Dietary Manipulation of Mouse Metabolism

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ABSTRACT

The maintenance of metabolic homeostasis relies on the balanced intake of nutrients from food. Consequently, diet composition strongly impacts whole-body physiology. Dietary formulations with strong nutrient imbalances can lead to metabolic disorders, with lipids and simple sugars playing a prominent role. This unit describes how diet formulation can be modified to generate mouse models of human metabolic pathologies, and it details methodological procedures linked to dietary manipulations, including caloric restriction and introduction of a test compound. *Curr. Protoc. Mol. Biol.* 84:29B.5.1-29B.5.12. © 2008 by John Wiley & Sons, Inc.

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INTRODUCTION

Nutrition strongly modulates risk factors in the development of metabolic disorders and chronic diseases, including obesity, metabolic syndrome, type 2 diabetes, and cardiovascular diseases. In order to gain a greater understanding of human metabolic disorders, mice are commonly used models because they will readily develop these pathologies when provided with an appropriate diet. Basic Protocol 1 presents the nutritional composition guidelines of a standard chow diet and of specialized diets commonly used to induce metabolic disorders. This is followed by protocols for administering a test compound through the diet (Basic Protocol 2) and performing a caloric restriction experiment (Basic Protocol 3).

INDUCTION OF METABOLIC PATHOLOGIES VIA DIETARY MANIPULATION

BASIC PROTOCOL 1

Standard chow diet is formulated to closely match the daily caloric requirements of mice (10 to 15 kcal/day) when provided ad libitum and to respect the animals' nutritional needs. Chow diets are generally formulated by commercial animal food suppliers, to which the reader is referred for a detailed composition. Because these diets are manufactured mainly from agricultural products, their composition is not tightly defined. Nevertheless, the main components include cereals as the major source of carbohydrates and fiber (~85% by weight), soy and yeast extracts as sources of vegetable proteins (~8% by weight), fish extracts as sources of animal protein and fatty acids (~3% by weight), ash for mineral supply (~4% by weight), and traces of mineral and vitamin complements formulated to match the animals' metabolic needs. The total fat content is composed of roughly equivalent amounts of saturated and unsaturated fatty acids, with palmitic, oleic, and linoleic acids being the most abundant lipids. The caloric intake from standard chow is ~2900 kcal/kg; the approximate energetic composition is specified below as percent of the total energy content:

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Fat ~5% to 12%
Proteins ~20% to 25%
Carbohydrates ~65% to 70% (~65% starch and 2% to 3% simple carbohydrates).

As summarized in Table 29B.5.1, diet modifications can be used to induce a variety of pathologic conditions in laboratory mice. These studies are typically carried out in the C57BL/6 background, which is relatively susceptible to dietary manipulation, or in genetically manipulated animals in the C57BL/6 background. If other strains or animals on a mixed background are used, it is essential to include appropriately matched control animals, restricted to wild-type littermates from a heterozygous breeding for animals on a mixed background.

In a typical experiment, animals of different genotypes or in different experimental conditions are challenged with one or more experimental diets, and their biological responses under these diets are compared to those of animals on a control chow. While it is not possible to outline a single protocol to cover all such experiments, key parameters to consider include using appropriate numbers of age- and gender-matched mice (see Critical Parameters for more details) and maintaining the diets for sufficient times to induce the desired responses. Typical times required for the induction of various pathologies are indicated in Table 29B.5.1.

Most of the diets described in Table 29B.5.1 are generally formulated by commercial animal food suppliers. Alternatively, experimenters with specific requirements may want to have custom diets formulated either by commercial food suppliers that offer this service or by academic facilities that specialize in animal nutrition. In any case, it should be kept in mind that most of these diets are only partially defined diets, and variations in the composition of certain constituents can modify the biological response in vivo. For example, a high-fat diet is most commonly made from animal fat using lard, but the nature of lard triglycerides can vary according to the specific origin and can thereby affect the biological outcome.

BASIC PROTOCOL 2

ADMINISTRATION OF A TEST COMPOUND THROUGH THE DIET

Incorporating a compound in the diet to test for its physiological actions is an easy and efficient way to achieve reproducible daily exposure with minimal stress to the animals. The type of diet in which the compound is mixed should be chosen, on the one hand, according the appropriate diet for the purpose of the study and, on the other hand, according to the chemical properties and bioavailability of the compound itself. Indeed, the polarity of the compound can influence its bioavailability. For example, in the case of a lipophilic molecule, the fat content of the diet can modify its intestinal absorption by increasing its incorporation into lipid micelles. In addition, technical considerations relating to the amount of compound to incorporate, its polarity, and the type of diet chosen should be evaluated in order to achieve a homogeneous incorporation, which will ensure constant and homogeneous dosing of the animals (see Critical Parameters and Fig. 29B.5.3).

Materials

Appropriate mouse strain (e.g., C57BL/6 or see Commentary)
Mouse diet (standard or high-fat diet) in powdered form
Test compound
Vehicle to dissolve compound (if required)

Group housing cages for mice
Scale with 0.1 g accuracy (to weigh food and mice)
Container to accommodate mice during weighing
Scale with 0.1 mg accuracy (to weigh compound)

Table 29B.5.1 Mouse Diets Commonly Used to Induce Metabolic Disorders

Induced pathology	Type of diet	Composition guidelines[a]	Appropriate mouse strain	Duration to develop pathology[b]	Reference
Obesity	High fat	Fat ~40%[c]	C57BL/6	8-12 weeks	West et al., 1992; Surwit et al., 1995
	Very high fat	Fat ~60%[c]		8-12 weeks	Surwit et al., 1995; Rossmeisl et al., 2003
Hypertriglyceridemia + insulin resistance (IR)	High sucrose diet	Sucrose ~50%-60%	Mice develop sucrose-induced IR very slowly	30-55 weeks	Surwit et al., 1995; Sumiyoshi et al., 2006
Metabolic syndrome (obesity + IR)	High fat/high sucrose	Fat ~40% Sucrose ~30%[d]	C57BL/6	14-18 weeks	Surwit et al., 1995; Parekh et al., 1998
Ketoacidosis	Very high fat, low carbohydrate	Fat ~84% Carbohydrate 0%	C57BL/6	4-8 weeks	Kennedy et al., 2007
	High fat/high protein, low carbohydrate	Fat ~65%/protein ~35% Carbohydrate ~0%	C57BL/6	4-8 weeks	
Hypercholesterolemia + atherosclerosis	High fat/high cholesterol + cholic acid	Fat ~15% Cholesterol ~1.25% Cholic acid ~0.25%-0.5%	C57BL/6	14-18 weeks	Paigen et al., 1985, 1987
	High cholesterol	~1.25%	LDLR KO Apo E KO	8-12 weeks	Getz and Reardon, 2006; Hartvigsen et al., 2007
Hypertension	High salt	NaCl ~8%	Mice develop salt-induced hypertension slowly	12-16 week	Yu et al., 2004
	High salt + DOCA + nephrectomy[e]	NaCl ~8% DOCA ~3.5 mg/kg/day	C57BL/6	2-4 weeks	Hartner et al., 2003; Monassier et al., 2006

[a]Composition guidelines are only given when differing from chow diet and are expressed in percentage of the total energetic content, except for NaCl which is a percentage of weight.

[b]The duration for inducing the pathologies described can vary according to the age at which the dietary intervention is started, with induction times being reduced in aged animals. In addition, disease progression will evolve gradually, and the importance of the pathologies developed will therefore be directly correlated to the length of the dietary intervention.

[c]It should be noted that high-fat and very-high-fat diets generally also have elevated sucrose content (from 7% to 20%), which also contribute to the induction of obesity. In addition, obesity and insulin resistance are tightly linked, and such diets will also promote insulin resistance, although the phenotype on glucose homeostasis will generally appear after the first signs of obesity.

[d]Fructose is also efficient at inducing insulin resistance and can replace sucrose.

[e]Deoxycorticosterone acetate (DOCA) is most generally administered subcutaneously by daily injection or by implantation of slow-releasing pellets, and NaCl can also be administered through drinking water at a concentration of 1% (w/w). In addition, DOCA-salt hypertension is maximized by unilateral nephrectomy.

Mortar and pestle for grinding the compound (if required)
Large container for mixing diet and test compound *or* automated mixing machine
Commercial pelleting machine (optional)
Desiccator (optional)

Determine daily food intake

The accuracy of the doses administered through food relies on the correct measurement of food intake (FI), which directly controls the amount of compound ingested. Therefore, FI should be evaluated, always housing the same number of animals per group.

1. At a given hour of the day, change mice to clean cages and weigh the amount of food given.
2. Two to seven days later, at the same hour of the day, weigh the food remaining, including any chunks of pellets in the cage.
3. Calculate FI in g/mouse/day according to the formula:

$$\mathrm{FI}\left(\mathrm{g/mouse/day}\right)=\frac{w_0-w_{n\mathrm{d}}}{n_{\mathrm{d}}\times n_{\mathrm{m}}}$$

Equation 29B.5.1

where w_0 is the weight (g) of the amount of food given;

$w_{n\mathrm{d}}$ is weight (g) of the food remaining in the cage at the end of the feeding period;

n_{d} is the number of days over which FI is calculated;

n_{m} is the number of mice per cage.

4. Repeat the measurement every two days during the first week of treatment to verify that the compound does not cause food aversion, and on a weekly basis thereafter.

Calculate test compound dose

A test compound is generally administered at a constant dose normalized to body weight (mg compound/kg body weight). Since accomplishing this for each individual animal would require individual housing and preparation of a diet with a concentration of compound adapted to each mouse, a widely accepted simpler approach consists of using the average body weight (BW) and food intake (FI) as an estimate for each animal. Alternatively, in the experimental case where both the diet and the treatment are expected to minimally affect body weight, the compound can be administered at a constant concentration in the diet. The concentration should be calculated the same as for a constant dose, but only once, using the BW and FI calculated before the beginning of the treatment or from estimates of these parameters derived from pilot studies performed in the exact same experimental setting.

5a. *To calculate test compound as a constant dose in diet (mg/kg body weight)*: Adjust the quantity of compound to incorporate into the diet (QCD) given on a weekly basis by calculating for week n the QCD_n in mg compound/g diet according to the following equation:

$$\mathrm{QCD}_n=\frac{\mathrm{LE}\times\mathrm{BW}_{n-1}}{\mathrm{FI}_{n-1}}$$

Equation 29B.5.2

where LE is the desired level of exposure (mg/kg BW/day);

BW_{n-1} is the average body weight (kg) from week the previous week (n-1);

FI_{n-1} is the food intake (g/mouse/day) from week the previous week (n-1).

The incorporation of the compound into the diet requires weekly adaptation to adjust for variations in FI and BW. Thus, these parameters should be recorded on at least a weekly basis, at a controlled time of day, as described in steps 1 to 4.

5b. *To calculate test compound as a constant concentration (mg/kg diet):* Calculate the concentration of test compound to be incorporated into the diet, using Equation 29B.5.2 and the BW and FI calculated before the beginning of the treatment or from estimates of these parameters derived from pilot studies performed in the exact same experimental setting.

After validating the stability (in the diet) of the compound to be incorporated, this alternative has the advantage of allowing the preparation of a large stock of pellets for long treatment periods, and therefore it has the potential to outsource the pelleting process.

Prepare pellets containing test compound

6a. *To incorporate a compound into a high-fat diet:* Mix the compound directly with the high-fat diet powder, using either manual mixing in a large container or an automated mixing machine.

The high lipid content of high-fat diet enables direct compaction.

Incorporating low amounts (<100 mg/kg) of compound will require using an appropriate vehicle to ensure homogeneous incorporation (see Critical Parameters and Fig. 29B.5.3 for details).

6b. *To incorporate a compound into a standard diet:* Incorporate 600 ml/kg autoclaved distilled water in standard chow diet powder. Mix in the powdered compound, using either manual mixing in a large container or an automated mixing machine.

Adding the water enables compaction.

Incorporating low amounts (<100 mg/kg) of compound will require using an appropriate vehicle to ensure homogeneous incorporation (see Critical Parameters and Fig. 29B.5.3 for details).

7. Form pellets manually (by tightly packing the mixture) or using a commercial pelleting machine (recommended).

Alternately, animals can be fed the powdered diet (see step 9 annotation).

8. Store high-fat diet pellets up to 2 months at 4°C (or frozen according to the manufacturer's instruction) until use. Air-dry chow diet pellets in a desiccator or in a ventilated hood for at least 48 hr and store up to 6 months at room temperature or according to the stability of the compound (see Critical Parameters).

Administer test compound in diet

9. Give a large excess of pellets (at least 20% more than the amount required according to FI on week n-1) in a clean cage, at a weekly frequency (minimum).

Although animals can be fed the powdered diet from step 1, it is essential that specialized equipment be used, as mice will spill large quantities of food and rapidly contaminate open troughs. For example, special troughs with grids have been designed to limit these problems, and one is illustrated in Figure 29B.5.1. To avoid these problems entirely, it is best to restrict this feeding of powdered diet to specialized applications where powdered diet is absolutely required (e.g., monitoring food consumption in metabolic cages; see Basic Protocol 4 of UNIT 29B.1).

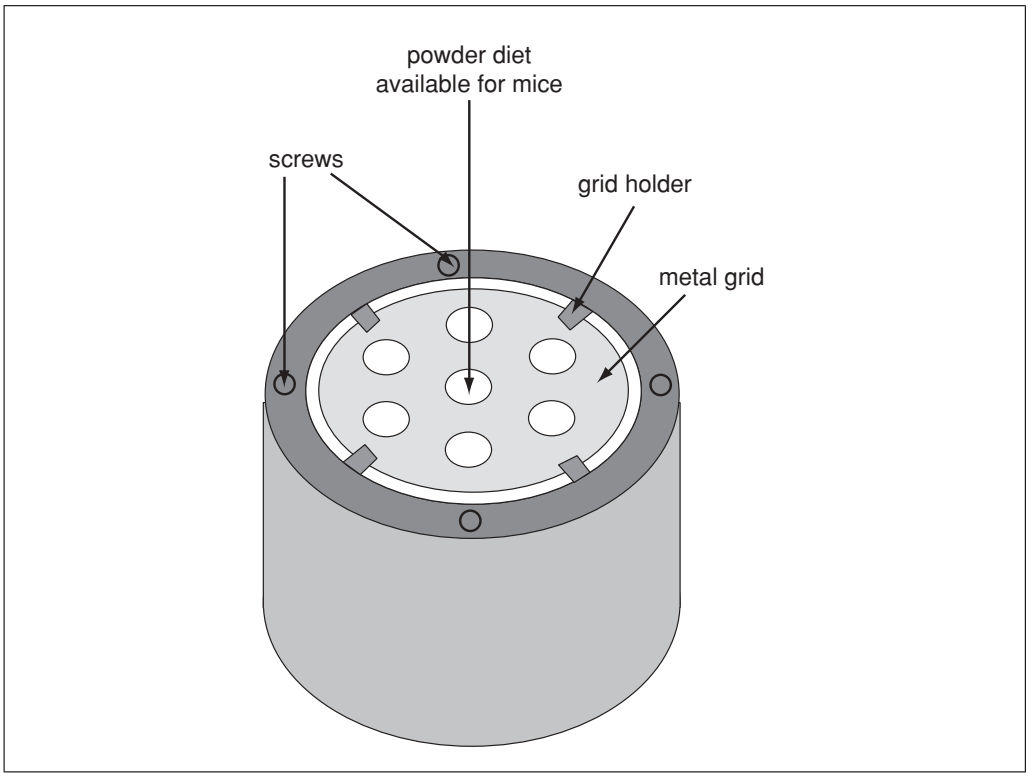

Figure 29B.5.1 Example of a food trough for powdered diet. Powdered diet is placed in the barrel-shaped container and the metal grid (which limits food spoiling and contamination) is positioned on top of the diet and secured with screws and a grid holder. Mice access food via the small holes of the grid.

BASIC PROTOCOL 3

CALORIC RESTRICTION

Caloric restriction (CR) is the main nongenetic manipulation known to extend lifespan in a wide variety of animal models. Although various CR strategies have been experimentally tested, with different levels of feeding restriction, CR is typically defined as a 33% to 50% decrease in total caloric intake. In order to maintain a balanced nutritional supply, diet composition is unchanged to ensure that all sources of nutrients, vitamins, and minerals are provided, and only the quantity of food the animals can access daily is held at levels lower than ad libitum.

The altered metabolic rate induced by this dietary manipulation has been demonstrated to produce a metabolic profile desirable for treating diseases of aging in both animal models and humans (e.g., metabolic disorders or neurodegeneration; Koubova and Guarente, 2003; Masoro, 2005). CR has also been shown to reduce the incidence and progression of spontaneous and induced tumors (Weindruch and Walford, 1988). It is thus not surprising that caloric-restricted mice are a model used extensively to understand the fundamental mechanisms underlying these prevalent human pathologies, and that developing pharmacological caloric restriction mimetics is a current research strategy for antineurodegenerative and anticancer interventions (Roth et al., 2005; Elliott and Jirousek, 2008).

An easily applicable method for restricting caloric intake is to feed the CR group with the same diet as a control group fed ad libitum, but to fast them for 24 hr on alternate days. This method leads to a 30% to 40% decrease of the global caloric intake (Weindruch et al., 1986). However, it generates cycles of lipolysis and fat storage that are not representative of a constant reduction in caloric intake.

The most reliable method for restricting caloric intake consists of pair-feeding CR mice at 50% to 67% of a control group fed ad libitum by restraining their daily food intake. A caloric restriction of 40% is recommended (i.e., pair-feeding at 60% of the ad libitum control). Ideally, mice should be housed in individual cages. If this is not possible, they should at least be housed at the same number of animals per cage. The quantity of food to be administered to the CR group should be evaluated daily as described in the steps below.

Materials

Mice (see Commentary)
Appropriate diet

Housing cages (individual housing, recommended)
Scale with 0.1 g accuracy (to weigh mice and food)
Container to accommodate mice during weighing

1. Monitor daily the food intake (FI) of the control (C) group, fed ad libitum, as described in Basic Protocol 2.
2. On day *n*, at a given hour of the day, change mice of both the C and the caloric restriction (CR) groups to clean cages. Feed the C group ad libitum and adjust the quantity of food administered to the CR group based on the FI of the C group on the preceding day and the percentage of restriction desired (generally 40%).
3. Calculate the quantity of food administered to the CR group on any given day ($QFA_{CRd\mathit{n}}$) in g/cage/day as follows:

$$QFA_{CRd\mathit{n}} = \frac{(100 - PRD)FI_{Cd\mathit{n}-1}}{100} n_m$$

Equation 29B.5.3

where PRD is the percentage of restriction desired;

$FI_{Cdn\text{-}1}$ is the food intake of the control group on the preceding day in g/mouse/day;

n_m is the number of mice per cage.

4. Repeat the FI measurements and the pair-feeding of the C and CR group daily at a constant hour of the day.

COMMENTARY

Background Information

Obesity is a multifactorial disorder that results from an imbalance between caloric intake and expenditure, where the excess of energy is stored as triglycerides, primarily in the adipose tissue. Under such circumstances, triglycerides also accumulate in peripheral tissues (e.g., the liver or the muscle) and exert deleterious consequences on insulin signaling, which leads to a broader pathology where glucose homeostasis is also altered. The C57BL/6 mouse strain is prone to develop obesity when challenged by a diet enriched in fat. Typical high-fat diets contain between 40% and 60% of energy as fat (Table 29B.5.1), and the ability of a diet to promote weight gain directly correlates with its fat content (Fig. 29B.5.2).

As explained above, the action of high-fat and very-high-fat diets on obesity will consequently lead to insulin resistance and ultimately hyperglycemia (Surwit et al., 1988), although it is important to note that several mouse strains are partially resistant to obesity-induced hyperglycemia (Surwit et al., 1988). Alternately, a wider metabolic impact with a more striking hyperglycemic phenotype can be achieved by combining a high-fat with a high-sucrose (or other simple carbohydrates such as fructose) content in the diet (Surwit et al., 1995). This combination is often referred to

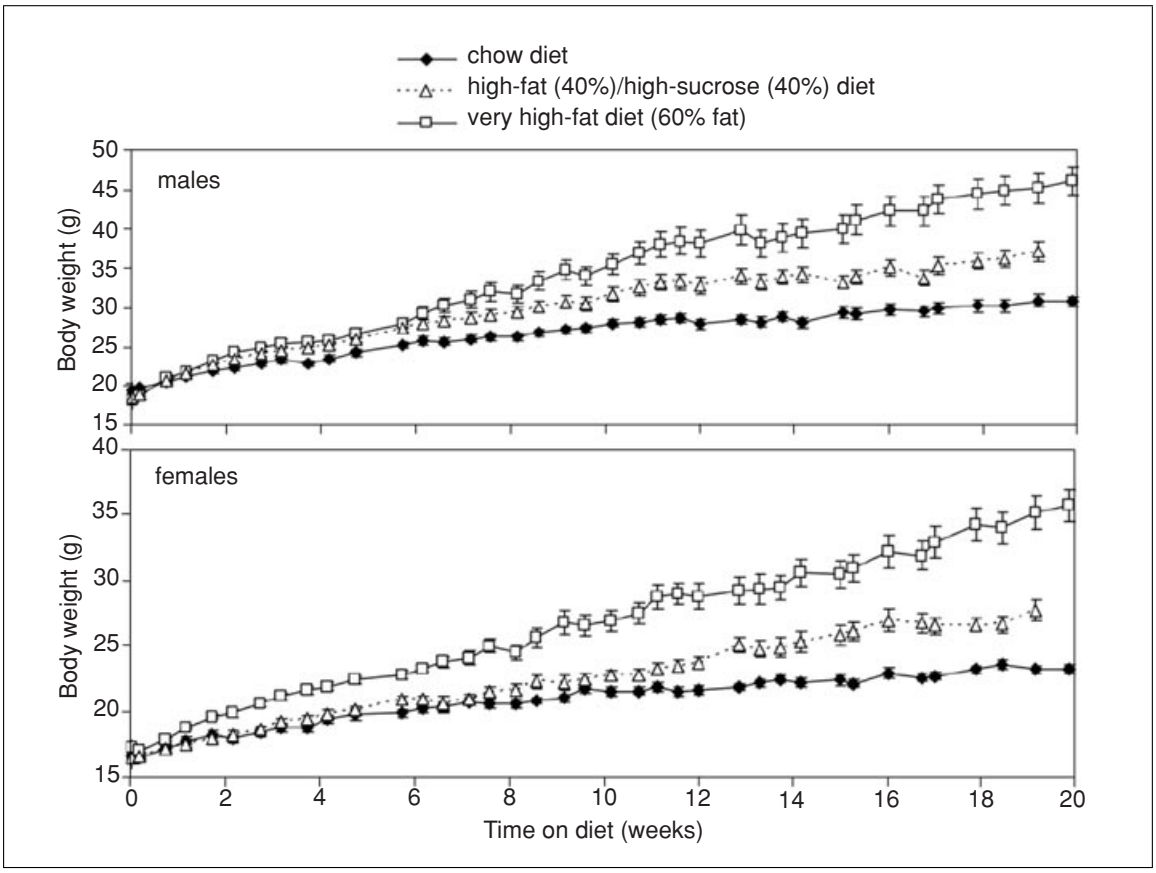

Figure 29B.5.2 Effects of chow and high-fat diets on body weight. Three groups of 7-week male and three groups of 7-week female C57BL/6J mice were housed at five animals per cage and fed ad libitum with chow diet (D04, Safe Diets), high-fat/high-sucrose diet (40% of energy as fat and 40% as sucrose; D12327, Research Diets), or very-high-fat diet (60% energy as fat; D12492, Research Diets). Body weight was recorded twice weekly over twenty weeks on ten animals per group.

as a "western diet" because of its similarity to lipid- and sugar-rich fast-food meals, one of the causes of the increasing prevalence of metabolic diseases in western societies.

Ketoacidosis is an acidification of the plasma resulting from an excessive production of acidic ketone bodies (acetoacetate, β-hydroxybutyrate, and acetone) when cells use fatty acids preferentially over glucose. This pathology can arise when glucose utilization is impaired (e.g., in diabetic patients with a higher prevalence in type 1 as opposed to type 2, diabetics). Features recapitulating this pathology can be induced in mice by totally removing carbohydrates from the diet. Calories are then provided by elevating the fat content, which is the primary source of ketone bodies, but also possibly by enhancing protein concentrations, as amino acid deamination also leads to ketone body production.

Another facet of metabolic research relates to cholesterol homeostasis, as hypercholesterolemia is a major cause of atherosclerosis and its cardiovascular complications. Mice are not a representative model of human cholesterol homeostasis because, unlike in humans, cholesterol is predominantly associated with high-density lipoproteins and can be cleared more efficiently from the bloodstream through conversion to bile acids and subsequent intestinal elimination. Since standard rodent diets are generally poor in cholesterol, high-cholesterol diets are necessary to study cholesterol homeostasis in a setting more relevant to human physiology.

Hypertension is a parameter often correlated with obesity and insulin resistance, and it contributes to the accelerated development of cardiovascular complications associated with metabolic disorders. However, hypertension can be induced in the absence of other metabolic abnormalities by using high-sodium diets and/or chemical models such as mineralocorticoids or nitric oxide synthase inhibitors.

Critical Parameters

Animal housing

The number of animals per cage is a critical parameter in dietary intervention studies. Housing in individual cages is advantageous in certain situations where dominant behavior can be problematic. For example, the precision of food intake measurements and test compound dosing will benefit from individual housing. Moreover, individual housing is particularly recommended for pair-feeding experiments to induce caloric restriction, as a dominant animal can otherwise eat ad libitum and restrain the food intake of its cage mates to levels lower than those desired. In the choice of individual housing, it should be kept in mind that mice are social animals, for whom isolated housing can lead to elevated levels of stress. When collective housing is chosen, the number of animals per cage should always be matched for all the cages of the experiment, as density can profoundly affect behavior, feeding habits, and metabolic homeostasis (Champy et al., 2004). Depending on cage size, three to five animals per cage is generally considered optimal.

Number of animals per group

Dietary manipulations, like many metabolic experiments, display high levels of interindividual variability, essentially because they involve a systemic response of the organism that involves a complex inter-organ cross-talk. Strictly controlled animal housing and experimental conditions are definitely important parameters to limit this variability. Nevertheless, a minimum of eight animals per group is recommended to delineate subtle biological variations from background variability.

Chow diet composition

Given the wide diversity of animal food suppliers, the composition of chow diet can vary slightly according to individual research and development optimization. Changes in chow diet supplier should therefore be minimized, and the composition and batch reference of a diet should be archived, as it constitutes an important experimental parameter. In addition, chow diets have only a partially defined composition, as their main constituents are agricultural cereals of complex composition, which allow relatively inexpensive production costs.

While the global nutritional content is generally well controlled, some specific variations may arise at similar crude composition depending on the source of the primary products. For example, phytoestrogen content can vary greatly depending on the proportion and the source of soy (Thigpen et al., 2004). Consequently, the use of chow diet as a reference for direct comparison with the experimental diets listed in Table 29B.5.1, which are most often formulated according to different and better-defined compositions, should be considered only with these limitations in mind, and diet compositions should be stated as an integral part of any experimental procedure (Warden and Fisler, 2008).

The composition of chow diet indicated in Basic Protocol 1 can be slightly modulated according to the special needs of the animals due to their growing phase. For instance, growing, breeding, and nursing mice, as well as some sensitive mouse strains, can benefit from a chow diet containing ~3500 kcal/kg, in which protein and lipid levels represent about 23% and 5.5% of caloric intake, respectively, and mineral and vitamin content is consequently optimized.

Induction of metabolic disorders

It should be noted that the nature of the fat contained in the diet can considerably impact weight gain and metabolic alterations, according to the proportion of saturated to unsaturated fat and the composition in fatty acids. In that respect, high-fat diet made from animal fat is a widely used challenge that will generally give the strongest metabolic alterations because of the high saturated fat content (Buettner et al., 2007). Context-specific considerations may, however, lead to the use of alternate diets made from vegetable oils chosen according to their fatty acid composition. In any case, the action of a high-fat diet on weight gain will give rise, even on a pure genetic background, to some inter-individual variability, with some mice being very sensitive while others are resistant.

Even on a pure genetic background, the strain of mice is an important parameter to consider for the induction of diet-induced metabolic disorders. 129SvPas mice, commonly used for gene targeting strategies, are resistant to age-induced obesity and insulin resistance (Champy et al., 2008). In contrast, C57BL/6 mice are more susceptible to both diet-induced obesity and hyperglycemia (Surwit et al., 1988), the latter of which has recently been attributed to a reduced capacity to secrete insulin due to a mutation of the

nicotinamide nucleotide dehydrogenase, an enzyme controlling mitochondrial metabolism in pancreatic β cells (Freeman et al., 2006).

It should be kept in mind, however, that high-fat diet–induced hyperglycemia occurs over relatively long periods in most mouse strains because of the capacity of mouse pancreatic β-cells to maintain high secretory levels of insulin despite peripheral insulin resistance. When using high-fat diet to induce global metabolic disorders, it is therefore important to select diets with a very high fat content (60% of energy supplies) and to plan long treatments (>10 weeks) in order to induce both obesity and its negative consequences on glucose homeostasis. A wider metabolic impact with a more striking hyperglycemic phenotype can be achieved by combining high-fat and high-sucrose content in the diet (Surwit et al., 1995). It should be noted, however, that the use of high-sucrose diet alone is rather inefficient at impairing glucose homeostasis in mice. Ketogenic diets lead to weight loss in mice as they strongly stimulate fat oxidation.

High-cholesterol diets are not sufficient to promote cholesterol-related diseases such as atherosclerosis because of the resistance of wild-type mice to hypercholesterolemia. A classical way of overcoming this resistance is to combine high cholesterol with high cholic acid in the diet, because this bile acid promotes uptake of dietary cholesterol and inhibits the rate limiting enzyme that catalyzes the conversion of cholesterol to bile acids through a feed-back loop, thereby reducing its clearance (Houten et al., 2006). In addition, atherogenic lesions can be amplified by combining elevated fat content with high cholesterol and cholic acid levels in a diet generally referred to as the Paigen diet (Paigen et al., 1985, 1987; Nishina et al., 1990). Alternatively, a high-cholesterol diet alone can be efficient in genetic models predisposed to atherosclerosis, such as apolipoprotein E (apoE) or low-density lipoprotein (LDL) knock-out mice.

Mice are not very sensitive to diet-induced hypertension. For example, C57BL/6 mice exhibit elevated blood pressure only after several months of feeding with a diet high in NaCl. The use of high-salt diets in combination with surgical (nephrectomy) and chemical (mineralocorticoids such as deoxycorticosterone acetate—DOCA) models therefore proves more useful for rodent models of hypertension. In addition, hypertension can also be induced by salt-independent procedures such as administration of the nitric oxide synthase inhibitor *Nω*-nitro-L-arginine-methyl ester (L-NAME).

Incorporation of a test compound in the diet

Ensuring homogenous incorporation of the compound in the diet is key to constant dosing of the animals. The method of incorporating the compound differs according to the amount of compound used in mixing and its chemical nature (Fig. 29B.5.3). If the QCD value exceeds 100 mg compound/kg diet for incorporation in chow diet, or 500 mg compound/kg diet for incorporation in high-fat diet, the compound can be directly mixed into powder diet. Grinding of the compound may prove necessary in some cases to ensure homogenous incorporation.

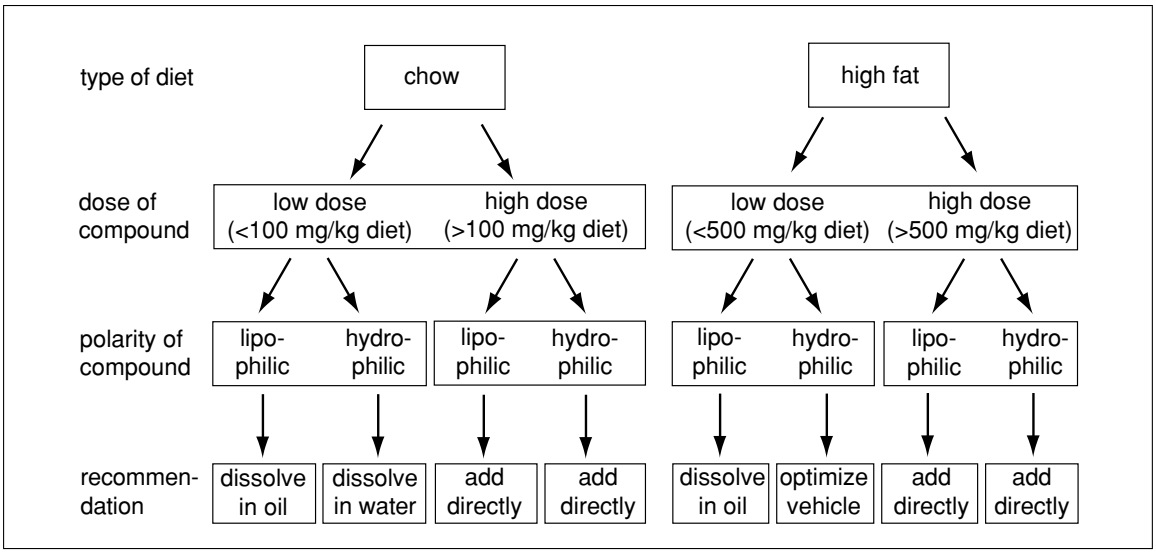

Figure 29B.5.3 Decisional flowchart for test compound incorporation in a diet.

If the QCD values are lower than indicated above, the compound should be dissolved in a vehicle adapted to its polarity to ensure homogenous incorporation to the powder diet. When a hydrophilic compound is to be incorporated in chow diet, water is the vehicle of choice at a volume of 600 ml/kg diet (then replacing step 6b of Basic Protocol 2), but the vehicle and its concentration need to be optimized according to polarity when a hydrophilic compound is to be incorporated in high-fat diet. In contrast, when a hydrophobic compound is to be incorporated in either chow or high-fat diet, oil becomes the best vehicle and should be added to the diet at 10 ml/kg. However, the oil used as vehicle can increase the caloric density of the diet and should be taken into account when analyzing the results.

Administration of compounds at a constant concentration over the entire treatment with high-fat diets is not recommended, as the dosing normalized to body weight will vary during treatment because of weight gain. Similarly, administration at a constant concentration should be avoided when the compound itself affects body weight or feeding behavior.

Diet preservation

While regular chow diet can be stored at room temperature for several months, the elevation of fat content in diets used for metabolic intervention render these diets much more perishable. All diets with a high-fat content should be stored at 4°C over a short term (<2 months) and frozen for longer preservation, according to the manufacturer's recommendations. When a test compound is incorporated in the diet, the time and conditions of storage should be adapted to the stability of the compound.

Caloric restriction

It is best to start CR at 6 weeks of age to avoid any developmental defects.

Anticipated results

Diet-induced metabolic disorders

The actions of very-high-fat and high-fat/high-sucrose diets on body weight are depicted in Figure 29B.5.2.

Caloric restriction

CR can promote an extension of lifespan by up to 50% in rodent models (Koubova and Guarente, 2003), but lifespan extension can vary according to the genetic background, with C57BL/6 mice being more responsive than DBA mice (Ferguson et al., 2007). In addition, CR will prevent weight gain without inducing weight loss in lean adult mice (Ferguson et al., 2007).

Time Considerations

The impacts of dietary manipulations are typically both time dependent and progressive. Typical time frames for inducing different pathologies in the C57BL/6 background are indicated in Table 29B.5.1. However, the duration of the dietary intervention must be adapted to the degree of pathological severity pursued, according to the literature and to the experimenter's experience. For example, a high-fat diet will induce mild glucose intolerance after only 4 weeks, but this intolerance will gradually increase with time and the final symptoms of type 2 diabetes (fasting hyperglycemia, pancreatic dysfunction) will only be observed at later times, after at least 10 weeks on the diet. In addition, the time course of the impact of diet on different strains or on genetically manipulated animals can vary greatly and must be evaluated based on the literature and personal observations.

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Jérôme N. Feige and Marie Lagouge contributed equally to this work.

Literature Cited

Buettner, R., Scholmerich, J., and Bollheimer, L.C. 2007. High-fat diets: Modeling the metabolic disorders of human obesity in rodents. *Obesity* 15:798-808.

Champy, M.F., Selloum, M., Piard, L., Zeitler, V., Caradec, C., Chambon, P., and Auwerx, J. 2004. Mouse functional genomics requires standardization of mouse handling and housing conditions. *Mamm. Genome* 15:768-783.

Champy, M.F., Selloum, M., Zeitler, V., Caradec, C., Jung, B., Rousseau, S., Pouilly, L., Sorg, T., and Auwerx, J. 2008. Genetic background determines metabolic phenotypes in the mouse. *Mamm. Genome* 19:318-331.

Elliott, P.J. and Jirousek, M. 2008. Sirtuins: Novel targets for metabolic disease. *Curr. Opin. Investig. Drugs* 9:371-378.

Ferguson, M., Sohal, B.H., Forster, M.J., and Sohal, R.S. 2007. Effect of long-term caloric restriction on oxygen consumption and body temperature in two different strains of mice. *Mech. Ageing Dev.* 128:539-545.

Freeman, H.C., Hugill, A., Dear, N.T., Ashcroft, F.M., and Cox, R.D. 2006. Deletion of nicotinamide nucleotide transhydrogenase: A new quantitive trait locus accounting for glucose

intolerance in C57BL/6J mice. *Diabetes* 55:2153-2156.

Getz, G.S. and Reardon, C.A. 2006. Diet and murine atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 26:242-249.

Hartner, A., Cordasic, N., Klanke, B., Veelken, R., and Hilgers, K.F. 2003. Strain differences in the development of hypertension and glomerular lesions induced by deoxycorticosterone acetate salt in mice. *Nephrol. Dial. Transplant.* 18:1999-2004.

Hartvigsen, K., Binder, C.J., Hansen, L.F., Rafia, A., Juliano, J., Horkko, S., Steinberg, D., Palinski, W., Witztum, J.L., and Li, A.C. 2007. A diet-induced hypercholesterolemic murine model to study atherogenesis without obesity and metabolic syndrome. *Arterioscler. Thromb. Vasc. Biol.* 27:878-885.

Houten, S.M., Watanabe, M., and Auwerx, J. 2006. Endocrine functions of bile acids. *Embo J.* 25:1419-1425.

Kennedy, A.R., Pissios, P., Otu, H., Xue, B., Asakura, K., Furukawa, N., Marino, F.E., Liu, F.F., Kahn, B.B., Libermann, T.A., Maratos-Flier, E., and Roberson, R. 2007. A high-fat, ketogenic diet induces a unique metabolic state in mice. *Am. J. Physiol. Endocrinol. Metab.* 292:E1724-E1739.

Koubova, J. and Guarente, L. 2003. How does calorie restriction work? *Genes Dev.* 17:313-321.

Masoro, E.J. 2005. Overview of caloric restriction and ageing. *Mech. Ageing Dev.* 126:913-922.

Monassier, L., Combe, R., and Fertak, L.E. 2006. Mouse models of hypertension. *Drug Discov. Today Dis. Models* 3:273-281.

Nishina, P.M., Verstuyft, J., and Paigen, B. 1990. Synthetic low-and high-fat diets for the study of atherosclerosis in the mouse. *J. Lipid Res.* 31:859-869.

Paigen, B., Morrow, A., Brandon, C., Mitchell, D., and Holmes, P. 1985. Variation in susceptibility to atherosclerosis among inbred strains of mice. *Atherosclerosis* 57:65-73.

Paigen, B., Morrow, A., Holmes, P.A., Mitchell, D., and Williams, R.A. 1987. Quantitative assessment of atherosclerotic lesions in mice. *Atherosclerosis* 68:231-240.

Parekh, P.I., Petro, A.E., Tiller, J.M., Feinglos, M.N., and Surwit, R.S. 1998. Reversal of diet-induced obesity and diabetes in C57BL/6J mice. *Metabolism* 47:1089-1096.

Rossmeisl, M., Rim, J.S., Koza, R.A., and Kozak, L.P. 2003. Variation in type 2 diabetes-related traits in mouse strains susceptible to diet-induced obesity. *Diabetes* 52:1958-1966.

Roth, G.S., Lane, M.A., and Ingram, D.K. 2005. Caloric restriction mimetics: The next phase. *Ann. N.Y. Acad. Sci.* 1057:365-371.

Sumiyoshi, M., Sakanaka, M., and Kimura, Y. 2006. Chronic intake of high-fat and high-sucrose diets differentially affects glucose intolerance in mice. *J. Nutr.* 136:582-587.

Surwit, R.S., Kuhn, C.M., Cochrane, C., McCubbin, J.A., and Feinglos, M.N. 1988. Diet-induced type II diabetes in C57BL/6J mice. *Diabetes* 37:1163-1167.

Surwit, R.S., Feinglos, M.N., Rodin, J., Sutherland, A., Petro, A.E., Opara, E.C., Kuhn, C.M., and Rebuffe-Scrive, M. 1995. Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. *Metabolism* 44:645-651.

Thigpen, J.E., Setchell, K.D., Saunders, H.E., Haseman, J.K., Grant, M.G., and Forsythe, D.B. 2004. Selecting the appropriate rodent diet for endocrine disruptor research and testing studies. *ILAR J.* 45:401-416.

Warden, C.H. and Fisler, J.S. 2008. Comparisons of diets used in animal models of high-fat feeding. *Cell Metab.* 7:277.

Weindruch, R. and Walford, R.L. 1988. The Retardation of Aging and Disease by Dietary Restriction. Charles C. Thomas, Springfield, Ill.

Weindruch, R., Walford, R.L., Fligiel, S., and Guthrie, D. 1986. The retardation of aging in mice by dietary restriction: Longevity, cancer, immunity and lifetime energy intake. *J. Nutr.* 116:641-654.

West, D.B., Boozer, C.N., Moody, D.L., and Atkinson, R.L. 1992. Dietary obesity in nine inbred mouse strains. *Am. J. Physiol.* 262:R1025-R1032.

Yu, Q., Larson, D.F., Slayback, D., Lundeen, T.F., Baxter, J.H., and Watson, R.R. 2004. Characterization of high-salt and high-fat diets on cardiac and vascular function in mice. *Cardiovasc. Toxicol.* 4:37-46.