COMMENTARY

Nutrient density: an important and useful tool for laboratory animal studies

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Nutritional deficiencies or imbalances are suspected contributory factors to several types of human cancers, and perhaps other human diseases related to long-term metabolic derangements. In order to study these more effectively in laboratory animals, it is suggested that the laboratory diets more closely mimic the nutrient density of suspect human diets. Toxicology and carcinogenic data obtained in animal study using diets based on human nutrient density might be more readily applicable in relation to humans.

Dietary considerations in animal studies

Nutritional factors are of growing interest in relation to human diseases, particularly those that are seemingly related to long-term metabolic derangements such as those which occur in cancer. For example, epidemiologic studies in humans have revealed a few correlations between dietary habits and increased risk of cancer. Thus a diet rich in fat and meat, but low in cereals, fiber, fruits and yellow and green vegetables correlates with increased risk for colorectal and mammary cancer in humans (1). Studies of this type seem to identify one or more dietary factors that are associated with an increased risk for certain kinds of cancer.

Once identified by epidemiologic studies, investigators often wish to study these suspected dietary factors in laboratory animals. This poses problems, long known to the early pioneers in nutrition research, of translating nutritional requirements and nutrient interactions from humans to the species used in the laboratory studies. A few of these problems, and methods of dealing with them, are discussed below.

Metabolic rate differences between species

Even adult animals of different species may have large differences in metabolic rate. This is evident by the calories of energy required per kg of body weight to maintain body weight while engaged in ‘normal’ activities, which can vary from 80 kcal/day for a 200-g rat (400 kcal/kg), to 2400 kcal/day for a 60-kg human (40 kcal/kg body wt). This illustrates an ~ 10-fold difference in energy requirement between an adult rat and human when expressed on a body weight basis.

Age differences

In all species, nutrient requirements for young animals are greater than those for adults due to the requirements for production of new tissues for growth. In addition, younger animals usually have a higher metabolic rate than adults, further increasing energy and nutrient requirements on a body weight basis.

Body surface area

Body surface area has been used as a means of calculating nutrient intake and drug dosage for young animals (or humans) (2). The metabolic rate of homothermal animals is more nearly proportional to the body surface area than to the weight, although not strictly proportional (2,3). It has also been used as a means of calculating nutrient intake and drug dosage for young animals (or humans) (2). The metabolic rate of homothermal animals is more nearly proportional to the body surface area than to the weight, although not strictly proportional (2,3). It has also been found that the metabolic rate, divided by 3/4 the power of the body wt in kg, is independent of the body size. Under normal conditions this quotient, from mice to cattle, averages 70 kcal per 3/4 the power of kg (body wt) per day (2,3). This represents another alternative in calculating inter-species nutrient requirement, but is complex and seldom used for comparison of nutrient requirements between species.

Nutrient density

This is probably the simplest and most useful method of comparing adequacy of nutrient requirements between species, and between young and old animals. This method relates the quantities of all nutrients to the energy content of the diet, usually expressed as mg or units of nutrient per kcal of diet. Increased dietary intake of an animal due to higher metabolic rate, thus requiring more energy for balance, then results in proportionately increased intake of all nutrients. This method is a common tool in many areas of nutrition research and in domestic farm animal production. Nutrient density is frequently used in calculations and definition of nutrient requirements, particularly of nutrients that are not extensively stored in mammals. The human US Recommended Dietary Allowances for thiamin and riboflavin nutrition requirements are defined in terms of mg per 1000 kcal of diet (4). The concept of nutrient density has long been applied to protein intake, and it has been proposed as a basic concept for zinc as well (5). In animal nutrition, the energy (caloric) intake can be expressed as ‘gross energy’, ‘digestible energy’ or ‘metabolizable energy’ (6). For laboratory animals metabolizable energy is generally acceptable (4 kcal/g for protein and carbohydrate; 9 kcal/g for fat; 7 kcal/g for alcohol). Alternatively, it may be sufficiently useful to use the approximate easily obtained values for gross energy, minus those fiber components (cellulose, lignin) known not to be metabolized by the animals used in the laboratory.

Relation of nutrient density to carcinogenesis

Applications of the concept of nutrient density of nutrients in studies of carcinogenesis can be very useful. Laboratory animal diets, particularly for rodents (mice and rats), are frequently designed to be optimum for growth of young animals, and are probably ‘too rich in protein, vitamins and minerals for adult animals, resulting in unnatural obesity in adults’ (7). Obesity itself may magnify carcinogenesis in laboratory studies (8). Laboratory animal experiments with adult animals in carcinogenesis studies should preferably adjust the levels of suspect key nutrients to their density in the diet of the human population considered at risk. Sometimes this is done, as with studies on high dietary fat using 20–30% (by weight) of dietary fat in an experimental
animal feed to simulate the 40–60% of dietary calories present in some high-fat Western diets. However, other nutrients in the experimental laboratory feeds are usually left at the (high) density levels considered optimum for growth of the young animal. This may result in the feeding of ‘unnatural’ levels of nutrients which could result in laboratory artefacts.

(i) The human RDA (Recommended Daily Allowances) for nutrients (protein, carbohydrate, energy, vitamins and minerals) for adults is based on the requirements for optimum health of the adult (4). In contrast, laboratory animal RDA values are based on optimum growth of the young animal (9). When dietary intakes of laboratory rodents are compared to humans on the basis of amount per kg of body wt, some bizarre results can emerge. In Table 1 a summary is presented of calcium intake comparisons between mice, rats and humans. On the basis of mg of calcium intake per kg body wt there are 30- to 40-fold differences between rodents and man. In the same table are calculations based on nutrient density, or mg per kcal, which are much more reasonable and comparable.

On the basis of nutrient density, calcium intake is still several fold higher (3–8 times) in laboratory rodents than in human diets in the US. A review of nutrient requirements for domestic farm animals including poultry, swine, dairy cattle, beef cattle, horses and dogs, indicates that all have an adult animal requirement for calcium ≥ 1.5 mg per kcal (10). In addition, diets for a wide variety of zoo animals also require calcium additions > 1.5 mg/kcal (11). It would thus seem that calcium intake in humans in the US is significantly lower, on a nutrient density basis, than that in laboratory and farm animals.

In a recent study in mice, Bird et al. (12,13) reduced the calcium level to 0.1% of the diet, resulting in a nutrient density of ~0.3 mg/kcal, roughly comparable to current human recommended intakes. On oral administration of cholic acid, the irritation and resulting stimulation of epithelial cell proliferation was much greater on the lower calcium level, as compared with the 0.5% calcium level normally used in mice. The data suggest that the higher level of calcium normally used in laboratory rodent feeds may mask a basic characteristic of high fat human foods, namely the capacity of increased free bile and fatty acids to irritate the colon and act as promotion agents in colon carcinogenesis. Since there is a significant segment of the human population in the US that has a calcium intake of ~200–250 mg/day (4), representing a nutrient density of 0.1 mg/kcal, carcinogenic studies in laboratory animals with feeds at 3.5–4.0 kcal/g of feed should contain 0.04% calcium to mimic the human diet in terms of calcium intake. This is about one-tenth the RDA level of 0.5% of calcium in normal laboratory rodent feeds.

(ii) The human requirement for ascorbic acid has been calculated by Pauling (14) based on a study in adult normal rats which established a rate of biosynthesis of ~26 mg/day per kg of body wt to normally saturate its tissues (15). Extrapolation to human adults (60–70 kg) on a body weight basis suggested to Pauling a requirement of up to 1.8–4.1 g/day (14). However, calculated on a nutrient density basis, the rat biosynthesis of 26 mg/day of ascorbic acid per kg of body wt would be calculated against the ~400 kcal/day of dietary energy per kg of body wt required by the rat, equivalent to ~0.065 mg/kcal (diet) per day. Extrapolation of this value to estimates of human ascorbic acid requirements based on nutrient density suggests values of 115–175 mg of ascorbic acid for human diets ranging from 1800 to 2700 kcal per day. These calculations are close to the level of intake of 100 mg/day which was experimentally found to maintain the maximum tissue pool size of ascorbic acid in humans, indicating the utility of species extrapolation using the principle of nutrient density (16).

(iii) In a recent study in female rats, Jacobson et al. (17) varied the calcium intake of the diet from 1.5 mg/kcal (i.e. the rodent RDA) down to 0.25 mg/kcal (i.e. equivalent to the human RDA) and also 0.1 mg/kcal (equivalent to a low adult human intake of 250 mg calcium on a 2500 kcal daily diet), on low fat (3% by weight) and high fat (20% by weight, or 40% of calories) diet. After 12 weeks on these diets, the mammary epithelial proliferation rate as measured by [3H]thymidine-labelling index was several-fold higher in the rats on the lower calcium levels that mimic the human nutrient density levels. This was the case within both the low and high fat groups. In a parallel preliminary experiment with DMBA-induced mammary tumors, similar large differences in tumor production were noted after feeding the diets for 29 weeks. These results strongly suggest that utilization of nutrient density can be a useful tool in studies of dietary modulation of mammary carcinogenesis. In another recent study in female mice in another laboratory, Zhang et al. (18) have also found that variation of dietary calcium significantly affects the proliferation status of mammary glands measured by [3H]thymidine-labeling index. The labelling index for the terminal ducts on high fat (corn oil) diets decreased from 14.1, 11.9 to 8.5, as the calcium levels were increased from 0.1% of diet (nutrient density of ~0.25 mg/kcal, equivalent to human RDA), to 0.5% of diet (~1.25 mg/kcal), and to 1% of diet (~2.5 mg/kcal). Mitotic indices followed the same trend. These studies, which mimic the human diet by controlling the fat and calcium levels to mimic the human diets utilizing the principle of nutrient density, by percent of total calories, or amount of nutrient per kcal of diet, have demonstrated the implications of dietary calcium as a modulator of the known effects of high fat diets in breast carcinogenesis.

Toxicology

In long-term toxicity studies, similar problems probably occur. At least one investigator has suggested that diet restriction be regarded as the norm and more scientific than the currently used ad lib feeding of rich diets designed for growing young animals (7).

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<th>Table I. Daily calcium dietary intake</th>
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<sup>a</sup>Data from recommended daily allowances, RDA, 9th edn 1980 (4).
<sup>b</sup>Mouse data based on an adult mouse of 20 g consuming 2 g/day of AIN-76 rodent feed, containing 3.6 kcal/g and 0.5% calcium (9).
<sup>c</sup>Rat data based on an adult rat of 200 g consuming 20 g/day of AIN-76 rodent feed, calculated similarly to mouse (footnote b).

<sup>d</sup>The numbers in the columns headed by calcium are based on the RDA values. The numbers in parenthesis are based on estimates of actual intakes.
<sup*e</sup>The estimate of actual intake is from ref. 19.
<sup>f</sup>Laboratory rodent feeds contain a minimum of 0.5% calcium (9). However, many rodent feeds contain considerably more (up to 0.9%) (20). The estimate of actual intake chosen here was 0.6%.
Summary and recommendations

In recent years, long-term studies in laboratory animals, particularly in tests for carcinogenesis or toxicology, use laboratory feeds that have been 'standardized' or 'optimized' for maximum growth of young animals. If continued uniformly through adult life in long-term studies, such diets can produce abnormal effects such as obesity, and may produce data with little relevance for application to humans.

It is suggested that diets for animals in laboratory studies in carcinogenesis and toxicology be adjusted to mimic the human nutrient density of key nutrients (e.g. vitamins and minerals). This may require a change in diet during the study from a 'rich' nutrient density required for young growing animals, to the 'leaner' nutrient density of an adult.

The use of nutrient density in designing laboratory animal feeds to obtain information relative to human conditions is an old established nutrition concept. Unfortunately, it is often ignored in current studies, possibly due to the ready commercial availability of diets designed for optimum growth of young animals. A return to diets that mimic the human in terms of nutrient density of essential nutrients would produce results more readily useful and applicable to an understanding of effects in humans.

References


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