Nutrition & Dietary Regimens in Laboratory Animals
(Mice & Rats)

Georgia Chatzinikolaou

IMBB, FORTH & Department of Biology, UOC

georgiach@imbb.forth.gr

10th International Course, Care & Use of Laboratory Animals

17 May 2024, Heraklion, Crete, Greece

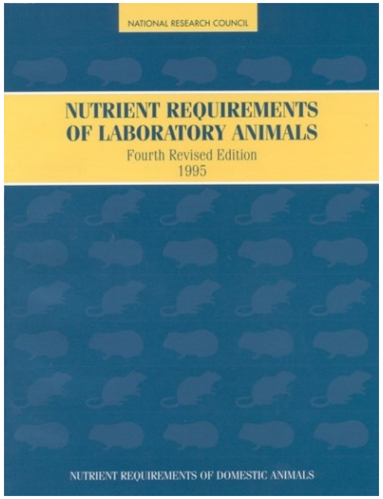
Nutrient Requirements

- Dietary composition need to fulfill each species specific needs (e.g vitamin C is an essential nutrient for the guinea pig, and not for rats)

- Nutrient requirements change according to developmental state, reproductive activity and age

- National Research Council has established detailed nutrient requirements of laboratory animals :

  https://www.nap.edu/read/4758/chapter/1



Why nutrition in lab animals is important?

- Feed is a source of raw materials that influences animals genetic potential for growth, reproduction and longevity

- Nutritionally balanced diet is important for:

  - the welfare of laboratory animals

  - obtaining reliable experimental results

Nutrients - Safety margins

- Toxic effects – important to keep nutrient concentrations within the "recommended" levels

  e.g Methionine dietary levels of : 2.2-2.4% ⟶ toxic effect (reduced growth, premature death)

  1.4% ⟶ no signs of toxicity

- Poor nutrition :
  - Dietary vitamin (B6, B12) and mineral deficiencies ⟶ skin lesions
  - Fatty acid imbalance ⟶ developmental defects
  - High phosphorus levels ⟶ soft tissue calcification (kidney, heart)

The Nutrients

- Macronutrients
  - Proteins (or aminoacids) - Suitable protein content for breeding is 17-20 %
  - Lipids (fats)
  - Carbohydrates

- Micronutrients
  - Vitamins ⟶ fat-soluble vitamins (A, D, E & K) - stored in several organs
    water-soluble vitamins (C & B-complex group) - stored only in very limited quantities
  - Minerals ⟶ sodium, potassium, chloride, calcium, phosphorus, magnesium and sulfur
    Trace elements (zinc, copper, cobalt, iron, iodine, manganese, chromium, selenium, fluorine, molybdenum)

- Clean, fresh water!

The Nutrients

- Organisms require energy for growth, reproduction and maintenance

    Sources of energy: fats, carbohydrates and proteins

    Calories : amount of heat required to raise 1 gram of water 1^{o}C

- Need for essential and nonessential nutrients

    If animals can't produce sufficient quantities, supply through diets

- A loss of approximately 10 to 20% of body water can result in death

Factors Affecting Nutrient Requirements

- Genetics (differences among species, breeds, strains, sexes and individuals)
- Stage of life (growth, pregnancy, lactation)
- Environmental impacts (diurnal or seasonal variation in temperature, light cycle or other environmental conditions)
- Microbiological status (rodents practice coprophagia - a source of vitamins H, K and B7 biotin)
- Research conditions (stress, altered food intake)
- Nutrient interactions (competition for absorption sites among certain minerals that share common active transport systems)

Diet types

- I. Natural ingredient (“chow”, “standard”) diets
- II. Purified (synthetic) diets
- III. Chemically defined diets

I. Natural ingredient (“chow”, “standard”) diets

- Formulated with :
  - agricultural products and by-products :
    - whole grains (ground corn & wheat),
    - mill by-products (wheat bran),
    - high protein meals (soybean meal)
  - fortified with minerals and vitamins
  - additives (dried molasses, fishmeal)

- Frequently used
- Composition makes pelleting possible
- Inexpensive to manufacture
- Palatable for most laboratory animals

- Variation in the composition of the individual ingredients ⟹ NOT possible to predetermine the concentration of each nutrient

II. Purified (synthetic) diets

- Formulated with relatively pure and restricted ingredients :
  - casein, albumin, soybean (protein)
  - sugar, starch (carbohydrates)
  - vegetable oil, lard (fats, essential fatty acids)
  - chemically extracted form of cellulose (fiber)
  - chemically pure inorganic salts & vitamins

- Less variable and easier controlled nutrient concentrations

- Not readily consumed by all species

- More expensive to produce

- Often used in studies of specific nutritional deficiencies

III. Chemically defined diets

- Formulated with the most elemental ingredients:
  - individual aminoacids
  - specific sugars
  - chemically defined triglycerides
  - essential fatty acids
  - inorganic salts
  - vitamins
- Strict control over nutrient concentrations
- Not readily consumed by most species of laboratory animals (" not good taste")
- Too expensive for general use

Comparative benefits of the types of diets

	Natural ingredients	Purified	Chemically- defined
Cost	Moderately low	High	Very high
Availability	Standard diet	Purpose made	Purpose made
Batch-to-batch variability	Variable	Low	Very low
Background nutrient levels	Moderately high	Low	Low
Flexibility	Low	High	High

Problems with natural ingredient diets

- variation between brand
- variation between batch
- certificate analysis strongly recommended

PNAS

Variation in commercial rodent diets induces disparate molecular and physiological changes in the mouse uterus

Haibin Wang[*†], Susanne Tranguch[*†‡], Huirong Xie[*], Gregory Hanley[§], Sanjoy K. Das[*¶], and Sudhansu K. Dey[*‡‖**]

Departments of [*]Pediatrics, [§]Pathology, [¶]Cancer Biology, [‡]Cell and Developmental Biology, and [‖]Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232

Edited by George E. Seidel, Jr., Colorado State University, Fort Collins, CO, and approved May 13, 2005 (received for review March 1, 2005)

Impact on research

0023-6837/01/8105-735$03.00/0
Laboratory Investigation

Vol. 81, No. 5, p. 735, 2001
Printed in U.S.A.

Animal Models Impacted by Phytoestrogens in Commercial Chow: Implications for Pathways Influenced by Hormones

Nadine M. Brown and Kenneth D. R. Setchell

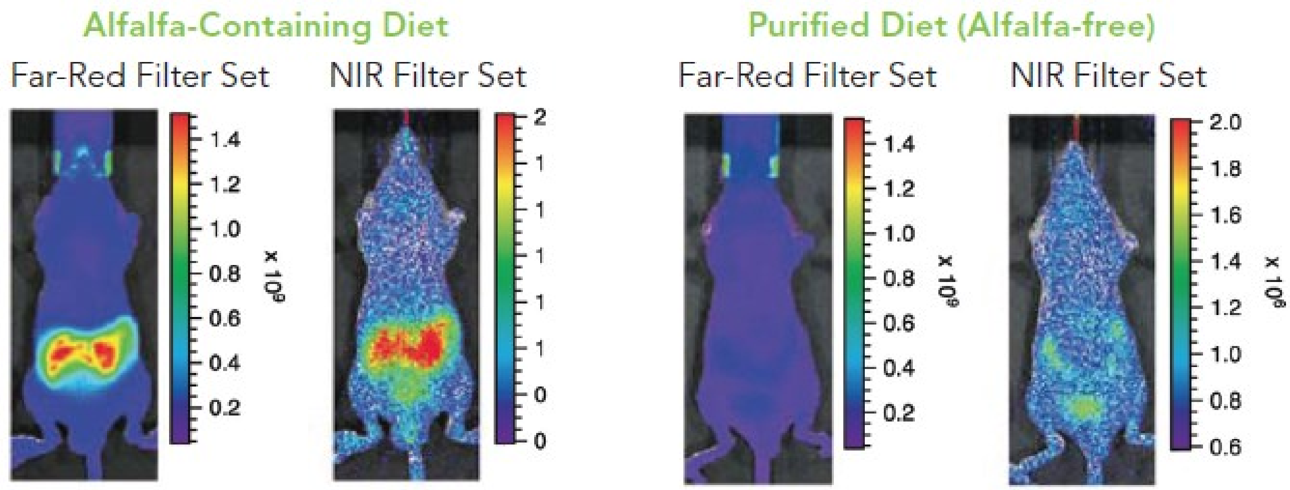
© Laboratory Animals Ltd. *Laboratory Animals* (2007) **41**, 1–18

How isoflavone levels in common rodent diets can interfere with the value of animal models and with experimental results

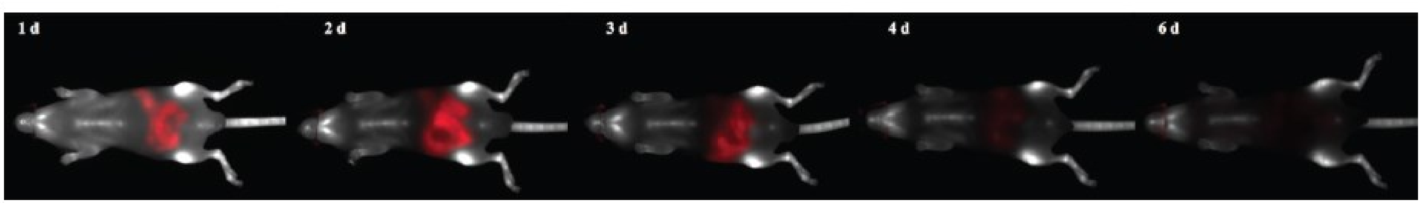
M Nygaard Jensen and M Ritskes-Hoitinga
Central Animal Facility, University Medical Center, St Radboud, PO Box 9101, NL-6500 HB Nijmegen, The Netherlands

Diet Impacts Fluorescent Imaging

Alfalfa-free diets minimize autofluorescence & improve imaging clarity



Optimal signal clearance may require washout of up to 4 days



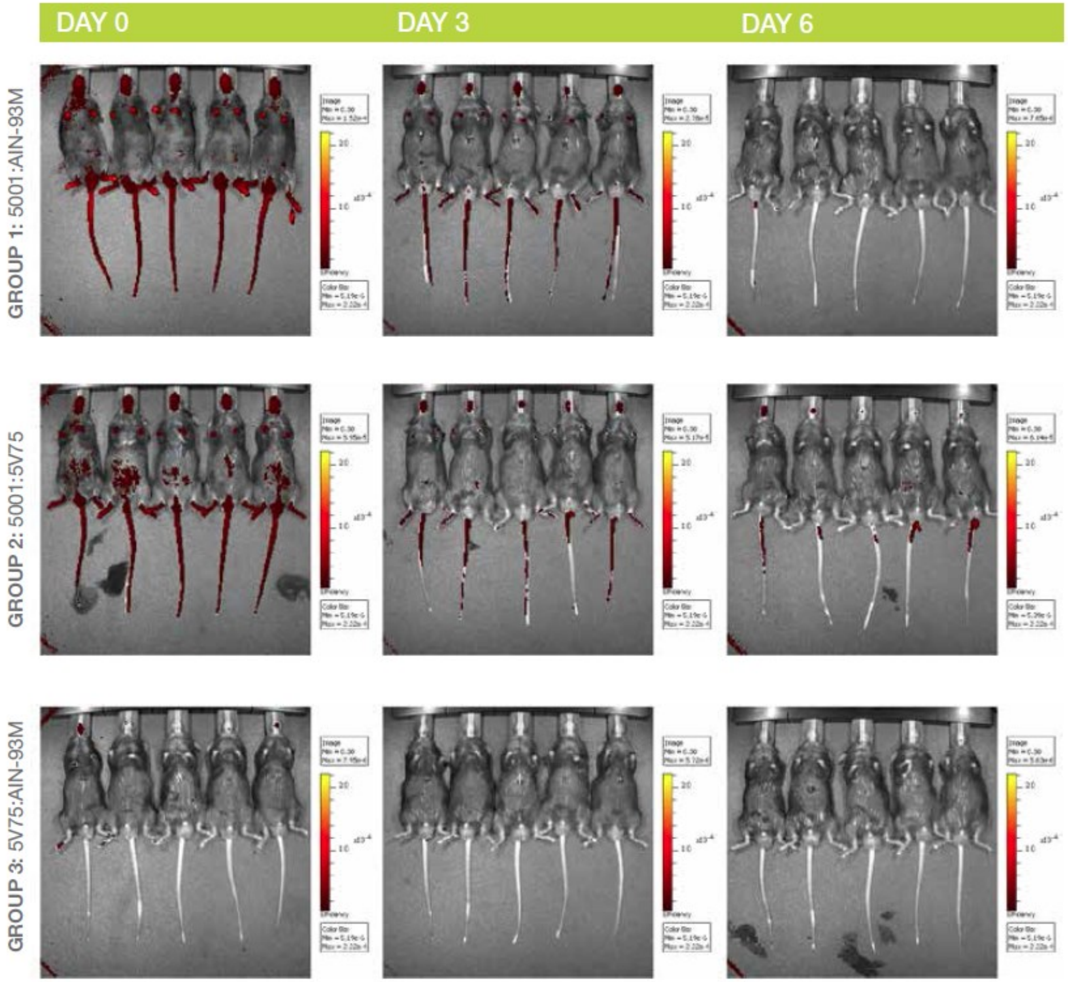
Grain based and purified diets reduce auto-fluorescence *in vivo*

GROUP #	PRE-IMAGING DIET	IMAGING DIET
1	LabDiet 5001	AIN-93M
2	LabDiet 5001	LabDiet 5V75
3	LabDiet 5V75	AIN-93M

5001: Ground corn, dehulled SBM, dried beet pulp, fish meal, ground oats, dehydrated alfalfa meal

5V75: Ground corn, ground wheat, wheat middlings, corn gluten meal, cane molasses

AIN-93M: Cornstarch, dextrin, casein, sucrose



High Fat Diets & Diet-Induced Obesity Models

- In laboratory research typically contain 32 to **60%** of calories from fat
- May contain: saturated fat (lard, beef tallow, coconut oil) or
  omega-3 fatty acids (fish oil)
- Increased fat intake is associated with body weight gain that can lead to obesity and other related metabolic diseases
- Used in research to study: atherosclerosis, obesity, diabetes
- Some strains are more susceptible to obesity when fed high-fat diets (e.g C57Bl6 or AKR mouse)

How do I choose a feed for my research animals?

- Choose a diet that reduces rather than introduces the potential for variation in research results

- Try to choose a diet that aligns with :
  - experimental animals' nutritional requirements
  - the type of research
  - the experimental design
  - the endpoints being measured

- Consider the formula - does it stay the same over time or can it change?

- Evaluate the diet manufacturer (quality control, technical support, diet availability, warehouse biosecurity, sales support and customer service)

Physical forms of diets

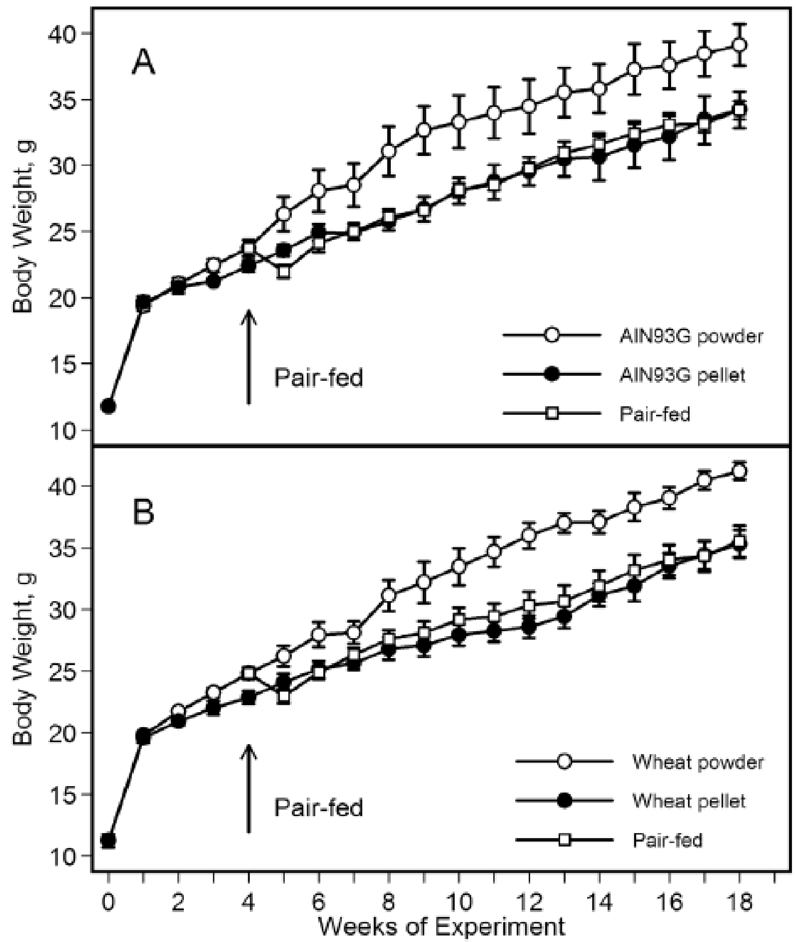
- Pelleted diet :
  - usually heat treated (60-85^{o}C)
  - easy to handle, store and use
  - prevent animals from selecting choice ingredients
  - reduces dust in animal facilities
- Extruded diet :
  - expanded with high heat and steam
  - increased wastage during feeding
  - not commonly used for laboratory rodents
  - higher production cost
- Powdered diet :
  - incorporation of additives & test compounds
  - large amounts may be wasted
  - specially designed feeders are needed
- Gels :
  - requires refrigeration to retard microbial growth
  - must be fed daily to maintain moisture content
- Liquid diets :
  - filter sterilization possible

Journal of the American Association for Laboratory Animal Science
Copyright 2011
by the American Association for Laboratory Animal Science

Vol 50, No 4
July 2011
Pages 488–494

Effects of the Physical Form of the Diet on Food Intake, Growth, and Body Composition Changes in Mice

Lin Yan,* Gerald F Combs Jr, Lana C DeMars, and LuAnn K Johnson



Diet consumption

- Mice eat approximately 3-5 grams of food per day and consume 1.5 mL water per 10 g of body weight per day
- Rats eat approximately 15-20 grams of food per day and consume 10ml water per 100g bodyweight per day
- How do we measure?

  Diet used = eaten + spillage

  (difficult to measure in a normal cage)
- For group housed animals ⟶ get averages
- For reliable measurements ⟶ metabolic cage

Metabolic cages

- measurement of :
  - food intake
  - energy expenditure
  - physical activity
  - water intake
  - O^2 consumption and CO^2 production (respiratory exchange ratio to reflect energy consumption)

- only one animal/time
- used in awake mice

Diet Quality (Assurance & Potential Contaminants)

- Chemical (pesticides, heavy metals)
- Hygiene (microbial content: bacteria, bacterial toxins & mycotoxins)
- Nutritional:
  - assured by the producer → quality control
  - right choice of diet → growth, maintenance; autoclaved or not
  - quality certification → important

Factors affecting diet quality

- Manufacturers:
  - Same manufacturer - different batch quality
  - Different manufacturers
- Transportation
- Storage: Hygienic and environmental conditions
- Handling/treatment:
  - Autoclaving - excessive exposure ⟶ destroys vitamins and affects protein quality
    under vacuum ⟶ reduced of nutrient losses
  - Irradiation - less damage to nutrients & less moisture
- Dosing
- Animal

Impact on research

- Contents in the diet could change the metabolic characteristic of the test substance

- The test substance could:
  - affect the taste of the feed
  - change the animals eating & drinking habits
  - change the nutrient absorption, metabolism or secretion

- Recommended to use the feed from the same batch during experiments

Types of feeding

- *Ad libitum* (free feeding)
- Restricted feeding :
  - Meals (restricted feeding time - day or night)
  - Amount limited
- Pair feeding (same amount for study and control groups) :
  - weighing
  - coupling of food dispensers
  - gavage – permanent stomach cannula
  - feeding machine

Ad libitum

- Food is available at all times
  (rodents eat for energy, mostly in the dark period)
- Easy (practical) to administer
- Possible for group feeding
- Can lead to overfeeding

Problems associated with *ad libitum* feeding

Higher risk of :

- Obesity
- Kidney, liver, heart degenerative disease
- Tumors
- Reduced life-span
- “Boredom” as feed is always available

Restricted feeding

- Time or amount limited
- Difficult in grouped animals
- Different ways in different species :
    - through feeding machine
    - hidden
    - toys
- Animals “work for food”

Beneficial effects of restricted feeding

- Weight gain is controlled
- Nutrition-associated diseases are reduced
- Life expectancy increases
- Fewer spontaneous tumors

The influence of food restriction versus *ad libitum* feeding of chow and purified diets on variation in body weight, growth and physiology of female Wistar rats

M Moraal, P P A M Leenaars, H Arnts, K Smeets, B S Savenije, J H A J Curfs and M Ritskes-Hoitinga

Central Animal Laboratory, Radboud University Nijmegen Medical Centre, PO Box 9101, NL-6500 HB Nijmegen, The Netherlands
Corresponding author: M Ritskes-Hoitinga. Email: M.Ritskes-Hoitinga@cdl.umcn.nl

Abstract

Ad libitum (AL) supply of standard chow is the feeding method most often used for rodents in animal experiments. However, AL feeding is known to result in a shorter lifespan and decreased health as compared with restricted feeding. Restricted feeding and thus limiting calorie intake prevents many health problems, increases lifespan and can also increase group uniformity. All this leads to a reduced number of animals needed. So-called standard chows are known to be prone to variation in composition. Synthetic foods have a more standard composition, contributing to group uniformity which, like diet reduction, may decrease the number of animals necessary to obtain statistical significance. In this study, we compared the effects of AL versus restricted feeding (25% reduction in food intake) on standard chow versus synthetic food of three different suppliers on body weight (BW), growth, several blood parameters and organ weights in growing female Wistar rats over a period of 61 days. Diet restriction led to a decreased growth and significantly reduced variation in BW and growth as compared with AL feeding. AL feeding on synthetic diets caused a significantly higher BW gain than on chow diets. Due to experimental design, this same effect occurred on food restriction. Blood parameters and organ weights were affected neither by diet type nor by amount. Incidentally, variations were significantly reduced on food restriction versus AL, and on synthetic diets versus chow diets. This study demonstrates that food restriction versus AL feeding leads to a significantly reduced variation in BW and growth, thereby indicating the potential for reduction when applying this feeding schedule.

Keywords: Diet restriction, food type, variation, reduction

Laboratory Animals 2012; **46**: 101–107. DOI: 10.1258/la.2011.011011

© Laboratory Animals Ltd. *Laboratory Animals* (1999) **33**, 101–107

The effects of feeding and housing on the behaviour of the laboratory rabbit

T. C. Krohn, J. Ritskes-Hoitinga & P. Svendsen

Biomedical Laboratory, Odense University, Winsloewparken 21, DK-5000 Odense C, Denmark

Summary

The effects of housing, feeding time and diet composition on the behaviour of the laboratory rabbit were examined. The animals were caged individually in single or double metal cages with perforated metal floors, metal walls, and bars in the front, or kept as a group in floor pens. The light/dark cycle was 12/12 h with light from 04:00 to 16:00 h and 30 min twilight. One experiment compared feeding equal energy levels of a high energy diet (10.1 MJ/kg) and with a low energy diet (7.0 MJ/kg) at 08:00 h. The second experiment compared feeding the high energy diet at 08:00 h and at 14:00 h. In both studies the behaviour of the rabbits was recorded between 08:00 and 14:00 h and between 16:00 and 22:00 h. Feeding the animals at 14:00 h reduced abnormal behaviour during the dark period compared to feeding at 08:00 h, whereas no difference in behaviour could be detected between feeding a high-energy and a low-energy diet at 08:00 h. Animals in floor pens generally showed less abnormal behaviour than caged animals. The results indicate that the welfare for caged rabbits can be improved by feeding the animals in the afternoon rather than in the morning.

Dietary restriction

Important :

achieve caloric restriction of test animals,
without producing unintended
nutrient deficiencies

Caloric restriction & aging

- Dietary restriction (without malnutrition) in rodents → ↓ serum IGF-1 concentration (~40%) → ↑ maximal lifespan and health span in rodents

- IGF-1 receptor-deficient mice & klotho transgenic mice (decreased IGF-1 signaling) → involved in the delayed aging phenotype

- LETTER

15 SEPTEMBER 2016 | VOL 537 | NATURE | 427 doi:10.1038/nature19329

Restricted diet delays accelerated ageing and genomic stress in DNA-repair-deficient mice

W. P. Vermeij[1]*, M. E. T. Dollé[2]*, E. Reiling[1,2], D. Jaarsma[3], C. Payan-Gomez[1,4], C. R. Bombardieri[1], H. Wu[5], A. J. M. Roks[5], S. M. Botter[1,6], B. C. van der Eerden[7], S. A. Youssef[8], R. V. Kuiper[8]†, B. Nagarajah[2], C. T. van Oostrom[2], R. M. C. Brandt[1], S. Barnhoorn[1], S. Imholz[2], J. L. A. Pennings[2], A. de Bruin[8,9], Á. Gyenis[1], J. Pothof[1], J. Vijg[10], H. van Steeg[2,11] & J. H. J. Hoeijmakers[1,12]

Fasting

- Short-term food restriction periods:
  - greatest effect in dark phase
  - possible during daytime (6-8 hours)

- Leads to: bodyweight loss, significant changes in multiple cardiovascular, hormonal and metabolic parameters

- In rats, food deprivation for:
  - 6 hours → stomach is empty
  - 12 hours → shift from carbohydrate to fat metabolism
  - > 24 hours → used all glycogen deposits

- Metabolism in rodents is high → fasting has a huge effect ↷ blood sugars reduction, then liver glycogen is used

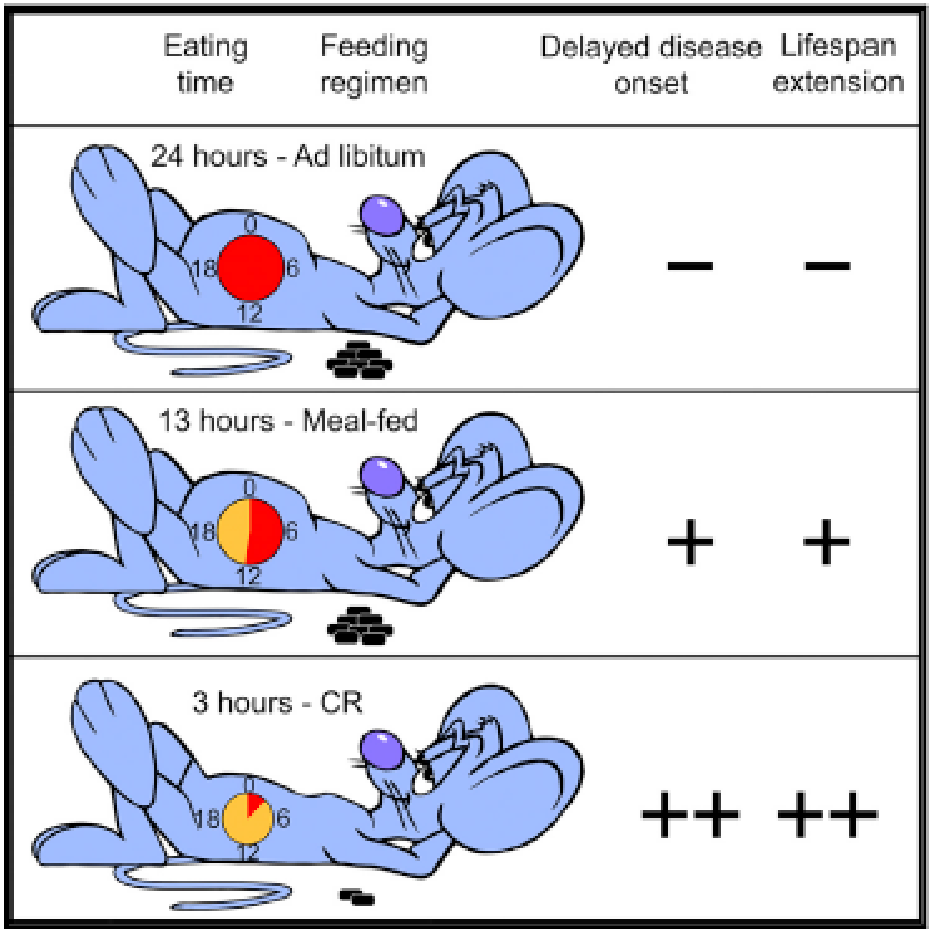
- Not recommended for rodents (cannot vomit)

Cell Metabolism
Mitchell et al., 2019, Cell Metabolism 29, 221–228
January 8, 2019 Published by Elsevier Inc.
https://doi.org/10.1016/j.cmet.2018.08.011

Short Article

Daily Fasting Improves Health and Survival in Male Mice Independent of Diet Composition and Calories

Sarah J. Mitchell,[1,8] Michel Bernier,[1] Julie A. Mattison,[1] Miguel A. Aon,[1,2] Tamzin A. Kaiser,[1] R. Michael Anson,[1,3] Yuji Ikeno,[4] Rozalyn M. Anderson,[5,6] Donald K. Ingram,[7] and Rafael de Cabo[1,9,10,*]



Highlights

- The duration of eating/fasting varies based on diet type and feeding protocol
- Meal feeding and CR, unlike AL, show high metabolic flexibility in male mice
- Eating patterns rather than diet composition influence longevity regulation
- A prolonged daily fasting is associated with delayed onset of liver pathologies
- Increasing the fasting time may provide benefits similar to CR without the need to dramatically reduce the amount of calories,

In Brief

Mitchell et al. show that a long daily period of fasting improves the health and survival of male mice, regardless of caloric intake, diet composition, and body weight.

Research fields of nutrition

- Nutrigenomics & nutrigenetics :

  use of biochemistry, physiology, nutrition, genomics, proteomics, metabolomics, transcriptomics and epigenomics

  ⇩

  gene-nutrient interaction at a molecular level

  ⇩

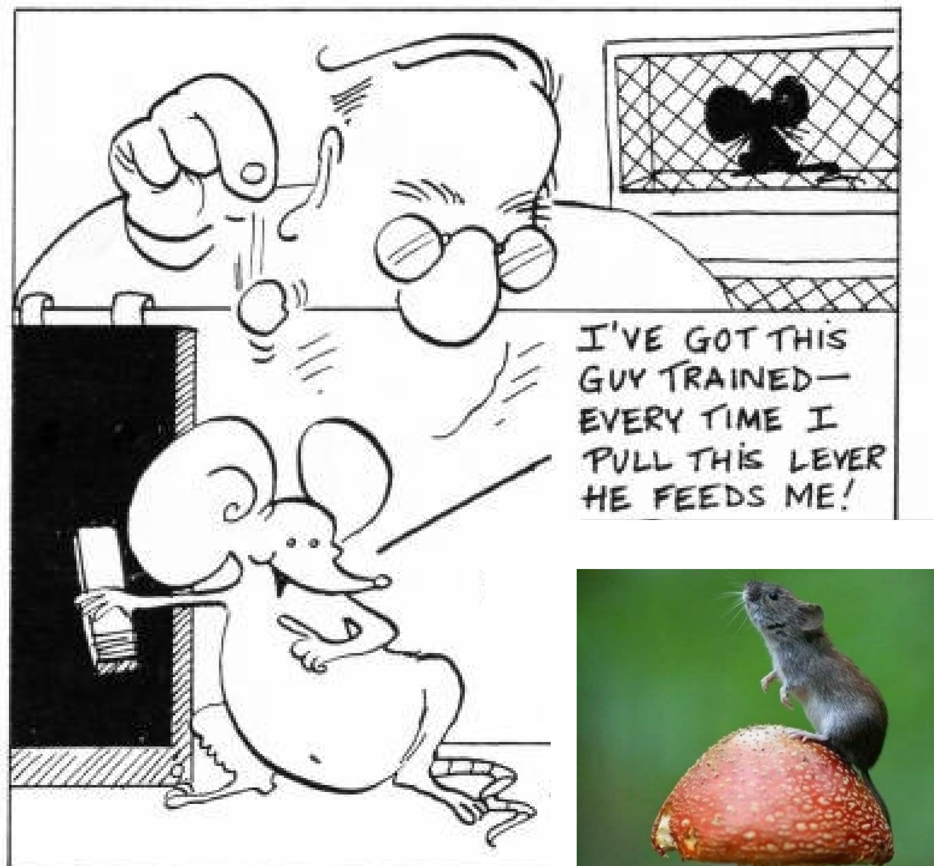
  health optimization via nutritional intervention

- Nutrigerontology :

  studies the impact of nutrients, foods, macronutrient ratios & diets on lifespan, ageing process & age-related diseases

  ⇩

  ↓ risk of ageing-related diseases & ↑ healthy lifespan


I'VE GOT THIS GUY TRAINED— EVERY TIME I PULL THIS LEVER HE FEEDS ME!