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COMMENTARY



Chow diet in mouse aging studies: nothing regular about it

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Abstract Chow diet is used in the majority of rodent studies and, although assumed to be standardized for dietary source and nutritional contents, it varies widely across commercial formulations. Similarly, current approaches to study aging in rodents involve a single-diet formulation across the lifespan and overlook age-specific nutritional requirements, which may have long-term effects on aging processes. Together, these nutrition-based disparities represent major gaps in geroscience research, affecting the interpretation and reproducibility of the studies. This perspective aims to raise awareness on the importance of rodent diet formulation and proposes that geroscientists

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include detailed descriptions of all experimental diets and feeding protocols. Detailed reporting of diets will enhance rigor and reproducibility of aging rodent studies and lead to more translational outcomes in geroscience research.

Keywords Chow diet · Dietary source · Aging

Introduction

Rodent models of aging are critical for advancing the field of geroscience but biological heterogeneity has long hampered the reproducibility of aging rodent studies [1, 2]. It is known that diet has a major impact on aging and that dietary intake of macronutrients modulates the aging process [3, 4]. Furthermore, subtle variations in dietary composition can have substantial effects leading to variable rodent phenotypes that impact metabolism and aging-associated outcomes [2–4]. Despite its importance, the role of diet heterogeneity is particularly underappreciated when interpreting results of aging studies. We propose that enhancing awareness regarding the importance of diet among geroscience investigators and requiring more rigorous descriptions of diets in funding applications and in peer-reviewed publications will improve reproducibility in aging rodent studies. While acknowledging that the same principles apply to all animal models of aging, this review will be focused on rodent models.



There is no such thing as a "regular chow diet"

Most peer-reviewed publications using rodent models of aging include little, if any, description of the diet used in the study design. Indeed, the majority contain terms such as "food and water" or "chow." The assumption behind this lack of detail on the diet is that all "standard" chow diets are the same. However, careful comparison of the nutritional values of two commonly used diets such as LabDiet® 5K0G and Teklad 2018 (Table 1, Supplemental Data) reveals major differences between the two. For example, gross energy (kcal/g) is 4.18 in LabDiet® 5K0G versus 3.1 in Teklad 2018, while levels of vitamin A and vitamin D₃ are, respectively, 4.44- and 117.3fold greater in the former compared to the latter. By contrast, levels of vitamin E and iodine are, respectively, 1.64- and 2.8-fold greater in Teklad 2018 compared to LabDiet® 5K0G. Notably, nine dietary constituents found in LabDiet® 5K0G are absent or not reported in Teklad 2018 (taurine, arachidonic acid, omega-3 fatty acids, carotene, cholesterol, sulfur, fluoride, cobalt, and chromium; Table 1), all of which have known bioactive properties that may impact metabolism. These differences may contribute to diverse rodent metabolic phenotypes and aging outcomes, lack of reproducibility, and erroneous conclusions even in highly controlled experimental setups involving genetically identical inbred rodents.

It has been long appreciated within the nutrition field that standardization of rodent research diets is critical for reproducibility of results across all disciplines [5]. Nutritional guidelines can reduce variability among rodent studies, and thus prevent "wasted effort and money caused by failure to duplicate research findings and faulty conclusions in nutritional, toxicological, behavioral, and cancer studies" [6]. While commercially produced chow diets have consistently supported growth in rodent models, their historically uncontrolled variability in minerals and vitamins has sometimes resulted in nutritional inadequacies [5]. This prompted the American Institute of Nutrition (AIN), now known as the American Society of Nutrition (ASN), to develop formula rodent diets in 1976, with clearly stated percentages and/or quantities of ingredients that met established rodent nutrient requirements at that time. This initial formulation did provide a critical framework to develop standardized diets for rodents but was not without challenges. In fact, the diet was designed to meet nutritional needs for all rodents but was found to induce ectopic calcification of the kidneys in female Sprague Dawley rats [7].

The AIN diet was revised in 1993 with a focus on weight gain over 3-4 months. The final adopted formulation(s), named AIN-93, resulted in a 13% weight increase in Swiss-Webster mice but not in Sprague–Dawley rats [8]. Of particular relevance to this commentary and the field of geroscience is the fact that these diets and their efficacy were designed to maximize bodyweight during growth of relatively young mice. Subsequent studies, which further examined the effects of micronutrient manipulation in mice, were performed on mature mice but not specifically aged mice. For example, dietary copper requirements were determined in 20-week-old male mice fed AIN-93 diets with variable levels of copper [9]. Thus, our understanding of nutrient requirements remains limited within the context of aging.

The current AIN-93 diet is assumed by many to be a "regular" chow diet. However, AIN-93 is a defined diet formulated from purified ingredients, while "chow" generally comprise a variety of grain or cereal-based diets, including soybean meal, corn, fish meal, and animal byproducts in either openly disclosed (open) or proprietary (closed) formulations (Table 1). This lack of dietary information poses issues when trying to reproduce and/or compare results among rodent studies. In addition, batch effects, which are impacted by factors including season-dependent nutritional quality of the crops, may result in distinct compositions of the diets within and across commercial manufacturers. In the absence of a direct analysis of the ingredients for each batch of diet, substantial variability in the formulation can be inadvertently introduced over the course of an aging rodent study. Diet variability can confound the results of studies that compare phenotypes of interest at different points in the lifespan, thus, heavily impacting the interpretation of research outcomes.

The different compositions of chow diets can induce a shift in macronutrient ratios that can in turn impact research results and create challenges in reproducibility across studies. For example, the carbohydrate component is 63% (10% sucrose and approximately 53% cornstarch) in the AIN-93G formula, while is 62% (cornstarch only) in the AIN-93 M formula (5). Importantly, the AIN-93G is formulated for



growth, pregnancy, and lactation, whereas the AIN-93 M is formulated for maintenance. The Open Formula NIH-31 diet (62% carbohydrates from ground corn and corn gluten meal), which is the standard rodent reference diet for the National Institutes of Health (NIH), is formulated for maintenance in addition to growth, reproduction, and lactation in rodents. No diet has been developed for the specific purpose of aging, yet every nutritional component in rodent diets may have distinct biological effects that impact aging processes.

Chow diet variability extends beyond that of absolute macro- and micronutrient concentrations. In fact, although autoclaving and irradiation are intended to sterilize diets to meet animal barrier facility regulations, heat treatment (heat, pressure, steam) conditions are variable and directly affect nutrient bioavailability [10]. For example, the autoclaving process is known to alter the levels of heat-labile vitamins such as vitamin K [11], and the bioavailability of soy protein [10]. As a way to circumvent this issue, the NIH-31 Open Formula Autoclavable diet contains additional amounts of vitamins to compensate for losses upon steam sterilization (Table 1).

What is the appropriate diet for aging rodent studies?

In the absence of systematic long-term studies on the dietary requirements of aging rodents, it has been assumed that chow diets developed for the maintenance of younger animals are also suitable for studies on aging. However, the effects of such diets on aging in mice remain unclear, largely because it is hard to discern physiological changes associated with aging (mitochondrial dysfunction, cellular senescence, and neurodegeneration) from diet-induced effects.

Long-term studies, such as The Study of Longitudinal Aging in Mice (SLAM), will ultimately provide unique insight into normative aging (1). Here, 8-week-old inbred C57BL/6 J and outbred UM-HET3 mice of both sexes were purchased from The Jackson Laboratory (Bar Harbor, ME) and fed LabDiet 5K0G (22% protein, 16% fat, and 62% carbohydrates). During 1 month of acclimatization, mice were fed Envigo 2018SX (24% protein, 18% fat, and 58% carbohydrate) before being transitioned to the open source NIH-31 formula (24% protein, 14% fat, and 62%

carbohydrate) (Envigo, Table 1) for the duration of the study. In addition, fiber content varied between 4.2% in LabDiet® 5K0G, 3.5% in 2018SX, and 4% in NIH-31 (Table 1). Fiber content impacts gut microbiome function and host metabolism [12]. Although it is likely that differences in fiber levels across diets will impact aging processes [12], it is still unknown whether these multiple modifications in macronutrient ratios (and their source) during the first 3 months of life will have an impact later in life. Regardless, we commend the SLAM investigators for publishing data on the diet manufacturers and formulations as well as timelines for transition of diets, as these data will be critical when comparing aging rodent studies that use different diet formulations.

Caloric restriction

Caloric restriction (CR), through a reduction in total diet intake, has been demonstrated to extend longevity in rodents [13, 14]. In a unique rodent study comparing longevity between ad libitum fed and dietaryrestricted rats (31% less overall energy intake), Duffy et al. reported that survival rates for the ad libitum fed were lower than those of the dietary-restricted group, consistent with findings from other studies [14, 15]. Both groups of rats were fed a purified AIN-93 M diet (casein as source of protein) that had the same content of vitamins and mineral per gram of diet, such that the calorie-restricted animals had a commensurate reduction in micronutrient intake. The consequences of inadequate vitamin and mineral intake in dietary restricted rats were not analyzed in this work. The authors then compared their results to a prior rodent study in which rats were fed the cereal-based NIH-31 Open Formula, either ad libitum or at 25% or 40% dietary restriction [14]. In contrast to the AIN-93 M diet regimen which did not adjust for vitamins and minerals, the rats fed the NIH-31 Open Formula were fed a formulation that contained 1.67 x additional vitamin mix. While survival was no different between the rats that were fed either diet ad libitum, the rats fed the AIN-93 M diets at 31% dietary restriction had lower survival compared to those fed the NIH-31 formula at both 25% and 40% dietary restrictions. Although there were no differences in survival when compared to NIH-31-fed rats, ad libitum consumption of AIN-93 M by Sprague Dawley



Table 1 Comparison of the nutritional values of commonly used diets

Diet 8 Diet Name Company Protein (% from kcal) Fat (% from kcal) Carbohydrate (% from kcal)	Nutrient ranges	1 5K0G Lab DietB 22.206 15.008	2 5K52 LabDiet0 22,085 16,618	3 Formulab Diet 5008 Lat-Cleff8 26.849 16.71	4 NIH-31 Open Formula Envigo 24 14	5 7012 (LM-485 Mouse/Rat Steritzable Diet) Envigo 25	6 2018 (Teklad Global 18% protein redient diet) Envigo 24 18	7 2016 (Tekiad Global 16% protein rodent diet) Envigo 22 12	8 8604 (Teklad Rodent Diet) Envigo 24.3 4.7	9 5053 (PicoLab® Rodent Diet 28; irradiated) LabDet® PicoLab® 24,651 3,205	10 PicoLab Diet 5058 (irradiated) LabOattli PicoLabili 23.189 21.635	SAFE® A04 (Europe) Scientific Diets 18.1	12 AIN-03M BioServ 12.8	13 AIN-93G BioServ 18.1
Carbohydrate (% from kcaf) Fiber (Crude, %) Goos Energy, kcald) Physiological Fuel Value, kcallg Metabolizable Energy, kcallg	3.07 - 3.56	61.892 4.2 4.18 3.48 3.13	61.294 4.1 4.21 3.5 3.14	58.441 3.8 4.15 3.5 3.31	62 4 3	58 4.6 3.1	58 3.5 3.1 CONUTRIENTS	68 3,3 3	40.2 4 3	62.144 4.7 4.07 3.41 3.07	55.176 2.2 4.6 3.75 3.56	60.4 3.9 3.339	67.5 4.8 3.58	59.3 4.8 3.74
Asginina (%) Cysterne (%) Cysterne (%) Citycina (%) Histodrina (%) Inolescrina (%) Laucina (%) Lucina (%)	0.45-1.44 0.22-0.4 0.3-1.3 0.34-0.6 0.67-1.2 1.02-1.9 0.72-1.4	1.09 0.36 0.96 0.45 0.79 1.54	1.08 0.35 0.95 0.45 0.77 1.53	1,44 0.35 1,23 0.58 1,2 1,87 1,4	1 0.3 1 0.4 0.8 1.4 0.8	1.2 0.3 0.8 0.5 0.8 1.7	1 0.3 0.8 0.4 0.8 1.8	0.8 0.3 0.7 0.4 0.7 1.9	1.5 0.4 1.3 0.6 1 1.9	1,22 0,28 0,96 0,5 0,97 1,56 1,16	1.15 0.31 0.93 0.5 1.02 1.82	0.9 0.25 0.81	0.46 0.22 0.3 0.34 0.67 1.02	0.64 0.35 0.43 0.48 0.96 1.48
Methiconine (%) Phensylaterine (%) Tyrosine (%) Threacrine (%) Thysophen (%) Tryptophen (%) Valine (%)	0.28-0.7 0.55-1.1 0.5-1 0.54-0.9 0.14-0.3 0.8-1.19	0.62 0.87 0.58 0.89 0.23 0.91	0.63 0.86 0.57 0.69 0.22 0.9	0.43 1.08 0.68 0.9 0.28 1.19	0.4 0.8 0.7 0.7 0.2 0.8	0,4 0,9 0,8 0,8 0,3 0,3	0.4 1 0.6 0.7 0.2 0.9	0.3 0.9 0.5 0.6 0.2 0.8	0.4 1.1 0.9 0.9 0.3 1.1	0.7 0.9 0.59 0.77 0.26	0.67 0.97 0.64 0.79 0.25 1.03	0.28	0.31 0.55 0.7 0.54 0.14 0.8	0.45 0.78 1 0.77 0.2 1.14
Aspartic acid (%) Glutarric acid (%) Glutarric acid (%) Alarine (%) Profine (%) Taurine (%) Fiber (Crude, %)	0.79-2.13 2.49-4.77 0.32-1.4 1.26-1.8 0.02-0.05	1.88 4.4 1.16 1.42 0.05	1.84 4.2 1.17 1.4 0.05	2.6 4.77 1.59 1.63 0.62	1.5 3.2 1.1 1.5	1.8 2.8 1 1.4	1,4 3,4 1,1 1,6 SOHYDRATES	1 3.3 0.9 1.5	2.3 4.1 1.4 1.8	2.19 4.34 1.15 1.47 0.62	2.13 4.47 1.34 1.54 0.02		0.79 2.49 0.32 1.26	1.12 3.56 0.46 1.8
Neutral detergent fiber (%) Acid detergent fiber (%) Acid detergent fiber (%) Nitrogen-free schact (%) Sacrose (%)	None 10.8-16.4 6-Mar 49.4-60.4 33.9-43.5 0.69-3.18	15.5 5.2 53.8 35.6 0.89	15.3 5.1 53.8 53.9 0.79	11.3 4 49.4 34.9 2.517	13.6	13.7	14.7	15.2	12.4	16.4 6 52.9 33.9 3.18	10.8 3 51.8 52.3 0.71	60.4 43.5	4.0	4.0
Guecosa (%) Pructosa (%) Pructosa (%) Luctosa (%) Lincelario acid (C18:2; %) Lincelario acid (C18:2; %) Arachiderno acid (%)	0.12-0.22 0.16-0.24 0-1.34 1.37-3.57 0.09-0.48 <0.01-2	0.12 0.17 0 2.66 0.38 0.02	2.87 0.4 0.02	0.24 0.24 0.39 1.37 0.09 0.01	1.9 0.2	2.6 0.3	3.1 0.3	2 0.1	1.9 0.2	0.19 0.23 1.34 2.19 0.26	0.16 0.18 0.78 2.32 0.21 2	3.2% 3.2% 1.5 0.12	2.04 0.27	3.57 0.48
Cmega-3 fatty acids (%) Total sisturated fatty acids (%) Potal sisturated fatty acids (%) Steamic acid (%) Yotal monourosisturated fatty acids (%) Total polyurosisturated fatty acids (%) Total polyurosisturated fatty acids (%)	0.29-0.63 0.6-2.72 0.5-0.7 0.06-0.2 0.7-2.88 0.48-1.3 2.1-4.04 50-280	1.14	0.83 1.17	2.52 2.51	0.9 0.7 0.1 1.2 1 2.1	0.8 0.6 0.2 1.3 1.3 2.9	0.9 0.7 0.2 1.3 1.2 3.4	0.6 0.5 0.1 0.7 0.7 2.1	0.9 0.7 0.1 1.1 0.9 2.1	0.33	2.52 2.72 2.83	0.59 0.06 0.48	0.63 0.91 2.31	1.1
Carotane (ppm) Carotane (ppm) Vitamin K3 (menadone) (ppm) Thiamin (81) (ppm) Riboflavin (82) (ppm)	trace-4 2.5-80 5.0-95 5.0-15	15 20 79.4 2.1	1.5 20 80 9	4 3.2 16 5	22 76 7	80 95 14	ONUTRIENTS ATAMINS 50 17 15	50 17 15	40 27 8	1.5 3.3 17 8	Trace 3.1 15 8	2.5 5 6.5	0.82 (K1; phylloquinone) 5.3 6	0.88 (K1; phylloquinone) 6 6
Pantofamic acid (85) (ppm) Pantofamic acid (85) (ppm) Choline (ppm) Folic acid (89) (ppm) Pyridoxine (86) (ppm) Biotin (ppm) Vitamin B ₁₂ (ppm)	30-109 10.0-87 1028-2530 0.35-7 3.0-18 0.08-0.77 0.01-51	37 2,001 19 10 0.3 51	97 2000 1.9 10 0.3 51	15 2000 3 8 0.2 20	39 1890 2 10.8 0.3	100 87 2200 7 17 0.77 0.09	33 1200 4 18 0.4 0.08	33 1030 4 18 0.4 0.08	21 2530 3 13 0.38 0.05	200 17 2000 3 9.8 0.3 0.051	21 2200 2.9 9.8 0.3 0.051	10 1800 0.35 3 0.08 0.01	14.7 1028 2 5.8 0.2 0.025	14.7 1028 2 5.8 0.2 0.025
Valamin 4 (ppm) Vlamin D, (ppm) Vlamin E (ppm or IU/kg) Ascorbic acid (mg/gm) Ash (%) Calcium (%)	1.23-20 0.025-4.4 20.1-100.5 not reported 2.2-7.4 0.5-1.4	44 45 0 65	4.4 45 0 6.5 1.32	3.3 3.3 55 not reported 6.8	0.105 27.47 6.2 1.1	0.06 100.5 6.1	5.3 1	4.9 4.9	0.06 80.4 7.4 1.4	0.055 68.33 not reported 6.1 0.81	0.0825 38.19 not reported 5 0.81	0.025 20.1 4.8 0.73	0.025 53.533 not reported 22 0.5	1,242 0,025 55,945 not reported 2,2 0,51
Prosprorus, non-prysiss (%) Phosphorus (%) Potassium (%) Magnessum (%) Subtu (%) Sodium (%) Chlorine (%)	0.33-0.95 0.28-1.1 0.36-1.1 0.051-0.3 0.0301-0.34 0.103-0.3 0.4-0.51	0.96 0.89 0.23 0.3 0.29 0.49	0.95 0.71 0.66 0.22 0.23 0.28 0.49	0.85 1.1 0.2 0.24 0.28 0.48	0.6 0.6 0.2 0.3	0.7 0.8 0.2 0.3 0.5	0.4 0.7 0.6 0.2 0.2	0.4 0.7 0.8 0.2 0.2	1.1 1 0.3 0.3 0.5	0.33 0.83 1.07 0.22 0.34 0.3	0.8 0.7 0.18 0.27 0.25 0.42	0.55 0.8 0.16 0.25 0.4	0.28 0.38 0.051 0.0301 0.1032	0.28 0.36 0.051 0.0301 0.103
Florida (spen) Florida (spen) Iden (spen) Zinc (spen) Margariasa (spen) Copper (spen) Cobell (spen) Ledias (spen)	1.0-36 36.6-360 35.4-120 10.5-160 6.0-25 0.4-0.82	35.4 359 84 156 11 0.82 2.14	36 360 82 100 10 0.82	19 230 73 71 13 0.4	270 47 155 13	240 63 93 23	200 70 100 15	200 70 100 15	300 80 100 25	10 220 87 85 13 0,71	12 200 120 120 17 0.55	270 56 70 16	1 36.6 35.4 10.5 6	37.2 37.7 10.5 6
Chromium (ppm) Sekinium (ppm)	0.21-6 0.01-1.4 0.16-0.39	0.01 0.39 Whole wheat, ground corn, ground oats, wheat middlings, dehulled soybean meal, fish meal, soybean oli, dehydrated	0.01 0.39 Ground wheat, ground corn, ground cets, wheat middlings, fish meat, dehulfed soybean meat, soybean oil, dehydariafed	1.4 0.23 Ground corn, dehulled soybean meal, ground wheat, fish meal, wheat middlings, porcine animal fat creasined with BHA, care	0.3 Ground wheat, ground corn, ground cets, wheat middlings, fish meat, dehalted sovbean meat.	Ground corn, dehulled soybean meal, ground oats,	0.23 RY INGREDIENT LIST Ground wheat, ground corn, wheat middlings, dehulled stoybean meal, corn gluter meal, soybean oil, calcium	0.23 Ground wheat, ground com, wheat middlings, com gluter meat, calcium carbonate, dicalcium	0.34 Dehulled soybean meat, wheat middings, flaked corn, ground corn, fish meat, care molesses.	0.81 0.3 Ground Corn, Dehulled Soybean Meal, Wheat Middings, Ground Wheat, Fish Meal, Dried Plain Beet Pulo.	0.58 0.3 Ground wheat, ground corn, dehulled soybean meal, wheat germ, fish meal, brewers dried yeast, corn oldern meal.	Barley, wheat, maize, soybean meal, wheat bran, hydrolyzed faih	0.17 Corn syrup solids, dextrose, corn starch, casein, sucrose, soybean oil, cellulose, mineral mix.	1 0.17 Com starch, casein, maltodextrin, sucrose, soybean oil, cellufose, mineral mix. vitamin mix.
		dehalled soybean meat, fish meat, soybean oil, dehydrated alfalfa meat, com glutar meat, dicalcium phosphate, browers dried yeast, calcium carbonate, menadione, diresthylpytriedinck baulfile (source of Vitamin, salt, DL-methionine, choline chloride, megrassium codde, thiamine monochrate, remeiline betworkboride	maal, soybsan oli, dehydrated affalfa maal, com glutan maal, dicalcium phosphate, bewers dried yeast, calcium carbonate, manadione dienethylyprimolib isulfate (vitamin K), sait, DL- matricinine, choline chloride, magnasium code, thiemine mononitrate, pyridoxine	wheat middings, porcine animal fat preserved with BHA, care molesses, brewers died yeast, porcine meat meal, wheat germ, ground costs, dried beet pulp, dehydrafed affalfa meal, calcium carbonate, dried whey, salt, menadione diresthylopyrindinol bisulfae, choline chloride, cholecacifero, chiamin A sensite midrorine.	middlings, fish meal, dehulids objean meal, dehydrated alfalfa meal, corn glutan meal, sophean oil, dicalcium phosphate, brawers dried yasalt, caloium carborales, iodiaed salt, choline chicride, magnesium oode, kaolin, ferrous sudfac, menadione sodium bisulfac, menadione sodium bisulfac	wheat middings, dehydrated alfalfa meal, acybean oil, com gluten meal, calcium carbonate, dicatoium phosphate, brewers dried yeast, iodized salt, choline chloride, kaolin reagessium code, L-heine, DL-methionine, femous sulfate, manualone sodium hiselifte remover (sevene di- tieut).	soybean meal, corn glaten meal, soybean oil, calcium carbonate, dicalcium phosphate, brevers dried yeast, iodized salt, L. lysine, DC-methiorine, choline chloride, ksolin, magnesium code, vitamin E acetate, measdices sodium bisulfite complex (source of vitamin & articit). measurements rick	corn gluten meal, calcium carbonate, dicalcium phosphate, asybaun oil, brewers dried yeast, iodized sait, 1-typine, DLL- methiocine, choline chloride, magnesium oxide, vitarnin E acetate, menadione sodium bisulfite compliax (source of vitarnin K artichin) magnesorus.	corn, ground com, fish meal, cane molasses, ground wheat, disid whey, soybaan oil, brewen dried yeast, dicalcium phosphate, calcium carbonate, iodized salt, choline chloride, kaolin, magnesium oxide, femous suffate, vitamin E acetate, memarfirma extrem hissifite	Middings, Ground Wheat, Fish Meat, Dried Plain Beet Pulp, Cane Molasses, Wheat Germ, Ersower, Dried Yeast, Ground Oats, Dehydrated Alfaffa Meat, Soybaan Oli, Dried Whey, Calcium Carbonate, Salt, Di.— Mathionina, Menadona Dimathlypprindinol Bisuffle (Vlasmin K), Choline Chloride, Portificine hydrochrotide,	gams, fish meat, browers dried yeast, own gluten meat, porcine smirred fat preserved with BHA, sopbean oil, catcium phosphate, monocalcium phosphate, choline chloride, menedione dimethylprimidnol bisuffic, DC-methorisme, vitamin A sociate, cholosealciant, prafetricine	meat, wheat brain, hydrolyzed fish proteins, dicakcium phosphate, pre- midure of minerals, calicium carbonate, pre- midure of vitamins.	casen, sucreas, seybaen oil, celulose, mineral mix, vitamin mix, calcium silicate, magnesium steanste, choline bitartrate L-cysteine	mineral mix, viternin mix, L-cysteine, choline bitertrate, IBHQ
ALL INGREDIENTS		cholecatolferol (form of Vitamin D3), Vitamin A acetate, calcium pantotherate, ferrous sulfate, biotin, manganous oxide, calcium lodate. DL-alcha	minonnitrate, pyridosine hydrochloride, cholecatolirol (vitamin D3), vitamin A acetate, minganous oxide, calcium, particibranish, ferrous sulfate, calcium lodate, DL-alpha tocopheryl acetate (vitamin E), folio acid, vitamin B12 supplement, riboflavin-5- chophate, inc caride, ferrous	choine chloride, cholecalofferol, vitamin A acetate, pyridoxine hydrochloride, di-alpha tocopheryl acetate, thiamin monorsitrate, folic acid, Di- methionine, nicotinic acid, calcium partiotherate, riboflavin, vitamin B12	menadione aodium bisulfite complico (aource of vitamin Ki activity), manganous ocide, thiamin monocilinate, riscin, calcium pantotherusie, vitamin E aoutate, vitamin A acutate, copper susfate, zinc oxide, riboflavin, pyridotoine hydrochloride, vitamin B12 sanedament vitamin B12	sulfate, merudione sodium bisulfite complex (source of vitamin K activity), vitamin E acetate, thiamin mononitrate, calcium partiothenate, manganous coide, niacin, copper sulfate, zinc coide, vitamin A acetate, pyridoxine	complex (source of vitamin K activity), manganous oxide, ferrous sulfate, zinc oxide, niacin, calcium pantothenate, copper sulfate, pyridoxine hydrochloride, riboflavin,	oxide, ferrous sulfate, zinc oxide, nisclin, calcium pantothenate, copper sulfate, pyridoxine, hydrothronia rhofissin	complex (source of vitamin K activity), manganous oxide, copper sulfate, zinc oxide, niacin, thiamin monoprinate, vitamin A	(Vitamin K), Choline Chloride, Pyridoxine Hydrochloride, Cholecalolierd (Vitamin D3), Vitamin A Acetate, (DAlpha Tocophery Acetate (Vitamin E), Folic Acid, Thiamine Monorilate, Manganous Oxide, Vitamin B12 Supplement, Zinc Oxide, Femous Carbonate, Microline and Ribridger	hydrochloride, dried whey, folic acid, di-alpha tocopheryl acetate, biotin, thiamin monoritrate, calcium pantothenate, lecitrin.			
		tocophenol acetate (form of Vitamin E), folic acid, Vitamin B- 12 supplement, ribotilavin supplement, nicotinic acid, cobalt carbonate, zinc oxide, femous carbonate, opper suffate, zinc sutfate	folic acid, vitamin B12 supplement, riboflavin-5- phoshate, zinc oxide, femous carbonate, nicofinic acid, copper sulfate, zinc sulfate, cobalt carbonate, biotin	riboflavin, vitarrin B12 supplament, manganous oside, zinc oside, ferrous cirbonate, copper sulfate, zinc sulfate, calcium iodate, cobalt carbonate	oxide, riboflavin, pyridoxinse hydrochloride, vitamin B12 supplement, vitamin D3 supplement, calcium iodate, biotin, folic acid, cobalt carbonate	sulfate, zinc oxide, vitamin A acetate, pyridoxine hydrochloride, riboflarin, vitamin D3 supplements, vitamin B12 supplement, folic acid, biotin, calcium iodate, cobalt carbonate	thiamin mononitrate, vitamin A sostate, calcium iodate, vitamin B12 supplement, folic acid, biotin, vitamin D3 supplement, cobatt carbonate	thiamin mononitrate, vitamin A acetate, calcium locate, vitamin B-12 supplement, folic acid, biotin, vitamin D-3 supplement, cobalt carbonate	acetate, vitamin D3 supplement, calcium pentotheriate, pyridoxine hydrochloride, ribollavin, vitamin B12 supplement, calcium iodate, folic acid, biotin, cobalt carbonate.	Vitamin B12 Supplament, Zinc Oxide, Femous Carbonate, Nicotinic Acid, Ribollavin Supplament, Cabcium Pantothemate, Copper Sulfate, Zinc Sulfate, Calcium Iodate, Cobalt Carbonate, Biotin, Sodium Salanite	riboflavin, nicotinic scid, casein, vitamin B12 supplement, manganous oride, zino oxide, ferrous carbonate, copper suffate, zino suffate, calcium iodate, cobatt carbonate, sodium selenite			
Ground wheat Wheat variety Wheat middlings Ground corn Corn gluten meal						ітем/2е	D INGREDIENTS							
Dehulled acobean meal Soybean oil Ground oats Fish meal Dehydrated alfalar meal Brewer's dried yeast Barky														
Dried best pulp Dried whey/casein Animal fat varietal Animal fat varietal Sucrose Cane molasses						LISTED INDIVIDUAL MICRO	NUTRIENTS, MINERALS, VI	AMINS						
calcium carbonate dicalcium phosphate menocalcium phosphate salt DL-methionine vitamin k choline chloride														
L-bysine Kaolin magnesium oxide manganous oxide vitamis E calcium pandotherate vitamin D3														
vitamin A vitamin B12 thiamine mononitrate pyridoxine hydrochioride ferrous sulfate ferrous carbonate niacin														
copper suffate zinc oxide zinc suffate zinc suffate ribotlavin folic acid nicotinic acid cobalt carbonate biolin												premix of vitamins; premix of minerals (undefined)		
calcium lodate choline chloride choline chloride cobal carbonate sodium selenite Alum Lake Lodex Solta Floc Solta Floc														
Solia Floc V160091C choline bitartrate Dye cholesterol ethoxyquin locithin														
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Green indicates that the dietary component is present in the diet. Black indicates that the dietary component is not found in the diet

rats resulted in approximately a 10% increase in bodyweight at 96 weeks of age as compared to ad libitum

consumption of NIH-31 [16]. This study highlights the importance of documenting the exact composition



of diets when manipulating macronutrients to extend longevity in murine models. Of note, despite the importance of micronutrients (vitamins and minerals) in regulating metabolic processes and their likely impact on aging outcomes, alterations in their levels are generally not accounted for in CR rodent studies.

The team of LeCouteur and colleagues elegantly leveraged rodent aging studies that have complete diet information to demonstrate the importance of macronutrient ratios [3, 17]. These studies reported that female and male mice that were fed ad libitum with higher protein and lower carbohydrate diets had the greatest reproductive potential. In contrast, female and male mice fed ad libitum with lower protein and higher carbohydrate diets had longer lifespans, independent of their caloric intake. Several conclusions can be drawn from these studies. It is critical to better define and report macronutrient ratios and compositional subtypes (i.e., unsaturated versus saturated fats [18]) instead of simply reporting the calories and their degree of restriction in rodent studies. Perhaps more importantly, the fact that a protein-rich diet is beneficial during reproduction but detrimental to aging indicates that rodent diet formulations and their macronutrient ratios should be adjusted over the lifespan instead of being kept throughout, as in the current practice. Furthermore, it has been shown that circadian alignment impacts the effect of caloric restriction [19]. Therefore, it is important to include details on the exact time of feeding as related to the light cycle. Lastly, much more research is needed to determine whether it would be beneficial to adjust nutrient by age (age-specific diets).

Looking forward

In order to improve scientific rigor and reproducibility, the geroscience research community needs to better define the composition of rodent diets used in aging studies, preferably across the lifespan. We propose two ways to enhance this awareness: (1) review process of grant applications submitted to the National Institute of Aging (NIA); and (2) review process of manuscripts submitted to geroscience journals.

NIH provides clear guidance on how to address rigor and reproducibility for all grant applications submitted, including a mandatory section entitled, "Consideration of relevant biological variables." Currently, biological variables are defined as sex, age, weight, and underlying health conditions. We propose that the NIA considers diet as an additional mandatory biological variable. Applicants should be required to describe the exact diet formulations and provide details of diet modifications to be made within the study design. This will raise awareness on the importance of rodent diet formulation in research studies and improve the assessment of rigor in prior research (20).

We propose that geroscience journals that publish rodent studies require a detailed description of both the control and experimental diets. When a diet composition is published for the first time in a journal, complete information on all the components, including any modifications to published diet compositions, should be presented in a table. Nutrition journals, such as *Journal of Nutrition*, have already implemented this requirement and it is our opinion that research progress in other fields would benefit from a standardization in the reporting of this important yet overlooked experimental variable.

In summary, detailed reporting of diets in aging rodent studies will enhance reproducibility and lead to more translational outcomes, thus improving the use and relevance of animal models in geroscience research.

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Declarations

Conflict of interest The authors declare no competing interests.

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