6	2	HFD	Absolute (g)	Equal	Higher ⁵	Equal ⁷

(Continues)

Table 1 (Continued)

First author, year of publication (subgroup)	Species (strain)	Sex	Age at start of isolation (weeks)	Duration of isolation (weeks)	#animals in social group	Obesogenic diet?	Measure of body weight	Isolated animals' body weight compared with socially housed?	Isolated animals' food intake compared with socially housed?	Isolated animals' white adipose tissue compared with socially housed?
Bibancos, 2007(61)	C57BL/6J	M	3	6	4	Normal	Change (g)	Equal		
Brain, 1970(62)	Mice (TT albino)	M	13	3	2	Normal	Absolute (g)	Equal ⁹		
Brain, 1970(62)	Mice (TT albino)	M	13	3	4	Normal	Absolute (g)	Equal ⁹		
Brain, 1970(62)	Mice (TT albino)	M	13	3	8	Normal	Absolute (g)	Equal ⁹		
Brain, 1970(62)	Mice (TT albino)	M	13	3	16	Normal	Absolute (g)	Equal		
Breslin, 2010(63)	Mice (Crl: CD1[1CR])	M	6	12	4	Obesity	Absolute (g)	Equal		Higher ⁴
Chvedoff, 1980b(64)	Mice (CD1)	M	3	80	2	Normal	change (%)	Higher		Higher ¹
Chvedoff, 1980b(64)	Mice (CD1)	M	3	80	4	Normal	Absolute (g)	Higher		Higher ¹
Chvedoff, 1980b(64)	Mice (CD1)	M	3	80	8	Normal	Absolute (g)	Higher		Higher ¹
Chvedoff, 1980b(64)	Mice (CD1)	F	3	80	2	Normal	Absolute (g)	Equal		Higher ¹
Chvedoff, 1980b(64)	Mice (CD1)	F	3	80	4	Normal	Absolute (g)	Equal		Equal ¹
Chvedoff, 1980b(64)	Mice (CD1)	F	3	80	8	Normal	Absolute (g)	Higher		Higher ¹
Cowley, 1965(65)	Rats (Lister hooded)	M	3	9	3	Normal	Absolute (g)	Higher		Higher ²
Cowley, 1965 (handling)(65)	Rats (Lister hooded)	M	3	9	3	Normal	Absolute (g)	Higher		Higher ²
Cruz, 2016(66)	Rats (Wistar)	M	8.5	3	4	Normal	Absolute (g)	Equal		
Cruz, 2016(66)	Rats (Wistar)	M	3	3	4	Normal	Absolute (g)	Lower		
Dawson 1981(67)	Mice (CF-1)	M	16	4	Not specified	Normal	Absolute (g)	Lower		
Dawson 1981(67)	Mice (CF-1)	F	16	4	Not specified	Normal	Absolute (g)	Equal		
Fisher, 2012(68)	Rats (Flinders Resistant)	M	10	5.5	2	Normal	Absolute (g)	Higher		Higher ²
Gangrade, 1984, exp 1(69)	Mice (Parkes)	F	11	3	15	Normal	change (g)	Equal		
Gilbert-Juan, 2013(70)	Rats (Lister hooded)	M	3	8	3	Normal	Absolute (g)	Higher		
Goodrick, 1974(71)	Mice (A/J)	M	5	10	2	Normal	Absolute (g)	Equal		
Goodrick, 1974(71)	Mice (C57BL6j)	M	5	10	2	Normal	Absolute (g)	Equal		
Goodrick, 1974(71)	Mice (A/J/C57BL6j)	M	5	10	2	Normal	Absolute (g)	Equal		
Guo, 2004(72)	Mice (Swiss-Kunming)	M	3	16	5	Normal	Absolute (g)	Lower		
Guo, 2004(72)	Mice (Swiss-Kunming)	F	3	16	5	Normal	Absolute (g)	Lower		

(Continues)

Table 1 (Continued)

First author, year of publication (subgroup)	Species (strain)	Sex	Age at start of isolation (weeks)	Duration of isolation (weeks)	#animals in social group	Obesogenic diet?	Measure of body weight	Isolated animals' body weight compared with socially housed?	Isolated animals' food intake compared with socially housed?	Isolated animals' white adipose tissue compared with socially housed?
Hahn, 1965(73)	Rats (SD)	M	8.5	2	12	Normal	Absolute (g)	Equal		
Haller, 1999(74)	Rats (Wistar)	M	10	0.5	6	Normal	Change (g)	Equal		Equal ⁷
Harris, 1997(38)	Rats (Wistar)	M	10	3	4	Normal	Absolute (g)	Equal		
Harris, 2008, exp 1(35)	Rats (Lister hooded)	M	10	12	2	Normal	Descriptive only	Equal		
Harris, 2008, exp 2(35)	Rats (Lister hooded)	M	10	3	2	Normal	Descriptive only	Equal		
Harris, 2008, exp 3(35)	Rats (Lister hooded)	M	5	12	2	Normal	Descriptive only	Equal		
Harris, 2008, exp 3(35)	Rats (Lister hooded)	F	5	12	2	Normal	Descriptive only	Equal		
Harris, 2008, exp 1(35)	Rats (Lister hooded)	F	10	12	2	Normal	Descriptive only	Equal		
Harris, 2008, exp 2(35)	Rats (Lister hooded)	F	10	3	2	Normal	Descriptive only	Equal		
Hoffman-Goetz, 1992(75)	Mice (C3He)	M	4	4	4	Normal	Absolute (g) change (g)	Equal		
Hoyenga, 1969(9)	Rats (Holtzman)	M	3	11	4	Normal	Change (%)	Lower		
Hughes, 1973(76)	Rats (black-hooded)	M	3	14	2	Normal	Absolute (g)	Higher ⁹		
Hughes, 1973(76)	Rats (black-hooded)	M	3	14	4	Normal	Absolute (g)	Higher ⁹		
Innos, 2012(77)	Mice (WT 129SvEvTac × C57BL/6)	M	9	5	8	Normal	Absolute (g)	Lower		
Ishida, 2003(78)	Mice (HOS:HR-1, hairless)	F	12	13	5	Normal	Change (g)	Equal	Higher ¹	
Jahng, 2012(79)	Rats (SD)	F	4	3	3	Normal	Absolute (g)	Higher	Higher ¹	
Jennings, 1986(31)	Mice (C57BL/IOScSn)	F	20	3	2	Normal	Absolute (g) gain (g)	Higher ⁹		
Jennings, 1986(31)	Mice (C57BL/IOScSn)	F	20	3	3	Normal	Absolute (g) gain (g)	Equal ⁹		
Jennings, 1986(31)	Mice (C57BL/IOScSn)	F	20	3	6	Normal	Absolute (g) gain (g)	Higher ⁹		
Kalinichev, 2005(32)	Rats (Wistar, SD)	F	14	1	5	Normal	Absolute (g)	Lower		
Ko, 2015(80)	Rats (SD)	M	3	4	2	Normal	Absolute (g)	Equal		

(Continues)

Table 1 (Continued)

First author, year of publication (subgroup)	Species (strain)	Sex	Age at start of isolation (weeks)	Duration of isolation (weeks)	#animals in social group	Obesogenic diet?	Measure of body weight	Isolated animals' body weight compared with socially housed?	Isolated animals' food intake compared with socially housed?	Isolated animals' white adipose tissue compared with socially housed?
Ko, 2015(80)	Rats (SD)	F	3	4	2	Normal	Absolute (g)	Equal		
Ko, 2016 (gavaged) (81)	Rats (SD)	M	3	9	2	Normal	Absolute (g)	Equal		
Ko, 2016(81)	Rats (SD)	M	3	9	2	Normal	Absolute (g)	Equal		
Ko, 2016 (gavaged) (81)	Rats (SD)	F	3	9	2	Normal	Absolute (g)	Equal		
Ko, 2016(81)	Rats (SD)	F	3	9	2	Normal	Absolute (g)	Equal		
Krolov, 2013(82)	Rats (Wistar)	M	3	1	4	Normal	Change (g)	Equal		Equal ³
Krolov, 2013(82)	Rats (Wistar)	F	3	1	4	Normal	Change (g)	Equal		Equal ³
Krolov, 2013(82)	Rats (Wistar)	M	3	1	4	HSD	Change (g)	Equal		Higher ³
Krolov, 2013(82)	Rats (Wistar)	F	3	1	4	HSD	Change (g)	Higher		Higher ³
Krugel, 2014(83)	Rats (Wistar)	M	9	4	4	Normal	Absolute (g)	Higher		
Lamkin, 2011(47)	Mice (C57B1/6J)	F	11	14	4	Normal	Descriptive only	Equal		
Lopak, 2000(48)	Rats (SD)	M	10	3	7	Normal	Absolute (g)	Equal		Equal ¹
Lopez, 2015 (enriched)(45)	Mice (C57B1/6J)	M	3	5.5	4	Normal	Absolute (g)	Equal		
Lopez, 2015 (enriched)(45)	Mice (C57B1/6J)	F	3	5.5	4	Normal	Absolute (g)	Equal		
Lopez, 2015(45)	Mice (C57B1/6J)	M	3	5.5	4	Normal	Absolute (g)	Equal		
Lopez, 2015(45)	Mice (C57B1/6J)	F	3	5.5	4	Normal	Absolute (g)	Equal		
Marcollin, 2012(43)	Rats (Wistar)	M	3	1	3	HSD	Change (g)	Equal		Equal ²
Marcollin, 2012 (handling)(43)	Rats (Wistar)	M	3	1	3	Normal	Change (g)	Equal		Equal ²
Marcollin, 2012(43)	Rats (Wistar)	M	3	1	3	Normal	Change (g)	Equal		Equal ²
Marcollin, 2012 (handling)(43)	Rats (Wistar)	M	3	1	3	HSD	Change (g)	Equal		Equal ²
Marks-Kaufman, 1984(84)	Rats (SD)	M	3	5.5	2	Normal	Descriptive only	Equal		
Marks-Kaufman, 1984(84)	Rats (SD)	M	3	5.5	4	Normal	Descriptive only	Equal		
Maslova, 2010(85)	Rats (Wistar)	M	3	6,	4	Normal	Absolute (g)	Equal		Higher ¹
Morgan, 1975(86)	Rats (Hooded Lister)	F	3	5.5	5	Normal	Absolute (g)	Higher		
Morinan, 1980(87)	Rats (Wistar)	M	3	3	4	Normal	Absolute (g)	Equal		Higher ¹
Motoyama, 2009(88)	Mice (BALB/c)	M	5	4	3	Normal	Absolute (g)	Equal		

(Continues)

Table 1 (Continued)

First author, year of publication (subgroup)	Species (strain)	Sex	Age at start of isolation (weeks)	Duration of isolation (weeks)	#animals in social group	Obesogenic diet?	Measure of body weight	Isolated animals' body weight compared with socially housed?	Isolated animals' food intake compared with socially housed?	Isolated animals' white adipose tissue compared with socially housed?
Nagy, 2002(33)	Mice (C57Bl/6J)	M	3	9	5	Normal	Absolute (g)	Lower	Higher ¹	Equal ^{7,8}
Nakhate, 2011(30)	Rats (SD)	M	7	6	3	Normal	Absolute (g)	Higher		
Nonogaki, 2007(39)	Mice (C57Bl/6J)	M	5	3	3	Normal	Change (g)	Equal		Equal ⁷
O'Connor, 2009(49)	Rats (SD)	M	7	3	2	Normal	Change (g)	Equal		
Perez, 1997(89)	Rats (Wistar)	F	13	1	5	Normal	Absolute (g)	Equal	Higher ¹	
Pradier, 2015(90)	Mice (C57Bl/6J)	M	8	20	2	Normal	Absolute (g)	Equal	Equal ¹	
Ryu, 2009(91)	Rats (SD)	M	3	6	3	Normal	Absolute (g)	Equal	Equal ¹	
Sakakibara, 2012(92)	Mice (C57Bl/6J)	M	5.5	13	5	Normal	Absolute (g)	Higher		Higher ⁷
Shelly, 1965, exp 1(93)	Rats (SD)	M	3	11	2	Normal	Absolute (g)	Equal		
Shelly, 1965, exp 1(93)	Rats (SD)	M	3	11	10	Normal	Absolute (g)	Higher		
Shelly, 1965, exp 2(93)	Rats (SD)	M	3	11	2	Normal	Absolute (g)	Equal		
Shelly, 1965, exp 2(93)	Rats (SD)	M	3	11	10	Normal	Absolute (g)	Higher	Higher ¹	
Silva, 2003(94)	Rats (Wistar)	M	11	3	5	Normal	Change (g)	Equal		
Skalicky, 2001(95)	Rats (SD)	M	20	72	4	Normal	Absolute (g)	Lower		
Stern, 1960(96)	Rats (SD)	M	3	8	15	Normal	Absolute (g)	Equal		
Sun, 2014(42)	Mice (C57Bl/6J)	M	12	8	4	Normal	Absolute (g)	Equal		Higher ⁷
Takemoto, 1975(97)	Mice (dd-D)	M	11	13	4	Normal	Absolute (g)	Higher ⁹		
Takemoto, 1975(97)	Mice (dd-D)	M	11	13	4	Normal	Absolute (g)	Higher ⁹		
Takemoto, 1975(97)	Mice (dd-D)	M	29	12	4	Normal	Absolute (g)	Higher	Equal ¹	
Takemoto, 1975(97)	Mice (dd-D)	F	32	12	4	Normal	Absolute (g)	Higher	Equal ¹	
Takemoto, 1975(97)	Mice (dd-D)	M	8	12	4	Normal	Absolute (g)	Higher	Equal ¹	
Takemoto, 1975(97)	Mice (dd-D)	F	8	12	4	Normal	Absolute (g)	Equal	Equal ¹	
Thorsell, 2005(98)	Rats (Wistar)	M	7	13	2	Normal	Absolute (g)	Lower		Higher ⁷
Tsudukui, 2015(40)	Mice (C57Bl/6J)	M	3	8	6	Normal	Absolute (g)	Equal	Higher ¹	Higher ⁷
Tsudukui, 2015 (offspring HFD)(40)	Mice (C57Bl/6J)	M	3	8	6	HFD	Absolute (g)	Equal	Higher ¹	Higher ⁷
Tsudukui, 2015 (maternal + offspring HFD)(40)	Mice (C57Bl/6J)	M	3	8	6	HFD	Absolute (g)	Equal	Higher ¹	Higher ⁷
Tsudukui, 2015 (maternal HFD)(40)	Mice (C57Bl/6J)	M	3	8	6	HFD	Absolute (g)	Equal	Higher ¹	Higher ⁷
Weitman, 1966(99)	Mice (albino)	F	4	16	2	Normal	Absolute (g)	Equal	Higher ¹	Higher ⁷

(Continues)

Table 1 (Continued)

First author, year of publication (subgroup)	Species (strain)	Sex	Age at start of isolation (weeks)	Duration of isolation (weeks)	#animals in social group	Obesogenic diet?	Measure of body weight	Isolated animals' body weight compared with socially housed?	Isolated animals' food intake compared with socially housed?	Isolated animals' white adipose tissue compared with socially housed?
Wiberg, 1966(100)	Rats (SD)	M	4	15	10	Normal	Descriptive only	Lower		
Wiberg, 1966(100)	Rats (SD)	F	4	15	10	Normal	Descriptive only	Lower		
Wiberg, 1966(100)	Rats (Wistar)	M	4	16	10	Normal	Absolute (g)	Lower		
Wiberg, 1966(100)	Rats (Wistar)	F	4	16	10	Normal	Absolute (g)	Lower		
Wyndham, 1983(101)	Rats (Wistar)	M	7	71	7	Normal	Absolute (g)	Higher	Higher ¹	
Yamada, 2000(102)	Mice (WT unknown background)	M	6	19	4	Normal	Absolute (g)	Lower	Lower ¹	
Yamada, 2015(103)	Mice (C57Bl/6)	M	8	2	3	Normal	Change (%)	Equal	Higher ¹	
Yamada, 2015(103)	Mice (C57Bl/6)	F	8	2	3	Normal	Change (%)	Equal	Higher ¹	
Zaias, 2008(104)	Rats (SD)	M	3	4	2	Normal	Change (%)	Higher ⁹		
Zaias, 2008(104)	Rats (SD)	M	3	4	3	Normal	Change (%)	Equal ⁹		
Zaias, 2008(104)	Rats (SD)	M	3	4	3	Normal	Change (%)	Higher ⁹		
Zaias, 2008(104)	Rats (SD)	M	3	4	2	Normal	Change (%)	Equal ⁹		

Results in bold are included in the appropriate meta-analysis. HFD, high fat diet; HSD, high sucrose diet; M, male; F, female; SD, Sprague Dawley; WT, wild type.

¹g/day;

²g/week(s);

³kcal or KJ/week;

⁴kcal or KJ/week;

⁵kcal/g bw;

⁶Fold change from baseline;

⁷Absolute (g);

⁸Percentage of body mass (%);

⁹Synthesized variable included in meta-analysis.

of the summary estimates for body weight and meta-regression for number of animals per cage in the subsets of studies that also reported food intake and visceral adiposity. In addition, the summary estimates for food intake were calculated separately for the subsets of studies reporting higher and those reporting equal or lower body weights.

Results

The database search yielded 3,751 articles, and an additional 52 articles were identified via reference lists and Google search (Fig. 1). Title/abstract screening resulted in 699 eligible articles. After full text screening, a total of 71 articles were included for review, describing 134 independently matched groups of animals housed socially or individually. Table 1 describes the experimental details of each matched group, including species, strain, sex, age, exposure period, number of animals per cage and diet. Additional details regarding cage and environmental conditions and biotechnical procedures (e.g. ambient temperature, cage size, cage materials and enrichment, handling frequency) were not consistently reported.

There were 23 matched groups classified as young-young, 68 matched groups classified as young-adult and 43 matched groups classified as adult-adult. The duration of social isolation applied varied from 0.5 to 80 weeks (median duration = 5.5 weeks). The number of animals housed per cage in socially housed groups varied between 2 and 16 (median = 4). One article did not provide details regarding

the number of animals in the socially housed group (67) and was excluded from the meta-analysis. Rats and mice were equally represented in the included articles (71 rat, 63 mouse matched groups), but there were more male (96) than female matched groups (38). Thirteen matched groups were subject to an obesogenic diet (high fat or high sucrose), whilst the remaining matched groups were on standard rodent diet.

Author reported results

Body weight

Effects of housing condition on body weight were reported for 134 matched groups from 71 articles (Table 1). Across these articles, authors reported higher body weight as a result of individual housing in 30 matched groups, lower body weight as a result of individual housing in 26 matched groups and no differences due to housing on body weight in 76 matched groups. Higher body weight due to individual housing was reported most often in the young-adult life stage (25% of all matched groups in this life stage) compared with the young-young (17%) and adult-adult (21%) life stages.

Food intake

Of the studies reporting body weight, food intake was reported in 27 articles, representing 55 matched groups

Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram of study selection process. [Colour figure can be viewed at wileyonlinelibrary.com]

(Table 1). Individual housing resulted in higher food intake in 29 matched groups, lower food intake in 3 matched groups and equal food intake in 23 matched groups. A life stage-specific pattern was observed for higher food intake due to individual housing that was quite similar to differences in body weight; 69% of the young–adult matched groups reported higher food intake in individually housed animals, compared with just 38% of the young–young and 31% of the adult–adult matched groups.

Visceral white adipose tissue

There were 8 articles reporting on effects of individual housing versus social housing on visceral adipose tissue mass for a total of 12 matched groups; 7 matched groups demonstrated higher visceral adipose tissue mass as a result of individual housing, whilst 1 reported lower visceral adipose tissue mass (Table 1). For the matched groups in the young–adult life stage, 70% reported higher visceral white adipose tissue mass, whilst none of the young–young life stage matched groups reported this outcome and 50% reported it in the adult–adult life stage. Due to the low number of matched groups, no statistical analysis could be performed.

Meta-analyses

The final dataset for meta-analyses comprised 94 ES estimates for body weight derived from a total of 59 articles (83% of the original included articles, Table 1 and Fig. S1); 45 ES estimates for food intake derived from 23 articles (Table 1 and Fig. S2); 12 ES estimates derived from 8 articles for visceral fat mass (Table 1 and Fig. S3); and 45 ES estimates for body weight derived from the 23 articles that also reported on food intake (Table 1 and Fig. S4).

Effects of individual housing on body weight

Meta-analysis revealed no significant effect of individual housing on body weight (Hedges' g ES = 0.11, 95% CI = −0.05, 0.26, p = 0.17; Table 2 and Fig. S1). There was moderate to high heterogeneity (Q = 290.1, degrees of freedom [df] = 93, I^2 = 67.9). The life stage during which animals were housed individually and during which data were collected (a categorical variable) did not significantly affect the outcome (Table 2). The considerable heterogeneity could not be explained by effects of species, sex or diet on overall mean ES (Table 2), nor was the effect influenced by the duration of the individual housing paradigm. Meta-regression analysis revealed, however, that cage density did affect the outcome, with number of animals per cage negatively related with ES for body weight (slope = −0.08, 95% CI = −0.17, 0.01, p = 0.04; Fig. 2), meaning that increased number of animals in a cage would actually *increase* body weight relative to individually housed animals. However, exclusion of one study with a very high cage density (i.e. 15 animals per cage (69)) from the regression analysis resulted in loss of significance for this effect.

Effects of individual housing on food intake

Data on food intake of individually and socially housed animals were usually presented as (change) in absolute value (e.g. average kcal/day) and, except for one article, (41) data were not adjusted for body size. The ESs for unadjusted food intake were not correlated with ESs for body weight (R^2 = 0.08), indicating that the current results on food intake were not confounded by differences in body weight between groups. Meta-analysis indicated that individual housing resulted in a 'medium' and significant increase in food intake (ES = 0.57, 95% CI 0.36, 0.77,

Table 2 Effect sizes for body weight, food intake and white adipose tissue mass by moderator variables in the meta-analysis.

		Body weight					Food intake					White adipose tissue mass				
		95% CI					95% CI					95% CI				
		<i>n</i>	ES	Lower	Upper	Qb	<i>n</i>	ES	Lower	Upper	Qb	<i>n</i>	ES	Lower	Upper	Qb
Overall mean		94	0.11	−0.05	0.26		45	0.57**	0.36	0.77		12	0.92*	0.34	1.51	
Species	Mice	43	0.05	−0.19	0.28	0.50	16	0.60**	0.26	0.93	0.05	6	0.84	−0.01	1.70	0.11
	Rats	51	0.16	−0.05	0.38		29	0.55**	0.29	0.81		6	1.05*	0.15	1.94	
Sex	Male	65	0.10	−0.08	0.31	0.01	30	0.45**	0.21	0.69	2.83					
	Female	29	0.15	−0.18	0.39		15	0.82**	0.46	1.17						
Diet	Normal	81	0.10	−0.07	0.27	0.15	32	0.50**	0.26	0.73	1.29	7	0.70	−0.06	1.45	0.94
	Obesogenic	13	0.19	−0.26	0.64		13	0.76**	0.37	1.16		5	1.30**	0.35	2.24	
Life stage	Adult–adult	32	0.08	−0.19	0.35	0.16	12	0.50*	0.10	0.89	1.66	5	0.58	−0.27	1.43	3.92
	Young–adult	41	0.15	−0.10	0.39		20	0.70**	0.41	1.00		6	0.98*	0.15	1.81	
	Young–young	21	0.09	−0.25	0.43		13	0.40*	0.01	0.79		1	3.08*	0.75	5.41	

* p < 0.05,

** p < 0.01.

Figure 2 Bubble plot of body weight meta-regression analysis for the full data set. Effect sizes (Hedges' g , $n = 98$) represent differences in body weight between individually and socially housed animals, plotted against the number of animals per cage in the socially housed groups. Positive effect size indicates that individually housed animals had higher body weight than socially housed animals, negative effect size indicates the reverse. The size of bubbles (indicated by volume) represents the precision of Hedges' g estimate; large bubbles are most precise. The regression line fitted to the raw data shows the constant (Y intercept) and slope of the relation between number of the animals in the cage and the Hedges' g effects size.

$p < 0.01$, Table 2 and Fig. S2). The heterogeneity was moderate ($Q = 84.3$, $df = 44$, $I^2 = 47.8$). Similar to body weight, the effect of housing condition on food intake was not significantly influenced by life stage or the moderator's species, sex and diet (Table 2). Meta regression indicated that cage density did not affect food intake (Fig. 3). Also, duration of individual housing did not affect outcomes.

Effects of individual housing on visceral adipose tissue mass

Weights of visceral adipose tissue depots were reported unadjusted for body mass in 11 of the 12 selected matched

groups, whilst one article also reported the outcomes as percentage of body mass (33). The ES of unadjusted fat mass was not correlated to the ES of body weight in this data subset (Pearson's $R^2 = 0.49$, $p = 0.11$, $n = 12$). The meta-analysis indicated that individual housing resulted in a strong and significant increase in visceral adipose tissue mass (ES = 0.92, 95% CI = 0.34, 1.51, $p = 0.002$; Table 2 and Fig. S3), although heterogeneity was high ($Q = 44.7$, $df = 11$, $I^2 = 75.4$). The life stage during which individual housing and data collection took place had no significant effect on the outcome, nor did species or diet (Table 2). A moderator analysis for sex was not performed, as there was only one article that reported data from female subjects. Meta-regression did not provide any evidence that

Figure 3 Bubble plot of food intake meta-regression analysis. Effect sizes (Hedges' g , $n = 45$) represent the differences in food intake between individually and socially housed animals, plotted against the number of animals per cage in the socially housed groups. Positive effect size indicates that individually housed animals had higher food intake than socially housed animals, negative effect size indicates the reverse. The size of bubbles (indicated by volume) represents the precision of Hedges' g estimate; large bubbles are most precise. The regression line fitted to the raw data shows the constant (Y intercept) and slope of the relation between number of the animals in the cage and the Hedges' g effects size.

the high heterogeneity could be explained by the duration of the housing intervention or the cage density of the socially housed animals.

Stratification

Housing condition did not significantly influence body weight across the included studies. However, when only the 45 matched groups for which food intake data were also available ($n = 45$ matched groups) were analysed for the effect of individual housing versus social housing on body weight, there was a significant effect ($ES = 0.23$, 95% $CI = 0.02, 0.44$, $p = 0.03$, Fig. S4), with higher body weight in individually housed animals compared with socially housed animals. Meta-regression analysis of this subset revealed a tendency for the number of animals per cage to be negatively correlated to body weight (slope = 0.14, 95% $CI = -0.01, 0.29$, $p = 0.07$). In this subset, 23 matched groups showed a higher body weight in individually housed animals compared with socially housed animals (i.e. positive ES for body weight, Fig. S4), and this was in conjunction with an increase in food intake (meta-analysis of food intake in 23 matched groups, $ES = 0.59$, 95% $CI = 0.38, 0.81$, $p < 0.01$). Although body weight was not higher due to individual housing in the remaining 22 matched groups (Fig. S4), food intake was still significantly higher in the individually housed animals compared with socially housed animals in this subset (meta-analysis of food intake in 22 matched groups, $ES = 0.44$, 95% $CI = 0.08, 0.80$, $p = 0.02$).

In the subset of studies that reported on visceral fat mass (12 ES s), meta-analysis did not reveal a significant effect of individual housing on body weight ($ES = 0.24$, 95% $CI = -0.19, 0.66$, $p = 0.28$). In this subset, there were 7 matched groups that also reported on food intake. In these cases, the ES s for visceral adiposity and food intake were in the same direction, however, no analysis was performed because of the limited number of records.

Quality and risk of bias of included studies

The quality of the studies included in this review was assessed using the Systematic Review Center for Laboratory Animal Experimentation risk of bias tool (52) (Fig. 4). There was a high risk of bias due to incorrect use of the unit of analysis (i.e. the socially housed animals living together in one cage were used as individual units of analysis), with only 7% of studies using the correct unit of analysis. Whilst over 50% of studies controlled for baseline differences at the beginning of the study, and a further 19% corrected or controlled for differences at baseline, initial randomization of animals was poorly reported (65% of articles did not describe any sequence allocation). Methods for blinding of experimenters and randomization of outcome measurements were rarely reported (Fig. 4).

Discussion

We have systematically collated, evaluated and analysed the available rodent studies on effects of individual versus social housing on body weight, food intake and visceral fat mass, for rats and mice living under well-accepted and commonly used laboratory conditions. Analysis of the included 94 matched groups revealed that housing condition had no overall effect on body weight. Alterations in body weight by individual housing were not universally observed in our stratification of the meta-analysis; in a subset that reported food intake, individual housing was associated with an increase in food intake and an increase in body weight. Further subdivision of this group revealed that food intake was significantly increased in individually housed animals in those studies that reported a positive and those that reported an equal or negative ES for body weight. Studies that reported visceral adiposity demonstrated that individual housing strongly increased visceral adiposity without an associated increase in body weight. The independence of body weight and food intake or visceral adiposity was underlined by the fact that their ES s were unrelated. The differences in

Figure 4 Risk of bias for body weight measure reported in 71 included articles. The quality and risk of bias of body weight data in the included studies were independently assessed by two reviewers using a modified version of the Systematic Review Center for Laboratory Animal Experimentation risk of bias tool. 'High' indicates that there was clear indication of potential bias. 'Low' indicates that the risk of bias was minimal. 'Unclear' indicates that the risk of bias could not be judged due to incomplete reporting.

outcomes of the overall and subset analysis for body weight may be the result of differences in maximal cage density; i.e. there is the risk of potential crowding in studies using high cage density, which may have confounded the observed results. However, collectively, these data suggest that individual housing occasionally increases body weight, but increases in food intake and visceral adiposity, that are independent of changes in body weight, are a consistent consequence of individual housing.

To the best of our knowledge, this is the first systematic review and meta-analysis to compare the effects of individual and social housing conditions in rats and mice on body weight, food intake and visceral adiposity. Several reviews have assessed the effects of rearing rodents in social isolation on brain development and function, and the use of social isolation as a model for neuropsychiatric disease, e.g. (21,22). Although these reviews were aimed at evaluating behavioural phenotypes rather than metabolic health, they did report that social isolation did not alter body weight (22) and resulted in higher food intake (21), which is in line with our analysis. Higher body weight due to individual housing have been reported in a few studies evaluating large datasets comprising multiple studies conducted over several years (108–112). However, these studies did not control for individual studies introducing potential statistical bias (112), or for additional differences between individual and socially housed groups in terms of treatments, research facilities, experimental design, cage material and food presentation (108–111), all of which are known to affect body weight in rats and mice (113).

Effects of experimental factors

There was high heterogeneity for body weight and moderate to high heterogeneity for food intake and visceral fat mass as the experimental designs of the included studies showed considerable variation in terms of species, sex and dietary background. We included both rats and mice in our analysis. Yet, although species differences in response to stress (114,115) and metabolic challenges (116,117) have been reported, we found no influence of species on the effects of individual housing on body weight, food intake or visceral fat mass. Similarly, whilst some evidence suggests sex specific differences in the responses to metabolic challenges (118,119) and hypothalamic-pituitary-adrenal (HPA) axis activation in response to social isolation (120,121), the results of our meta-analysis did not confirm that sex contributed significantly to the effects of housing on body weight, food intake or visceral fat mass. We did include studies in our analysis that used high fat diets or high sugar diets to induce metabolic disturbances and hypothesized that individual housing on this dietary background could induce stronger changes in metabolic phenotype, but did not detect such effects in

the moderator analysis. Nevertheless, there are studies showing that mice exposed to individual housing for 4 weeks experienced substantially greater weight gain when fed a high fat diet compared with normal chow fed controls (44). Yet overall, our moderator analyses did not detect any contribution of species, sex or diet to the observed heterogeneity.

The inclusion of studies using a high cage density for socially housed groups may have confounded the observed effects of individual versus social housing on body weight. A number of studies comparing housing densities have reported population-dependent effects on body weight gain, with animals from higher density cages gaining less weight (31,64,78,93,104). In contrast, however, social overcrowding of rodents has been proposed as a model for obesity and metabolic dysfunction (122). Inclusion of crowding in our dataset may explain the inverse (or, after removal of one study (69), lack of) relationship we observed between cage density and body weight. We were unable to identify crowding in the included articles, unless specified by the authors, also because a clear definition for crowding does not exist and details about cage size/surface area per animal were rarely reported. Moreover, optimal population density for rodents depends on many factors, including procedures, strain, sex, age, familiarity of the animals and previous social experience (123). Comparing effects of different forms of social housing on body weight or metabolic health parameters was beyond the scope of this review, but it remains an interesting angle to be investigated.

Other experimental/environmental factors and outcome variability

Other experimental/environmental factors potentially increased the variability in reported outcomes, but could not be included in the meta-analysis due to incomplete reporting. Ambient temperature modulates food intake and thermoregulation in mice, and has been reported to interact with cage density (124). In addition, cage materials and other inanimate environmental factors such as cage size, shelter and enrichment objects can influence the temperature within the cage (125). These factors affect many other aspects of rodent behaviour and physiology, including the level of anxiety and stress (126–128), home cage activity (129), body weight (130), energy expenditure, adiposity and the browning of white adipose tissue (131); indeed, some have been shown to interact with the effects of individual housing on these outcomes (45,54,97,104). However, many studies did not report ambient temperature and/or cage size, materials and (enrichment) objects, and hence, could not be used as a variable.

The effects of physical activity are also important in the interpretation and applicability of our findings. For

instance, studies in which individually housed animals had access to a running wheel were excluded from the review, because running wheel activity may alter the outcome of housing on energy balance and metabolic health. Indeed, physical activity requires energy and is integral in the regulation of body weight (132). Although reduced levels of home cage activity in individually housed animals might be expected, due to a lack of interaction with cage mates (133), studies report higher levels of activity during behavioural tests in individually housed animals (61,72,79,134,135). Yet, home cage activity may not be comparable with activity during testing conditions in a novel environment. The evidence for increased home cage activity in individually housed animals is more limited (47).

We did not include or exclude articles based on inanimate environmental conditions (except exercise paradigms) because detailed information was often not reported. The frequency/intensity of animal handling (routine or experimental) may also influence metabolic health and potentially interact with housing conditions (43,74,91,135,136), but, again, detailed information was mostly lacking. In addition, whilst we excluded data obtained from mutants and strains with specific metabolic deficits (39,137), we did not correct for genetic background (e.g. strain). Some variability in weight gain would be expected based on strain-dependent susceptibility to stress (138,139) or diet induced obesity (140–142), however, there was no evidence for strain-specific effects in the two included studies that compared multiple rodent strains (71,100).

A hierarchical relationship exists between rodents living in the same cage (143), and subordinate and dominant animals can show contrasting patterns in weight gain, food intake and adiposity (11,44,144). We excluded studies that deliberately induced a social hierarchy within socially housed groups, e.g. chronic social stress or social defeat models, but social status was not specifically tested in the included articles. It is likely though that social dynamics may have increased variance in outcomes. Indeed, higher variation in body composition was observed in socially housed compared with individually housed animals (33), and a dominant/subordinate relationship has been shown to be reflected in higher adrenal weights in subordinate animals (62).

The level of perceived isolation during individual housing can be influenced by prior exposure to different social contexts and the degree of visual, auditory or olfactory interaction between animals, which is in turn dependent on cage design, cage material and placement of the cage in the room. Some of the studies indicated that the caging system allowed some degree of visual, auditory or olfactory interaction between 'isolated' animals (61,63,66,70,79,84). These housing systems may result in lower levels of stress compared with true isolation (145–147). Of the included studies, only

two applied true social isolation by placing cages within polystyrene boxes (88,92). Most studies provided no details as to what extent social contact between isolated animals was possible. Improved reporting of such housing details are necessary to understand the impact of visual, auditory and olfactory cues on observed differences between individual and socially housed animals.

Effects of life stage

The second aim of this review was to determine whether the age at which mice and rats experience isolation housing would influence their body weight, food intake and visceral fat mass at a later stage. We report that ESs were higher in the young-adult age life stage compared with the young-young (for body weight and food intake) and adult-adult (for body weight, food intake and visceral fat mass) stages, although the differences between life stages did not reach statistical significance.

In rodents, adolescence (from weaning until sexual maturation) is a period of vulnerability to isolation stress, which can lead to anxiety and cognitive dysfunction (148). Some of the neurobiological and endocrine systems affected are also involved in controlling food intake (e.g. hypothalamus, amygdala (30,149)), and these systems continue to mature in the post-weaning period (150,151). Adolescence is a period of fast lean body growth (26,27) and adipose tissue maturation (152–154), which is linked to increased energy requirements and can be influenced by glucocorticoids (155). Consequently, adolescence is a period of increased vulnerability to changes in energy distribution and stress, challenging the maturing metabolic system, resulting in programming of later life metabolic health. In general, animals in the young-adult life stage experienced the longest exposure to individual housing, but regression analysis to assess duration of individual housing as a modulator showed no such relationship.

Proposed mechanisms by which individual housing affects energy balance and metabolic health

Several mechanisms can be proposed to contribute to increased food intake and visceral fat deposition in individually housed compared with socially housed animals. Rats and mice are social species that minimize energy expenditure by sharing body heat; individual housing increases their energy costs. As changes in body weight are the result of imbalances between energy intake and energy expenditure, group differences in energy intake without differences in body weight must be due to changes in energy expenditure (assuming that absorption is not affected by housing condition). Thus, the increased energy intake that we report in individually housed animals is likely necessary to support the demands of increased thermogenesis (7,8).

The debate regarding this phenomenon has increased over recent years (see e.g. (36,156)), and some studies indeed report increases in brown adipose tissue (BAT) stores and thermogenic capacity and activity in individually housed rodents, which may also depend on the ambient temperature (31,42,157). A study by Himms-Hagen and Villemure showed that even at 28°C (i.e. which is close to thermoneutrality for the mouse), the occurrence of social thermoregulation (huddling) reduces the requirement for BAT thermogenesis, and actually results in a reduction in its thermogenic capacity (31,42,157). The BAT is able to increase thermogenesis in response to cold stress through activation of uncoupling protein 1 (UCP-1), which is mediated via activation of the sympathetic nervous system (158). In addition, cold stress may induce feelings of hunger, leading to initiation of feeding behaviour to fuel cold-induced BAT thermogenesis (159) and stimulation of diet-induced thermogenesis (160). Visceral adipose tissue can also undergo 'browning' as a response to cold, and engage UCP-1 activity (161). As physical activity also increases body temperature, the increased activity level of individually housed animals has also been proposed to be a compensatory mechanism to generate body heat in the absence of social thermoregulation (63).

Individual housing can also induce chronic emotional stress (13,162). Remodelling of brain neural circuitry, combined with hormones released in response to isolation stress, may affect the neurobiological control of motivational and hedonic aspects of food intake (163,164), as well as impaired homeostatic regulation of energy balance, leading to leptin resistance, hyperphagia and increased (visceral) adiposity (15,16,30,165,166). In addition, stress-induced activation of the HPA axis and alterations in glucocorticoid functioning can affect the brain-adipose axis, resulting in increased visceral fat storage (17,18,155,167). Indeed, individual housing has been found to disrupt plasma glycaemic and lipid profiles (80,81,89,137), adipocyte morphology and hepatic/adipocyte gene expression profiles involved in lipid metabolism (40,42,88), even in the absence of concurrent changes in body weight. In turn, increased fatty acid overflow from the visceral depot towards the liver stimulates the sympatho-adrenal system and the HPA axis, (168) which can ignite a vicious circle, worsening metabolic syndrome characteristics (169).

Although the here reported effects of individual housing on increased food intake and visceral adiposity independent of body weight came from different stratifications within the initial data set, it is tempting to speculate that these phenomena can actually take place within the same animal. The question remains whether, and to what extent, individually housed animals with increased visceral fat deposition largely engage BAT thermogenesis or even browning of visceral fat. Increased ectopic expression of UCP-1 in visceral depots reduces visceral adiposity (170), which

is in contrast to what we report. Adverse health consequences of visceral adiposity are insulin resistance and inflammatory activation, which can impair cardiometabolic health (171). It has been suggested in the human literature that adverse health consequences of obesity are metabolically costly and could increase basal metabolic rate (BMR) (172–174). An increase in BMR would increase heat production independent of physical activity, thermic effects of food, and adaptive thermogenesis through UCP1 activation. Such a mechanism may also have contributed to the finding by the group of van Marken-Lichtenbelt (175), showing that obese humans rely less on BAT activation to maintain core temperature than lean individuals. Obese and metabolically deranged individuals might engage BAT activation in more extreme cold gradients, when BMR buffering becomes insufficient. Insufficient BMR-mediated thermogenesis may also be the case in rodents that live solitarily, and thus lack social thermoregulation and are exposed to more extreme cold (as is suggested by the work of Jennings *et al.* (31)). Methodologies used to study the different components of energy expenditure and body temperature regulation in individually housed animals, such as those employed in Fischer *et al.*, Abreu-Vieira *et al.* and van der Vinne *et al.* (176–178), should be validated for use in group-housed animals to reconcile the behavioural energetics of housing conditions in rodents at sub-euthermic temperatures.

Some of the proposed mechanisms underlying visceral adiposity and thermoregulatory control that may play a role in the effects of individual housing are depicted in Fig. 5. The thermoregulatory control itself may differ extensively between obese and non-obese animals (179), which would obviously complicate this picture further, and is considered beyond the scope of this review.

Limitations

Our initial screening criteria resulted in the inclusion of articles that mentioned body weight in the title/abstract. Thus, we did not include articles that may have reported body weight or other metabolic health measures in the full text only. This was an important compromise that was necessary to secure feasibility of the review-process; however, studies mentioning body weight in the title/abstract are likely to be those that evaluated metabolic health outcomes as a main aim of the study, which will have contributed to publication bias in our dataset. The use of the standardized ES Hedges' *g* allows for the inclusion of data reported in different units (e.g. absolute body weight or relative body weight gain). However, it cannot correct for cases in which discrepancies exist between absolute and relative values, such as differences in body weight at the start of a study. In most studies, authors reported either absolute or body weight gain at the end of the experiment,

Figure 5 Proposed mechanisms on how (chronic) individual housing may affect energy balance and visceral adiposity. At standard room temperature, individual housing induces a combination of emotional stress and cold. Chronic emotional stress (solid lines) induces changes in neuroendocrine activity, including activation of the hypothalamic-pituitary-adrenal (HPA) and changes in regulation of energy balance (among others associated with leptin resistance, lowered satiety and altered reward pathways), which collectively can lead to increased visceral adiposity. This is a risk factor for causing deterioration of cardiometabolic health. The lack of social thermoregulation could induce cold stress (dashed lines), which activates the sympathetic nervous system (SNS), leading among others to brown-adipose tissue (BAT) thermogenesis. In addition, visceral adiposity itself may undergo browning, which would counter visceral adiposity. Cold stress also stimulates food intake, which (together with the increased emotional stress-induced food intake) contributes to diet-induced thermogenesis. Finally, the cardiometabolic derangements are in itself energy-consuming processes, which are proposed to stimulate basal metabolic rate. Collectively, these mechanisms all contribute to increased production of body heat that compensate for the lack of social thermoregulation and fall of body temperature. These processes may occur even without changes in body weight. Contributions of spontaneous activity are not clear, and may depend on experimental paradigm.

yet body weight at the start of the intervention was not reported.

Recommendations for future research

Based on the current review and meta-analysis, we propose four recommendations for future studies. First, unless as a primary experimental manipulation, changes in social housing conditions within an experiment should be avoided. Switching animals (temporarily) from social to individual housing for the purpose of data collection should be applied with care, taking into account short-term and long-term metabolic changes. Second, whilst standardization of cage and environmental conditions would be ideal, a less challenging goal would be to improve reporting of environmental and experimental conditions and basic experimental data, e.g. following Animal Research: Reporting of In Vivo Experiments guidelines (180) to allow replication of results and reduce impact of confounding factors. Third,

experiments designed to understand the influence of life stage on the metabolic consequences of individual housing are needed. There are currently only few studies that directly compare the metabolic phenotypes following individual housing during adolescence and adulthood. Fourth, more mechanistic studies, which investigate *how* individual housing affects energy balance regulation and metabolic phenotype, are urgently required.

The findings presented in this review are relevant for obesity and metabolic (programming) research using rodents. In humans, the prevalence of obesity, metabolic syndrome and related non-communicable diseases has increased to epidemic proportions in industrialized societies (181,182). Whilst our analysis indicates that individual housing in mice and rats could be considered as a model for obesity or metabolic programming, the basis for the metabolic phenotype induced by individual housing in rodents differs considerably from that in the human situation. Consequently, pharmaceutical, nutritional or other interventions designed to

repair or rescue metabolic health may be influenced by the chosen housing situation. Moreover, interventions in individually housed animals may not be directly translatable or comparable with effects of similar interventions in socially housed animals. Data collected during the temporary individual housing of an animal cannot be used to explain the metabolic phenotype established in a preceding period in which animals were socially housed. Indeed, the same considerations may apply to other experimental outcomes sensitive to changes in metabolic health status such as behaviour and cognition. The use of different social housing paradigms may help explain some of the variation reported in these outcomes. These considerations are of particular importance when evaluating efficacy of drugs, diet or other interventions on metabolic health or other outcomes. This includes (systematic) reviews; housing conditions are seldom taken into account as a possible moderator and confounder.

Conclusions

In summary, the results of this systematic review support the understanding that individual housing of laboratory rats and mice influences energy balance regulation and metabolic health, with visceral adiposity and increased food intake as key features, which we hypothesize to be connected to increased thermogenesis and emotional stress. The results show considerable heterogeneity that could not be explained by species, sex or diet, which is likely caused by high variation in environmental and experimental factors. To minimize housing condition-dependent variation in study outcomes, quality of future studies may be improved by standardization and more detailed reporting of such factors in order to facilitate interpretation and allow between-study comparisons. The influence of life stage on long-term metabolic health as a result of individual housing could not be substantiated by the current meta-analysis; future studies should focus on age specificity and the underlying mechanisms involved in individual housing-induced metabolic changes described here. Finally, programmed metabolic disturbances will contribute to interpretation of outcomes of any study investigating effects of drugs, diets or other interventions where individual housing is applied, and should be taken into account when designing and describing such studies.

Conflict of interest statement

No conflict of interest was declared.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article. <https://doi.org/10.1111/obr.12663>

Figure S1: Forest plot of effect of individual housing on body weight, $n = 94$ effect sizes M, male; F, female; HFD, high fat diet; HSD, high sugar diet; CI, confidence interval

Figure S2: Forest plot of effect of individual housing on food intake, $n = 45$ effect sizes M, male; F, female; HFD, high fat diet; HSD, high sugar diet; CI, confidence interval

Figure S3: Forest plot of effect of individual housing on fat mass, $n = 12$ effect sizes HFD, high fat diet; WATv, visceral White Adipose Tissue; CI, confidence interval

Figure S4: Forest plot of effect of individual housing on body weight (in groups also reporting food intake), $n = 45$ effect sizes. M, male; F, female; HFD, high fat diet; HSD, high sugar diet; CI, confidence interval

References

1. Ebensperger LA. A review of the evolutionary causes of rodent group-living. *Acta Theriol* 2001; **46**: 115–144.
2. Terrien J, Perret M, Aujard F. Behavioral thermoregulation in mammals: a review. *Front Biosci (Landmark Ed)* 2011; **16**: 1428–1444.
3. Council NR. Guide for the Care and Use of Laboratory Animals, 8th edition, 8th edn. National Academic Press: Washington D.C., 2011.
4. Jones CA, Watson DJ, Fone KC. Animal models of schizophrenia. *Br J Pharmacol* 2011; **164**: 1162–1194.
5. Golden SA, Covington HE 3rd, Berton O, Russo SJ. A standardized protocol for repeated social defeat stress in mice. *Nat Protoc* 2011; **6**: 1183–1191.
6. Nollert M, Le Guisquet AM, Belzung C. Models of depression: unpredictable chronic mild stress in mice. *Curr Protoc Pharmacol* 2013; **61** Chapter 5: 5.65.1 - 5.65.17
7. Heldmaier G. The influence of the social thermoregulation on the cold-adaptive growth of BAT in hairless and furred mice. *Pflügers Arch* 1975; **355**: 261–266.
8. Gordon CJ, Becker P, Ali JS. Behavioral thermoregulatory responses of single- and group-housed mice. *Physiol Behav* 1998; **65**: 255–262.
9. Hoyenga KT, Aeschleman S. Social facilitation of eating in the rat. *Psychon Sci* 1969; **14**: 239–239.
10. Galef BG Jr, Giraldeau LA. Social influences on foraging in vertebrates: causal mechanisms and adaptive functions. *Anim Behav* 2001; **61**: 3–15.
11. Moles A, Bartolomucci A, Garbugino L *et al.* Psychosocial stress affects energy balance in mice: modulation by social status. *Psychoneuroendocrinology* 2006; **31**: 623–633.

12. Nyuyki KD, Beiderbeck DI, Lukas M, Neumann ID, Reber SO. Chronic subordinate colony housing (CSC) as a model of chronic psychosocial stress in male rats. *PLoS One* 2012; 7: e52371.
13. Koolhaas JM. The laboratory rat. In: Hubrecht R, Kirkwood J (eds). *The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals*. Wiley-Blackwell: Chichester, United Kingdom, 2010, pp. 311–326.
14. Martin AL, Brown RE. The lonely mouse: verification of a separation-induced model of depression in female mice. *Behav Brain Res* 2010; 207: 196–207.
15. Sefton C, Harno E, Davies A *et al*. Elevated hypothalamic glucocorticoid levels are associated with obesity and hyperphagia in male mice. *Endocrinology* 2016; 157: 4257–4265.
16. Gyengesi E, Liu ZW, D'Agostino G *et al*. Corticosterone regulates synaptic input organization of POMC and NPY/AgRP neurons in adult mice. *Endocrinology* 2010; 151: 5395–5402.
17. Peckett AJ, Wright DC, Riddell MC. The effects of glucocorticoids on adipose tissue lipid metabolism. *Metabolism* 2011; 60: 1500–1510.
18. Lee MJ, Pramyothin P, Karastergiou K, Fried SK. Deconstructing the roles of glucocorticoids in adipose tissue biology and the development of central obesity. *Biochim Biophys Acta* 1842; 2014: 473–481.
19. Makinodan M, Rosen KM, Ito S, Corfas G. A critical period for social experience-dependent oligodendrocyte maturation and myelination. *Science* 2012; 337: 1357–1360.
20. Lukkes JL, Mokin MV, Scholl JL, Forster GL. Adult rats exposed to early-life social isolation exhibit increased anxiety and conditioned fear behavior, and altered hormonal stress responses. *Horm Behav* 2009; 55: 248–256.
21. Lapid MD, Fulford A, Muchimapura S, Mason R, Parker T, Marsden CA. Influence of postweaning social isolation in the rat on brain development, conditioned behavior, and neurotransmission. *Neurosci Behav Physiol* 2003; 33: 13–29.
22. Fone KC, Porkess MV. Behavioural and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders. *Neurosci Biobehav Rev* 2008; 32: 1087–1102.
23. Whitaker LR, Degoulet M, Morikawa H. Social deprivation enhances VTA synaptic plasticity and drug-induced contextual learning. *Neuron* 2013; 77: 335–345.
24. Ros-Simo C, Valverde O. Early-life social experiences in mice affect emotional behaviour and hypothalamic-pituitary-adrenal axis function. *Pharmacol Biochem Behav* 2012; 102: 434–441.
25. Lander SS, Linder-Shacham D, Gaisler-Salomon I. Differential effects of social isolation in adolescent and adult mice on behavior and cortical gene expression. *Behav Brain Res* 2017; 316: 245–254.
26. Malik RC. Genetic and physiological aspects of growth, body composition and feed efficiency in mice: a review. *J Anim Sci* 1984; 58: 577–590.
27. Gargiulo S, Gramanzini M, Megna R *et al*. Evaluation of growth patterns and body composition in C57BL/6J mice using dual energy X-ray absorptiometry. *Biomed Res Int* 2014; 2014: 253067.
28. Nutrition NRCUSoLA. Nutrient Requirements of the Mouse. In: Overton JM (ed.). *Nutrient Requirements of Laboratory Animals: Fourth Revised Edition*, 1995. National Academies Press: (US); Washington D.C., 1995.
29. Bergman RN, Kim SP, Catalano KJ *et al*. Why visceral fat is bad: mechanisms of the metabolic syndrome. *Obesity (Silver Spring)* 2006; 14(Suppl 1): 16S–19S.
30. Nakhate KT, Kokare DM, Singru PS, Subhedar NK. Central regulation of feeding behavior during social isolation of rat: evidence for the role of endogenous CART system. *Int J Obes (Lond)* 2011; 35: 773–784.
31. Jennings G, Richard D, Trayhurn P. Effect of caging singly or in groups of different sizes on the thermogenic activity of interscapular brown adipose tissue in mice. *Comp Biochem Physiol A Comp Physiol* 1986; 85: 583–586.
32. Kalinichev M, Rourke C, Daniels AJ *et al*. Characterisation of olanzapine-induced weight gain and effect of aripiprazole vs olanzapine on body weight and prolactin secretion in female rats. *Psychopharmacology (Berl)* 2005; 182: 220–231.
33. Nagy TR, Krzywanski D, Li J, Meleth S, Desmond R. Effect of group vs. single housing on phenotypic variance in C57BL/6 J mice. *Obes Res* 2002; 10: 412–415.
34. Kamakura R, Kovalainen M, Leppaluoto J, Herzig KH, Makela KA. The effects of group and single housing and automated animal monitoring on urinary corticosterone levels in male C57BL/6 mice. *Physiol Rep* 2016; 4: e12703.
35. Harris AP, D'Eath RB, Healy SD. Sex differences in spatial cognition are not caused by isolation housing. *Behaviour* 2008; 145: 757–778.
36. Karp CL. Unstressing intemperate models: how cold stress undermines mouse modeling. *J Exp Med* 2012; 209: 1069–1074.
37. Bjørndal B, Burri L, Staalesen V, Skorge J, Berge RK. Different adipose depots: their role in the development of metabolic syndrome and mitochondrial response to hypolipidemic agents. *J Obes* 2011; 2011: 490650.
38. Harris RB, Zhou J, Youngblood BD, Smagin GN, Ryan DH. Failure to change exploration or saccharin preference in rats exposed to chronic mild stress. *Physiol Behav* 1997; 63: 91–100.
39. Nonogaki K, Nozue K, Oka Y. Social isolation affects the development of obesity and type 2 diabetes in mice. *Endocrinology* 2007; 148: 4658–4666.
40. Tsuduki T, Yamamoto K, Shuang E, Hatakeyama Y, Sakamoto Y. High dietary fat intake during lactation promotes the development of social stress-induced obesity in the offspring of mice. *Forum Nutr* 2015; 7: 5916–5932.
41. Benite-Ribeiro SA, dos Santos JM, Duarte JAR. Moderate physical exercise attenuates the alterations of feeding behaviour induced by social stress in female rats. *Cell Biochem Funct* 2014; 32: 142–149.
42. Sun M, Choi EY, Magee DJ, Stets CW, During MJ, Lin E-JD. Metabolic effects of social isolation in adult C57BL/6 mice. *Int Sch Res Notices* 2014; 2014: 9.
43. Marcolin ML, Benitz AND, Arcego DM, Noschang C, Krolow R, Dalmaz C. Effects of early life interventions and palatable diet on anxiety and on oxidative stress in young rats. *Physiol Behav* 2012; 106: 491–498.
44. Bartolomucci A, Cabassi A, Govoni P *et al*. Metabolic consequences and vulnerability to diet-induced obesity in male mice under chronic social stress. *PLoS One* 2009; 4: e4331.
45. Lopez MF, Laber K. Impact of social isolation and enriched environment during adolescence on voluntary ethanol intake and anxiety in C57BL/6J mice. *Physiol Behav* 2015; 148: 151–156.
46. Ben Menachem-Zidon O, Goshen I, Kreisel T *et al*. Intrahippocampal transplantation of transgenic neural precursor cells overexpressing interleukin-1 receptor antagonist blocks chronic isolation-induced impairment in memory and neurogenesis. *Neuropsychopharmacology* 2008; 33: 2251–2262.
47. Lamkin DM, Lutgendorf SK, Lubaroff D, Sood AK, Beltz TG, Johnson AK. Cancer induces inflammation and depressive-like behavior in the mouse: modulation by social housing. *Brain Behav Immun* 2011; 25: 555–564.
48. Lopak V, Eikelboom R. Pair housing induced feeding suppression: individual housing not novelty. *Physiol Behav* 2000; 71: 329–333.
49. O'Connor R, Eikelboom R. The effects of changes in housing on feeding and wheel running. *Physiol Behav* 2000; 68: 361–371.

50. Abramov U, Raud S, Kõks S *et al.* Targeted mutation of CCK(2) receptor gene antagonises behavioural changes induced by social isolation in female, but not in male mice. *Behav Brain Res* 2004; 155: 1–11.
51. Ali AM, Hakami FA. Comparative studies on the effect of Prozac drug and barely grains on the skeletal muscles of mice: behavioral and histological studies. *J Appl Pharm Sci* 2012; 2: 45–51.
52. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol* 2014; 14: 43.
53. Collaboration TC. Cochrane Handbook for Systematic Reviews of Interventions. John Wiley & Sons Ltd: Chichester, UK, 2008.
54. Abou-Ismaïl UA, Darwish RA, Ramadan SGA. Should cages of laboratory rats be enriched physically or socially? *Global Veterinaria* 2014; 13: 570–582.
55. Ader R, Kreutner A Jr, Jacobs HL. Social environment, emotionality, and alloxan diabetes in the rat. *Psychosom Med* 1963; 25: 60–68.
56. Amir S, Galina HZ, Amit Z. Chronic naltrexone administration reverses the suppressive effect of crowding on body weight gain in rats. *Neuropharmacology* 1979; 18: 905–907.
57. Arcego DM, Krolow R, Lampert C *et al.* Isolation during the prepubertal period associated with chronic access to palatable diets: effects on plasma lipid profile and liver oxidative stress. *Physiol Behav* 2014; 124: 23–32.
58. Baker S, Bielajew C. Influence of housing on the consequences of chronic mild stress in female rats. *Stress* 2007; 10: 283–293.
59. Barnhart JE, Pizzi WJ. The Monosodium L-glutamate (MSG) syndrome in mice develops independently of housing condition. *Neurobehav Toxicol Teratol* 1982; 4: 549–556.
60. Bartolomucci A, Parmigiani S, Gioiosa L, Ceresini G, Palanza P. Effects of housing social context on emotional behaviour and physiological responses in female mice. *Scand J Lab Anim Sci* 2009; 36: 87–95.
61. Bibancos T, Jardim DL, Aneas I, Chiavegatto S. Social isolation and expression of serotonergic neurotransmission-related genes in several brain areas of male mice. *Genes Brain Behav* 2007; 6: 529–539.
62. Brain PF, Nowell NW. The effects of differential grouping on endocrine function of mature male albino mice. *Physiol Behav* 1970; 5: 938–943.
63. Breslin WL, Strohacker K, Carpenter KC, Esposito L, McFarlin BK. Weight gain in response to high-fat feeding in CD-1 male mice. *Lab Anim* 2010; 44: 231–237.
64. Chvedoff M, Clarke MR, Irisarri E. Effects of housing conditions on food intake, body weight and spontaneous lesions in mice. A review of the literature and results of an 18-month study. *Food Cosmet Toxicol* 1980; 18: 517–522.
65. Cowley JJ, Widdowson EM. The effect of handling rats on their growth and behaviour. *Brit J Nutrition* 1965; 19: 397–406.
66. Cruz FC, Duarte JO, Leão RM, Hummel LFV, Planeta CS, Crestani CC. Adolescent vulnerability to cardiovascular consequences of chronic social stress: immediate and long-term effects of social isolation during adolescence. *Dev Neurobiol* 2016; 76: 34–46.
67. Dawson R Jr, Lorden JF. Behavioral and neurochemical effects of neonatal administration of monosodium L-glutamate in mice. *J Comp Physiol Psychol* 1981; 95: 71–84.
68. Fischer CW, Liebenberg N, Elfving B, Lund S, Wegener G. Isolation-induced behavioural changes in a genetic animal model of depression. *Behav Brain Res* 2012; 230: 85–91.
69. Gangrade BK, Dominic CJ. Evaluation of the involvement of the adrenals in the pheromonal influences on the oestrous cycle of laboratory mice. *Exp Clin Endocrinol* 1984; 84: 13–19.
70. Gilabert-Juan J, Belles M, Saez AR *et al.* A “double hit” murine model for schizophrenia shows alterations in the structure and neurochemistry of the medial prefrontal cortex and the hippocampus. *Neurobiol Dis* 2013; 59: 126–140.
71. Goodrick CL. The effect of protein malnourishment and caging on growth and behavior of laboratory mice. *Dev Psychobiol* 1974; 7: 249–256.
72. Guo M, Wu CF, Liu W, Yang JY, Chen D. Sex difference in psychological behavior changes induced by long-term social isolation in mice. *Prog Neuro-Psychopharmacol Biol Psychiatry* 2004; 28: 115–121.
73. Hahn WW. Some effects of group size on behavior and physiology of the rat. *J Psychosom Res* 1965; 8: 455–465.
74. Haller J, Halász J. Mild social stress abolishes the effects of isolation on anxiety and chlordiazepoxide reactivity. *Psychopharmacology (Berl)* 1999; 144: 311–315.
75. Hoffman-Goetz L, Arumugam Y. Effects of housing condition on natural killer cell activity in mildly energy restricted mice. *Nutr Res* 1992; 12: 747–755.
76. Hughes PC, Nowak M. The effect of the number of animals per cage on the growth of the rat. *Lab Anim* 1973; 7: 293–296.
77. Innos J, Philips M-A, Raud S, Lilleväli K, Kõks S, Vasar E. Deletion of the Lsamp gene lowers sensitivity to stressful environmental manipulations in mice. *Behav Brain Res* 2012; 228: 74–81.
78. Ishida H, Mitsui K, Nukaya H, Matsumoto K, Tsuji K. Study of active substances involved in skin dysfunction induced by crowding stress. I. Effect of crowding and isolation on some physiological variables, skin function and skin blood perfusion in hairless mice. *Biol Pharm Bull* 2003; 26: 170–181.
79. Jahng JW, Yoo SB, Ryu V, Lee J-H. Hyperphagia and depression-like behavior by adolescence social isolation in female rats. *Int J Dev Neurosci* 2012; 30: 47–53.
80. Ko C-Y, Liu Y-P. Isolation rearing impaired sensorimotor gating but increased pro-inflammatory cytokines and disrupted metabolic parameters in both sexes of rats. *Psychoneuroendocrinology* 2015; 55: 173–183.
81. Ko C-Y, Liu Y-P. Disruptions of sensorimotor gating, cytokines, glycemia, monoamines, and genes in both sexes of rats reared in social isolation can be ameliorated by oral chronic quetiapine administration. *Brain Behav Immun* 2016; 51: 119–130.
82. Krolow R, Noschang C, Arcego DM *et al.* Sex-specific effects of isolation stress and consumption of palatable diet during the prepubertal period on metabolic parameters. *Metab Clin Exp* 2013; 62: 1268–1278.
83. Krugel U, Fischer J, Bauer K, Sack U, Himmerich H. The impact of social isolation on immunological parameters in rats. *Arch Toxicol* 2014; 88: 853–855.
84. Marks-Kaufman R, Lewis MJ. Early housing experience modifies morphine self-administration and physical dependence in adult rats. *Addict Behav* 1984; 9: 235–243.
85. Maslova LN, Bulygina VV, Amstislavskaya TG. Prolonged social isolation and social instability in adolescence in rats: immediate and long-term physiological and behavioral effects. *Neurosci Behav Physiol* 2010; 40: 955–963.
86. Morgan M, Einon D. Incentive motivation and behavioral inhibition in socially-isolated rats. *Physiol Behav* 1975; 15: 405–409.
87. Morinan A, Leonard BE. Some anatomical and physiological correlates of social isolation in the young rat. *Physiol Behav* 1980; 24: 637–640.

88. Motoyama K, Nakai Y, Miyashita T *et al.* Isolation stress for 30 days alters hepatic gene expression profiles, especially with reference to lipid metabolism in mice. *Physiol Genomics* 2009; **37**: 79–87.
89. Perez C, Canal JR, Dominguez E, Campillo JE, Guillen M, Torres MD. Individual housing influences certain biochemical parameters in the rat. *Lab Anim* 1997; **31**: 357–361.
90. Pradier B, Erxleben E, Markert A, Rácz I. Interaction of cannabinoid receptor 2 and social environment modulates chronic alcohol consumption. *Behav Brain Res* 2015; **287**: 163–171.
91. Ryu V, Yoo SB, Kang D-W, Lee J-H, Jahng JW. Post-weaning isolation promotes food intake and body weight gain in rats that experienced neonatal maternal separation. *Brain Res* 2009; **1295**: 127–134.
92. Sakakibara H, Suzuki A, Kobayashi A *et al.* Social isolation stress induces hepatic hypertrophy in C57BL/6J mice. *J Toxicol Sci* 2012; **37**: 1071–1076.
93. Shelley HP. Eating behavior: social facilitation or social inhibition. *Psychon Sci* 1965; **3**: 521–522.
94. Silva RCB, Santos NR, Brandão ML. Influence of housing conditions on the effects of serotonergic drugs on feeding behavior in non-deprived rats. *Neuropsychobiology* 2003; **47**: 98–101.
95. Skalicky M, Narath E, Viidik A. Housing conditions influence the survival and body composition of aging rats. *Exp Gerontol* 2001; **36**: 159–170.
96. Stern JA, Winokur G, Eisenstein A, Taylor R, Sly M. The effect of group vs. individual housing on behaviour and physiological responses to stress in the albino rat. *J Psychosom Res* 1960; **4**: 185–190.
97. Takemoto TI, Suzuki T, Miyama T. Effects of isolation on mice in relation to age and sex. *Tohoku J Exp Med* 1975; **117**: 153–165.
98. Thorsell A, Slawewski CJ, Khoury A, Mathe AA, Ehlers CL. Effect of social isolation on ethanol consumption and substance P/neurokinin expression in Wistar rats. *Alcohol (Fayetteville, NY)* 2005; **91–36**, 7.
99. Weltman AS, Sachler AM, Sparber SB. Endocrine, metabolic and behavioral aspects of isolation stress on female albino mice. *Aerosp Med* 1966; **37**: 804–810.
100. Wiberg GS, Airth JM, Grice HC. Methodology in long-term toxicity tests: a comparison of individual versus community housing. *Food Cosmet Toxicol* 1966; **4**: 47–55.
101. Wyndham JR, Everitt AV, Everitt SF. Effects of isolation and food restriction begun at 50 days on the development of age-associated renal disease in the male Wistar rat. *Arch Gerontol Geriatr* 1983; **2**: 317–332.
102. Yamada K, Ohki-Hamazaki H, Wada K. Differential effects of social isolation upon body weight, food consumption, and responsiveness to novel and social environment in bombesin receptor subtype-3 (BRS-3) deficient mice. *Physiol Behav* 2000; **68**: 555–561.
103. Yamada C, Saegusa Y, Nahata M, Sadakane C, Hattori T, Takeda H. Influence of aging and gender differences on feeding behavior and ghrelin-related factors during social isolation in mice. *PLoS One* 2015; **10**: e0140094.
104. Zaias J, Queeney TJ, Kelley JB, Zakharova ES, Izenwasser S. Social and physical environmental enrichment differentially affect growth and activity of preadolescent and adolescent male rats. *J Am Assoc Lab Anim Sci* 2008; **47**: 30–34.
105. Hedges LV, Olkin I. Statistical Methods for Meta-Analysis. Academic Press, Elsevier Inc: London, 1985.
106. Cohen J. Statistical Power Analysis for the Behavioral Sciences, 2nd edn. Lawrence Erlbaum Associates: Hillsdale, NJ, 1988.
107. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**: 557–560.
108. Haseman JK, Bourbina J, Eustis SL. Effect of individual housing and other experimental design factors on tumor incidence in B6C3F1 mice. *Fundam Appl Toxicol* 1994; **23**: 44–52.
109. Haseman JK, Ney E, Nyska A, Rao GN. Effect of diet and animal care/housing protocols on body weight, survival, tumor incidences, and nephropathy severity of F344 rats in chronic studies. *Toxicol Pathol* 2003; **31**: 674–681.
110. Marino DJ. Absolute and relative organ weight trends in B6C3F1 mice. *J Toxicol Environ Health A* 2012; **75**: 148–169.
111. Rao GN, Crockett PW. Effect of diet and housing on growth, body weight, survival and tumor incidences of B6C3F1 mice in chronic studies. *Toxicol Pathol* 2003; **31**: 243–250.
112. DeFeudis FV. Differences in brain and body weights of mice caused by differential housing. *Experientia* 1974; **30**: 521–522.
113. Tabata H, Ikegami H, Kariya K. Comparison of age-related peripheral nerve changes in mice housed in either plastic cages with sawdust-covered solid flooring or wire-mesh-floor cages. *Exp Anim* 2000; **49**: 147–151.
114. Armario A, Castellanos JM. A comparison of corticoadrenal and gonadal responses to acute immobilization stress in rats and mice. *Physiol Behav* 1984; **32**: 517–519.
115. Bain MJ, Dwyer SM, Rusak B. Restraint stress affects hippocampal cell proliferation differently in rats and mice. *Neurosci Lett* 2004; **368**: 7–10.
116. Witte MM, Resuehr D, Chandler AR, Mehle AK, Overton JM. Female mice and rats exhibit species-specific metabolic and behavioral responses to ovariectomy. *Gen Comp Endocrinol* 2010; **166**: 520–528.
117. Haouzi P, Bell HJ, Notet V, Bihain B. Comparison of the metabolic and ventilatory response to hypoxia and H2S in unsedated mice and rats. *Respir Physiol Neurobiol* 2009; **167**: 316–322.
118. Shi H, Strader AD, Woods SC, Seeley RJ. Sexually dimorphic responses to fat loss after caloric restriction or surgical lipectomy. *Am J Physiol Endocrinol Metab* 2007; **293**: E316–E326.
119. Nohara K, Zhang Y, Waraich RS *et al.* Early-life exposure to testosterone programs the hypothalamic melanocortin system. *Endocrinology* 2011; **152**: 1661–1669.
120. McCormick CM, Robarts D, Kopeikina K, Kelsey JE. Long-lasting, sex- and age-specific effects of social stressors on corticosterone responses to restraint and on locomotor responses to psychostimulants in rats. *Horm Behav* 2005; **48**: 64–74.
121. McCormick CM, Smith C, Mathews IZ. Effects of chronic social stress in adolescence on anxiety and neuroendocrine response to mild stress in male and female rats. *Behav Brain Res* 2008; **187**: 228–238.
122. Lin EJ, Sun M, Choi EY, Magee D, Stets CW, During MJ. Social overcrowding as a chronic stress model that increases adiposity in mice. *Psychoneuroendocrinology* 2015; **51**: 318–330.
123. Foltz C, Carbone L, DeLong D *et al.* Considerations for determining optimal mouse caging density. *Lab Anim (NY)* 2007; **36**: 40–49.
124. Toth LA, Trammell RA, Ilsley-Woods M. Interactions between housing density and ambient temperature in the cage environment: effects on mouse physiology and behavior. *J Am Assoc Lab Anim Sci* 2015; **54**: 708–717.
125. Gaskill BN, Gordon CJ, Pajor EA, Lucas JR, Davis JK, Garner JP. Heat or insulation: behavioral titration of mouse preference for warmth or access to a nest. *PLoS One* 2012; **7**: e32799.
126. Meijer MK, Sommer R, Spruijt BM, van Zutphen LF, Baumans V. Influence of environmental enrichment and handling

- on the acute stress response in individually housed mice. *Lab Anim* 2007; **41**: 161–173.
127. Belz EE, Kennell JS, Czambel RK, Rubin RT, Rhodes ME. Environmental enrichment lowers stress-responsive hormones in singly housed male and female rats. *Pharmacol Biochem Behav* 2003; **76**: 481–486.
 128. Weiss IC, Feldon J, Domeney AM. Isolation rearing-induced disruption of prepulse inhibition: further evidence for fragility of the response. *Behav Pharmacol* 1999; **10**: 139–149.
 129. Gordon CJ. Effect of cage bedding on temperature regulation and metabolism of group-housed female mice. *Comp Med* 2004; **54**: 63–68.
 130. Tsai PP, Pachowsky U, Stelzer HD, Hackbarth H. Impact of environmental enrichment in mice. 1: effect of housing conditions on body weight, organ weights and haematology in different strains. *Lab Anim* 2002; **36**: 411–419.
 131. Cao L, Choi EY, Liu X *et al.* White to brown fat phenotypic switch induced by genetic and environmental activation of a hypothalamic-adipocyte axis. *Cell Metab* 2011; **14**: 324–338.
 132. Tou JC, Wade CE. Determinants affecting physical activity levels in animal models. *Exp Biol Med (Maywood)* 2002; **227**: 587–600.
 133. Spangenberg EM, Augustsson H, Dahlborn K, Essen-Gustavsson B, Cvek K. Housing-related activity in rats: effects on body weight, urinary corticosterone levels, muscle properties and performance. *Lab Anim* 2005; **39**: 45–57.
 134. Garzon J, Del Rio J. Hyperactivity induced in rats by long-term isolation: further studies on a new animal model for the detection of antidepressants. *Eur J Pharmacol* 1981; **74**: 287–294.
 135. Sahakian BJ, Burdick C, Luckhurst H, Trayhurn P. Hyperactivity and obesity: the interaction of social isolation and cafeteria feeding. *Physiol Behav* 1982; **28**: 117–124.
 136. Holson RR, Scallet AC, Ali SF, Turner BB. “Isolation stress” revisited: isolation-rearing effects depend on animal care methods. *Physiol Behav* 1991; **49**: 1107–1118.
 137. Miyajima K, Torinawa Y, Motohashi Y *et al.* Effect of isolation stress on glucose/lipid metabolism in spontaneously diabetic torii (SDT) fatty rats. *Br J Med Res* 2015; **8**: 588–594.
 138. Razzoli M, Carboni L, Andreoli M, Ballottari A, Arban R. Different susceptibility to social defeat stress of BalbC and C57BL/6J mice. *Behav Brain Res* 2011; **216**: 100–108.
 139. Pardon MC, Gould GG, Garcia A *et al.* Stress reactivity of the brain noradrenergic system in three rat strains differing in their neuroendocrine and behavioral responses to stress: implications for susceptibility to stress-related neuropsychiatric disorders. *Neuroscience* 2002; **115**: 229–242.
 140. Nishikawa S, Yasoshima A, Doi K, Nakayama H, Uetsuka K. Involvement of sex, strain and age factors in high fat diet-induced obesity in C57BL/6 J and BALB/cA mice. *Exp Anim* 2007; **56**: 263–272.
 141. West DB, Boozer CN, Moody DL, Atkinson RL. Dietary obesity in nine inbred mouse strains. *Am J Physiol* 1992; **262**: R1025–R1032.
 142. Marques C, Meireles M, Norberto S *et al.* High-fat diet-induced obesity Rat model: a comparison between Wistar and Sprague-Dawley Rat. *Adipocyte* 2016; **5**: 11–21.
 143. Blanchard DC, Blanchard RJ. Behavioral correlates of chronic dominance-subordination relationships of male rats in a seminatural situation. *Neurosci Biobehav Rev* 1990; **14**: 455–462.
 144. Reber SO, Birkeneder L, Veenema AH *et al.* Adrenal insufficiency and colonic inflammation after a novel chronic psycho-social stress paradigm in mice: implications and mechanisms. *Endocrinology* 2007; **148**: 670–682.
 145. Hurst JL, Barnard CJ, Tolladay U, Nevison CM, West CD. Housing and welfare in laboratory rats: effects of cage stocking density and behavioural predictors of welfare. *Anim Behav* 1999; **58**: 563–586.
 146. Boggiano MM, Cavigelli SA, Dorsey JR, Kelley CE, Ragan CM, Chandler-Laney PC. Effect of a cage divider permitting social stimuli on stress and food intake in rats. *Physiol Behav* 2008; **95**: 222–228.
 147. Harris AP, D'Eath RB, Healy SD. A cage without a view increases stress and impairs cognitive performance in rats. *Anim Welf* 2010; **19**: 235–241.
 148. McCormick CM, Mathews IZ. HPA function in adolescence: role of sex hormones in its regulation and the enduring consequences of exposure to stressors. *Pharmacol Biochem Behav* 2007; **86**: 220–233.
 149. Adams T, Rosenkranz JA. Social isolation during postweaning development causes hypoactivity of neurons in the medial nucleus of the male rat amygdala. *Neuropsychopharmacology* 2016; **41**: 1929–1940.
 150. Grove KL, Grayson BE, Glavas MM, Xiao XQ, Smith MS. Development of metabolic systems. *Physiol Behav* 2005; **86**: 646–660.
 151. Gos T, Becker K, Bock J *et al.* Early neonatal and postweaning social emotional deprivation interferes with the maturation of serotonergic and tyrosine hydroxylase-immunoreactive afferent fiber systems in the rodent nucleus accumbens, hippocampus and amygdala. *Neuroscience* 2006; **140**: 811–821.
 152. Gaben-Cogneville AM, Swierczewski E. Studies on cell proliferation in inguinal adipose tissue during early development in the rat. *Lipids* 1979; **14**: 669–675.
 153. Ochi M, Sawada T, Hattori T. Tritiated thymidine autoradiographic study on postnatal development of epididymal adipose tissue in the normal mouse. *Anat Embryol* 1987; **177**: 139–145.
 154. Greenwood MR, Hirsch J. Postnatal development of adipocyte cellularity in the normal rat. *J Lipid Res* 1974; **15**: 474–483.
 155. Bujalska IJ, Kumar S, Hewison M, Stewart PM. Differentiation of adipose stromal cells: the roles of glucocorticoids and 11 β -hydroxysteroid dehydrogenase. *Endocrinology* 1999; **140**: 3188–3196.
 156. Speakman JR, Keijer J. Not so hot: optimal housing temperatures for mice to mimic the thermal environment of humans. *Mol Metab* 2013; **2**: 5–9.
 157. Himms-Hagen J, Villemure C. Number of mice per cage influences uncoupling protein content of brown adipose tissue. *Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine (New York, NY)* 1992; **200**: 502–506.
 158. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev* 2004; **84**: 277–359.
 159. Melnyk A, Himms-Hagen J. Temperature-dependent feeding: lack of role for leptin and defect in brown adipose tissue-ablated obese mice. *Am J Physiol* 1998; **274**: R1131–R1135.
 160. Armbruszt S, Garami A. The short- and long-term effects of food intake on thermogenesis. *Temp Multidiscip Biomed J* 2014; **1**: 96–96.
 161. Rosell M, Kafrou M, Frontini A *et al.* Brown and white adipose tissues: intrinsic differences in gene expression and response to cold exposure in mice. *Am J Physiol Endocrinol Metab* 2014; **306**: E945–E964.
 162. Dixon AK. The social behaviour of mice and its sensory control. In: Hedrich HJ, Bullock G (eds). *The Laboratory Mouse*. Elsevier Academic press: London, San Diego, 2004, pp. 287–300.

163. Dallman MF, Pecoraro N, Akana SF *et al.* Chronic stress and obesity: a new view of “comfort food”. *Proc Natl Acad Sci U S A* 2003; **100**: 11696–11701.
164. Teegarden SL, Bale TL. Effects of stress on dietary preference and intake are dependent on access and stress sensitivity. *Physiol Behav* 2008; **93**: 713–723.
165. Thorsell A, Slawecki CJ, El Khoury A, Mathe AA, Ehlers CL. The effects of social isolation on neuropeptide Y levels, exploratory and anxiety-related behaviors in rats. *Pharmacol Biochem Behav* 2006; **83**: 28–34.
166. Liu XY, Shi JH, Du WH *et al.* Glucocorticoids decrease body weight and food intake and inhibit appetite regulatory peptide expression in the hypothalamus of rats. *Exp Ther Med* 2011; **2**: 977–984.
167. Rosmond R. Role of stress in the pathogenesis of the metabolic syndrome. *Psychoneuroendocrinology* 2005; **30**: 1–10.
168. Benthem L, Kuipers F, Steffens AB, Scheurink AJ. Excessive portal venous supply of long-chain free fatty acids to the liver, leading to hypothalamus-pituitary-adrenal-axis and sympathetic activation as a key to the development of syndrome X. A proposed concept for the induction of syndrome X. *Ann N Y Acad Sci* 1999; **892**: 308–311.
169. van Dijk G, de Vries K, Benthem L, Nyakas C, Buwalda B, Scheurink AJ. Neuroendocrinology of insulin resistance: metabolic and endocrine aspects of adiposity. *Eur J Pharmacol* 2003; **480**: 31–42.
170. Poher AL, Veyrat-Durebex C, Altirriba J *et al.* Ectopic UCP1 overexpression in white adipose tissue improves insulin sensitivity in *lou/C* rats, a model of obesity resistance. *Diabetes* 2015; **64**: 3700–3712.
171. Ritchie SA, Connell JM. The link between abdominal obesity, metabolic syndrome and cardiovascular disease. *Nutr Metab Cardiovasc Dis* 2007; **17**: 319–326.
172. Horgan GW, Stubbs J. Predicting basal metabolic rate in the obese is difficult. *Eur J Clin Nutr* 2003; **57**: 335–340.
173. Sabounchi NS, Rahmandad H, Ammerman A. Best fitting prediction equations for basal metabolic rate: informing obesity interventions in diverse populations. *Int J Obes (2005)* 2013; **37**: 1364–1370.
174. Soares MJ, Cummings NK, Ping-Delfos WL. Energy metabolism and the metabolic syndrome: does a lower basal metabolic rate signal recovery following weight loss? *Diabetol Metab Syndr* 2011; **5**: 98–101.
175. Vijgen GH, Bouvy ND, Teule GJ, Brans B, Schrauwen P, van Marken Lichtenbelt WD. Brown adipose tissue in morbidly obese subjects. *PLoS One* 2011; **6**: e17247.
176. Fischer AW, Csikasz RI, von Essen G, Cannon B, Nedergaard J. No insulating effect of obesity. *Am J Physiol Endocrinol Metab* 2016; **311**: E202–E213.
177. Abreu-Vieira G, Xiao C, Gavrilova O, Reitman ML. Integration of body temperature into the analysis of energy expenditure in the mouse. *Mol Metab* 2015; **4**: 461–470.
178. van der Vinne V, Riede SJ, Gorter JA *et al.* Cold and hunger induce diurnality in a nocturnal mammal. *Proc Natl Acad Sci U S A* 2014; **111**: 15256–15260.
179. Landsberg L, Young JB, Leonard WR, Linsenmeier RA, Turek FW. Do the obese have lower body temperatures? A new look at a forgotten variable in energy balance. *Trans Am Clin Climatol Assoc* 2009; **120**: 287–295.
180. Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG. Animal research: reporting in vivo experiments: the ARRIVE guidelines. *Br J Pharmacol* 2010; **160**: 1577–1579.
181. Wang YC, McPherson K, Marsh T, Gortmaker SL, Brown M. Health and economic burden of the projected obesity trends in the USA and the UK. *Lancet* 2011; **378**: 815–825.
182. Muller-Riemenschneider F, Reinhold T, Berghofer A, Willich SN. Health-economic burden of obesity in Europe. *Eur J Epidemiol* 2008; **23**: 499–509.