International Journal of Medical Microbiology 306 (2016) 328–333

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.com/locate/ijmm

Mini review

Nutritional influences of overfeeding on experimental outcomes in laboratory mice: consequences for gut microbiota and other functional studies

Stephan C. Bischoff*, Valentina Volynets

Dept. of Nutritional Medicine, University of Hohenheim, Stuttgart, Germany

ARTICLE INFO

Article history:
Received 30 January 2016
Received in revised form 2 May 2016
Accepted 30 May 2016

Keywords:
Overfeeding
Ad libitum diet
Caloric restriction
Intestinal microbiota
Gut permeability
Inflammation

ABSTRACT

Data from literature suggests that laboratory mice are often overfed and malnourished. This might have several reasons, including: (i) we usually offer an ad libitum diet, which is not the natural way of feeding for a wild mouse; (ii) many commercial diets we use contain rather high amounts of carbohydrates, particularly of sugars, and low amounts of fat; and (iii) laboratory mice live in a warm and constricted environment in which energy expenditure is lower than in the wild. Such selective or global overfeeding in laboratory mice, which resembles the widespread overfeeding in humans, although it does not always result in overweight, likely affects a number of outcome variables analyzed in laboratory mice, such as microbiota composition and function, metabolic alterations, longevity, intestinal permeability and inflammation. Therefore, a careful selection of experimental diets and their way of administration, as well as detailed documentation, is mandatory in order to understand and compare scientific data obtained from different mouse experiments.

1. How do we feed our laboratory mice?

Mice are the most commonly used laboratory animals worldwide. They have clear advantages compared to other animals, such as small size, low price, availability of genetically largely homogenous strains, short reproduction times, possibility of manifold genetic alterations, and usually easy breeding conditions. The combination of advantages mice offer is quite unique and prevails serious disadvantages, such as their dissimilarity to human beings. Nevertheless, mice are frequently used as model organisms for human physiology and diseases with more or less successful outcomes, depending on the area of research.

While many experimental conditions are described in detail when mouse experiments are published in scientific journals, the description and discussion of feeding conditions often remain vague and superficial. Phrases such as “mice were fed a standard chow and had permanent and ad libitum access to food and drinking water” are often the only indication of the feeding process presented to the reader.

The composition of the food can be looked up at best in the catalogues of the respective animal food suppliers. When doing so, one finds that laboratory mice are fed with diets containing high amounts of carbohydrates which provide the majority of energy (usually 50–70 kJ%), followed by protein (usually 24–36 kJ%), whereas the fat fraction is relatively small (9–15 kJ%), with some variations (Table 1). Major mouse food suppliers offer standard diets for growing/breeding mice (e.g., SD-B and AIN-B in Table 1), or for maintenance (SD-M and AIN-M in Table 1). The “breeding diets” contain more fat and sometimes more protein, whereas the “maintenance diets” contain more carbohydrates and sometimes also more sugars. The sugar fraction expressed in g% is most variable when comparing different control diets (from 4.7 to 25.7 g%), which will likely influence the mouse physiology, namely metabolic pathways or gut microbiota function.

The standard diets proposed by the American Institute of Nutrition (AIN) differ from those preferred in Europe with regard to the protein/carbohydrate ratio. The “AIN diets” (AIN-B and AIN-M in Table 1) have higher portions of carbohydrates, particularly of sugars, and lower portions of protein than the others (CD-B and SC-M in Table 1). However, some other “standard diets” proposed as “control diets” (SD-C and CD-WSD in Table 1) when using particularly modified experimental diets show similar compositions as the “AIN diets”. The latter were adapted by the AIN in the 1990s in order to achieve relatively lower sugar contents and a different fatty acid composition (Reeves, 1997). The “AIN 76A” diet used earlier contained as much as 51 g% sugar, whereas the “AIN 93G” and “AIN 93M” diets that replaced the “AIN 76A” diet in the 1990s

* Corresponding author at: Department of Nutritional Medicine, University of Hohenheim, Fruwirthstr. 12, D-70593 Stuttgart, Germany.
E-mail address: bischoff.stephan@uni-hohenheim.de (S.C. Bischoff).

http://dx.doi.org/10.1016/j.ijmm.2016.05.018
1438-4221/

Table 1
Common diets used for feeding of laboratory mice.

Diet	Standard diet breeding (SD-B)		Standard diet maintenance (SD-M)		Standard diet for rodents		Standard diet control (SD-C)		Standard diet breeding (AIN-B)		Standard diet maintenance (AIN-M)		"Control diet" to the WSD (CD-WSD)		Western-style diet (WSD)	
Product	ssniff® M-Z Ereich chow		ssniff® R/M-H chow		LabDiet® Laboratory Rodent Diet 5001		ssniff® EF R/M Control		ssniff® EF R/M AIN 93G = LabDiet® 58M1		ssniff® EF R/M AIN 93 M = LabDiet® 57W5		ssniff® EF R/M CD88137		ssniff® EF R/M TD88137	
Diet specification	High-protein low- sugar moderate-fat diet		High-protein low- sugar low-fat diet		High-protein low- sugar moderate-fat diet		High-protein moderate-sugar low-fat diet		Moderate-protein moderate-sugar moderate-fat diet		Moderate-protein high-sugar low-fat diet		Moderate-protein high-sugar low-fat diet		Moderate-protein high-sugar high-fat diet	
	g%	kJ%	g%	kJ%	g%	kJ%	g%	kJ%	g%	kJ%	g%	kJ%	g%	kJ%	g%	kJ%
Protein	23.0	36	19.0	33	25.0	30	20.8	30	17.7	24	12.3	24	17.1	24	17.5	20
Fat	6.0	15	3.3	9	6.4	13	4.2	9	7.1	15	4.1	9	5.1	11	21.2	39
Fibers	3.3		4.9		5.3		5.0		5.0		5.0		5.0		5.0	
Carbohydrate	49.2	49	54.1	58	47.5	57	59.4	61	62.0	61	71.9	73	64.5	65	48.8	41
– Starch	34.4		36.5		21.0		46.8		37.2		44.7		39.0	–	14.6	–
– Sucrose	5.2		4.7		6.3[b]		10.8		11.2		25.7		23.3	–	33.2	–
Total	81.5	100	81.3	100	84.2	100	89.4	100	91.8	100	93.9	100	91.7	100	92.5	100
Energy density[a] (MJ/kg)	14.3		12.8		12.2		15.4		16.3		15.6		15.7		18.6	

Examples are shown from ssniff Spezialdiäten GmbH (Soest, Germany) and from LabDiet® (St. Louis, MO 63144, USA).

[a] Metabolizable energy (ME) calculated according to the "pig formula" (see Annex 4 of the German feed regulation). EF, experimental food. Data were obtained from the manufacturer (sniff GmbH, Soest, Germany).

[b] Sucrose, Lactose, Fructose, Glucose.

contain 11.2 and 25.7 g% sugar, respectively (Reeves, 1997). The rationale for such a high sugar administration remains unclear until at present.

The fact that a single mouse diet supplier offers at least six different so-called "standard" or "control diets" may confuse the scientist and requires them to carefully note which chows are being used in an experimental study. If different pellets have been used, it also limits the comparability of results derived from different mouse studies.

2. Do we meet the needs of a laboratory mouse?

A conclusive definition of what is normal and healthy eating/feeding is lacking for mice as well as for humans. Usually, approaches to define healthy eating focus on diet composition and energy amount. The composition should be versatile and balanced; the amount should be adapted to the caloric needs defined by the resting and the exercise-related energy expenditure. Both are difficult to assess, particularly for free-living humans and mice. One might assume that wild mice need higher amounts of energy than laboratory mice, because wild mice have to move more in order to acquire sufficient food. At least for rats, an altered motor activity has been documented when comparing inbred strains and wild animals (van den Brandt et al., 1999). However, equations to estimate caloric needs of mice have been published that suppose the opposite (Fig. 1). The definition of overfeeding and underfeeding can vary also depending on the definition of overweight and underweight. Overfeeding does not necessarily mean a global overfeeding with all substrates, but a selective overfeeding, e.g. with sugars, if a sugar-rich diet is presented. Such a selective overfeeding does not automatically result in enhanced energy intake and weight gain, if the total amount of diet that is consumed is reduced in a compensatory way, but in malnutrition. Even in mice, in which overfeeding results in enhanced energy intake not necessarily experience weight gain, because – compared to humans – rodents can more efficiently regulate body weight through thermogenesis. The reason for this is that mice can increases energy expenditure to resist diet-induced obesity via the uncoupling protein-1 (UCP1) pathway in the brown adipose tissue, which explains also why susceptibility to diet-induced obesity in mice is correlated with

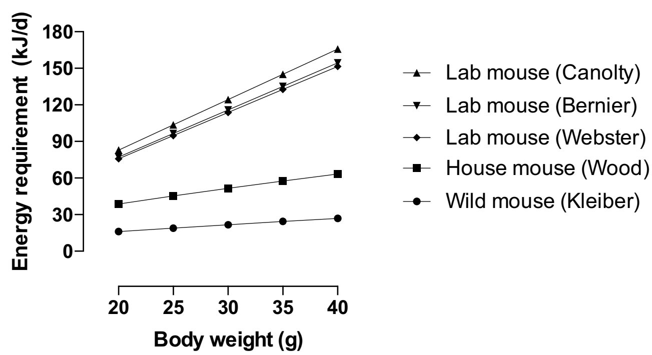

Fig. 1. Comparison of energy requirements of wild mice, house mice and laboratory mice. Energy requirement (expressed in kJ/d) of wild mice was calculated according to the Kleiber equation (301*[BW in kg]$^{0.75}$), that of house mice, according to the Wood equation (622.6*[BW in kg]$^{0.71}$), that of laboratory (lab) mice, according to the Canolty and Koong equation ([BW in g]*736/[kg]$^{0.75}$), the Bernier equation ([BW in g]*686/[kg]$^{0.75}$), and the Webster equation ([BW in g]*673/[kg]$^{0.75}$). References for the equations are indicated in the text.

the induction of brown adipocytes in traditional white fat depots (Kozak et al., 2010). In humans, the definition of overweight and underweight was occasionally related to the mean body weight of a given population. For example, normal nutritional status in children is usually assessed by defining a percentile range referring to the percentiles of the general population. However, when using this approach, the definition of the range of 'normal nutritional status' may increase to a higher values in times of general overfeeding in large parts of the world's population. On the other hand, 'underfeeding' may then just mean a return to the levels previously considered as the normal ones. In Germany, the percentiles therefore refer now to the population's 1994 data, and are no more adapted to the population's nutritional status (Kromeyer-Hauschild and Zellner, 2007). This random fixing of the reference population avoids a further shift of the definition of overweight and underweight.

In wild and laboratory mice, data are scarce on resting and exercise-related energy expenditure. However, scientifically justified equations exist that allow one to calculate at least approximately an animal's caloric needs. According to the Kleiber equation