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REVIEW

Model organisms in molecular nutrition research

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The complexity of food organism interactions necessitates the use of model organisms to understand physiological and pathological processes. In nutrition research, model organisms were initially used to understand how macro and micronutrients are handled in the organism. Currently, in nutritional systems biology, models of increasing complexity are needed in order to determine the global organisation of a biological system and the interaction with food and food components. Originally driven by genetics, certain model organisms have become most prominent. Model organisms are more accessible systems than human beings and include bacteria, yeast, flies, worms, and mammals such as mice. Here, the origin and the reasons to become the most prominent models are presented. Moreover, their applicability in molecular nutrition research is illustrated with selected examples.

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1 Why do we need model organisms in molecular nutrition research?

A model organism is an organism in which biology can be studied and in which parts or the whole biological process resembles that occurring in humans or other species. Nutrient and non-nutrient diet components of foods can affect every step in the processes from gene to protein altering metabolic pathways. The complexity of these interactions makes necessary the use of more accessible systems than human beings, such as model organisms, to elucidate these mechanisms. Bacteria, yeast, flies, fishes, worms and mammals, such as cows or mice, are valid model organisms to study the interactions of non-nutrients and nutrients with the organism, elucidating biological processes and pathways and extrapolating this knowledge to humans (Fig. 1).

At its beginning, nutrition research used model organisms to understand how macronutrients are handled in the organism [1]. Most of the nutrient-nutrient interactions, bioavailability and toxicity of e.g. minerals, vitamins and amino acids have been obtained by studying animal models. For example, the pig and the rat were used to prove that excess dietary methionine could spare and even eliminate the need to consume preformed choline, as the organism is able to synthesise choline from methionine in the liver via the so-called one-carbon metabolism pathway [2, 3]. Further examples include the use of animal models to enhance our understanding of bioavailability of minerals supplements consumed as salts, or the toxicity of vitamin C when ingested repetitively in high amounts [4].

Dietary intervention studies in animal models as opposed to humans are less limited due to ethical aspects and reduced costs. In addition, the consumption of a defined diet is more manageable than in humans, the ability to induce disease states like the development of cancer or diabetes is speeded up and there is an easier accessibility to tissue samples. Besides these advantages the generation of mutants in model organism allows the study of loss-of-function or gain-of-function of determined genes facilitating the understanding of their biological role. Recently, with the advent of nutrigenomics, the understanding of physiological and pathological processes triggered by nutrients requires model organisms in which invasive techniques are applicable in a cost-effective and ethic-driven way. In model organisms sampling and subsequent application of high-throughput *omics* technologies is facilitated.

In nutrition research, an emerging field is nutritional systems biology. In this new approach to understanding biology, models of increasing complexity are needed in order to determine the global organisation of a biological system. Conclusions about the biological systems are ideally first obtained by studying single cell organisms, confirmed in multicellular simple organisms, validated in mammals and finally in humans [5]. Therefore, in molecular nutrition research, we aim to gain knowledge by drawing conclusions from cell to society with the application of different model organisms.

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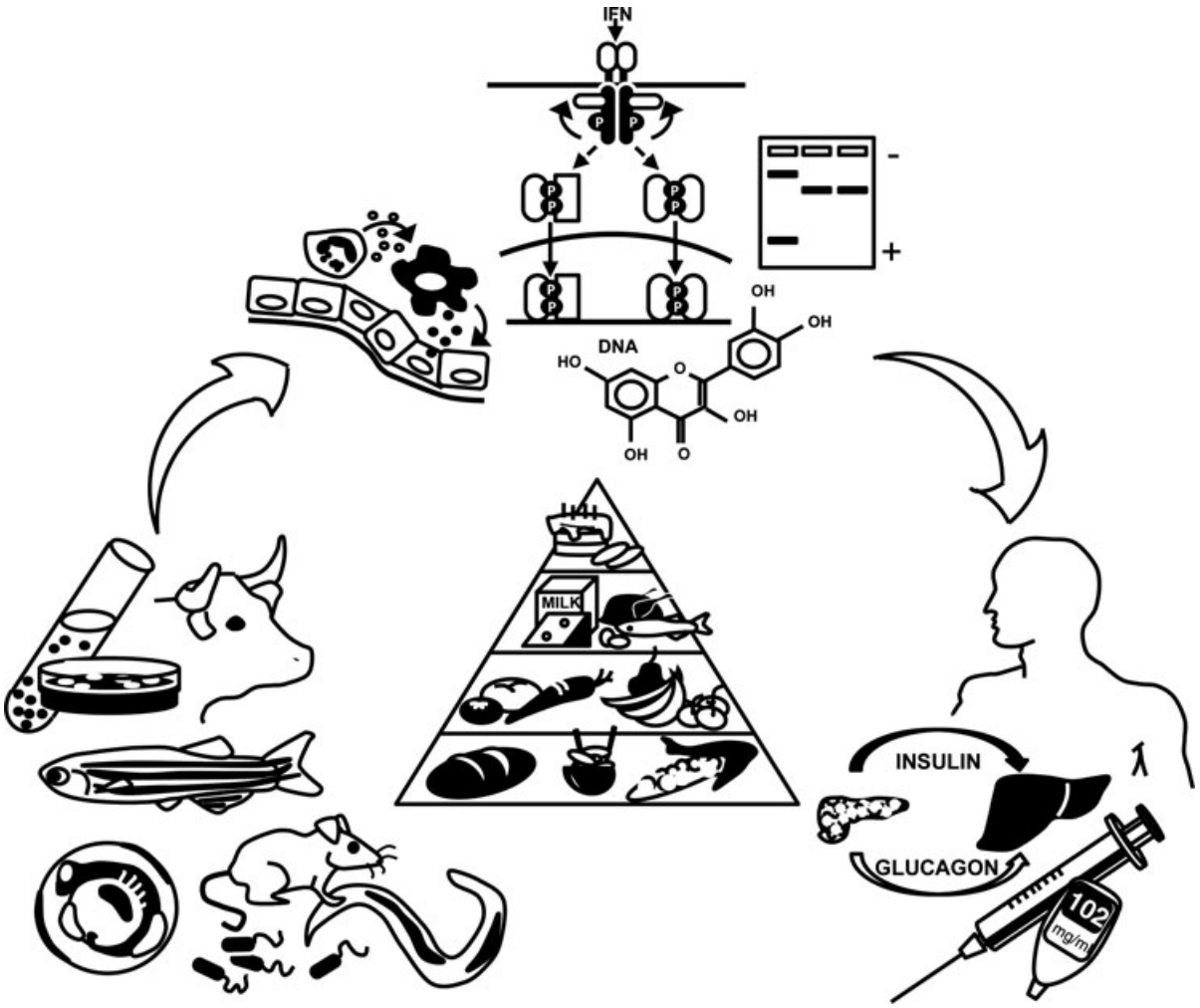

Figure 1. Model organisms in molecular nutrition research. Model organisms like mice, worms, fish, and mammals help in the understanding of mechanisms of actions of food on the organism.

The most prominent model organisms emerged primarily in genetics and have been applied to other research fields, like molecular nutrition research. Here the origin of model organisms in biomedical research, selected model organisms and examples in nutrition research are presented.

2 The history of model organisms

In the latter half of the 19th century, researchers started not only to observe but to experiment on the lives of animals and plants to understand phenomena, making biology an organism-oriented science. A well-known approach to understand phenomena is to use the simplest and most accessible system in which the problem can be addressed, i.e. a model organism. Several models have been employed in the last 150 years ranging from unicellular models like bacteria and yeast to mammalian models like mice [6]. One well-known example of the use of models in order to understand biology is Gregor Mendel's work on inheritance in peas in 1855. Mendel used different varieties of *Pisum sativum* to establish the basic rules of inheritance. Mendel focused on understanding the causes for the observed difference in colours in peas and postulated his inheritance laws. Colour is determined by a single gene, whereas if he had focused his research to study size in plants, as size is dependent on several genes, and therefore not observable by "simple" genetic crosses, he would have not been able to postulate his theory [7]. This example instructs us about both the strengths and the weakness of studying model organisms to gain biological knowledge.

After the advent of classical genetics in 1900 with the rediscovery of Mendel's theory, researcher's attention was diverted from studying inheritance of the underlying mutated genes to study their physiological function. One crucial example to understand gene function and physiological processes or metabolic pathways with model organisms was the isolation of nutritional mutants of the fungi *Neurospora* in 1941 [8]. Beadle and Tatum created several nutritional mutants

by x-ray and demonstrated that nutritional requirements are caused by mutations in genes of metabolic pathways, linking for the first time genes to proteins and function initiating molecular biology as a new research field [9].

Model organisms emerged in three phases [8]. During the first phase, from 1900 to 1937, inheritance was mainly studied in the mouse (*Mus musculus)*, corn (*Zea mays)*, the fruit fly (*Drosophila melanogaster*), and the prokaryote *Paramecium*. From 1938 to 1952, when molecular mechanisms and gene function were main focus of research, microorganisms were clearly the most accessible systems to study these processes. Therefore, microorganisms were introduced and the most prominent were the fungi *Neurospora*, *Aspergillus nidulans*, *Saccharomyces cerevisiae*, the algae *Chlamydomonas*, the bacteria *Escherichia coli,* and the viruses T phages and phage lambda. Due to the success of microbial models in genetics and molecular biology, after 1960, researchers searched for further animal and plant models to understand physiological and biological processes. In this third phase, the worm *Caenorhabditis elegans* and the plant *Arabidopsis thaliana* emerged most prominently, accompanied by the renewed interest in mouse and the fruit fly.

The publication of the human genome in 2000 reflected the so-called "phenotype gap" and caused the progress of functional genomics by developing high-throughput technologies [10]. For most of the genes sequenced, the function is still unknown. Although the emergence of epigenetics overruled the principle one gene ≡ one protein, the generation of genetic mutants is a valuable tool to understand biological processes and disease development. At present, several genetic projects are running using different animal organisms with the aim to create a model organism for every gene disruption or gain-of-function facilitating the understanding of their physiological function and the development of diseases [11–14]. The importance of model organisms is highlighted by the fact the European Commission has allocated € 180 million to the generation of model organisms to model disease states [15]. Similar efforts have been committed in different parts of the world including US and Canada.

3 Features of model organisms

The Krogh Principle states that for any physiological question, there is a species in which the research questions can be best studied [16]. There are millions of species in the world [6]; therefore, it is justifiable to ask whether it is sensible to restrict the model organisms to approximately a dozen. Nevertheless, model organisms have to fulfil minimum requirements to be useful in biomedical research and not all species meet these requirements.

Most model organisms are cost-effective: they have short regeneration times, their maintenance and reproduction in the laboratory is easy and standardised, they have small size and require little space. Moreover, model organisms have been chosen for their different basic biological properties and should reflect the underlying human-derived questions, i.e. have similar pathways and physiological/pathological responses. Most models have been chosen due to their practicability or physiological tractability. A good understanding of their metabolism and biochemistry facilitates data interpretation and extrapolation to humans and therefore their applicability [17].

A very important requirement of a model organism is the availability of powerful tools of traditional and molecular genetics, making it possible to manipulate and study the organism genetically. Manipulating the genomes in organisms has proven as a powerful tool to associate physiological and biochemical functions to genes. Moreover, the generated mutants could serve as model organisms for different disease states. Forward and reverse genetics are powerful tools to generate mutants [18]. In forward genetics, mutant lines are selected by their variant phenotype and the underlying mutated gene has to be identified. These mutants have spontaneously appeared in laboratory strains by breeding or are usually generated by x-ray or chemical mutagenesis. In classical forward genetics, mutations are caused "randomly" and the progeny is screened for a variant phenotype of interest (e.g. increased body weight or high cholesterol levels in plasma) in a high-throughput manner. Forward genetics have proven to be a powerful tool to generate disease models, but the bottleneck is still the identification of the underlying mutation. Reverse genetics is the targeted disruption or mutation of a selected gene followed by the investigation of the physiological and pathological consequences. Reverse genetics are mainly performed by homologous recombination and have demonstrated to be a powerful tool in the elucidation of gene function. Several papers have appeared in the last years, in which the different genetic screens that can be performed in the different model organisms are presented [18–22].

An advantage for a model organism is the availability of its sequenced genome. The comparison of its genome to other species reinforces the use of the model organism by allowing a better prediction of the evolutionary conservation of metabolic processes. Phylogeny is rarely or never a factor in the choice of model organisms. Nevertheless, phylogeny still matters as we study models to extrapolate and our generalisations ability depends on their exemplifying the taxa to which they belong [23].

Last but not least, the use of a model organism is reinforced by a large scientific community behind it, which promotes the interchange of knowledge and tools, such as mutant or bioinformatic resources.

The most popular model organisms meet these criteria; nevertheless, a careful selection of the model organism is still critical to properly resolve the research matter. Ideally, to study a biochemical process or the function of a gene, several model organisms should be investigated to gain robust knowledge. As an example, the peptide transporter family is crucial for amino acid homeostasis in the organism. The discovery that this nutrition-related family of proteins was highly conserved between species, helped to investigate their

features using different model organisms [24]. Yet, using different model organisms can also create confusion in the elucidation of the function of a gene. Disruption of the peptide transporter PEPT1 in mice caused a mild and partially different phenotype than disruption of its ortholog in *C. elegans*, clarifying only partially the role of this peptide transporter in humans [25, 26].

4 Viruses and bacteria

Viruses and bacteria offer the simplest organism to study the basic processes of life. Among viruses, bacteriophages have been extensively applied in molecular biology. They all lack the metabolic machinery and their genomes, generally small, are replicated when the bacteriophages infect the host cells and use their metabolic machinery. Bacteriophages have not contributed explicitly to nutrition research, but have extensively contributed in molecular biology to understand DNA replication, gene expression and recombination.

Bacteria are single-cell living organisms with no nuclear membrane and intracellular compartments. They can be grown and manipulated very easily, have short regeneration times (about 20 min) and a genetically homogenous population of cells (clones) can be obtained fairly easily. The recombination in bacteria by Lederberg paved the way for generations of researchers to devise simple and effective means for the isolation and detailed characterisation of a wide range of mutants. Moreover, bacteria allow the identification of rare mutants among large populations of the order of 10^{10} individuals as phenotype screening is easy and affordable [22].

The gut bacterium *Escherichia coli* is the most common prokaryote model. *E. coli* constitutes 0.1% of the human intestinal flora, has a facultative anaerobic life style, is rod-shaped, ca. 1–2 μm long and 0.1-0.5 μm thick. Bacteria are valuable tools in molecular biology, e.g. for the amplification of foreign DNA in cloning vectors [27]. In nutrition, besides contributing to the basic principles of biology such as in genetics or structural biology, bacteria are important in food technology. Recently, *E. coli* and others have become model organisms for other bacteria to understand how the gut microbiota interacts with the host genome (examples in [28]). This knowledge may help in the understanding of the modulation caused by pro and prebiotics.

5 Yeast

Yeast are the workhorses of cell biology because of the ease of genetic manipulation [19]. Yeast are eukaryote cells with nuclear membrane and cellular compartments. They have a relatively small genome compared to other eukaryote cells and can be easily and rapidly grown in the laboratory with regeneration times of about 90 min. Among yeast, the baker yeast, Saccharomyces c*erevisiae,* has been the most popular experimental system. It was first identified by Louis Pasteur as the catalyst for fermentation.

Yeast have an important role in food technology, like for brewing, and in molecular biology and biochemistry. Biochemical pathways such as glycolysis and fatty acids biosynthesis were first characterised in yeast. *S. cerevisiae* was the first organism to have their genome sequenced (in 1993) and the first to be manipulated through gene targeting by homologous recombination. Yeasts have extensively contributed to our understanding of protein–protein interactions by the so-called two-hybrid technique (Y2H). Y2H is a genetic procedure to analyse the physical interactions of two proteins, elucidating protein–protein interactions [29].

The versatility and tractability of yeast makes them valuable tools to understand genetic networks by combining different genetic and metabolic mutants in systems biology [12]. In (nutritional) systems biology, an important drawback of yeast is the lack of cellular specialisation and their simpler interplay of extracellular signalling processes controlling metabolic adaptation than in multicellular organisms [5]. Nevertheless, they are valuable in understanding basic processes such as the prime metabolic pathways and this modular understanding can be translated to mammalian organisms. In addition, a well-organised yeast research community makes yeast a valuable model organism.

6 Plants

Arabidopsis thaliana was suggested as a plant model in 1943 by Friedrich Laibach [8]. *A. thaliana* is a small flowering plant that can be cultured in large numbers in greenhouses or laboratories. It is characterised by its small size, rapid regeneration time, ability to grow well under optimum conditions, high fecundity and the ease with which mutant lines can be maintained and outcrossed. As it is the smallest known plant genome with five chromosomes and with fewer repetitive sequences than any higher plant, molecular studies are greatly facilitated. Before 1980, only plants economically important as corn were extensively investigated. Later it was recognised that despite the high diversity of plants and their high capacity to adapt to numerous environments, *A. thaliana* is the model system of choice for plant biologists which allows efficient analysis of plant function combining classical genetics with molecular biology [21]. Nevertheless, plants are not usually chosen as model organisms in molecular nutrition research. They have contributed to the understanding of animal development and genome structure and in nutrition plants are sources of food and plant secondary metabolites.

7 Worms

Within worms *Caenorhabditis elegans* is the most popular model organism. *C. elegans* became a very powerful genetic model since the Nobel laureate, Sydney Brenner, published his pioneering genetic screen in 1974, a powerful tool for

the discovery of the molecular basis of the nervous system development [30]. Since then, *C. elegans* has led to seminal discoveries in development, signal transduction, cell death and ageing. Moreover, it was the first multicellular organism to have its genome sequenced and 60% of the genes have orthologs in humans. A further major contribution of worms to biology was the elucidation of the molecular mechanism of RNA interference (RNAi) and the discovery of microRNAs. Moreover, the mechanism behind RNAi, in which double-stranded RNA silences its homologous gene, appears to be universal, occurring in most plants, fungi and animals investigated [31]. RNAi is a powerful tool to generate genetic mutants in *C. elegans*, but as well in *Drosophila melanogaster* and animal and human cells. RNAi has facilitated functional studies in genes by generating loss-of-function phenotypes by depletion of the corresponding transcript.

The success of *C. elegans* as a model organism is mainly due to its simplicity. These worms have an invariant number of cells. Adults are males or hermaphrodites with 1031 and 959 somatic cells, respectively. The lineage of all these cells is precisely known from the fertilised egg to the adult animal. These cells form a nervous system, a digestive tract with a mouth, pharynx and anus, and a reproductive system that produces both sperm and eggs. Worms possess transparent bodies, have a small genome size, a rapid life cycle and are easy to maintain and reproduce in the laboratory. Adults are 1 mm long and 65 μm thick and the laboratory strain is fed by *E. coli*. Worms have a rapid life cycle (3 days) and a total lifespan of 20 days. Another factor that has highly contributed to the success of *C. elegans* as a model organism is the extensive freely shared range of tools which has emerged from a culture of close collaboration between worm researchers. Recently, a review listed all these resources to guide worm researchers and invite newcomers to use this model organism [14, 30].

Only a few examples show the applicability of *C. elegans* in molecular nutrition and biomedical research. The majority of these studies are concerned with understanding the molecular mechanisms of how caloric restriction and insulin signalling mutations increase lifespan. Nutritionists have been reluctant to use this model organism due to insufficient physiological and metabolic knowledge available, the difficulty to collect tissues in biochemical relevant quantities and the difficulty to perform dietary interventions as the worms are fed by *E. coli*. Nevertheless, a compilation of the knowledge on intermediary metabolism in *C. elegans* should help to diminish this reluctance [32]. Worms use carbohydrates, lipids and amino acids in similar ways to mammals. Moreover, some vitamins have been described as essential in worms such as thiamine and riboflavin. Furthermore, knowledge on biochemical processes could be gained easier than in mammals combining several RNAi models, as the complete RNAi library is commercially available. Further efforts within the worm community are focused on defining a chemical medium, in which worms are able to grow (and reproduce) as on agar plates, allowing performance of dietary intervention studies. Although *C. elegans* is a powerful model organism, it has some limitations. The worms do not possess blood and therefore do not have a central distribution for a complex interorgan metabolism. In addition, they do not possess liver or kidneys, although their intestine structure and function makes them a valuable model in nutrition research [32].

8 Flies

The fruit fly has been used for more than 100 years as a model organism, especially in genetics and developmental studies, to study rhythmic behaviour and neurodegeneration. In 1908, Thomas Hunt Morgan and colleagues at Columbia University searched for a small, quickly reproducing animal, useful to study quantitative traits such as eye colour [8]. *Drosophila melanogaster* emerged as the animal of choice. The fruit fly genome was published in 2000 and it was shown that 61% of the human genes implicated in disease had homologs and orthologs in the fly genome [33].

The success of *D. melanogaster* as a model organism is largely due to the power of classical forward genetic screens in this organism to identify genes involved in biological processes. These screens were first successfully performed, to study development, by two Nobel laureates, Christiane Nüsslein-Volhard and Eric Wieschaus. Later, a large number of developmental processes have been shown to be conserved between flies and vertebrates. Other advantages of *D. melanogaster* as a model organism: flies are small (3 mm), easy and cheap to maintain in the laboratory, have a 10-day regeneration time and produce many progenies. Females can produce approximately 800 eggs, which can be injected with DNA to create transgene flies. In addition, like with yeast and worms, a big community of researches is working with flies.

Most of the studies performed with *D. melanogaster* in nutritional research are on caloric restriction and its effects on life-span extension. A recent publication highlights the potential of *D. melanogaster* and encourages the nutrition community to use flies as a model organism in nutrigenomics [34]. Flies have contributed to the understanding of lipid storage by the perilipins genes. Flies have adipose-like tissues and lipid transport systems, making them a closer model to humans than yeasts and worms [35, 36]. *D. melanogaster* has a fat body filled with adipocytes and the metabolic and signalling pathways involved in fat metabolism, adipocyte development and insulin signalling are conserved in humans. Recent efforts applying *omics* technologies are focusing on the understanding of nutrient signalling in flies, which could make them a valuable model organism in molecular nutrition research [37, 38].

9 Fish

Among fish, the zebrafish (*Danio rerio*) is the most popular model organism. Zebrafish are 2.5–5-cm-long freshwater tropical cyprinid fishes native to India. *D. rerio* had been a

popular aquarium fish before George Streisinger introduced it as an animal model in late 1960 [39]. Zebrafish are easy to maintain in the laboratory, show high fecundity and economical husbandry requirements and large populations of zebrafish are generated quickly and cheaply facilitating large-scale forward genetics screens. Classical forward genetics is a powerful tool in this model to generate mutant lines. The zebrafish emerged as a prominent model organism with the publication of one issue of the journal *Development* dedicated exclusively to zebrafish in 1996 (volume 123). This issue included 37 papers reporting the results of a big forward genetic screen in developmental biology in which 4000 embyonic lethal mutants were recovered and characterised.

Zebrafish fills the gap between invertebrates and vertebrates in developmental research. Recently, they have become a useful model organism for disease modelling, mainly in cardiac and haematopoietic diseases. Besides its role in the elucidation of basic biological processes, the understanding of iron metabolism represents one of the few contributions of zebrafish to molecular nutrition research [40].

10 Mice

Mus musculus is probably the most popular model organism used in molecular nutrition and biomedical research. The main advantage in the use of mice is that like humans, they are mammals. Moreover, in comparison to other mammals they are perfectly suited for the laboratory environment and genetic experimentation. The mouse genome was sequenced in 2002 showing that 85% of the human genes have orthologs in mice. Their life cycle is short, 8–9 wk, when compared to other mammals. The advent of genetics in 1900 was marked by the demonstration that the inheritance laws were also valid in mammals using mice as model organism [8]. Lucien Cuénot showed that the inheritance of coat colour in mice was determined by the same laws as in peas.

Since the beginning of last century, several investigators based their work in creating the so-called inbred strains [41]. An inbred strain implies mating siblings for at least 20 generations. These inbred strains have therefore isogenic backgrounds, reducing individual variability that sometimes facilitates drawing conclusions. More than 300 inbred strains in mouse have been generated and are maintained at the Jackson Laboratory in the US. Most inbred strains are more susceptible to develop diseases due to the inbred strategy. This potential can be used for elucidating pathological states. As an example, the popular C57BL/6J mouse strain is susceptible to develop diet-induced obesity and therefore often used in high-fat dietary intervention studies [42]. Moreover, the breeding facilities allowed the identification of naturally occurring mutant lines and their maintenance. In nutrition research, one of the most prominent examples is the *ob/ob* mouse. These mice are extremely obese due to a mutation in leptin, a hormone secreted by the adipose tissue that is responsible for decreasing energy intake and increasing energy expenditure [43]. Although global disruption of genes in the leptin-response pathway rarely cause extreme obesity in humans, the discovery of leptin in mice revealed new aspects of energy metabolism in humans [44, 45].

Mice have become prominent model organisms due to their genetic manipulability. Classical forward genetics screens in mice are as powerful as in flies, fishes and worms, although the space required for these screens is more demanding and therefore not possible in all laboratories. Several laboratories are running big forward genetic screens using N-ethly-N-nitrosurea to generate chemically induced mutations. These laboratories focus on different variant phenotypes affecting behaviour or metabolism among other topics (references in review [18]). Several mutant lines have been successfully established, although in mice the bottleneck of forward genetics is still the identification of the underlying mutation. Nevertheless, a range of mutations have been detected providing valuable disease models to the research community [46]. A valuable example for nutrition research is the model for maple syrup urine disease or for cystinuria type I [47].

The ability to perform reverse genetics by homologous recombination in mouse has catapulted the mouse to the most prominent model organism in biomedical research. In the late 1980, the work of three Nobel laureates (Mario C. Cappechi, Sir Martin J. Evans and Olivier Smithies) allowed the isolation and maintenance of murine pluripotent embryonic stem cells (ES cells), homologous recombination in these cells using a targeting vector and the raise of mice with the targeted locus, generating the first knockout mouse line. Complete loss-of-function can lead to lethal or misleading phenotypes, therefore reverse genetics improved allowing not only complete loss-of-function, but also tissue specific or time-controlled loss- or gain-of-function [48, 49]. All these methodologies have contributed to the better understanding of gene function by using the mouse as a model organism. Currently, the aim of the research community is to develop a conditional and complete loss-of-function and gain-of-function model mouse for every gene [13].

The contribution of the mouse as model organisms in molecular nutrition research is enormous and therefore not possible to be listed in this review. Mice have been invaluable in identifying the mechanisms of food intake and energy regulation such as the pathways involving leptin and agouti. Several monogenic models have contributed to this understanding, like the *agouti* and *ob/ob* mice mentioned before, the *db/db*, *fat/fat* and *tub/tub* mouse [43, 50–52]. Some disparate results between mouse and human have been obtained when studying hormones controlling energy metabolism, which have hindered the development of therapies for obesity and the metabolic syndrome [53]. Nevertheless, mice have played a major role in the understanding of complex relationships between ageing, obesity and increased visceral fat with the metabolic syndrome, insulin resistance and diseases associated with these diseases [54]. Moreover, further studies are focused on using the genetic variability of the different well-established mouse strains to understand obesity and the

metabolic syndrome by performing crosses between these strains. Mice have been suggested for the study of the transgenerational epigenetics on obesity-related traits because of their rapid regeneration and their relative ease of experimental pliability [55]. Moreover, mice have contributed to the study how the intestinal flora can alter the host organism [56], among several other topics.

11 Other rodents

Rattus norvegicus is primarily known as a physiological animal model and has been extensively used in biomedical research. The rat was the first mammalian species to become domesticated for laboratory research in the last half of the 19th century [8]. Similar to the mouse, several rat inbred strains have been generated. Rats are easy to handle and have a suitable size. Rats were chosen as a model organism due to their accessibility to physiological, learning and behavioral parameters, when the research community began to move from other mammals like cats and dogs. The rat was the most widely used organism in biomedical research in the last century, but in the last two decades its popularity decayed due to the limitation to perform reverse genetics in rats, and therefore the mouse became the most prominent mammalian model organism. The rat genome has been available since 2004. In 2008 major efforts of the rat scientific community led to the isolation of ES cells from rats [57], which probably will allow the generation of knockout rats by homologous recombination in the near future. Furthermore, since recently another powerful reverse genetics technique allows gene-targeted deletions or modification in rats by using zinc-finger nucleases [58]. Both findings will probably contribute to a more prominent use of rats as model organism in molecular nutrition research in the near future.

Despite these last two decades where rats were less prominent than other model organisms, rat strains bred in the laboratory have provided valuable model diseases and have contributed to molecular nutrition research. The *fa/fa* rat is one of the monogenic models extensively used in obesity research [59]. The controversial link between ageing and diet is known over 50 years since the discovery in rats that caloric restriction extends lifespan [60]. Moreover, rats have been studied to elucidate the molecular mechanisms behind diabetes or the metabolic syndrome. Further advantages of using rats in molecular nutrition research include the following: rats have been comprehensively biologically characterised and rats show more complex feeding behaviours and food choices and eat a wider variety of foods in different forms than mice.

Cavia porcellu were the first rodents where inbred strains were generated. Guinea pigs were extensively used as animal models in the last two centuries, but this animal model has lost popularity due to its less potent genetic manipulation strategies, longer gestation times and less standardisation and higher costs of husbandry. Nevertheless, several nutritional research relevant studies have been performed with these rodents. Nowadays, the study of diet effects on cholesterol and lipoprotein metabolism seem to be more similar to human in guinea pigs than in mice. In guinea pigs like in humans most cholesterol in plasma is transported as LDL, and they show similar lipoprotein distributions to that of other vertebrates [61]. Moreover, they show similar plasma lipids pattern in response to a diet intervention or drug treatment, and are able to develop atherosclerosis.

Hamsters have been also used as models in nutrition research. These seasonal animals have contributed to understanding body weight regulation in humans [44, 62].

12 Other mammals

In physiological research, dogs and cats have been valuable models until 50 years ago when these models started to fall into disuse due to ethical reasons and suitability for laboratory use. Nevertheless, they have provided important findings to the biomedical community. Dogs for example, contributed to the discovery of the cause of diabetes type I. In early 1920 Frederick Banting and collaborators showed that removing the pancreas of dogs lead to the development of diabetes type I and administrating pancreas extract treated the disease [63].

Some scientists promote the use of farm animals in biomedical research as selective breeding (purifying selection) has led to the accumulation of mutations with no pathological consequences, but with modified gene function [64]. For example, mutants in lean-to-fat mass ratio could elucidate aspects of body composition in humans [65, 66]. Pigs have contributed to the understanding of obesity and metabolic syndrome. Nevertheless, restricted feeding is necessary in diet intervention studies with pigs as they become markedly obese when ad libitum access of food is given [67]. Cows have contributed to the understanding of milk fat synthesis revealing its biochemistry [68]. As the lactating cow synthesizes easily measurable quantities of lipid, they could contribute to understanding the acute and chronic regulation of milk fat synthesis.

Due to economical, husbandry and genetic amenability, rodents will remain the animal model of choice in molecular nutrition research. Nevertheless, specific questions, like homeostatic and hedonistic signalling mechanisms in the brain that regulate food intake and energy balance, can only be answered using different animal models.

13 *Homo sapiens*

Some areas of molecular nutrition research require work in humans, like the final stages of functional food testing or aroma and taste perception. In these cases, work with humans is necessary and irreplaceable.

Another approach in biomedical research is the use of mammalian (human) cell culture as a model organism. This methodology has emerged as a suitable screening system to

elucidate gene function [69]. Mammalian cell cultures have been maintained in the laboratory for 40 years after the formulation of an adequate cell culture media for the maintenance and growth of usually transformed cells. In the last years, major efforts have been focused in the maintenance of primary isolated cell cultures. These cells provide a reductionist view of cells in a two-dimensional instead of three-dimensional environment. Despite its limitations, this model provides advantages in high-through put screens for example to test compounds (nutraceuticals) for activity and translate the findings to animal models our humans. Yeast are a good eukaryotic model but some biological processes are not present, like apoptosis, oncogenic transformation, defined nutrient transporters or receptors, making mammalian cell culture an adequate model.

14 Considerations when employing model organisms

The most significant problem that investigators face when using model organisms, is the question of the applicability of the results to humans. The underlying assumption in their use is that the basic aspects of metabolism and physiology are the same. Choosing the appropriate animal model is one of the most important steps when planning an experiment, in order to be able to translate the information to humans avoiding misinterpretations [70]. There are examples where differences in metabolism or physiology have led to different results between species. As an example, lung and pancreatic anatomy between humans and mice are divergent and probably the reason why mouse cystic fibrosis models lack pathological lesions [71]. Therefore, it is necessary to choose the appropriate model that will deliver most translational information.

Most animal experiments require the approval of their experimental designs by a local ethics committee. Governments have considered the importance of regulating animal experiments by controlling the pain and distress caused to the animals. The laws are different between countries, but most are based on the three R's principle postulated by Russel and Burch [72]. The three R's, reduction, refinement and replacement, should be considered when working with model organisms. Reduction does not imply using fewer animals for an experiment, but using the adequate number for any experiment. Refinement is the process of eliminating and/or minimising any pain or distress in a protocol. And replacement involves, when practicable, the replacement of the animal model with in vitro methodology or a non-animal model.

15 Modelling in molecular nutrition research

Finally, in nutrition and biomedicine research models are not only uni or multicellular but also mathematical. In nutritional systems biology, bioinformatics and especially mathematical tools allow the modelling of nutrition-related processes [5]. These modelling approaches usually rely on mechanistic data collected at different time points in different model organisms or in the same organism subjected to different perturbations. Usually, differential equations are generated that explain a biological process or mechanistic pathway. 'Omics' technologies performed in the last years will probably provide valuable data for these approaches, as currently the datasets are scarce. For most nutritional components, the mechanisms about how they affect body function is unknown and even less information has been collected how they can modulate the conversion from health-to-disease status and vice versa. Despite the scarceness of data, few examples already demonstrate the usefulness of mathematical modelling in nutrition research. Thus, folate and copper metabolism have been modelled allowing the reassessment of known biochemical responses as well as the estimation of how perturbations affect gene expression, protein synthesis or metabolite concentrations [73, 74]. Nevertheless, not only mechanistic data are necessary to model nutritional phenomena. Mathematical modelling of macronutrient metabolism has proven to predict fairly accurately energy requirements [75]. In this case, differential equations considering macronutrient intake and metabolism could predict body weight outcomes.

16 Conclusion

Organisms are clearly much more than the sum of their parts and the behaviour of complex physiological and pathological processes triggered by food components cannot be understood simply by knowing how the different parts work in isolation. Therefore, model organisms where general principles of functions can be established due to practicability and ethical reasons have demonstrated to be a powerful tool in molecular nutrition and other biomedical sciences. Most model organisms have become prominent because of their power in genetics and developmental biology and less because of their physiological tractability. Nevertheless, most nutrition researchers have focused on studying mammalian models, and probably the utilisation of other prominent organism like worms, flies and fish could facilitate formulate hypotheses in molecular nutrition and nutritional systems biology research. Finally, these hypotheses have to be validated in humans with e.g. nutritional intervention studies.

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17 References

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