"Control" laboratory rodents are metabolically morbid: Why it matters

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Failure to recognize that many standard control rats and mice used in biomedical research are sedentary, obese, glucose intolerant, and on a trajectory to premature death may confound data interpretation and outcomes of human studies. Fundamental aspects of cellular physiology, vulnerability to oxidative stress, inflammation, and associated diseases are among the many biological processes affected by dietary energy intake and exercise. Although overfed sedentary rodents may be reasonable models for the study of obesity in humans, treatments shown to be efficacious in these animal models may prove ineffective or exhibit novel side effects in active, normal-weight subjects.

cancer | clinical trials | exercise | insulin resistance | obesity

Mice and rats are the most widely used animal models in biomedical research. When housed under standard laboratory conditions these rodents are sedentary, have continuous access to food, and have virtually no environmental stimulation. Animals maintained in this manner are widely used as "standard" controls in basic and translational biomedical research studies, including preclinical drug testing. Compared to those that are fed less, exercise more, and have a stimulating environment, animals maintained under the usual standard laboratory conditions are relatively overweight, insulin resistant, hypertensive, and are likely to experience premature death (1–5). Indeed, simply reducing daily food intake 20–40% below the ad libitum amount, or providing food intermittently, rather than continuously, has been shown to significantly reduce the risk of developing diseases such as cancer, type 2 diabetes, and renal failure and can extend lifespan by up to 40% in rats and mice (3, 6, 7). Currently, one of the greatest health concerns is the rise in obesity and its associated pathologies, such as metabolic syndrome, diabetes, cardiovascular disease, and cancer. In the United States, more than 30% of the adult population is obese, and some studies estimate that by 2030, more than 366 million people worldwide will develop type 2 diabetes, with obesity being an important factor responsible for this increase (8, 9). Although dietary energy restriction can increase the maximum lifespan of laboratory animals and is therefore hailed as an "antiaging" intervention (10), its major effect is to increase the average lifespan by preventing or delaying the development of various diseases that are the primary cause of death in overweight rodents (11).

The use of overweight and unstimulated animals as standard controls may bias the measured experimental outcomes. We therefore suggest that new guidelines with regards to laboratory animal husbandry should be developed and implemented that ensure that control animals are fed in portions rather than ad libitum and are provided environmental stimulation. At the very least, institutional animal care and use committees should make investigators aware of the fact that their control animals are overfed and relatively sedentary. In the present article, we provide an overview and discuss how experimental outcomes and data interpretation may be altered depending on the type of control animal that is used, i.e., healthy nonobese "lean" control compared to an overweight unhealthy standard control.

Health of Laboratory Animals Is Poor by Human Standards

Laboratory mice and rats are typically housed either singly or in groups of 2–5 per cage with 1–3 square feet of floor space covered with bedding. The animals have continuous access to food, which typically consists of dry pelleted food provided in an overhead bin. Under these conditions the animals gain weight progressively during their adult life; some strains of laboratory rats achieve body weights upwards of 1 kg and fat mass that accounts for 30–50% of the body weight (12). Regular exercise (voluntary exercise on an in-cage running wheel, for example) and/or dietary energy restriction have been shown to cause significant reductions in body weight and body fat mass (~20–40%) (12, 13). In addition to being overweight, laboratory rodents maintained under standard laboratory conditions exhibit a physiological profile consistent with increased disease susceptibility, compared to animals maintained on lower energy diets and/or animals with higher physical activity levels (see Table 1 for overview). Thus, relative to their leaner counterparts on reduced energy diets, typical overweight ad libitum-fed rodents exhibit elevated levels of energy regulatory hormones and factors such as glucose, insulin, triglycerides, low-density lipoprotein (LDL) cholesterol and leptin, and decreased levels of adiponectin and ghrelin (3, 14). Additionally, the general cardiovascular health of laboratory rats has been shown to be improved when their food intake is reduced; i.e., their plasma lipid profiles are improved, blood pressure and resting heart rate are reduced, and the ability of their cardiovascular system to recover from stress is enhanced (15, 16). Consistent with an adverse effect of the standard housing conditions on the overall health and well-being of laboratory animals, it has been demonstrated that wild mice eat less and live longer than domesticated laboratory mice (17).

Studies in which the dietary energy intake of rats or mice was reduced have clearly demonstrated that the standard ad libitum feeding paradigm fosters poor health outcomes and premature death. In one study, the average lifespan of rats was increased from 2.4 years for those housed under standard conditions to 4 years for those maintained on a reduced energy diet; the longest-lived control ad libitum rat lived for 2.9 years, whereas the

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oldest energy-restricted rat lived for 4.6 years (18). The activity levels of rodents that eat a reduced calorie diet have been shown to be increased compared to their ad libitum-fed counterparts (15), which may contribute to their increased lean/fat mass ratio. The insulin sensitivity of muscle and liver cells increases when dietary energy intake is decreased or animals are provided with the opportunity to exercise, indicating that animals housed under standard laboratory conditions are relatively insulin resistant (15, 19). In humans, such a metabolic profile is a harbinger of future dysfunction and disease in multiple organ systems (20). Not only are ad libitum-fed animals less healthy than those that eat less and exercise more, they also have reduced cognitive function. Thus, the cognitive abilities of rats maintained on restricted feeding schedules (15) or provided access to a running wheel (21) are superior to rats maintained under standard housing conditions.

Insulin Resistance and Diabetes

Insulin resistance is defined by impaired sensitivity to insulin of its main target organs, adipose tissue, liver, and muscle. Insulin regulates glucose uptake and circulating free fatty acid (FFA) concentrations. Insulin decreases lipolysis and FFA efflux from adipocytes, inhibits gluconeogenesis in liver cells by reducing key enzyme activities, and induces glucose uptake in skeletal muscle cells by stimulating the translocation of the GLUT4 glucose transporter to the plasma membrane. Obesity, and particularly central obesity, is the most common risk factor for the development of type 2 diabetes and other features of the metabolic syndrome such as dyslipidemia and hypertension. Numerous studies have demonstrated that dietary energy restriction promotes euglycemia and increases insulin sensitivity (22, 23). One longitudinal study of rats showed that dietary energy restriction decreases the mean 24-h plasma glucose levels by ≈15 mg/dL and the circulating insulin levels by ≈50% (24). It has been proposed that the significant effects of dietary energy restriction on circulating levels of glycemic hormones could play a role in the beneficial life-extending actions of dietary energy restriction. However, as control laboratory rats and mice tend to become overweight and insulin resistant under standard housing conditions, the question arises: Is the lifespan of the restricted animals extended only because it has been compared to the lifespan of the relatively unhealthy standard control animals?

Immune Function and Inflammation

There are strong functional connections between the metabolic and immune systems (25–27). Metabolic disruption and obesity involve overactivation of an inflammatory process in metabolically active sites such as adipose tissue, liver, and immune cells. The consequence of this effect is a potent increase in circulating levels of proinflammatory cytokines and other inflammatory markers (28). Activation of the immune system in response to obesity is mediated by several specific and conserved signaling pathways, with Jun N-terminal kinase (JNK) and IκB kinase β/nuclear factor κ-light-chain-enhancer of activated B (IKKβ/NFκB) pathways being the most well studied (29). It is known that the immune activation of these signaling pathways can modify insulin signaling and result in the development of insulin resistance, which exacerbates systemic physiological failure. In obese patients as well as standard control experimental animals, a chronic low-grade inflammation occurs which is characterized by increased plasma levels of C-reactive protein, inflammatory cytokines such as TNF-α, IL-6, MCP-1, IL-8, and multifunctional proteins such as leptin (30) and osteopontin (31). Adipose tissue produces large quantities of inflammatory cytokines and chemokines (collectively called adipokines) and adipose tissue is one of the main contributors to elevated systemic TNF-α concentrations in obesity (32). Long-term obesity-induced systemic metabolic disruption also generates a chronic stressful state response (33) that can suppress humoral immunity. This stress can be causally linked to obesity through increased levels of plasma glucocorticoids, which can stimulate the development and differentiation of preadipocytes (34). The biological activity of the stress-responsive glucocorticoids is tightly controlled by the expression of the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) (35). Protection from diet-induced obesity and insulin resistance was found in mice lacking 11β-HSD1 (36), whereas mice with adipose tissue-specific overexpression of 11β-HSD1 developed an obese phenotype when fed a high calorie diet (37). Studies in obese humans resulted in similar findings (38, 39) and, most importantly, suggested that the ability to regulate the activity of 11β-HSD is lost in type 2 diabetic patients and is compromised in nondiabetic, obese individuals. Proinflammatory mediators, (TNF-α, IL-6, MCP-1, and IL-8) intracellular processes (stress response) and signaling pathways (JNK and NFκB activation) have all been linked to obesity and inflammation in experimental animals, demonstrating the importance of consideration of the metabolic-immune status of the control animal. In humans, several functional similarities with the animal findings have been found (40, 41). However, there are disparities between animal and human therapeutic responsiveness, which may be due to the use of the standard animal control. Obesity-related immunotherapeutics based on mouse studies, such as neutralization of TNF-α, have not transferred successfully to humans. Obese humans administered TNF-α monoclonal antibodies do not show similar responses to those of mice with respect to a decrease in body weight and the reversal of insulin resistance (42–44). As the standard control animal may possess altered immune responses, activation of adipokines, and stress status, it is likely that this may impact the apparent efficacy of lifestyle or pharmacotherapeutic interventions for disorders that are influenced by inflammatory and immune activity.

Cancer

The standard living conditions of laboratory animals might be considered a good reflection of an increasingly large proportion of populations in industrialized countries who are sedentary and overfed. With respect to the induction of tumors in experimental animal conditions, standard controls develop more spontaneous tumors than do those that eat less (45, 46). Many different types of tumors grow more rapidly in animals fed ad libitum compared to those on reduced energy diets (47–49). In addition, some carcinogens are less effective in inducing cancers in animals maintained on reduced energy diets, compared to overweight animals on the standard ad libitum diet (50, 51). Certain aspects of the mechanisms of carcinogenesis, tumor growth, and metastasis may be different in obese mice compared to more slender mice that eat less. Indeed, the effects of energy intake and exercise on carcinogenesis may result from changes in the expression of p450 enzymes that metabolize carcinogens, or in the amounts of oxidative DNA damage and apoptosis (50–53).

Although the risk of several types of cancers is increased in individuals who are overweight and sedentary, many cancers can strike otherwise healthy people at any age. It is therefore important to know whether potential cancer therapies that are efficacious in reducing tumor growth in overweight and unstimulated animals are equally effective in more physically fit animals. There have been more than 500 phase II clinical trials in cancer patients involving drugs that fall into only a few general types with regard to their mechanisms of action, including cytotoxic, antiproliferative, and antiangiogenic agents (54). The majority of these drugs, although exhibiting efficacy in animal studies are often ineffective in the human clinical trials. It is reasonable to consider that some of these drugs might have failed because they were evaluated in preclinical rodent models in which the subjects are uniformly

in a metabolically morbid state. For example, angiogenesis may play a much more prominent role in the growth of tumors in obese individuals (55), and so antiangiogenic drugs may have relatively little effect on the same type of tumor in a slender and fit individual. In addition, numerous hormones and growth factors whose levels are affected by energy intake (testosterone, estrogen, leptin, IGF1, VEGF, etc.) also affect the growth of cancers (45, 46, 49–58). A given cancer drug might therefore be more (or less) effective in overweight individuals compared to their slender counterparts.

Neurological Disorders

What are the arguments, both pro and con, of standard ad libitum animal models for major neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases, and stroke? The most widely used models of Alzheimer's disease are transgenic mice that express a mutant form of human amyloid precursor protein alone, or in combination with mutant presenilin-1 and tau (59). Such "Alzheimer's mice" develop progressive accumulation of amyloid β-peptide, and associated dysfunction of synapses, in brain regions that play a role in regulating learning and memory processes. The progression of the latter pathological processes is retarded by dietary energy restriction (60) and environmental enrichment (61), and is hastened by diabetes (62). Similarly, dietary energy restriction (63, 64) and exercise (65, 66) have been reported to delay disease onset and slow progression in mouse models of Parkinson's and Huntington's diseases. A potential advantage of the standard ad libitum laboratory housing conditions is that the disease process is accelerated, and so experimental interventions can be tested more rapidly. A possible disadvantage is that drugs that show good efficacy in the overweight animal models may be less effective or ineffective in active normal weight animals. For example, because levels of oxidative stress are elevated in brain cells of sedentary obese animals, antioxidant therapies may be very effective in animals housed under standard laboratory conditions, but not in more healthy animals that eat less food and exercise more (67, 68). The failure of several different drugs to improve outcome in human stroke patients, despite their clear effectiveness in animal models of stroke, might be due to the fact that the animal models involved young sedentary overfed rats and mice (69, 70). Indeed, neurons in the brains of overfed animals are more vulnerable to ischemic stroke than are neurons in the brains of their more healthy counterparts (64). Thus, drugs such as glutamate receptor antagonists (71) and antiinflammatory agents (72) may be effective in animal models, but ineffective in human clinical trials, because they modify processes associated with a sedentary gluttonous lifestyle instead of, or in addition to, stroke-specific processes.

A Comparison of Various Genomic and Physiological Parameters in an ad Libitum Overfed Standard Control Laboratory Rat Model to a Healthy Body Weight Control

Using a standardized animal model, the Sprague–Dawley rat, we compared how usage of an overweight standard control or a lean control can affect various output data. In this experiment, multiple forms of animal data were recorded from Sprague–Dawley rats subjected to different amounts of energy intake and food availability (40% calorie restriction, alternate day fasting and a high fat/glucose diet) for a period of 4 months (from 4 to 8 months of age). The output variables measured, using comparison with standard or lean controls, included transcriptional regulation in multiple tissues including gonads (73) and hippocampus (74), whole body weight and food intake, plasma hormones, and performance in cognitive tests (15). In this scenario, we compared output data indices first to the standard overweight ad libitum control and then to a lean "mild calorie-restricted" control (20% calorie restriction compared to ad libitum control). First, we studied the differences in global transcription in two different tissues of female rats (similar results were also obtained for males). When comparing the genes significantly modulated by the implemented experimental diets to the two controls (standard or lean) it is clear from Fig. 1 that there is minimal similarity in the identity of the significantly regulated genes in both tissues for all of the experimental paradigms. Therefore, using either an ad libitum standard control or a lean control leads to distinct transcriptional outputs in both the tissues. Because physiological processes are regulated by the concerted actions of multiple genes in related groups, it may be possible that even though the gene identities appear different on the basis of which control animal is used, their predictive functional output may still be the same, albeit mediated via different genes. To assess the potential functional signaling output of these genes, we assessed their functional clustering using both gene ontology (GO: http://www.geneontology.org/) and KEGG (Kyoto Encyclopedia of Genes and Genomes: http://www.genome.jp/kegg/) pathway analysis. Using functional clustering of the output genes with GO terms, it is again clear in Fig. 2 that in each of the experimental cases the predicted significant GO term functional clusters are very different, depending on which control animals are used. In a similar manner to Figs. 1 and 2, using an even higher level of functional sophistication, i.e., signaling pathway analysis, it is clear in Fig. 3 that when one predicts the signaling behavior of the significantly regulated gene sets in the two tissues that there is very little similarity in the predicted physiological activity when using standard versus lean control animals. Therefore, if one were to attempt to assess the role of gene transcription between various tissues it seems that divergent data could be obtained depending on which control was employed.

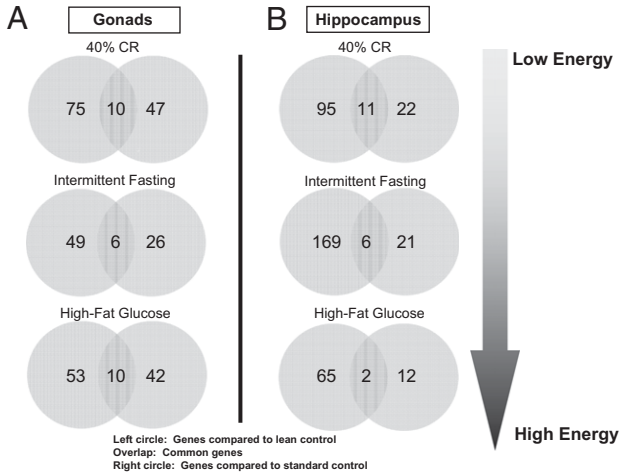

Fig. 1. Differences and commonalities in gonadal and hippocampal gene expression for Sprague–Dawley rats maintained on low and high energy diets, compared with both healthy "lean" controls and standard ad libitum–fed overweight controls. (*A* and *B*) Common and unique significantly altered genes in gonadal tissue (ovaries; *A*) and hippocampal tissue (*B*) from rats on 40% caloric restriction, alternate day fasting, and a high fat/glucose diet, compared with standard ad libitum overweight controls and healthy lean controls (20% caloric restriction).

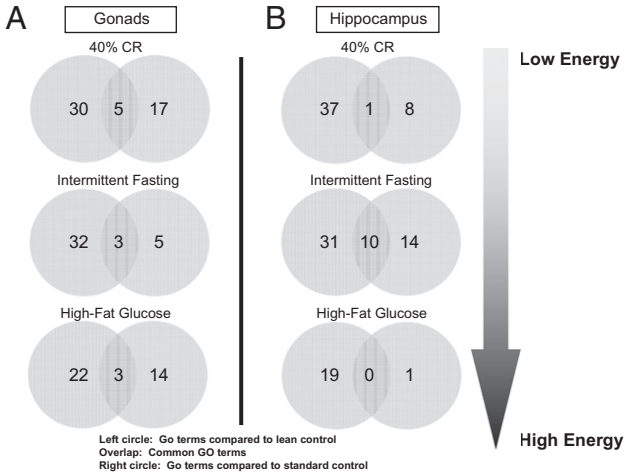

Fig. 2. Differences and commonalities in gonadal and hippocampal gene ontology (GO) functional groups for Sprague–Dawley rats maintained on low and high energy diets, compared with both healthy "lean" controls and standard ad libitum–fed overweight controls. (*A*) Common and unique significantly altered GO functional groups in ovaries (*A*) and hippocampus (*B*) from rats on 40% caloric restriction, intermittent fasting, and a high fat/glucose diet, compared with standard ad libitum overweight controls and healthy lean controls (20% caloric restriction).

As many scientists often subject experimental animals to a battery of physiological or behavioral tests, we also compared the differences in the data collected for several physiological variables depending on which control animals were used. Finally, we compared the percentage difference in the respective output index values for body mass, food consumption, plasma hormones, and maze-solving performance when using either lean or standard controls (Fig. 4). If the output index value was greater using the lean control than the output index value using the standard control, then a positive percentage difference was calculated. Negative percentage differences resulted when the output value for lean controls was less than the value for standard controls. It is clear that many of the measured indices (chemical, behavioral, and cognitive) show large percentage differences in their values when the two types of control animals are used, especially with respect to plasma hormones (leptin and growth hormone) and blood-borne energy-regulatory factors such as cholesterol and 3-hydroxybutyrate (Fig. 4). As the relative health status of lean versus standard control animals is divergent, it is not surprising that there are profound differences in experimental outcome, but it is surprising how dramatically distinct some experimental results can be (e.g., transcriptional responses). Therefore it is important for the extrapolation of data from animals to humans that the physiological health of the control animals be considered because important drug effects or physiological actions may be completely missed or wildly exaggerated.

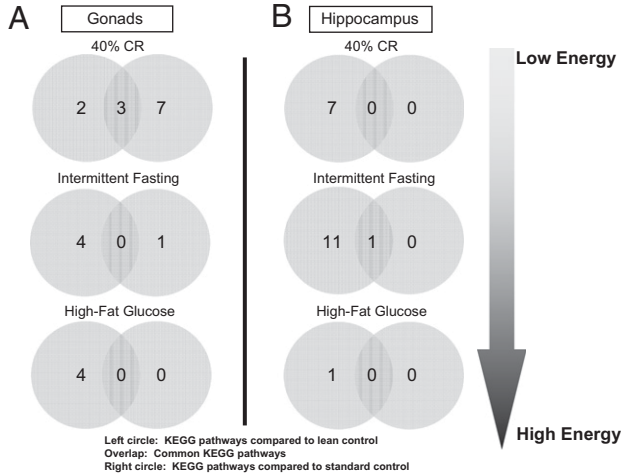

Fig. 3. Differences and commonalities in gonadal and hippocampal KEGG functional pathways for Sprague–Dawley rats maintained on low and high energy diets, compared with both healthy controls and standard ad libitum–fed overweight controls. (*A*) Common and unique significantly altered KEGG functional pathways in ovaries (*A*) and hippocampus (*B*) from rats on 40% caloric restriction, intermittent fasting, and a high fat/glucose diet, compared with standard ad libitum overweight controls and healthy lean controls (20% caloric restriction).

Research on Standard ad Libitum Overfed Laboratory Animals May Misinform the Design and Outcome of Human Studies

When maintained under the standard laboratory housing conditions of continuous food availability and minimal opportunity for exercise, laboratory animals exhibit increased vulnerability to cancers and neurodegenerative disorders. For example, in a mouse model of prostate cancer, reducing energy intake results in slower growth of the prostate tumors (47), and intermittent caloric restriction reduces the incidence of mammary tumors in a mouse model of breast cancer (75). In a mouse brain tumor model, dietary energy restriction reduces the growth of blood vessels in the tumors and slows the growth of the tumors (48). Compared to animals that eat more sparingly or exercise regularly, animals that overeat and live a sedentary lifestyle exhibit accelerated dysfunction and degeneration of neurons in the brain in experimental models of Alzheimer's disease (60, 61, 76), Parkinson's disease (63, 77) and stroke (78, 79). Moreover, cognitive function, synaptic plasticity, and neurogenesis (the production of new nerve cells from stem cells) are enhanced by exercise and dietary restriction and are compromised by a sedentary lifestyle (80–83). The adverse effects of a sedentary lifestyle on the brain results from an impaired ability to respond adaptively to stress as indicated by reduced production of neurotrophic factors, protein chaperones, and antioxidant proteins (56, 78, 81).

For many decades, countless studies have investigated the potential health benefits and mechanisms of action of dietary energy restriction regimens. Many studies have shown that caloric restriction can significantly extend lifespan in a variety of species, ranging from flies and worms to mice and rats (84–86). Dietary energy restriction is thought to prolong lifespan by impinging upon fundamental metabolic and cellular signaling pathways including insulin-like signaling, FoxO transcription factors, sirtuins, and peroxisome proliferator-activated receptor. These pathways stimulate the production of various protein chaperones, neurotrophic factors, and antioxidant enzymes, all of which help cells cope with stress and resist disease

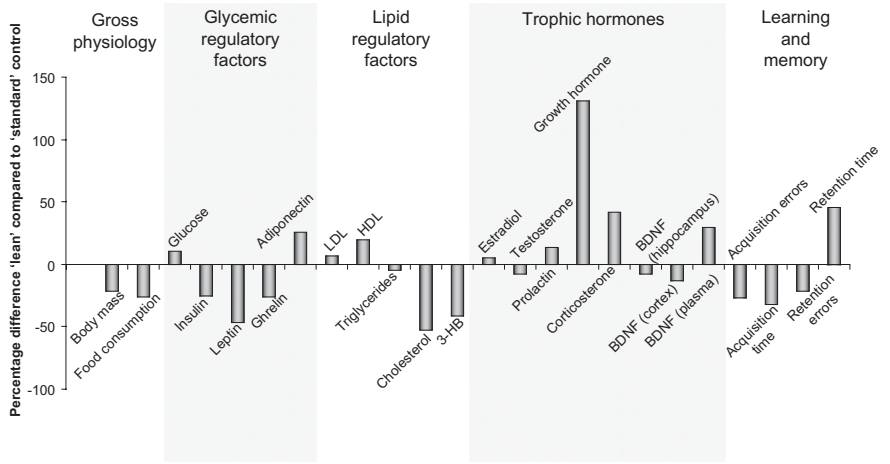

Fig. 4. Overview of various physiological differences between standard ad libitum–fed control animals and moderately fed lean animals. The percentage difference for numerous variables (gross physiology, glycemic regulatory factors, lipid regulatory factors, trophic hormones, and learning and memory) for "lean" control Sprague–Dawley rats (20% caloric restriction) compared to "standard" control Sprague–Dawley rats fed ad libitum. LDL, low density lipoprotein; HDL, high density lipoprotein; BDNF, brain-derived neurotrophic factor; and 3-HB, 3-β-hydroxybutyrate.

(see refs. 83, 87 for review). However, other studies have demonstrated that dietary energy restriction has no or even harmful effects on lifespan in different animal models (17, 84, 88–90). Further research is needed, using a standardized approach, to determine the pros and cons of long- and short-term dietary energy restriction. Moreover, it was recently reported that in recombinant inbred mice, there are striking variations in lifespan in response to food restriction, due to differences in genetic background (84). These findings suggest that the role of genetic background in the choice of experimental rodent models would also be important to consider, as different genetic backgrounds are likely to affect experimental outcomes.

Another factor to consider besides body weight, metabolic health, housing conditions, and genetic background of laboratory rodents is whether the rodent model in itself could have experimental drawbacks. Rodents are typically used for experimental research for their ease of use, relatively low cost, rapid breeding, high offspring numbers, and easy maintenance (91). In addition, mice and humans have considerable genome similarities, and more than 90% of the respective genomes can be grouped in terms of corresponding regions (92). However, it is well established that mice and humans have several physiological differences that may render the use of mouse models for the investigation of human physiology and pathophysiology imperfect (91). For example, there are significant differences between rodents and humans in olfactory function (93), reproductive function (94), and digestive function (95).

Two examples of animal models for which the standard housing conditions can be considered healthier than the standard conditions for rodents are canines and nonhuman primates. In both cases the animals are fed portioned meals two or three times daily and may exercise in large cages or open arenas; accordingly, they maintain lower levels of body fat compared to laboratory rats and mice. In contrast to rodents whose lifespans are shortened by the usual housing conditions, the lifespans of dogs (10–20 years for beagles) and monkeys (25–40 years for rhesus macaques) are believed similar to their wild counterparts (96, 97). On the other hand, some organ systems of dogs and monkeys are more sensitive than their rodent counterparts to adverse effects of excessive energy intake, with the cardiovascular system being one clear example, where rodents are resistant to atherosclerosis. This issue of human disease-relevant physiology has been, in part, circumvented in mice by generating transgenic animals that express mutated human genes that cause inherited diseases (cancers, diabetes, Alzheimer's disease, Parkinson's disease, and many others).

We suggest that all investigators who employ rodent models in their research should consider how housing conditions, particularly dietary energy intake and level of exercise, might affect the responses of the animals to experimental manipulations, as well as the outcomes measured and their interpretation. Both qualitative and quantitative features of many physiological processes are subject to modification by energy intake and exercise, with recent global gene expression studies (98, 99) indicating that the variables shown in Table 1 represent

Table 1. Comparisons of physiological and metabolic factors in rats maintained under standard housing conditions (overfed and sedentary) and more natural conditions (reduced energy intake or running wheel exercise)

	Housing conditions		
Factor	Standard	Diet restriction	Exercise
Body weight	600–700 g	350–500 g	500–600 g
Total body fat	25–40%	5–20%	10–20%
Mean blood pressure	110–130 mm Hg	80–90 mm Hg	115–125 mm Hg
Resting heart rate	350–400 bpm	250–300 bpm	280–300 bpm
Plasma glucose	150–160 mg/dL	110–130 mg/dL	125–135 mg/dL
Plasma insulin	125–140 nmol/L	70–80 nmol/L	NA
Plasma leptin	8–12 ng/mL	2–6 ng/mL	NA
Plasma adiponectin	9–11 ng/mL	14–16 ng/mL	NA
Total cholesterol	140–170 mg/dL	70–100 mg/dL	130–140 mg/dL
LDL cholesterol	15–25 mg/dL	10–15 mg/dL	NA
TNFα*	6–7 pg/mL	3–4 pg/mL	NA
IL-6*	6.5–7.5 pg/mL	4.5–5.5 pg/mL	NA

Unless indicated otherwise, all data were from studies of male Sprague–Dawley rats. The information in this table is based on data in refs. 15, 16, 100, 101, and 102. bpm, beats per minute; NA, data not available.
*Data from male Dahl-SS rats.

only the tip of the iceberg of a myriad of signaling and metabolic pathways influenced by energy intake and expenditure.

Conclusions

The beneficial effects of some drugs in animal models might result from their effects on processes associated with an unhealthy lifestyle (increased oxidative stress, inflammation, insulin resistance, etc.) rather than a specific effect of the drug on the disease process. This is a critical issue that should be addressed in future studies in many different branches of biomedical research. Ideally, the efficacy of interventions should be established in animals housed in both the usual overfed, sedentary conditions and more healthy conditions of reduced energy intake and increased exercise, i.e., lean control animals. The standard overfed sedentary control animal is a good model for an increasing fraction of human subjects who are overweight and sedentary, but may be inadequate for preclinical studies relevant to normal weight active humans. Many patients suffering from cancer, vascular disorders, and neurodegenerative disease may in many other ways be relatively fit and cognitively stimulated. Treatments for the latter patients should be tested in healthy animal models. It is not a major expense or hardship to reduce the food intake of laboratory rats and mice and raise them in cages with running wheels. In addition to better informing the development of therapeutic interventions, comparisons between sedentary and fit animals will undoubtedly reveal novel mechanisms by which diet and exercise affect basic biological processes and disease pathogenesis.

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9

"Control" laboratory rodents are metabolically morbid: why it matters.

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Recommendations:

Very Good 19 Apr 2010

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RECOMMENDATIONS 4 | ABSTRACT | COMMENTS collapse all

CONFIRMATION | CONTROVERSIAL | INTERESTING HYPOTHESIS

DOI: 10.3410/f.2954957.2625055

This important study and overview should remind us that appropriate controls are not always what we assume as conventional or status quo. Translation of basic science from bench to bedside is the constant drumbeat today. This study points out that conventional control husbandry conditions in key mammalian models produce anything but 'normal', optimal or perhaps even healthy animals.

This article presents a wide-ranging review/perspective of multiple previous studies that examined the evidence that typical housing conditions produce animals with clinically abnormal indicators of health (e.g. body weight, fat, blood pressure, plasma glucose, serum lipid profile and inflammatory mediator status). Standard husbandry is unlimited food with limited physical activity and produces an impressive profile in rats that bears a remarkable resemblance to the beginnings of the metabolic syndrome that is so common in most of the developed world populace -- overfed and sedentary. The authors present evidence that modest (20-40%) caloric restriction or alternate-day fasting alters reproductive (gonadal) and brain (hippocampal) gene expression in a continuum from fasting low energy states to high fat/glucose states. Table 1 in the paper is a particularly valuable comparison of standard practices, diet restriction and exercise interventions on clinically relevant measures. While not pointed out in this manuscript, however, veterinary staff at many academic sites view anything but ad lib feeding as 'stressful' and abnormal to the point of being reluctant to endorse anything except standard ad lib feeding under animal care and use reviews.

Disclosures
None declared

Add a comment

Very Good **04 May 2010**

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INTERESTING HYPOTHESIS

DOI: 10.3410/f.2954957.2762081

Laboratory rodents, housed under so-called standard conditions, are demonstrated here to present features of the metabolic syndrome, to be at an increased risk of developing cancer and to show a decreased lifespan in comparison to wild animals and rodents kept with food restriction and a stimulating environment. While limitations of the use of mice and rats for biomedical research has been widely discussed and evaluated in the past, housing conditions are usually not questioned and taken into account when interpreting study results. Researchers should be aware that not only the mouse itself has its restrictions as a model for human phenomena but the metabolic state of those animals also has limitations.

Laboratory rodents fed standard chow ad libitum are usually sedentary and lack a stimulating environment. They are metabolically abnormal (increased body weight, insulin resistance, chronic inflammatory state, disrupted blood lipid profile), are at an increased risk of developing cancer, show cognitive impairment and die earlier than wild animals and rodents housed with food restriction and stimulatory environment.
This paper elegantly shows massive differences in the transcriptional output in the gonads and hippocampus as well as in anthropometric data and biochemical parameters when comparing Sprague-Dawley rats fed a 40% calorie-restricted diet, alternate day fasting or a high fat/glucose diet with rats housed and fed under standard conditions, or lean rats (20% calorie restriction). Therefore, it is not only the genetic background of rats and mice that influences the experimental endpoints but also, probably more importantly, their metabolic state. When evaluating new drugs and therapies, the metabolic and inflammatory state of the rodents may essentially influence the study outcome and may partly explain why drugs often don't exert similar effects in humans as they do in rodents.
When discussing the metabolic state of laboratory rodents, one should take into account the gut flora as a potential link between nutrition and metabolism, immunity and response to therapeutic agents too. The composition of the gut microbiota can undergo drastic changes dependent on the diet, and overgrowth of certain bacteria or the presence of potential pathogens may critically influence the experimental outcome as well. Based on this report, we must consider the following: 1. the beneficial effects of some drugs in animal models might result from their effects on processes associated with an unhealthy lifestyle (increased oxidative stress, inflammation, insulin resistance, etc.) rather than a specific effect of the drug on the disease process; 2. ideally, the efficacy of interventions should be established in animals housed in both the usual overfed, sedentary conditions and more healthy conditions of reduced energy intake and increased exercise, i.e. lean control animals; 3. costs are probably a limiting factor; 4. the standard overfed sedentary control animal is a good model for an increasing fraction of human subjects who are overweight and sedentary but may be inadequate for preclinical studies relevant to normal weight, active humans.

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Disclosures
None declared

Add a comment

Exceptional **22 Jul 2010**

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REFUTATION

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Sometimes, there is a 800-pound silverback gorilla in the room and he is 'invisible'. This article exposes the gorilla and shows that the problem animal is not the gorilla but in fact a big fat rodent! An often overlooked aspect of research studies using rodents is that these animals tend to be sedentary, mentally stagnant and overfed. This life style results in obesity and a range of consequent pathologies, including cardiovascular disease, cancer, diabetes, accelerated neurodegeneration and renal failure. This article details the fact that animals in such an unhealthy state do not qualify as appropriate research models for anything other than the study of 'couch potatoes'.
This is a major wake-up call for the research community and easily addressed by portioned feeding of laboratory rodents and by putting a running wheel in their cages.

The authors of this paper point out that mice and rats used in experimentation are usually housed as 1-5 per cage with a floor space of 1-3 square feet.
Within this environment, the rodents typically have food continuously available, have no running wheel on which to voluntarily exercise and have little, if any, mental stimulation.
This situation contrasts with laboratory dogs and monkeys, who eat portioned meals and are exercised regularly.
As a result of these contrasting housing conditions, the physiology and life span of laboratory dogs and monkeys are similar to those of their wild counterparts, while laboratory rodents are comparatively obese and short-lived.
When overweight, overfed sedentary rodents are compared to rodents that are lean due to diet restriction and exercise, they exhibit elevated levels of a broad range of disease-susceptibility markers.
These include increased blood pressure, heart rate, plasma levels of glucose, insulin, triglycerides, cholesterol and the inflammatory cytokines IL-6 and TNF-alpha.
In addition, compared to lean animals, rodents housed under standard sedentary and ad libitum feeding conditions have impaired learning and memory capacity and show dramatic changes in gene expression profiles.
Taken together, these considerations indicate that rodents housed in the typical way are good models only for overweight and sedentary humans.
Such a life style predisposes to pathologies such as diabetes, cancer, atherosclerosis, hypertension, heart attack, stroke, renal failure, Huntington's, Parkinson's and Alzheimer's disease as well as disorders of the inflammatory and immune systems.
Consequently, treatments for these and other pathologies tested in obese and sedentary rodents may indicate efficacy not because they alleviate the specific entity under investigation but rather the underlying unhealthy way of life.

Disclosures
None declared

Add a comment

Very Good **13 Sep 2010**

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CONTROVERSIAL | INTERESTING HYPOTHESIS | NEW FINDING

DOI: 10.3410/f.2954957.5016075

This study confirms what many have already suspected: the standard housed, ad libitum fed, sedentary laboratory rat is in a morbid or pre-morbid metabolic state. The article challenges the overall value of our standard housing procedures for producing appropriate controls for pre-clinical modelling and screening.

There is a rich literature documenting positive effects of procedures such as caloric restriction and exercise on numerous body and brain functions, as well as on progression of diseases (e.g. cancers). This article turns the tables on these interpretations, suggesting that many of the myriad beneficial effects are associated with improving the overall health of what are essentially 'sick' animals, i.e. working to generate a metabolically 'normal' animal. The article presents both a review of the existing literature and new data comparing gene expression and physiological endpoints in sedentary, ad lib fed 'controls' with those of lean (20% calorie restricted) animals. The gene expression (microarray) studies, performed on hippocampal and gonadal tissues, demonstrate minimal overlap of 'regulated' genes or gene pathways in ad libitum fed and lean controls under various diet conditions (caloric restriction, every other day feeding and high fat-glucose diet). Functional studies confirmed that being lean had beneficial effects on multiple endpoints, including metabolic indices (e.g. circulating glycemic and lipid factors) as well as learning and memory. One factor not explicitly addressed is the issue of age of the control animals. Young adult rodents can be quite lean (<6-10% body fat in most cases), despite being on an ad libitum diet. Typically it takes several months for body fat to accumulate. Thus, in some cases young, non-obese animals may be appropriate controls (although it is still possible that metabolic problems may develop prior to frank obesity, and it is still the case that subjects would not have the possible positive benefit of exercise). Overall, this article raises critical questions regarding valid controls in rodent experiments. The use of ad libitum fed, sedentary animals may be of interest in modelling diseases associated with metabolic imbalance/obesity, but may not be universally optimal when wishing to model diseases/processes affecting individuals with otherwise 'normal' health. The authors suggest that moving forward, investigators need to consider the use of lean (e.g. mildly restricted, running wheel available) controls in further testing and development of animal models of human disease.

Disclosures
None declared

Add a comment

Abstract:

ABSTRACT

Failure to recognize that many standard control rats and mice used in biomedical research are sedentary, obese, glucose intolerant, and on a trajectory to premature death may confound data interpretation and outcomes of human studies. Fundamental aspects of cellular physiology, vulnerability to oxidative stress, inflammation, and associated diseases are among the many biological processes affected by dietary energy intake and exercise. Although overfed sedentary rodents may be reasonable models for the study of obesity in humans, treatments
shown to be efficacious in these animal models may prove ineffective or exhibit novel side effects in active, normal-weight subjects.

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Comments:

COMMENTS **add a comment**