IARC HANDBOOKS

ABSENCE OF EXCESS BODY FATNESS

VOLUME 16

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Cancer-Preventive Interventions, which met in Lyon, 5–12 April 2016

LYON, FRANCE - 2018

IARC HANDBOOKS OF CANCER PREVENTION

International Agency for Research on Cancer

World Health Organization

3. CANCER-PREVENTIVE EFFECTS IN EXPERIMENTAL ANIMALS

3.1 Methodological considerations

3.1.1 Definition of dietary/calorie restriction

Dietary/calorie restriction involves altering food intake qualitatively or quantitatively to control body weight gain and presumably maintain body composition. Several terms have been used to describe the different methodological approaches in which amount, nutrient, calorie, or energy intake is restricted or modulated to control body weight (Thompson et al., 2002, 2003; Thompson, 2015). These terms are not synonyms, and it is important to be aware of the methodological distinctions between the approaches when designing experiments.

Dietary restriction protocols refer to feeding a reduced amount of a complete diet, such that less total nutrients and dietary factors are ingested. If excessive, this approach can lead to an intake of micronutrients and/or macrocomponents that is incompatible with optimal health and survival. In early studies, this was the most commonly used approach to study cancer prevention. These studies have provided valuable insights, but the results should be interpreted with caution, because this approach does not allow the detection of effects that are specifically due to dietary modulation. Terms used to describe this approach include dietary restriction, food restriction, and total dietary restriction.

In energy restriction protocols, diets are formulated so that animals fed different amounts of calories still receive the same levels of other nutrients, such that the only variable is energy intake (Thompson et al., 2002, 2003; Thompson, 2015). In other words, there is selective reduction in energy intake (nutrient density) while feeding the same level of micronutrients as those of the control group. Nutrient density is altered in a manner that facilitates investigation of the effects of energy restriction without changing the amounts of nutrients and other dietary factors. These studies may provide information most relevant to understanding the mechanisms involved. Terms used to describe this approach include calorie restriction (CR), caloric restriction, dietary energy restriction, and energy restriction. In this section, the term dietary restriction (DR) will be used to include both dietary and calorie restriction.

3.1.2 Design issues in studies of dietary restriction

DR has been one of the most widely used methods for studying cancer prevention in experimental models. In DR protocols, control and carcinogen-treated animals are diet-restricted by amounts that result in lower body weights relative to those of controls fed ad libitum (AL), without weight loss. Also, diet-restricted animals

generally survive longer (Maeda et al., 1985; Yu et al., 1985).

However, inherent within DR protocols are factors that could confound the assessment of tumorigenesis. An important factor in carcinogenesis studies is that the sensitivity of bioassays to detect xenobiotics-induced carcinogenic responses may be altered by DR. A chemical that is observed to be toxic or carcinogenic in animals fed AL might not produce the same effects in diet-restricted or otherwise leaner animals, i.e. carcinogenic activity might be underestimated in the diet-restricted animals. In addition, DR may induce a variety of pleiotropic responses that affect metabolism, distribution, and disposition of xenobiotics. Furthermore, because diet-restricted animals live longer than those fed AL, evaluations performed at age 2 years result in comparisons at disproportionate times in the respective lifespans (NTP, 1997).

(a) Experimental systems

Animal models have been used to study how DR modulates the development and/or progression of cancer in humans. Typically, animals are fed diets that result in 20–40% reduction in daily food/calorie intake relative to controls fed AL while maintaining adequate nutrition (Klurfeld et al., 1989a; Kritchevsky, 1999; Hursting et al., 2010, 2013). The animals have lower body weight, proportionately smaller skeletal size, lower percentage of total body fat, and decreased weight of most internal organs (with the exception of the brain and testes) compared with animals fed AL, indicating that malnutrition is not involved (Keenan et al., 2013). DR models are designed to directly parallel and model conditions in humans. The range of DR controls body weight and is intended to resemble the range of energy balances that occur in healthy people.

(b) Selection of model

Characteristics that should be considered when choosing a model to study cancer in humans include the similarity of tumour morphology and biological traits (e.g. hormonal responsiveness) to those observed in humans. Such models are available for many organ sites. For example, in rat models for breast cancer, not only are the tumours in the rat morphologically similar to tumours in humans, but the majority are ovarian steroid-responsive. *N*-nitrosobis(2-oxopropyl) amine (BOP)-induced ductular pancreatic cancer in the Syrian hamster is a model that has been useful in studying aspects of pancreatic cancer in humans (Pour et al., 1993), whereas other carcinogen-induced pancreatic tumours are acinar cell carcinomas, which are not a common lesion in humans. There is a variety of animal models for cancers of the mammary gland, colon, liver, prostate, pancreas, skin, and pituitary gland, and cancers of the haematopoietic system, including lymphoma and leukaemia. In earlier studies, spontaneous tumour models were used. Increasingly in recent decades, researchers have used transgenic mice to model cancer in humans and to determine whether dietary interventions can prevent cancer development driven by genes known to be mutated in human cancer (Hursting et al., 2011). These models are described in the subsequent sections.

(c) Selection of intervention protocol

When a chemical carcinogen is used in DR models, the timing of carcinogen administration and dietary intervention needs to be taken into consideration. The intervention should generally be separate from the administration of the chemical carcinogen. However, it is important to assess the impact of the intervention both on the cancer induction phase (before and at the time of treatment with the carcinogen) and on the promotion/progression (development) of the cancer (after treatment with the carcinogen).

Models involving a short induction phase provide the ability to assess the impact of diet on early or late stages of promotion. If tumours are allowed to develop before the intervention, it is possible to assess the impact of the intervention on the regression or progression of the lesions. When spontaneous or genetically modified models are used, the dietary intervention can be applied at different ages, depending on the individual model (Thompson et al., 2002; Everitt & Alder, 2013).

(d) Selection of diet

Laboratory rodents can be fed three types of diets: (i) non-purified, (ii) purified or semi-purified, and (iii) chemically defined (Everitt & Alder, 2013; Lipman & Leary, 2015). Purified diets are the most widely used in cancer-related studies in rodents. These diets should meet and label the minimum requirements for protein and fat and maximum levels of fibre and ash; however, the percentages of the various macronutrients can vary. Purified diets (previously known as semi-synthetic or semi-purified diets) are formulated using refined ingredients, including sugars, proteins, carbohydrates, and fats, with added mineral and vitamin mixtures.

Non-purified and purified diets have been used in studies of dietary impact on tumorigenesis. In these types of studies, it is very important that the diet of both the intervention and the control group be adequate in all nutrients. Non-purified diets have the advantage that they are formulated using whole food ingredients. Purified diets have the advantage that each component can be changed independently of other constituents in a highly controlled fashion.

In most rodent models, DR administered throughout life appears to be more effective in controlling body weight than regimens started in adult animals (Ross & Bras, 1971; Hursting et al., 2010, 2013). There are three commonly used animal DR regimens that model approaches to and patterns of body weight regulation in adult humans. In the first regimen, animals are fed a restricted amount of energy (i.e. energy restriction to various extents) and continue to gain weight, but at a slower rate than animals fed AL. This DR does not usually cause weight loss, because restriction is started shortly after weaning. In the second regimen, when DR is started in older animals, restriction initially causes weight loss, but then diet-restricted animals maintain a body weight that is lower than that of animals that consume food AL. The third regimen mimics a cyclic pattern of dieting. Animals are subjected to intermittent and repetitive periods of DR or total fasting that result in alternating patterns of weight loss and weight gain (Thompson et al., 2002; Cleary & Grossmann, 2011).

3.2 Overview of the effects of excess body weight

3.2.1 Obesity models

The use of rodent models to study excess body weight and associated diseases in humans has steadily increased (Kanasaki & Koya, 2011). These models have several modulating factors that include species, strain, age of animals, type of diet, level of fat, and type of control diet; inflammation, metabolic status, and endocrine status may be associated confounding factors. Ray & Cleary (2013) and Cleary (2013) have published comprehensive reviews on the use of such animal models.

Most mouse models used to study obesity and cancer are genetically manipulated (transgenic) animals: animals are either genetically modified to induce carcinogenicity and fed a modified diet to induce obesity, or genetically modified to induce obesity and administered chemicals to induce cancer. There are several genetic mutations that result in obesity. One common disturbance is in the function of leptin, a critical anorexigenic adipokine that conveys information about adipose status; leptin levels

in serum/plasma are elevated in proportion to adipose tissue mass. Genetically obese mice include A^{vy} yellow obese mice, leptin-deficient C57BL/6L-*Lep*^{ob}*Lep*^{ob} (originally termed *ob/ob*) mice, and leptin receptor-deficient *Lepr*^{db}*Lepr*^{db} (originally termed *db/db*) mice. *Lep*^{ob}*Lep*^{ob} mice are homozygous recessive and do not produce leptin (Zhang et al., 1994), whereas *Lepr*^{db}*Lepr*^{db} mice have a defect in the leptin receptor (OB-R) and manifest high circulating levels of leptin (Frederich et al., 1995). Both strains are obese at a young age, and develop hyperinsulinaemia and insulin resistance. The A^{vy} yellow obese mouse has mutations in the *agouti* gene that cause ubiquitous expression of the agouti protein, which results in appetite stimulation, leading to hyperinsulinaemia (Wolff et al., 1999).

Genetically obese rat strains, such as the Zucker and Corpulent strains that have leptin receptor defects, have also been used in cancer studies. The Zucker "fatty" rat carries a mutation on the *Lepr* gene for obesity, which is inherited as a Mendelian recessive trait and leads to extreme, early-onset obesity by age 3 weeks (Zucker & Zucker, 1961).

The diet-induced obesity (DIO) model is also used to study the interplay between cancer and increased body weight in mice and rats. Although this model is generally considered to be closest to the development of obesity in humans, the utility of the model is limited by the fact that humans do not normally consume extremely high quantities of fat in their diets. In DIO studies, the fat content of the diets is increased from 10% of total calories to 30–60% of total calories. High-fat diets (HFDs) also contain higher calorie density than control or standard rodent chow, and therefore are in fact high-fat, high-calorie diets. Although the response is species- and strain-dependent, the animals gain weight rapidly and develop other health complications, such as glucose intolerance and insulin resistance or diabetes. This response may be species- and strain-specific, because not all species gain weight when placed on a HFD.

Other methods for inducing obesity in rodents include surgical removal of the ovaries to induce weight gain and a postmenopausal-like state (Nkhata et al., 2009), or damaging the hypothalamus by injection of gold thioglucose (GTG), which results in overeating, rapid weight gain, and subsequent obesity (Bergen et al., 1998). These models have been used to study mammary tumorigenesis (see Section 3.2.2).

3.2.2 Cancer of the mammary gland

See Table 3.1.

Various rodent models have been used to assess the association between obesity and cancer of the mammary gland, including genetic, diet-induced, and GTG-induced obesity models. The A^{vy} yellow obese mouse model was one of the first genetic obesity models used to study both spontaneous and chemically induced cancer of the mammary gland. In an early study, the time to tumour detection (latency period) for spontaneous mammary tumours initiated by the mouse mammary tumour virus (MMTV) was determined in breeding and virgin A^{vy} mice. The incidence of spontaneous mammary tumours was 96–100% by 8 months in virgin obese mice, whereas it reached 100% at 15 months in virgin lean mice (Heston & Vlahakis, 1961, 1962). The difference disappeared in the breeding mice, with approximately 100% incidence at 8 months in both lean and obese mice. In another study using A^{vy} mice, the time course of appearance of hyperplastic alveolar nodules and mammary tumours was determined in virgin "viable yellow" (A^{vy}/A) [obese] and non-yellow (A/a) (C3H/HeNIcrWf × VY/Wf)F_1 [lean] female mice. Hyperplastic alveolar mammary nodules occurred by age 16 weeks in virgin yellow obese mice compared with age 19 weeks in their non-yellow lean counterparts. In addition, the incidence of hyperplastic alveolar nodules was increased among yellow obese females compared with non-yellow lean females by age 36 weeks. Mammary adenocarcinomas

Table 3.1 Effect of obesity on the development of mammary tumours in mice and rats

Species	Obesity model	Cancer etiology	Results (obese vs lean animals)	Reference
Mouse	A^{vy} yellow obese	MMTV	Shortened latency until 100% incidence of tumours	Heston & Vlahakis (1961, 1962)
Mouse	A^{vy} yellow obese	Spontaneous	Shortened latency for hyperplastic alveolar nodules and adenocarcinomas	Wolff et al. (1979)
Mouse	A^{vy} yellow obese	DMBA	Shortened latency and increased incidence of tumours	Wolff et al. (1982)
Mouse	$Lep^{ob}Lep^{ob}$	Spontaneous	Shortened latency but decreased incidence of tumours	Heston & Vlahakis (1962)
Mouse	$Lep^{ob}Lep^{ob}$	Transgenic MMTV-TGF-α	No tumours; increased incidence of tumours in wild-type and heterozygous mice (see text)	Cleary et al. (2004c)
Rat	Zucker rat	MNU	No effect on latency of tumours; decreased incidence of carcinomas	Lee et al. (2001)
Rat	Zucker *fa/fa* rat; LA/Ncp corpulent rat	DMBA	Shortened latency and increased incidence of tumours	Klurfeld et al. (1991); Hakkak et al. (2005)
Rat	Zucker rat, ovariectomized	DMBA	Shortened latency and increased incidence of tumours (no tumours in lean animals)	Hakkak et al. (2007)
Mouse	GTG-induced	Spontaneous (C3H)	Shortened latency until 50% incidence of tumours	Waxler et al. (1953)
Mouse	GTG-induced	Implantation of T47-D human breast cancer cells	Increased incidence of tumours	Nkhata et al. (2009)
Mouse	Diet-induced (33% fat diet; mice divided into groups based on weight gain)	Transgenic MMTV-TGF-α (C57BL/6)	Shortened latency and increased incidence of palpable tumours; some high-grade adenocarcinomas	Cleary et al. (2004a); Dogan et al. (2007)
Mouse	Diet-induced (33% fat diet; mice divided into groups based on weight gain)	Transgenic MMTV-neu (FVB/N)	No effect on latency or incidence of tumours; earlier onset of second tumours, increased multiplicity	Cleary et al. (2004b); Khalid et al. (2010)
Mouse	Diet-induced (5.2 kcal/g or 3.8 kcal/g)	Implantation of mammary tumour cells from Wnt-1 transgenic mice	Significantly increased tumour volume and growth rate	Nuñez et al. (2008)
Mouse	Diet-induced, ovariectomized	Implantation of mammary tumour cells from Wnt-1 transgenic mice	Increased tumour volume	Rossi et al. (2016)
Rat	Obesity-prone Sprague-Dawley	MNU	Shortened latency; increased tumour incidence and tumour weight	Matthews et al. (2014)

DMBA, 7,12-dimethylbenz[*a*]anthracene; GTG, gold thioglucose; MMTV, mouse mammary tumour virus; MNU, *N*-methyl-*N*-nitrosourea; TGF-α, transforming growth factor alpha.

were first observed at an earlier age in yellow obese mice than in non-yellow lean mice (Wolff et al., 1979).

In a study of chemically induced mammary tumours, obese A^{vy} (BALB/c) mice aged 8 weeks treated with 1.5 mg/kg of 7,12-dimethylbenz[*a*] anthracene (DMBA) weekly for 2 weeks or with 6.0 mg/kg of DMBA weekly for 6 weeks had higher incidences of mammary tumours compared with the lean mice at both doses. In addition, the latency period was shorter for the A^{vy} mice than for the lean mice at both doses of DMBA (Wolff et al., 1982).

The $Lep^{ob}Lep^{ob}$ mouse is another model that has been used to study obesity and cancer. In an early study, obese $Lep^{ob}Lep^{ob}$ female mice had decreased incidences of spontaneous mammary tumours compared with lean mice; however, tumours were first detected at an earlier age, 10.7 months for obese mice versus 17.6 months for lean mice (Heston & Vlahakis, 1962).

In another study, obese $Lep^{ob}Lep^{ob}$ female mice crossed with transgenic mice overexpressing human transforming growth factor alpha (MMTV-TGF-α) were used as a model for postmenopausal mammary tumorigenesis. The MMTV-TGF-α strain develops 30% incidence of mammary tumours by age 16 weeks and is useful in assessing tumour incidence and latency. MMTV-TGF-α/$Lep^{ob}Lep^{ob}$ mice did not develop mammary tumours by age 2 years. However, the incidence was 50% for wild-type mice and 67% for heterozygous mice (Cleary et al., 2004c). Similar results were obtained with the leptin receptor-deficient ($Lepr^{db}Lepr^{db}$) model (Cleary et al., 2004d). [The lack of development of mammary tumours in these two genetically obese mouse strains has been attributed to problems with basic mammary gland development as well as the now-known involvement of leptin signalling in tumorigenesis.]

The Zucker rat is another genetic obesity model that has been used to study mammary tumours. Zucker rats developed fewer mammary tumours when treated with *N*-methyl-*N*-nitrosourea (MNU) compared with lean rats (Lee et al., 2001). However, two strains of genetically obese rats, the LA/Ncp Corpulent rat and the Zucker *fa/fa* rat, administered DMBA had higher incidences and significantly shorter latency of mammary tumours by approximately 112 days and 150 days after administration, respectively, compared with lean rats. Whereas multiplicity and tumour size were increased in Corpulent rats, such effects were not observed in Zucker *fa/fa* rats (Klurfeld et al., 1991; Hakkak et al., 2005).

Lean and obese ovariectomized Zucker rats were treated with DMBA at 50 days and killed at 135 days after DMBA treatment. Obese rats had higher incidences and shorter latency of mammary tumours compared with lean rats, which did not develop any mammary tumours (Hakkak et al., 2007).

Chemically induced obesity has been another approach to study increased body weight and cancer of the mammary gland. Injection of GTG to destroy the hypothalamus results in overeating and obesity (Bergen et al., 1998). Obese C3H mice injected with 10 mg of GTG at age 2–3 months to induce obesity developed 50% incidence of spontaneous mammary tumours by 295 days, whereas the incidence was only 19% in lean control mice (Waxler et al., 1953). In another study, ovariectomized mice aged 6 weeks were treated with GTG, followed 4 weeks later by implantation of the T47-D human breast cancer estrogen-positive cell line with and without estrogen implants. When assessed at 30 weeks, GTG-obese mice without estrogen implants had 100% tumour incidence, compared with 50% for GTG-lean controls, 20% for lean vehicle controls, and 0% for GTG-obese mice with estrogen implants (Nkhata et al., 2009).

HFDs have also been used to investigate mammary tumour development in rats and mice (Cleary, 2013; Ray & Cleary, 2013). In one approach, tumour-prone MMTV-TGF-α transgenic C57BL/6 mice aged 10 weeks were fed the

same HFDs and then divided into groups based on whether they gained weight (obesity-prone) or did not gain weight (obesity-resistant). All groups had incidences of mammary tumours between 72% and 82%; however, obesity-prone mice developed mammary tumours at an earlier age than obesity-resistant or control (low-fat) mice. In addition, some obesity-prone mice developed a more malignant variant of mammary adenocarcinoma (Cleary et al., 2004a; Dogan et al., 2007). In MMTV-neu mice, tumour latency was similar in mice fed a HFD compared with a low-fat diet (LFD) (Cleary et al., 2004b; Khalid et al., 2010); however, twice as many HFD mice developed a second tumour, compared with LFD mice (Khalid et al., 2010). In another study, C57BL/6 mice were fed either a high-calorie diet with 5.2 kcal/g (obese) or 3.8 kcal/g (overweight) or a 30% CR diet (lean). The mice were inoculated with mammary tumour cells from Wnt-1 transgenic mice. Tumour volume and growth rate were higher in obese mice and overweight mice than in lean animals (Nuñez et al., 2008).

Ovariectomized female C57BL/6 mice were fed a control diet or a DIO regimen, resulting in a normal weight or an obese phenotype, respectively. At week 24, mice were injected with MMTV-Wnt-1 mouse mammary tumour cells. At 36 months, mean tumour volumes were higher in DIO mice than in control animals (Rossi et al., 2016).

Matthews et al. (2014) used a somewhat different approach, with administration of MNU. Sprague-Dawley rats that had been bred to be obesity-resistant or obesity-prone were fed a moderately HFD from age 20 days and followed up for development of mammary tumours. Tumour incidence was significantly higher in the obesity-prone rats (91.1%) than in the obesity-resistant rats (65.1%). In addition, tumour weight was increased and latency was shortened in obesity-prone rats, compared with obesity-resistant rats.

3.2.3 Cancer of the colon

See Table 3.2.

Elevated body weight and obesity in association with cancers of the colon and intestine have been investigated in several transgenic and DIO rodent models. Genetically obese *Lep*^{ob}*Lep*^{ob} and *Lepr*^{db}*Lepr*^{db} mice treated with azoxymethane (AOM) or MNU to induce cancer of the colon had increases in the multiplicity of pre-neoplastic aberrant crypt foci in the colon compared with control lean mice (Hirose et al., 2004; Hayashi et al., 2007; Bobe et al., 2008; Ealey et al., 2008). Similar effects were observed in studies with obese gastrin gene knockout (GAS-KO) mice (Cowey et al., 2005), KK-*A*^{y} mice (derived from *A*^{vy} yellow obese mice) (Teraoka et al., 2011), and Zucker rats (Raju & Bird, 2003) administered AOM. In studies with Zucker obese (*fa/fa*) and lean (*Fa/Fa*) rats, tumours of the colon induced by administration of AOM or MNU were observed in the obese rats, whereas none occurred in the lean rats (Weber et al., 2000; Lee et al., 2001; Ray & Cleary, 2013).

Mice harbouring mutations in the adenomatous polyposis coli (*Apc*) gene develop tumours of the intestine and colorectum and have also been used to study the association between obesity and colorectal cancer. When *Apc*$^{1638N/+}$ mice were crossed with genetically obese *Lepr*^{db} mice, the resulting obese mice developed increased numbers of colon adenomas by age 6 months, compared with non-obese *Apc* mice, which did not develop tumours (Gravaghi et al., 2008).

In male and female C57BL/6 mice fed a high-calorie diet followed by subcutaneous injection of the MC38 colon carcinoma cell line, obese mice had increased numbers of palpable tumours and significantly higher average tumour size compared with non-obese mice (Yakar et al., 2006; Algire et al., 2010).

Table 3.2 Effect of obesity on the development of colon tumours and pre-neoplastic lesions in mice and rats

Species	Obesity model	Cancer etiology	Results (obese vs lean animals)	Reference
Mouse	*Lep*^{ob}*Lep*^{ob}	AOM	Increased multiplicity of ACF	Hirose et al. (2004); Hayashi et al. (2007); Bobe et al. (2008); Ealey et al. (2008)
Mouse	*Lepr*^{db}*Lepr*^{db}	AOM	Increased multiplicity of ACF	Hirose et al. (2004); Hayashi et al. (2007); Ealey et al. (2008)
Mouse	*Lepr*^{db}*Lepr*^{db}	MNU	Increased multiplicity of ACF	Ealey et al. (2008)
Mouse	Gastrin gene knockout (GAS-KO)	AOM	Increased multiplicity of ACF	Cowey et al. (2005)
Mouse	KK-*A*^{y}	AOM	Increased multiplicity of ACF at age 13 wk; increased tumour incidence at age 19 wk	Teraoka et al. (2011)
Mouse	*Lepr*^{db}*Lepr*^{db}	*Apc*$^{1638N/-}$	Increased incidence of colon tumours at age 6 mo (no tumours in lean animals)	Gravaghi et al. (2008)
Rat	Zucker	AOM	Increased multiplicity of ACF	Raju & Bird (2003)
Rat	Zucker (*fa/fa*)	AOM	Increased multiplicity of ACF and incidence of colon tumours	Weber et al. (2000)
Rat	Zucker (*fa/fa*)	MNU	Increased incidence of colon tumours	Lee et al. (2001)
Mouse	Diet-induced	s.c. injection of MC38 colon carcinoma cell line	Increased number and size of colon tumours	Yakar et al. (2006); Algire et al. (2010)

ACF, aberrant crypt foci; AOM, azoxymethane; mo, month or months; MNU, *N*-methyl-*N*-nitrosourea; s.c., subcutaneous; wk, week or weeks.
Adapted from Ray & Cleary (2013) by permission from Springer Nature, © 2013.

3.2.4 Cancer of the liver

See Table 3.3.

Several genetically modified animal models have been used to study the link between obesity and cancer of the liver. For example, genetically obese yellow *agouti* (*A*^{vy}) mice and *Lep*^{ob}*Lep*^{ob} mice had increased incidences of liver tumours, which also developed at a younger age in obese mice compared with lean mice (Heston & Vlahakis, 1961, 1962).

The genetically obese *Lep*^{ob}*Lep*^{ob} mouse is often used as an animal model for non-alcoholic fatty liver disease in humans. These mice have increased incidences of hepatocellular carcinoma (HCC) and of focal hepatocyte hyperplasia (considered to be a pre-neoplastic lesion) at an earlier age compared with lean littermates (Yang et al., 2001). The fatty liver Shionogi (FLS)-*Lep*^{ob}/*Lep*^{ob} mouse is a congenic obese strain that develops spontaneous hepatocellular adenomas and carcinomas at a younger age and a higher incidence than either parental strain (FLS and *Lep*^{ob}/*Lep*^{ob}) (Soga et al., 2010).

The outbred obese, diabetic, male Swiss-Webster mouse is another model for studying the link between obesity and cancer of the liver. These mice are polyuric, polydipsic, glucosuric, and hyperglycaemic. Compared with their lean counterparts, they develop a high incidence of late-onset HCC (Lemke et al., 2008). Strain–diet interactions are another important consideration for genetically controlled, diet-induced HCC. For example, male C57BL/6J mice made obese by feeding a HFD developed HCC, compared with none in mice fed a LFD; in contrast, a HFD had little effect on A/J mice similarly treated (Hill-Baskin et al., 2009).

Table 3.3 Effect of obesity on the development of liver tumours and pre-neoplastic lesions in mice and rats

Species	Obesity model	Cancer etiology	Results (obese vs lean animals)	Reference
Mouse	*A*^{vy}	Spontaneous	Shortened latency and increased incidence of tumours	Heston & Vlahakis (1961)
Mouse	*Lep*^{ob}*Lep*^{ob}	Spontaneous	Shortened latency and increased incidence of tumours	Heston & Vlahakis (1962)
Mouse	*Lep*^{ob}*Lep*^{ob}	Spontaneous	Shortened latency and increased incidence of tumours and of focal hepatocyte hyperplasia	Yang et al. (2001)
Mouse	*Lep*^{ob}*Lep*^{ob} crossed with fatty liver Shionogi (FLS)	Spontaneous	Increased incidence of hepatocellular adenoma and carcinoma at age 12 mo	Soga et al. (2010)
Rat	Obese, diabetic Swiss-Webster	Spontaneous	Increased incidence of late-onset hepatocellular carcinoma (male mice only)	Lemke et al. (2008)
Mouse	Diet-induced C57BL/6 (58% fat)	Spontaneous	Increased incidence of hepatocellular carcinoma (none in lean mice)	Hill-Baskin et al. (2009)
Mouse	Diet-induced A/J (58% fat)	Spontaneous	No effect	Hill-Baskin et al. (2009)
Mouse	*Lep*^{ob}*Lep*^{ob}	DEN	Increased incidence and number and size of tumours	Park et al. (2010)
Mouse	Diet-induced C57BL/6 (58% fat)	DEN	Increased incidence and number and size of tumours	Park et al. (2010)
Mouse	Diet-induced *IL6*$^{-/-}$; *TNFR1*$^{-/-}$ (59% fat)	DEN + phenobarbital promotion	Induction of hepatocellular carcinoma without phenobarbital promotion in mice fed high-fat diet but not in mice fed low-fat diet	Park et al. (2010)

DEN, diethylnitrosamine; mo, month or months.
Adapted from Ray & Cleary (2013) by permission from Springer Nature, © 2013.

Carcinogen-induced protocols involve induction of hepatocellular tumours by administration of diethylnitrosamine (DEN). After administration of DEN, DIO C57BL6 mice and genetically obese *Lep*^{ob}*Lep*^{ob} mice had increased incidence, numbers, and size of hepatocellular tumours, compared with lean mice fed a LFD. When IL-6-deficient (*IL6*$^{-/-}$) and tumour necrosis factor receptor-deficient (*TNFR1*$^{-/-}$) mice were fed either a LFD or a HFD and treated with DEN, the majority of the mice fed the HFD developed HCC without phenobarbital promotion. In contrast, DEN-treated mice fed the LFD did not develop HCC unless treated with phenobarbital (Park et al., 2010).

3.2.5 Cancer of the prostate

See Table 3.4.

There has been a lack of suitable experimental animal models that mimic the development and progression of cancer of the prostate in humans. Therefore, few published studies have reported the long-term effects of a HFD and/or obesity on the development of prostate cancer. In recent years, several genetically engineered mouse models of prostate cancer have been developed (Parisotto & Metzger, 2013). The model that is most commonly used is the transgenic adenocarcinoma of the mouse prostate (TRAMP) mouse model. When maintained on a C57BL/6 genetic background, male TRAMP mice develop prostatic intraepithelial neoplasia (PIN) (pre-neoplastic lesions) in all lobes between age

Table 3.4 Effect of obesity on the development of prostate tumours and pre-neoplastic lesions in mice

Obesity model	Cancer etiology	Results (obese vs lean animals)	Reference
Diet-induced, "Western diet" (40% fat vs chow)	TRAMP mice	More advanced disease; increased percentage of metastasis	Llaverias et al. (2010)
Diet-induced (33% fat)	TRAMP mice	More advanced disease; higher incidence of metastasis (see text for comment)	Bonorden et al. (2012)
GTG-induced	TRAMP mice	Less advanced disease; lower percentage of metastasis	Bonorden et al. (2012)
Diet-induced in C57BL/6	Implantation of TRAMP-C2 cells	Faster tumour growth	Bonorden et al. (2012)
Diet-induced (60% fat)	Transgenic Hi-Myc mice	More advanced disease	Blando et al. (2011)
Diet-induced	s.c. injection of RM1 prostate carcinoma cell line	Larger tumours	Ribeiro et al. (2010)
Lep^{ob}*Lep*^{ob}	s.c. injection of RM1 prostate carcinoma cell line	Larger tumours	Ribeiro et al. (2010)
Lepr^{db}*Lepr*^{db}	s.c. injection of RM1 prostate carcinoma cell line	Smaller tumours	Ribeiro et al. (2010)

GTG, gold thioglucose; s.c., subcutaneous; TRAMP, transgenic adenocarcinoma of the mouse prostate.
Adapted from Ray & Cleary (2013) by permission from Springer Nature, © 2013.

2 months and age 3 months, and progression to poorly differentiated neuroendocrine carcinoma occurs by age 4–7 months.

When fed a "Western-type" diet, enriched in both fat and cholesterol, TRAMP-C57BL6 mice had accelerated tumour incidence and burden compared with mice fed a control chow diet. Mice fed the Western-type diet had more advanced disease, characterized by highly invasive and less well differentiated tumours and fewer high-grade PIN, in contrast to the chow-fed mice, which had only high-grade PIN. Increased incidences of metastasis to the lung (67% vs 43%) were also observed in mice fed the Western-type diet (Llaverias et al., 2010).

In another study, TRAMP mice were fed a moderately HFD (33% of calories from fat) from age 7 weeks, and at age 18 weeks they were divided into obesity-resistant, overweight, and obesity-prone groups and were then followed up until age 50 weeks (Bonorden et al., 2012). An LFD group was also included. Obesity-prone mice tended to have more severe lesions, including a higher incidence of moderate and poorly differentiated tumours, than mice that weighed less, and a higher incidence of metastasis. [It should be noted that these results were not statistically significant and also that the aggressiveness of the development of prostate cancer in the TRAMP mouse affects its usefulness in this type of study.]

Implantation of TRAMP-C2 cells into diet-induced obese mice showed similar effects. In contrast, GTG-induced obesity in TRAMP mice led to a reduction in disease progression and metastasis (Bonorden et al., 2012).

The Hi-Myc transgenic mouse model of prostate cancer was used to study the effect of modulating dietary energy balance on the development and progression of prostate cancer. The mice were placed on one of three diets: 30% CR; a modified AIN-76A diet with 10% of calories from fat (overweight); or a DIO diet with 60% of calories from fat (obese). All three groups had similar incidences of hyperplasia and low-grade PIN at age 3 months and 6 months. The CR group had significantly reduced incidence of in situ adenocarcinomas at 3 months compared with the DIO

Table 3.5 Effect of obesity on the development of skin tumours in mice

Obesity model	Cancer etiology	Results (obese vs lean animals)	Reference
Lep^{ob}*Lep*^{ob}	s.c. injection of B16BL6 mouse melanoma cells	Increased number of metastatic tumour foci in the lung	Mori et al. (2006)
Lepr^{db}*Lepr*^{db}	s.c. injection of B16BL6 mouse melanoma cells	Increased number of metastatic tumour foci in the lung	Mori et al. (2006)
Lep^{ob}*Lep*^{ob}	s.c. injection of B16F10 melanoma cells	Significantly larger tumours	Brandon et al. (2009)
MC4R$^{-/-}$	s.c. injection of B16F10 melanoma cells	Significantly larger tumours	Brandon et al. (2009)
Diet-induced: HFD	s.c. injection of B16F10 mouse melanoma cells	Time to tumour formation similar, but tumours progressed more rapidly; increased tumour weight and volume	Pandey et al. (2012)
Diet-induced: pelleted + powdered (overweight) or powdered (obese)	SKH-1 hairless mice + UV radiation	Shortened latency and increased multiplicity: obese > overweight > lean	Dinkova-Kostova et al. (2008)

HFD, high-fat diet; s.c., subcutaneous; UV, ultraviolet.
Adapted from Ray & Cleary (2013) by permission from Springer Nature, © 2013.

group, and at 6 months compared with both the overweight group and the DIO group. The DIO regimen also significantly increased (P = 0.02) the incidence of invasive adenocarcinoma (95%), compared with the overweight group (65%) and with the 30% CR group (no invasive adenocarcinomas) (Blando et al., 2011).

Cancer cell lines have also been used to study the induction and progression of prostate cancer. Obese *Lep*^{ob}*Lep*^{ob} and *Lepr*^{db}*Lepr*^{db} mice, DIO mice, and control C57BL/6J mice were injected subcutaneously with RM1, a murine androgen-insensitive prostate carcinoma cell line, and evaluated 14 days after inoculation. The tumours induced in the obese *Lep*^{ob}*Lep*^{ob} mice and the DIO mice were significantly larger (P < 0.001), and those induced in the *Lepr*^{db}*Lepr*^{db} mice were significantly smaller (P = 0.047) than those in the controls (Ribeiro et al., 2010).

3.2.6 Cancer of the skin (melanoma)

See Table 3.5.

Several studies have been performed to assess the effect of obesity on the progression and metastasis of skin tumours. In one study, B16BL6 melanoma cells were injected into the tail vein of male genetically obese *Lep*^{ob}*Lep*^{ob} and *Lepr*^{db}*Lepr*^{db} mice to assess the effects of obesity on metastasis. At 14 days after injection, the number of metastatic tumour foci was significantly increased in the lungs of both obese strains compared with control C57BL/6 mice (Mori et al., 2006).

In another study, obese *Lep*^{ob}*Lep*^{ob} mice, obese melanocortin receptor 4 knockout MC4R$^{-/-}$ mice, lean wild-type mice, and pair-fed lean *Lep*$^{ob-/-}$ mice were injected subcutaneously with B16F10 melanoma cells. The resulting tumours were significantly larger in the obese *Lep*^{ob}*Lep*^{ob} (5.1 ± 0.9 g) and MC4R$^{-/-}$ mice (5.1 ± 0.7 g) than in the lean wild-type mice (1.9 ± 0.3 g) or the pair-fed *Lep*$^{ob-/-}$ mice (0.95 ± 0.2 g) (Brandon et al., 2009).

Table 3.6 Effect of obesity on the development of pancreatic tumours and pre-neoplastic lesions in mice

Obesity model	Cancer etiology	Results (obese vs lean animals)	Reference
Lep^{ob}*Lep*^{ob} and *Lepr*^{db}*Lepr*^{db}	s.c. injection of PAN02 pancreatic tumour cells	Larger tumours; increased incidence of metastases	Zyromski et al. (2009)
Diet-induced (60% fat)	s.c. injection of PAN02 pancreatic tumour cells	Larger tumours and increased tumour weight	White et al. (2010)
Diet-induced (HFHC diet)	*Kras*^{G12D}	More advanced PanIN lesions	Dawson et al. (2013)
Diet-induced (HFHC diet)	*Kras*^{G12D} + Ink4a deficiency: LSL-Kras/Pdx-1-Cre/Ink4a/Arf	Increased number of PanIN, more advanced PanIN, increased number of PDAC, and increased number of pancreatic desmoplastic (fibrotic) stroma	Lashinger et al. (2013)
Diet-induced (HFHC diet)	*Kras*^{G12D} with or without COX conditional knockout: LSL-Kras/Ela-CreERT; COXKO/LSL-Kras/Ela-CreERT and LSL-Kras/Pdx-1-Cre	Increased numbers of PanIN and PDAC in all 3 models	Philip et al. (2013)

HFHC, high-fat, high-calorie; PanIN, pancreatic intraepithelial neoplasia; PDAC, pancreatic ductal adenocarcinoma; s.c., subcutaneous.
Adapted from Ray & Cleary (2013) by permission from Springer Nature, © 2013.

In another study, male C57BL/6J mice made obese by feeding a HFD for 6 months and subsequently injected subcutaneously with B16F10 murine melanoma cells developed significantly larger tumours compared with LFD control mice (Pandey et al., 2012). Although there was no noticeable difference in the time of initiation of tumour formation between the two groups, tumours in HFD mice progressed more rapidly than those in controls. The average tumour weight was 3.52 g in HFD mice and 0.92 g in control mice, and the average tumour volume was 1920 mm^3 in HFD mice and 924 mm^3 in control mice.

One study involved the induction of skin cancer by whole-body exposure to ultraviolet radiation. SKH-1 hairless mice made obese by feeding a powdered AIN-76A diet exclusively from age 5 weeks to age 30 weeks, or made overweight by feeding a pelleted diet followed by a powdered diet, were exposed to ultraviolet radiation twice a week for 17 weeks (Dinkova-Kostova et al., 2008). A control group received the pelleted diet only. The obese group had a shortened tumour latency and an increased multiplicity of squamous cell carcinoma/papilloma compared with the control group; the overweight group had intermediate values.

3.2.7 Cancer of the pancreas

See Table 3.6.

After BOP treatment, increased incidence and multiplicity of cancer of the pancreas was reported in an early study in Syrian hamsters fed a HFD compared with those fed a LFD (Birt et al., 1981). More recent studies assessing the link between obesity and pancreatic cancer have used inoculation of cell lines and have examined progression rather than the induction and development of pancreatic tumours. Obese *Lep*^{ob}*Lep*^{ob} and *Lepr*^{db}*Lepr*^{db} mice injected subcutaneously with PAN02 murine pancreatic adenocarcinoma cells developed larger tumours, and a significantly greater number of them developed metastases compared with lean mice. Tumour weights at 5 weeks after inoculation were highest in the *Lep*^{ob}*Lep*^{ob} mice, intermediate in the

Table 3.7 Effect of obesity on the development of endometrial tumours in mice

Obesity model	Cancer etiology	Results (obese vs lean animals)	Reference
Diet-induced (AIN-93G-based; 58% fat)	Transgenic *Pten*$^{+/-}$	Increased incidence of glandular hyperplasia with atypia; 1 adenocarcinoma (0 in lean animals)	Yu et al. (2010)

Adapted from Ray & Cleary (2013) by permission from Springer Nature, © 2013.

Table 3.8 Effect of obesity on the development of acute lymphoblastic leukaemia in mice

Obesity model	Cancer etiology	Results (obese vs lean animals)	Reference
Diet-induced (60% fat)	Transgenic BCR/ABL	Shortened latency for development of B-cell-derived and T-cell-derived ALL	Yun et al. (2010)
Diet-induced (60% fat)	AKR/J	Shortened latency for development of B-cell-derived and T-cell-derived ALL	Yun et al. (2010)

ALL, acute lymphoblastic leukaemia.
Adapted from Ray & Cleary (2013) by permission from Springer Nature, © 2013.

Lepr^{db}*Lepr*^{db} mice, and lowest in the lean mice. Tumour weights were also positively correlated with body weights, and tumours from both obese groups exhibited higher proliferation rates than those from lean mice (Zyromski et al., 2009). DIO C57BL/6 mice injected with PAN02 murine pancreatic tumour cells also had significantly larger tumours compared with control mice, and tumour weights were positively correlated with body weights (White et al., 2010).

In mice, the *Kras* mutation combined with Ink4a/Arf deficiency induces development of pre-neoplastic pancreatic intraepithelial neoplasia (PanIN) lesions and their progression to invasive pancreatic ductal adenocarcinoma (PDAC). The association between obesity and pancreatic cancer was tested in three DIO studies using mice with acinar cell-specific expression of *Kras*^{G12D} alone, or mice crossed with the COX2 conditional knockout or with Ink4a deficiency. The mice were fed a control diet (12% of calories from fat), or a high-fat, high-calorie diet (40–60% of calories from fat) for 4–14 weeks. In all three studies, mice fed the high-fat, high-calorie diet had increased numbers of PanIN lesions, more advanced PanIN, increased numbers of PDAC, and shorter survival time than control mice (Dawson et al., 2013; Lashinger et al., 2013; Philip et al., 2013).

3.2.8 Cancer of the endometrium of the uterus

See Table 3.7.

Heterozygous phosphatase and tensin homologue deleted on chromosome 10 *Pten*$^{+/-}$ mice develop spontaneous multifocal glandular hyperplasia and endometrial cancer between age 28 weeks and age 52 weeks. Feeding *Pten*$^{+/-}$ mice a HFD increased the incidence of focal atypical glandular hyperplasia and malignant lesions from 58% in the *Pten*$^{+/-}$ mice fed a control diet to 78% in the obese *Pten*$^{+/-}$ mice (Yu et al., 2010).

3.2.9 Leukaemia

See Table 3.8.

Genetically modified models to study the link between obesity and haematological malignancies such as leukaemia and lymphoma are limited. The progression of acute lymphoblastic leukaemia (ALL) was tested in two animal models (transgenic BCR/ABL and AKR/J mice)

fed a HFD (60% of calories from fat). In both models, the obese mice developed both B-cell-derived and T-cell-derived acute lymphoblastic leukaemia significantly earlier than control mice (Yun et al., 2010).

3.3 Preventive effects of dietary/calorie restriction

3.3.1 Cancer of the mammary gland

The effect of dietary/calorie restriction on mammary tumorigenesis has been studied extensively over many decades. In the *IARC Handbook* on weight control and physical activity (IARC, 2002), the Working Group concluded that there was "*sufficient evidence* in experimental animals for a cancer-preventive effect of avoidance of weight gain by restriction of dietary energy intake" on tumours of the mammary gland. The models used for these initial investigations were primarily rats with carcinogen-induced mammary tumours or mice with spontaneous tumour development. Studies on the prevention of mammary tumours by dietary/calorie restriction are presented in Table 3.9 for mice and in Table 3.10 for rats, in chronological order. The text presents the key studies, by type of model and/or intervention.

(a) Mice

Tannenbaum (1945a) studied the effect of overall moderate CR, carbohydrate restriction, and DR with increased fat intake – all in the range of about 17% DR – in virgin DBA female mice from age 10 weeks until age 136 weeks. The mammary tumour incidence was lowest (47%) in the carbohydrate-restricted group, compared with 74% in the group fed AL, whereas the incidence was 65% in mice with overall CR and 87% in the high-fat group. In addition, tumour latency was extended for the carbohydrate-restricted group compared with the other three groups. [The basic diet had only 2% fat; no statistics were presented.]

In another study using the same mouse strain, a 27% DR or 36% DR compared with the AL mice was used (Tannenbaum, 1945b). No mice in the 36% DR group developed spontaneous mammary tumours, compared with 54% in the AL group and 12% in the 27% DR group. [No statistics were provided, and the diets fed were very low in fat (2–3%).] In a second study, parous DBA female mice were subjected to DR at various degrees (12–31% DR) beginning at age 21–25 weeks. A 50% reduction in incidence of spontaneous mammary tumours was observed for the 31% DR group. [No statistics were presented.]

Engelman et al. (1990) studied the effects of DR and/or increased fat levels in the diet on the development of spontaneous mammary tumours associated with MMTV in C3H/HeOu female mice. The mice were assigned to one of five experimental groups between age 6 weeks and age 8 weeks. This included four groups fed purified diets (AL LFD, 40% DR LFD, AL HFD, and 40% DR HFD) and an AL group fed regular laboratory chow. Mice were followed up until age 60 weeks. The chow-fed mice reached 100% incidence of spontaneous mammary tumours by age 46 weeks, whereas the AL HFD mice and the AL LFD mice did so by age 58 weeks and 64 weeks, respectively. At termination of the study, the incidence was 15% for the 40% DR HFD mice and 0% for the 40% DR LFD mice. Body weights were significantly reduced in the 40% DR groups compared with the AL groups ($P < 0.001$).

Engelman et al. (1994) also tried to identify potential critical periods for the impact of DR on the development of spontaneous mammary tumours in C3H/HeOu mice. Mice were separated into three groups: (i) fed AL, (ii) continuously 40% DR, or (iii) 40% DR only from age 4 weeks to age 12 weeks (40% DR4–12), after which they were fed a HFD AL [this was not described]. Mice were followed up until age 60 weeks; the incidence of mammary tumours was 83% in the

Table 3.9 Studies on the prevention of mammary tumours by dietary/calorie restriction in female mice

Strain Age at start Number of animals Duration of study Reference	Dose and duration of carcinogen administration	Type of diet, dosing regimen[a], and duration of intervention (if not until termination of study)	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance vs AL, unless otherwise specified	Comments
DBA 9–10 wk *n* = 200 126 wk Tannenbaum (1945a)	Spontaneous tumours	Cereal-based (fox chow), 2% fat 16% DR-CHO restricted 16% DR-all components 23% DR-18% fat	Mammary tumours: AL, 74% 16% DR-CHO restricted, 47% 16% DR-all components, 65% 23% DR-18% fat, 87%	NR	Diets low in fat; bw reduced in all DR groups Latency extended for the 16% DR-CHO restricted group
DBA 4 wk *n* = 150 96 wk Tannenbaum (1945b)	Spontaneous tumours	Cereal-based (fox chow) diluted with cornstarch, 2–3% fat DR: 27% or 36%, cornstarch removed	Mammary tumours [no indication of pathology]: AL, 54% 27% DR, 12% 36% DR, 0%	NR	Diets low in fat; bw of DR groups reduced Latency extended
DBA parous 21–25 wk *n* = 140 134–138 wk Tannenbaum (1945b)	Spontaneous tumours	Cereal-based (fox chow) diluted with cornstarch, 2–3% fat DR: 12%, 18%, 24%, or 31%, cornstarch removed	Mammary tumours [no indication of pathology]: AL, 73% 12% DR, 57% 18% DR, 63% 24% DR, 68% 31% DR, 36%	NR	Diets low in fat; bw reduced to 76% and 69% of AL DR started in adult animals
C3H/HeOu 6–8 wk *n* = 215 60 wk Engelman et al. (1990)	Spontaneous tumours (with MMTV)	Purified diets, either high-CHO (sucrose), low-fat (4.5%) or high-fat (60%), no-CHO, AL or 40% DR Chow-fed control	Mammary adenocarcinoma: Chow, 100% AL low-fat, 100% 40% DR low-fat, 0%* AL high-fat, 100% 40% DR high-fat, 15%**	Weibull distribution by survival analysis (*P* values NR) [*Significant vs AL low-fat] [**Significant vs AL high-fat]	Tumour latency: chow < AL high-fat < AL low-fat << 40% DR high-fat ≈ 40% DR low-fat Bw significantly reduced in DR groups vs AL groups ($P < 0.001$)
C3H/HeOu 4 wk *n* = 144 age 60 wk Engelman et al. (1994)	Spontaneous tumours	Purified diets 40% DR 40% DR age 4–12 wk	Mammary tumours: AL, 83% 40% DR, 13%*,** 40% DR 4–12 wk, 50%***	*$P < 0.000\,001$ **$P = 0.009$ vs 40% DR 4–12 wk ***$P = 0.004$	

Table 3.9 (continued)

Strain Age at start Number of animals Duration of study Reference	Dose and duration of carcinogen administration	Type of diet, dosing regimen[a], and duration of intervention (if not until termination of study)	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance vs AL, unless otherwise specified	Comments
$B6C3F_1$ 4 wk *n* = 102 Lifetime Sheldon et al. (1996)	Spontaneous tumours	NIH-31 diet 40% DR from age 14 wk	Mammary tumours (benign and malignant combined): AL, 27% 40% DR, 4%	NR	
C57BL6 4 wk *n* = 102 Lifetime Sheldon et al. (1996)	Spontaneous tumours	NIH-31 diet 40% DR from age 14 wk	Mammary tumours (benign and malignant combined): AL, 14% 40% DR, 0%	NR	
$B6D2F_1$ 4 wk *n* = 102 Lifetime Sheldon et al. (1996)	Spontaneous tumours	NIH-31 diet 40% DR from age 14 wk	Mammary tumours (benign and malignant combined): AL, 27% 40% DR, 0%	NR	
MMTV-TGF-α C57BL6 10 wk *n* = 93 age 79–80 wk Cleary et al. (2002)	Human TGF-α	AIN-93M CDR, mice pair-fed to IDR (21% DR) IDR, mice fed 3 wk of 50% DR + 3 wk of AL for 11 cycles (21% DR)	Mammary adenocarcinoma: AL, 77% CDR, 44%* IDR, 3%*	[*Significant, χ^2 test] (*P* values NR)	Final bw similar in all 3 groups, although IDR mice lost bw during the 50% DR periods Latency shorter in AL vs DR Only 1 tumour detected at necropsy for IDR (*n* = 1, hence no statistics)
HER2/neu 9 wk *n* = 96 age 80 wk Pape-Ansorge et al. (2002)	Transgene-heterozygous for HER2/neu	AIN-93M with 10% fat CDR, 25% CHO restriction IDR, mice fed 3 wk of 50% DR + 3 wk of AL for 11 cycles (~25% DR)	Mammary adenocarcinoma: AL, 37.5% CDR, 33.3% IDR, 22.5%	NS	Bw reduced in CDR and IDR vs AL

Table 3.9 (continued)

Strain Age at start Number of animals Duration of study Reference	Dose and duration of carcinogen administration	Type of diet, dosing regimen[a], and duration of intervention (if not until termination of study)	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance vs AL, unless otherwise specified	Comments
MMTV-TGF-α C57BL6 10 wk *n* = 76 age 85 wk Cleary et al. (2004a)	Human TGF-α	Chow, 33% fat; at age 34 wk, divided (based on weight status) into obesity-prone, overweight, and obesity-resistant groups; 1 group chow-fed, lean	Histologically confirmed mammary adenocarcinoma: AL, 72% Obesity-resistant, 82% Obesity-prone, 76%	NS	Latency shorter for obesity-prone mice ($P < 0.0001$); higher multiplicity per tumour-bearing animal ($P < 0.015$)
HER2/neu 8 wk *n* = 106 10 mo Anisimov et al. (2005)	Transgene	Diet NR AL AL-metformin (100 mg/kg bw in drinking-water 5 d/wk)	Mammary adenocarcinoma: 100% incidence in both groups Mean latency: 187 d vs 178 d Mean tumour size: 1.71 cm vs 1.59 cm	 NS $P < 0.05$ $P < 0.05$	Metformin used as a CR mimetic No effect on bw
MMTV-TGF-α C57BL6 10 wk *n* = 100 age 79–80 wk Cleary et al. (2007)	Human TGF-α	AIN-93M CDR, mice pair-fed to IDR (14% DR) IDR, mice fed 3 wk of 50% DR + 3 wk of AL for 11 cycles (11% DR)	Mammary adenocarcinoma: AL, 84% CDR, 27%* IDR, 15%*	*$P < 0.001$, χ^2 test	Final bw similar for AL and IDR (within 1 wk of refeeding); significantly higher than for CDR mice
MMTV-TGF-α C57BL6 10 wk *n* = 200 age 79–82 wk Rogozina et al. (2009)	Human TGF-α	AIN-93M CDR, mice pair-fed to IDR (27% DR) IDR, mice fed 3 wk of 50% DR + 3 wk pair-fed to AL for 11 cycles (25% DR)	Mammary adenocarcinoma: AL, 71% CDR, 35.4%* IDR, 9.1%*	*Significant, χ^2 test (*P* values NR)	Final bw for IDR and CDR significantly lower ($P < 0.001$) than for AL IDR mice limited to intake of AL mice during refeeding, thus higher DR; latency extended for both CDR and IDR vs AL
HER2/neu 8 wk *n* = 75 Lifetime Anisimov et al. (2010)	Transgene	Diet NR AL AL-metformin (100 mg/kg bw in drinking-water 5 d/wk)	Mammary adenocarcinoma: > 90% incidence in both groups Slower kinetic of tumour incidence with metformin Latency: 223 d vs 197 d	 NS $P < 0.001$ $P < 0.05$	No effect on bw

Table 3.9 (continued)

Strain Age at start Number of animals Duration of study Reference	Dose and duration of carcinogen administration	Type of diet, dosing regimen[a], and duration of intervention (if not until termination of study)	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance vs AL, unless otherwise specified	Comments
C57BL6 (OVX) 6–8 wk *n* = 120 14 wk Dunlap et al. (2012)	M-Wnt or E-Wnt cells implanted 8 wk after diets started, and mice followed up for 6 wk	AIN-76A, AL 30% DR HFD (60% fat)	Allograft: Tumour growth (area in mm^2) reduced by 30% DR for both cell lines	$P < 0.02$	Tumour growth enhanced by HFD for M-Wnt cells but not for E-Wnt cells
C57BL6 (OVX) 6 wk *n* = 45 4 wk Nogueira et al. (2012)	Wnt-1 cells implanted and mice followed up for 4 wk	AIN-76A 30% DR	Allograft: Tumour weight: 0.045 g vs 0.39 g Latency: 16.3 wk vs 15.6 wk	$P = 0.0002$ NS	Bw reduced in 30% DR vs AL
HER2/neu 10 wk *n* = 178 age 60 wk Mizuno et al. (2013)	Transgene-homozygous for HER2/neu	AL-CON, AIN-93M with 10% fat calories from soy oil CDR-CON, 25% CHO restriction IDR-CON, 3 wk of 50% DR + 3 wk pair-fed to AL for 11 cycles (25% DR) Same groups with EPA substituted for some of the soy oil	Mammary adenocarcinoma: AL-CON, 87% CDR-CON, 47%* IDR-CON, 59%* AL-EPA, 63% CDR-EPA, 40% IDR-EPA, 15%**	*$P < 0.05$ vs AL-CON **$P < 0.05$ vs CDR-EPA and AL-EPA	Bw reduced in CDR and IDR vs AL
MMTV-TGF-α C57BL6 10 wk *n* = 135 age 79–82 wk Rogozina et al. (2013) See Rogozina et al. (2009)	Human TGF-α	AIN-93M based diet with 22.7% fat CDR, mice matched to IDR (22% DR) IDR, mice fed 3 wk of 50% DR + 3 wk pair-fed to AL for 11 cycles (22% DR)	Mammary adenocarcinoma: AL, 66.7% CDR, 52.3%* IDR, 4.4%* Latency: AL < CDR* < IDR*	*$P < 0.0001$, χ^2 test *$P < 0.0001$, Kaplan–Meier	Bw of AL mice greater than CDR and IDR. IDR mice lost and regained weight with each cycle to values similar to those of CDR

Table 3.9 (continued)

Strain Age at start Number of animals Duration of study Reference	Dose and duration of carcinogen administration	Type of diet, dosing regimen[a], and duration of intervention (if not until termination of study)	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance vs AL, unless otherwise specified	Comments
C3(1)-TAg FVB/N 3 wk *n* = 40 27 wk Sundaram et al. (2014)	Transgenic C3(1)-TAg	LFD (10% fat) HFD (60% fat) HFD for 7 wk, switched to LFD for ~17 wk	Basal-like mammary tumours: Tumour volume: ~3-fold higher in HFD vs LFD HFD-7 vs LFD	 *P* = 0.0024 NS	Bw of HFD-7 decreased and remained at level of LFD mice; both significantly lower than in HFD mice (*P* = 0.019); latency and multiplicity not affected [Not clear whether effect due to weight loss or to change in diet composition]
HER2/neu/p53KO 60 d NR 11 mo Thompson et al. (2015)	Transgene	Teklad 4% mash AL AL-metformin (150 mg/kg)	Mammary adenocarcinoma: Tumour multiplicity Tumour weight	 NS NS	Metformin used as a CR mimetic [No bw data] Survival not affected

AL, ad libitum; bw, body weight; CDR, chronic dietary restriction; CHO, carbohydrate; d, day or days; CON, control; CR, calorie restriction; DR, dietary restriction; EPA, eicosapentaenoic acid; HFD, high-fat diet; IDR, intermittent dietary restriction; LFD, low-fat diet; mo, month or months; MMTV, mouse mammary tumour virus; NR, not reported; NS, not significant; OVX, ovariectomized; TGF-α, transforming growth factor alpha; vs, versus; wk, week or weeks.

[a] The (%) indicates the percentage of calories from fat.

Table 3.10 Studies on the prevention of mammary tumours by dietary/calorie restriction in female rats

Strain Age at start Number of animals Duration of study Reference	Dose and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention as appropriate	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
Wistar NR ~50/group 24 mo Tucker (1979)	Spontaneous tumours	AL, 20% DR after 1 mo for 23 mo	Mammary tumours: 17%, 3%	$P < 0.001$	
Sprague-Dawley 57 d $n = 104$ 26 wk Sylvester et al. (1981)	DMBA, 5 mg, once	Rat chow, 50% DR for 7 d before and for 30 d after injection of DMBA. One 50% DR group received various hormone treatments (e.g. E2)	Palpable mammary tumours: AL, 76% 50% DR, 29%* 50% DR+E2, 71%	*$P < 0.05$ vs AL or 50% DR+E2	Latency extended in 50% DR rats
Sprague-Dawley 57 d $n = 101$ 22 wk Sylvester et al. (1982)	DMBA, 5 mg, once	Rat chow: AL; 50% DR for 1 wk before and for 1 wk after DMBA; 50% DR for 2 wk starting 1 wk after DMBA; 50% DR for 2 wk starting 3 wk after DMBA; 50% DR for 4 wk starting 5 wk after DMBA	Palpable mammary tumours: 80.9%, 27.8%*, 76.2%, 75.0%, 75.0%	*$P < 0.05$	No significant effect on latency or tumour multiplicity
F344 52 d $n = 45$ 24 wk Boissonneault et al. (1986)	DMBA, 65 mg/kg bw, once	Purified diet, dextrose reduced in 30% fat diets, 1 d after DMBA: AL 30% fat, AL 5% fat, 14% DR 30% fat	Mammary tumours: 73%, 43%, 7%	NR	
Sprague-Dawley 50 d $n = 100$ 20 wk Klurfeld et al. (1989a)	DMBA, 5 mg, once	AIN-76A, sucrose reduced in DR diets, 1 wk after DMBA: AL, 10% DR, 20% DR, 30% DR, 40% DR	Histologically verified mammary tumours: 60%, 60%, 40%*, 35%*, 5%*	*$P < 0.005$	Tumour latency extended in 30% DR and 40% DR groups. Tumour multiplicity reduced in 40% DR group

Table 3.10 (continued)

Strain Age at start Number of animals Duration of study Reference	Dose and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention as appropriate	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
Sprague-Dawley 50 d n = 100 20 wk Klurfeld et al. (1989b)	DMBA, 5 mg, once	AIN-76A, sucrose reduced in DR diets, 1 wk after DMBA: AL 5% fat, AL 15% fat, AL 20% fat, 25% DR 20% fat, 25% DR 26.7% fat	Mammary tumours: 65%*, 85%, 80%, 60%*, 30%**	*P < 0.01 vs AL 15% fat and AL 20% fat **P < 0.0001 vs AL groups	Tumour multiplicity reduced to similar levels in both 25% DR groups
Sprague-Dawley 50 d n = 120 16 wk Kritchevsky et al. (1989)	DMBA, 5 mg, once	AIN-76A, sucrose reduced in DR diets, 1 wk after DMBA. 6 groups: (A) AL; (B) 25% DR, wk 1–16; (C) 25% DR, wk 1–4; (D) 25% DR, wk 1–8; (E) 25% DR, wk 5–12; (F) 25% DR, wk 9–16	Palpable mammary tumours: 50%, 20%*, 60%, 40%, 45%, 30%	*P < 0.001, group B vs A and group B vs C	Weight gain correlated with tumour incidence (r = 0.96) and with total calorie intake (r = 0.83)
Sprague-Dawley 50 d n = 110 20 wk Ruggeri et al. (1989a, b)	DMBA, 5 mg, once	AIN-76A, sucrose reduced in DR diets, 1 wk after DMBA: AL, 25% DR, 40% DR	Mammary tumours: 90%, 61%*, 20%*	*P = 0.007, χ^2 test	Multiplicity of palpable tumours reduced in 40% DR rats (P < 0.05)
LA/N 65 d n = 49 17 wk Klurfeld et al. (1991)	DMBA, 5 mg, once	AIN-76A, sucrose reduced in DR diets, 1 wk after DMBA: obese AL, obese 25% DR, lean AL	Mammary tumours: 100%, 27%, 21%	NR	Tumour multiplicity reduced in obese 25% DR rats; bw in DR significantly lower than in obese AL rats
Sprague-Dawley 50 d n = 83 30 wk Zhu et al. (1991)	MNU, 25 mg/kg bw, at age 50 d, once	Purified diet, 45% fat diet for ~10 wk (until tumour of 1 cm^3), then divided into 4 groups: AL 45% fat, 30% DR 45% fat, AL 25% fat, 30% DR 25% fat	Mammary tumours: Multiplicity: 2.43, 1.74*, 2.35, 0.95* Tumour/bw (%): 3.8, 2.2*, 2.5, 1.3*	*P < 0.05 vs corresponding AL group	100% incidence of mammary tumours; bw reduced by 10% in DR groups

Table 3.10 (continued)

Strain Age at start Number of animals Duration of study Reference	Dose and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention as appropriate	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
F344 14 wk *n* = 54/group (exp. 1); *n* = 114–116/group (exp. 2) Lifetime Thurman et al. (1994)	Spontaneous tumours	NIH-31; AL, 40% DR	Mammary adenocarcinoma: Exp. 1: 8%, 2% Exp. 2: 5%, 0% Mammary fibroadenoma: Exp. 1: 36%, 2% Exp. 2: 35%, 1%	[NS] [$P = 0.048$] [$P < 0.0001$] [$P < 0.0001$]	Extended survival; reduced bw; reduced tumour multiplicity [Low incidence of tumours in AL animals]
F344 50 d *n* = 132 20 wk Gillette et al. (1997)	MNU, 50 mg/kg bw, at age 50 d and 57 d, once	AIN-76A with cornstarch + cerelose; AL, 20% DR	Mammary adenocarcinoma: 23.3%, 6.7%*	*$P < 0.05$	Tumour multiplicity reduced in AL vs 20% DR; exercise by treadmill running not effective in any group
Sprague-Dawley 21 d *n* = 75 35 d Zhu et al. (1997)	MNU, 50 mg/kg bw, once	AIN-93G with cornstarch + cerelose, fed AL, 10% DR, 20% DR, 40% DR	Mammary carcinoma: 100%, 80%, 60%, 25%	$P_{trend} < 0.01$, dose-dependent reduction	Dose-dependent increased latency for DR Additional results presented (Zhu et al., 1999a, b)
ACI 49 d *n* = 84 220 d Harvell et al. (2002)	E2 treatment from age 59 d	Purified diet, 5% fat; AL, 40% DR in controls or mice treated with E2	Mammary tumours: AL, 0% 40% DR, 0% AL+E2, 100% 40% DR+E2, 59%*	*$P < 0.001$ vs AL+E2	Latency of palpable tumour: 69 d after E2 for AL+E2 vs 104 d for 40% DR+E2 Bw reduced in 40% DR groups
Sprague-Dawley 3 wk *n* = 66 90 d Zhu et al. (2002)	MNU, 50 mg, once	AIN-93G; AL, 40% DR (CHO reduced) for 6 wk, and then fed AL (DR-AL)	Mammary adenocarcinoma: Detectable tumour incidence at day 42 after MNU: AL, 61%; DR, 11%*	*$P < 0.001$, χ^2 test	Bw similar; incidence of tumours in DR-AL similar to AL by end of experiment

Table 3.10 (continued)

Strain Age at start Number of animals Duration of study Reference	Dose and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention as appropriate	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
Sprague-Dawley 3 wk *n* = 108 54 d Thompson et al. (2004a)	MNU, 50 mg, once	AIN-93G; 40% DR; 40% DR for 6 wk, and then fed AL (DR-AL)	Mammary adenocarcinoma: Tumour volume of DR rats significantly smaller than AL or DR-AL rats	$P < 0.001$	Bw reduced in DR rats 2 meals daily
Sprague-Dawley 3 wk *n* = 78 77–78 d Thompson et al. (2004b)	MNU, 50 mg, once	AIN-93G; 40% DR (CHO reduced) from age ~4 wk	Mammary adenocarcinoma: 96%, 59%* Multiplicity: 4.3, 1.0**	*$P < 0.01$ **$P < 0.001$	2 meals daily
Wistar 7 wk *n* = 90 50 wk Buison et al. (2005)	DMBA, 2 mg, once	AL, HFD (60% fat) IDR-HFD (50% DR CHO reduction), first cycle at age 15 wk to loss of 20% – 4 cycles	Mammary tumours: HFD, 17.6% IDR-HFD, 8.8%	NS	Bw significantly lower in IDR-HFD rats
Sprague-Dawley 3 wk *n* = 99 57–58 d Zhu et al. (2005)	MNU, 50 mg/kg bw, at age 21 d	AIN-93G; AL, 40% DR (CHO) at age 30 d for 6 wk, then divided into DR, DR-AL, DR+IGF-1	Mammary adenocarcinoma: AL, 96.6% DR, 56.7%* DR-AL, 80% DR+IGF-1, 60%	*$P < 0.0006$	
Sprague-Dawley 3 wk *n* = 60 7 wk Jiang et al. (2008a)	MNU, 50 mg/kg bw, at age 21 d	AIN-93G: AL, AL+0.03% 2-deoxyglucose	Mammary adenocarcinoma: 86.7%, 53.3% Multiplicity: 2.03, 1.37	$P < 0.005$ $P = 0.018$	2-deoxyglucose used as a CR mimetic No difference in bw

Table 3.10 (continued)

Strain Age at start Number of animals Duration of study Reference	Dose and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention as appropriate	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
Sprague-Dawley 21 d *n* = 90 52 d Jiang et al. (2008b)	MNU, 50 mg/kg bw, at age 21 d	AIN-93G: AL, 20% DR, 40% DR	Mammary adenocarcinoma: 96%, 60%*, 23%* Multiplicity: 2.1, 1.1*, 0.3*	*$P < 0.001$	Bw reduced in DR rats
Sprague-Dawley 21 d *n* = 103, 101 60 d Matthews et al. (2014)	MNU, 50 mg/kg bw, at age 21 d	SUMO32 diet (32% HFD corn oil-9.4% saturated fat) Obesity-prone (OP) Obesity-resistant (OR)	Mammary adenocarcinoma: OP, 91%; OR, 65%*	*$P < 0.01$	OP rats weighed 15% more than OR rats at study termination Tumour weight reduction of 80% for OR vs OP; tumour latency extended for OR vs OP
Sprague-Dawley 50 d NR 126 d Thompson et al. (2015)	MNU, 75 mg/kg bw, at age 50 d	Teklad standard diet (8% fat) Control; metformin, 50 mg/kg/d; metformin, 150 mg/kg/d	ER^+ mammary tumours: Tumour latency Tumour weight	 NS NS	Metformin used as a CR mimetic 2 meals daily Bw NR
Sprague-Dawley 21 d *n* = 120 (30/group) 51 d Zhu et al. (2015)	MNU, 50 mg/kg bw, once	AIN-93G Control; metformin, buformin, or phenformin	Mammary adenocarcinoma: 83.3%, 93.3%, 43.3%*, 76.7%	*$P < 0.003$	Compounds studied as CR mimetics Bw reportedly measured, but no data presented

AL, ad libitum; bw, body weight; d, day or days; CHO, carbohydrate; CR, calorie restriction; DMBA, 7,12-dimethylbenz[*a*]anthracene; DR, dietary restriction; E2, 17β-estradiol; ER, estrogen receptor; exp., experiment; F344, Fischer 344; HFD, high-fat diet; IDR, intermittent dietary restriction; IGF-1, insulin-like growth factor 1; MNU, *N*-methyl-*N*-nitrosourea; mo, month or months; NR, not reported; NS, not significant; OP, obesity-prone; OR, obesity-resistant; vs, versus; wk, week or weeks.

AL group, 13% in the 40% DR group, and 50% in the 40% DR4–12 group.

The effect of 40% DR on ageing and longevity in three different mouse strains was reported by Sheldon et al. (1996). DR was initiated at age 14 weeks and extended to 48 months in $B6C3F_1$, C57BL6, and $B6D2F_1$ mice. AL mice in the $B6C3F_1$, C57BL6, and $B6D2F_1$ groups had incidences of spontaneous mammary tumours of 27%, 14%, and 27%, respectively, compared with 4%, 0%, and 0% for the corresponding 40% DR groups.

More recently, transgenic mice have been used to evaluate the effect of DR on development of mammary tumours. Several studies were conducted using mice that overexpress human TGF-α, in which two modes of DR were compared (Cleary et al., 2002, 2007; Rogozina et al., 2009, 2013). In the initial experiments, mice received either intermittent DR (IDR) or chronic DR (CDR). IDR mice were subjected to 50% DR for 3-week intervals, followed by 3 weeks of refeeding AL. This resulted in an overall DR of 21%, because of overconsumption during refeeding compared with what the AL mice consumed. CDR mice were matched for calorie intake for each 6-week cycle of 50% DR/refeeding. The IDR mice had significantly lower mammary tumour incidence compared with the CDR mice, i.e. mammary tumour incidences of 3% and 15% in the two reports, compared with 77% and 84% for AL mice and 44% and 27% for the CDR mice (Cleary et al., 2002, 2007). In subsequent studies, IDR mice were pair-fed to the AL group during the refeeding phases, resulting in significantly lower body weights in both DR groups, with fluctuating body weights in the IDR group; both the CDR and IDR groups had lower tumour incidence than the AL mice (Rogozina et al., 2009, 2013). This was also observed when the fat content of the diets was moderately increased (Rogozina et al., 2013). IDR compared with CDR was also examined in the transgenic mouse strain HER2/neu. IDR resulted in lower tumour incidence than in CDR, although not significantly so (Pape-Ansorge et al., 2002; Mizuno et al., 2013).

In another transgenic mouse strain, C3(1)-TAg mice were fed either a LFD (10% of calories from fat) or a HFD (60% of calories from fat) from age 3 weeks (Sundaram et al., 2014), and a group of the HFD mice was switched to the LFD after 7 weeks on the HFD. The switch to the LFD from the HFD resulted in weight loss to the level of the LFD mice. The tumour volume in the HFD mice was 3 times that in the LFD mice, and switching from the HFD to the LFD resulted in tumour volumes similar to those in the LFD mice. [It is not clear whether the findings are due to weight loss or to the change in diet composition.]

Allograft models were also used to assess tumour progression in response to dietary intervention. For example, ovariectomized C57BL6 mice were fed AL, 30% DR, or a HFD (60% of calories from fat) for 8 weeks, before two types of Wnt cells (M-Wnt or E-Wnt cells) were implanted; the mice then continued on their diets for 6 weeks while tumour growth was monitored. DR reduced tumour growth for both cell lines compared with AL, whereas tumour growth was enhanced by the HFD only for the M-Wnt cells (Dunlap et al., 2012). In another study, Wnt-1 cells were implanted in AL or 30% DR mice. Tumour weight was lower in DR mice than in AL mice, but latency was not affected (Nogueira et al., 2012).

Another approach to assess the effects of body weight independent of diet is to use mouse or rat strains that respond to HFD feeding with a range of body weights. Cleary et al. (2004a) fed MMTV-TGF-α mice on a C57BL6 background a LFD or a moderately HFD (33% of calories from fat) and then divided the mice into three groups (obesity-prone, overweight, and obesity-resistant), based on body weight status at age 34 weeks. The heaviest group, obesity-prone, had the shortest mammary tumour latency, compared with obesity-resistant mice fed the

same diet, i.e. with body weights similar to those of the LFD mice. Furthermore, the heavier mice had more palpable tumours than mice that weighed less, although this was not statistically significant.

A murine model of basal-like breast cancer was used to assess whether the obesity-induced pro-tumour effects are reversed by weight normalization. Ovariectomized female C57BL/6 mice were fed a control diet or a DIO regimen for 17 weeks, resulting in a normal weight or an obese phenotype, respectively. After 17 weeks, mice on the DIO regimen were randomized to continue the DIO diet or switched to the control diet. The resulting formerly obese mice had body weights comparable to those of the controls. At week 24, the mammary pads of all mice were injected with MMTV-Wnt-1 mouse mammary tumour cells, and tumour growth was then measured twice per week until 36 months. Mean tumour volumes in the DIO and formerly obese mice were similar, and were higher than those in the controls (Rossi et al., 2016).

(b) Rats

In a longevity study, the incidence of mammary tumours was significantly lower in 20% DR Wistar rats (3%) than in the AL group (17%) (Tucker, 1979).

In one study (Thurman et al., 1994), groups of female Fischer 344 (F344) rats were fed AL or subjected to 40% DR from age 14 weeks and followed up for their lifetime in two experiments with a similar study design. In general, survival was extended by DR [the increase was modest, and no statistics were presented]. Incidence of spontaneous mammary adenocarcinoma was 8% and 5% in the AL rats and 2% and 0% [$P = 0.048$] in the 40% DR rats in the first and second experiment, respectively. Reductions in the incidence of mammary fibroadenoma were also reported, from 36% and 35% in the AL rats to 2% [$P < 0.0001$] and 1% [$P < 0.0001$] in the 40% DR rats. Reduced body weight was associated with extended survival and reduced multiplicity.

Female Sprague-Dawley rats were administered 5 mg of DMBA dissolved in corn oil at age 50 days (Klurfeld et al., 1989a). The rats treated with DMBA were then subjected to 10%, 20%, 30%, or 40% DR from age 57 days and followed up for 20 weeks. The 10% DR had no effect on tumour incidence, but the 20%, 30%, and 40% DR resulted in incidences of 40%, 35%, and 5%, respectively. Tumour multiplicity was significantly reduced in the 40% DR group, and latency was extended in the 30% DR and 40% DR groups.

In another study also using DMBA (Ruggeri et al., 1989a, b), only three groups of rats were included: AL, 25% DR, and 40% DR. The incidence of mammary tumours in these groups was 90%, 61%, and 20% ($P = 0.007$), respectively. In the 40% DR group, the majority of the tumours were small and non-palpable ($P < 0.05$). The authors also used rats treated with DMBA and fed diets combining increased fat levels with DR and determined effects on development of mammary tumours (Klurfeld et al., 1989b). The experimental groups included AL rats fed diets with 5%, 15%, and 20% of calories from fat, as well as 25% DR rats fed diets with 20% and 26.7% of calories from fat. The incidence of mammary tumours was significantly lower in the 25% DR, 26.7% fat group than in the other groups, for which the incidences were in the range of 60–85%. Although there was only a slight reduction in incidence for the 25% DR, 20% fat group, tumour weight and tumour multiplicity were reduced to similar levels as in the other DR group (i.e. 25% DR, 26.7% fat).

A similar study was reported by Boissonneault et al. (1986) using female F344 rats that were switched to experimental diets 1 day after DMBA treatment (at age 52 days); these groups included AL 30% fat, AL 5% fat, and 14% DR 30% fat. The groups were then followed up for 24 weeks after DMBA treatment. The incidence of mammary tumours was 73% for the AL 30% fat group

and 43% for the AL 5% fat group but only 7% for the 14% DR 30% fat group [no statistics were reported].

Klurfeld et al. (1991) also investigated the effects of DR on the development of DMBA-induced mammary tumours in genetically obese rats. After administration of DMBA at age 65 days, female LA/N Corpulent rats were fed purified diets either AL or 40% DR, and an AL lean group was also included. The body weight of the obese 40% DR rats remained at a level substantially lower than that of the AL obese rats but higher than that of the AL lean rats. The incidence of mammary tumours was 100% in the AL obese rats, compared with 27% in the obese 40% DR rats and 21% in the AL lean rats. [The Working Group noted that this study assessed DR in obese rats.]

Several investigations have focused on timing of DR interventions and development of mammary tumours.

One study examined the impact of 25% DR imposed at different times relative to the administration of DMBA (Kritchevsky et al., 1989). There were a total of six groups in the 16-week experiment: fed AL throughout (group A), fed 25% DR throughout (group B), fed 25% DR for the first 4 weeks (group C), fed 25% DR for the first 8 weeks (group D), fed 25% DR for the 8 weeks (weeks 5–12) in the middle of the experiment (group E), and fed 25% DR for the last 8 weeks (group F). The incidence of mammary tumours was 50% in the AL rats and 20% in the rats fed 25% DR throughout the study. The other groups had incidences of 30–60%; the incidence was 30% in the group fed 25% DR for the last 8 weeks (group F).

Sylvester et al. (1981, 1982) also investigated the effect of timing of DR in Sprague-Dawley rats treated with DMBA. In their first study, a 50% DR was imposed 1 week before and continued until 30 days after DMBA injection (Sylvester et al., 1981). After 26 weeks, the incidence of mammary tumours was 76% in the AL group and 29% in the 50% DR group. In the follow-up study, five groups of rats were used (Sylvester et al., 1982): AL control rats, 50% DR 1 week before and 1 week after DMBA treatment, 50% DR for 2-week periods starting 1 week or 3 weeks after DMBA treatment, and 50% DR for 4 weeks starting 5 weeks after DMBA treatment. All groups had similar incidences of mammary tumours (75.0–80.9%), except for the group subjected to 50% DR for 1 week before and 1 week after DMBA treatment, in which the incidence was only 27.8%. [The Working Group noted that DR started before administration of DMBA, and hence the effect of DR on DMBA metabolism is unknown and might have been partly responsible for the observed effect.]

Zhu et al. (1991) used Sprague-Dawley rats administered MNU at age 50 days to induce mammary tumours. The rats were then fed a 45% fat diet and followed up until the tumours reached a volume of 1 cm^3, which was 10 ± 2 weeks after administration of MNU. The rats were then divided into four groups: AL 45% fat (group 1), 30% DR 45% fat (group 2), AL 25% fat (group 3), and 30% DR 25% fat (group 4). The rats were then followed up for an additional 30 weeks, after which tumour progression was assessed. DR reduced the number of tumours per animal, the tumour weight, and the tumour weight per body weight, compared with AL. Body weight was reduced by 10%. [No statistics were reported.]

A rapidly developing carcinogen-induced mammary tumour model was developed in Sprague-Dawley rats to investigate the effect of DR on mammary tumorigenesis (Gillette et al., 1997; Zhu et al., 1997, 1999a, b, 2002, 2005; Thompson et al., 2004a, b). In this model, rats are administered MNU at age 21 days and then followed up until age 100 days or more as the tumours develop; they are subjected to 40% DR through carbohydrate restriction. This degree of DR consistently and significantly reduced body weight as well as mammary tumour development, as reflected by incidence and tumour volume.

In an additional study (Jiang et al., 2008b), 20% DR led to an incidence of 60%, compared with 96% in AL rats and 23% in the 40% DR rats. Multiplicity was also significantly reduced. [The Working Group noted that in this study model, tumours develop in pre-pubertal animals.]

Buison et al. (2005) used the DMBA mammary tumour model with the carcinogen administered at age 50 days and the rats followed up for 50 weeks. The intervention consisted of feeding the rats a 60% HFD followed by 50% DR (with carbohydrate restriction) for 4 cycles of 20% weight loss, followed by refeeding; this resulted in a 50% reduction in mammary tumour incidence, from 17.6% to 8.8% [not significant]. The body weight of the IDR rats fluctuated and at termination of the study was significantly lower than that of the HFD control rats. [The Working Group noted that a control group with chronic DR is missing.]

In another model (Matthews et al., 2014), ovary-intact female Sprague-Dawley rats were injected with 50 mg/kg of MNU at age 21 days. Obesity-resistant or obesity-prone animals were fed a purified diet containing 32% of calories from fat. At termination of the study, obesity-prone rats were approximately 15.5% heavier than obesity-resistant rats. Obesity-resistant rats had lower incidence, multiplicity, and burden of mammary carcinomas, with a concomitant increase in cancer latency compared with obesity-prone rats ($P < 0.01$ for all analyses).

Another model for breast cancer is the ACI rat; when supplementary estrogen is given to ovary-intact animals, this leads to development of mammary tumours (Shull et al., 1997). Harvell et al. (2002) determined the impact of 40% DR starting at age 7 weeks on mammary tumorigenesis in this model. By 216 days of estrogen treatment, 100% of the AL rats had at least one palpable mammary tumour, with the first tumour detected at 69 days. In contrast, the first palpable mammary tumour in the 40% DR group was not detected until 104 days of estrogen treatment, and at termination of the study, mammary tumour incidence was 59%. As expected, body weight was reduced for the 40% DR rats. No tumours were detected in ACI rats not treated with estrogen, whether they were fed AL or subjected to 40% DR.

(c) Calorie restriction mimetics

An additional approach to study the effect of CR on mammary tumour development has been the use of CR mimetics. Metformin, the most common CR mimetic, did not have an effect on mammary tumour development in the MNU rat model, when MNU was administered at age 50 days, or in transgenic HER2/neu/p53KO mice (Thompson et al., 2015). Several earlier studies using the HER2 mouse model of breast cancer had reported some effects of metformin on latency, but tumour incidence was not affected (Anisimov et al., 2005, 2010).

In the rapidly emerging tumour model in rats, treatment with 2-deoxyglucose (Jiang et al., 2008a) but not with metformin (Thompson et al., 2015; Zhu et al., 2015) decreased the incidence of mammary tumours; buformin and phenformin both reduced tumour incidence.

3.3.2 Cancer of the colon

See Table 3.11.

The early studies of the effect of DR on cancer of the colon used carcinogen-induced models in rats, whereas more recent studies used mouse models.

In one of the early studies, male Lobund Sprague-Dawley rats were administered methylazoxymethanol at 30 mg/kg at weaning, and about 25% DR started either 10 days or 63 days after and continued until 140 days after administration of methylazoxymethanol (Pollard et al., 1984). [It is not clear what the natural ingredient diet contained.] The long-term DR significantly reduced tumour incidence and multiplicity, whereas there was no effect when DR was initiated

Table 3.11 Studies on the prevention of colon tumours by dietary/calorie restriction in rats and mice

Species, strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
Rat					
Lobund Sprague-Dawley (M) Weanling *n* = 76 20 wk Pollard et al. (1984)	MAM, s.c., 30 mg/kg bw	Natural ingredient diet L-485; AL or 25% DR, starting at either 10 d or 63 d after MAM, or ADF from 8 d or 31 d after MAM (each intervention own AL group)	Tumours of colon and small intestine: AL, 90%; 25% DR-10, 30% AL, 85%; 25% DR-63, 100% AL, 60%; ADF-8, 60% AL, 90%; ADF-31, 67%	 $P < 0.0001$ NS NS NS	Small group sizes may have affected findings, and possibly components of diet had protective effect
F344 (M) 5 wk *n* = 60 32 wk Reddy et al. (1987)	AOM, 15 mg/kg bw, once a wk from age 7 wk for 2 wk	Semi-purified diet (23% fat); AL or 30% DR from 4 d after AOM, followed up for 32 wk	Colon adenoma or adenocarcinoma (combined): AL, 83%; 30% DR, 33% Colon adenocarcinoma: AL, 30%; 30% DR, 0%	$P < 0.05$ $P < 0.05$	Tumour multiplicity significantly reduced by DR
Sprague-Dawley (F, M) Neonatal *n* = 179 32 wk Newberne et al. (1990)	DMH, s.c., 10 mg/kg bw, once a wk for 10 wk from 1 mo after weaning	Two groups: pups raised 4/litter or 8/litter; weaned to semi-purified diet (20% fat) AL Third group: 4/litter pair-fed to 8/litter after weaning	Colon tumours: 8/litter: M, 48%; F, 42% 4/litter: M, 85%*; F, 60%* 4/litter pair-fed: M, 76%**; F, 52%	*$P < 0.01$ vs respective 8/litter M or F **$P < 0.01$ vs 8/litter M	[The Working Group considered that using litter size as an indicator of early-life access to nutrition made it difficult to evaluate the effect of DR on colon tumour development]
Zucker lean and obese (*fa/fa*) (F) 6 wk *n* = 32 obese, *n* = 16 lean 15 wk Raju & Bird (2003)	AOM, 10 mg/kg bw, at age 6 wk, once a wk for 2 wk	Lean rats, AL Obese rats, AL Obese rats, 20–25% DR	Multiplicity of ACF: All ACF Advanced ACF (≥ 7 crypts)	 NS $P < 0.001$ for DR vs obese AL	100% incidence of ACF in all groups; bw not affected by DR [Small *n* values; effect seen only when distinguishing between advanced and early foci, which is questionable]
Mouse					
C57BL6 *Apc*^{Min} (M) 10 wk 28–30/group 9 wk Mai et al. (2003)	Transgenic	AIN-76A 25% DR (CHO restriction)	Colon polyps: Mean number/mouse: AL, 5; 25% DR, 7	NS	Bw reduced in DR vs AL (19.4 g vs 25.9 g). No effect of feeding a HFD (30% of calories from corn oil) [Values reported in graph]

Table 3.11 (continued)

Species, strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
C57BL6 (M) 6 wk *n* = 80 (20/group) 23 wk Wheatley et al. (2008)	MC38 murine adenocarcinoma cells injected at wk 15	HFD (60% fat) for 7 wk, then divided into 4 groups: HFD (60% fat + 20% protein + 20% CHO) LCD (60% fat + 35% protein + 5% CHO) HCD (10% fat + 20% protein + 70% CHO) 30% DR (10% fat + 20% protein + 70% CHO)	Allograft tumour: Tumour latency: HFD, 11.2 d; LCD, 11.4 d; 30% DR, 20.1 d Tumour size : HFD, 397.2 mm^2; LCD, 351.6 mm^2; HCD, 474.6 mm^2; 30% DR, 162.4 mm^2*	NS *$P < 0.001$ vs all the other groups	Bw of HFD and LCD mice higher than other groups; HCD mice maintained bw; 30% DR mice lost bw and then maintained it
BALB/c (M) 4 wk 21/group 24 wk Park et al. (2012)	CT26 murine colon carcinoma cells inoculated at age 20 wk, and tumours harvested after 31 d	LFD (10% fat) for 16 wk HFD (60% fat) for 16 wk	Allograft tumour: Tumour volume increase from 3 wk after cell injection (measured in situ) Tumour weight (g): LFD, 1.2; HFD, 1.5	$P < 0.05$ $P < 0.05$	Metastasis to lung [number of tumour nodules] higher in HFD vs LFD ($P < 0.05$) Bw slightly higher, by 5.9%, in HFD vs LFD [BALB/c mice resistant to HFD]
C57BL6 (F) 7 wk 15/group 25 wk Harvey et al. (2013)	MC38 murine adenocarcinoma cells inoculated at age 29 wk, and tumours harvested after 24 d	AIN-76A 30% DR (CHO restriction) for 25 wk	Allograft tumour: Tumour volume: AL, 2286 mm^3; 30% DR, 515 mm^3	$P < 0.001$	Bw of DR mice remained stable over the 22 wk of restriction, whereas AL mice gained bw ($P < 0.05$)
FVB (M) 10 wk 12/group up to 20 wk Olivo-Marston et al. (2014)	AOM, 10 mg/kg bw, once a wk for 5 wk	AIN-76A 30% DR (CHO restriction) for 5, 10, or 20 wk HFD (45% fat)	Colon tumours: Multiplicity after 20 wk: AL, 9.5; 30% DR, 7.2*	*$P < 0.05$ vs AL	30% DR mice lost bw and then maintained it; increase in bw and tumour number in HFD group [Values read from graph]

ACF, aberrant crypt foci; ADF, alternate-day fasting; AL, ad libitum; AOM, azoxymethane; bw, body weight; CHO, carbohydrate; d, day or days; DMH, dimethylhydrazine; DR, dietary restriction; F, female; F344, Fischer 344; HCD, high-carbohydrate diet; HFD, high-fat diet; LCD, low-carbohydrate diet; LFD, low-fat diet; M, male; MAM, methylazoxymethanol; mo, month or months; NS, not significant; s.c., subcutaneous; vs, versus; wk, week or weeks.

in the older rats. Alternate-day fasting was also initiated at either 8 days or 31 days after administration of methylazoxymethanol, but there was no effect of this intervention. [The low number of animals per group in this experiment may have affected the study conclusions.]

Several other studies used the carcinogen AOM to induce cancer of the colon. For example, Reddy et al. (1987) fed F344 male rats a HFD AL or 30% DR from age 5 weeks. They were treated with an AOM regimen beginning at age 7 weeks and followed up until age 32 weeks. No adenocarcinomas were detected in the 30% DR group, compared with a 30% incidence in the AL rats. In a study with Zucker rats, AL lean and AL obese rats were used, as well as a 20–25% DR obese group, fed 75–80% of the consumption of the AL lean rats. After 8 weeks of DR, there was no effect on the multiplicity of total aberrant crypt foci in the colon (Raju & Bird, 2003). [The Working Group noted the small *n* values; an effect was seen when distinguishing between advanced and early foci, which is questionable.]

Another approach by Newberne et al. (1990) to study the effect of body weight on development of colon cancer was to use pups obtained from litter sizes adjusted to either four or eight. Dimethylhydrazine was administered from 1 month after weaning for 10 weeks. In addition to rats raised in litter sizes of four or eight, some pups from the litters of four were pair-fed to the pups from the litters of eight. Tumour incidence was significantly higher in male and female rats raised in the smaller litters when fed AL compared with the corresponding groups raised in the larger litters. Pair-fed male rats from litters of four had a higher incidence of colon tumours than the male rats from litters of eight. [The Working Group considered that the use of litter size as an indicator of food intake made interpretation difficult for the evaluation of the effect of DR on colon tumour development.]

More recent studies on the effect of DR on colon cancer development have focused on mice models. Transgenic *Apc*^{Min} mice that develop "spontaneous" tumours were subjected to 25% DR for 9 weeks and compared with AL mice (fed AIN-76A-based diets). DR had no effect on the number of colon polyps. In addition, feeding a HFD (30% of calories from fat) had no effect (Mai et al., 2003). In another study, AOM was used to induce colon tumours in FVB male mice. AL mice were fed the AIN-76A diet and compared with mice subjected to 30% DR (with carbohydrate restriction). Mean numbers of colon tumours were significantly reduced after 20 weeks of 30% DR (Olivo-Marston et al., 2014).

Allograft implants of colon cancer cell lines have also been used to assess the effects of DR on tumour growth. MC38 cells were used in two different studies (Wheatley et al., 2008; Harvey et al., 2013). In one study, female C57BL6 mice were fed the AIN-76A diet or were subjected to 30% DR (with carbohydrate restriction) from age 7 weeks (Harvey et al., 2013); the cells were implanted at age 29 weeks, and tumours were harvested 24 days later. Tumour volume was reduced significantly in the 30% DR mice compared with the AL mice (515 mm^3 vs 2286 mm^3). In the other study (Wheatley et al., 2008), male C57BL6 mice were fed a HFD (60% of calories from fat) for 7 weeks and then divided into four experimental groups, including a group subjected to 30% DR (10% of calories from fat). MC38 cells were injected at week 15. Tumour size was reduced in the 30% DR group compared with all the other experimental groups. [The Working Group noted that this study assessed DR in obese mice.]

The CT26 murine carcinoma cell line, which was developed from BALB/c mice, was used to evaluate tumour growth in LFD versus HFD mice (Park et al., 2012). The HFD group was fed from age 4–20 weeks; the cells were inoculated at age 20 weeks, and the mice continued on their respective diets for an additional 31 days. Body weight was only slightly higher in the HFD mice than in the LFD mice, whereas tumour volume

and weight were significantly higher in the HFD group. Metastasis to the lungs, as determined by the number of tumour nodules, was significantly higher in the HFD mice. [This study is of interest because – although it did not use DR – the BALB/c mice were resistant to the HFD.]

3.3.3 Cancer of the liver

See Table 3.12.

Comprehensive studies of nutrition and ageing, conducted in collaboration with the United States Food and Drug Administration's National Center for Toxicological Research and the United States National Institute on Aging provided pathological data on mice subjected to 40% DR (Blackwell et al., 1995; Sheldon et al., 1995, 1996). In scheduled-sacrificed female $B6C3F_1$ mice, no liver tumours were found in DR mice until age 30 months, whereas in AL mice, 4.9% (2 of 41 mice) had liver tumours at 24 months and 13.3% (2 of 15 mice) had liver tumours at 30 months (Sheldon et al., 1995). At 36 months, the incidences were similar between female AL and DR mice, at 42.9% (6 of 14 mice) and 33.3% (5 of 15 mice), respectively. In male $B6C3F_1$ mice, the incidence increased with advancing age in the AL group, and the incidence was significantly lower in the DR group than in the AL group at 24 months and 36 months. Necropsy data from mice that died spontaneously or were killed when moribund also showed that the incidence of liver tumours was lower in female and male DR mice, compared with the respective control AL mice. In C57BL6 mice, the incidence of liver tumours in scheduled-sacrificed male and female AL mice was less than 5%, and no tumours were found in the DR group (Blackwell et al., 1995). The incidence of liver tumours in male mice at necropsy was also lower in the DR group than in the AL group; there was no significant difference in female mice between the AL group and the DR group at necropsy.

In a study by the United States National Toxicology Program (NTP, 1997), administration of salicylazosulfapyridine (SASP) in the feed decreased the body weights of male $B6C3F_1$ mice by 15% in the 2-year bioassay. To eliminate a possible effect of the weight reduction by SASP on the occurrence of neoplasms, a weight-matched control group was included, in which the food intake was restricted by 13–22% to reduce the body weight to the level of AL mice treated with SASP (15% less than for the untreated AL group). The incidence of hepatocellular adenomas or of carcinomas was lower in the weight-matched control group, although this difference was not statistically significant. However, the incidence of adenoma and carcinoma combined was significantly lower in the weight-matched control group compared with the untreated AL group. Although the DR level in the 40% DR group was double (i.e. 40%), and therefore body weight was lower in the 40% DR group than in the weight-matched controls, the incidences of adenoma, of carcinoma, and of adenoma and carcinoma combined were not significantly lower even when compared with the untreated AL group. In contrast, in the animals treated with SASP, the 40% DR group had a significantly lower incidence of hepatocellular adenoma ($P < 0.001$), of carcinoma ($P < 0.05$), and of adenoma and carcinoma combined ($P < 0.001$), compared with the SASP AL controls.

In the National Toxicology Program feeding study of scopolamine hydrobromide trihydrate (SHT), the untreated weight-matched group (20% lower body weight compared with the AL group) and the DR group had lower incidences of hepatocellular adenoma, and of adenoma and carcinoma combined, although for carcinoma the difference was not statistically significant (NTP, 1997). In animals treated with SHT, the 40% DR group had a significantly lower incidence of hepatocellular adenoma ($P < 0.05$), of carcinoma ($P < 0.05$), and of adenoma and carcinoma combined ($P < 0.01$), compared with

Table 3.12 Studies on the prevention of tumours of the liver by dietary/calorie restriction in mice

Strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration, duration of study	Type of diet, dosing regimen, and duration of intervention	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
Swiss OF1 (M) Weanling (21 d) 14–20/group 48 wk Lagopoulos et al. (1991)	NDEA, 0.4 µmol/g bw, i.p., at birth	Nafag 857 diet, fed AL or 30% DR, from age 12 wk, killed at 24, 36, 48 wk	Liver G6Pd foci; adenoma; carcinoma; adenoma or carcinoma (combined):		
		Control (AL):			
		12 wk	17/20, 0/20, 0/20, 0/20		
		24 wk	17/20, 16/20, 0/20, 16/20		
		36 wk	11/18, 18/18, 5/18, 18/18		
		48 wk	2/14, 9/14, 6/14, 14/14		
		30% DR:			
		12 wk	7/19*, 0/19, 0/19, 0/19	*$P < 0.05$ vs AL group, χ^2 test	
		24 wk	16/20, 0/20*, 0/20, 0/20*	*$P < 0.001$ vs AL group, Fisher exact test	
		36 wk	16/17*, 12/17, 1/17, 12/17*	*$P < 0.05$ vs AL group, χ^2 test	
		48 wk	15/16*, 10/16, 0/16**, 10/16***	*$P < 0.001$, **$P < 0.01$, ***$P < 0.05$ vs AL group, χ^2 test or Fisher exact test	
C57BL6 (F, M) 4 wk 266/group SS at 12–36 mo, or lifelong Blackwell et al. (1995)	Spontaneous neoplasms	NIH-31 diet, fed AL or 40% DR, from age 14 wk	Hepatocellular tumours, mostly adenoma or carcinoma (%) (0–33 mo):	*$P < 0.01$ vs controls, Fisher exact test	Incidence of liver tumours was small; therefore, data of 0–27 mo and 28–33 mo were combined
		Control (AL)	**F:** 4/115 (*3.5%*), **M:** 18/156 (*11.5%*)		
		40% DR	**F:** 2/100 (*2%*), **M:** 2/106 (*1.9%*)*		

Table 3.12 (continued)

Strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration, duration of study	Type of diet, dosing regimen, and duration of intervention	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
$B6C3F_1$ (F, M) 4 wk 266/group SS at 12–42 mo, or lifelong Sheldon et al. (1995)	Spontaneous neoplasms	NIH-31 diet, fed AL or 40% DR; DR increased gradually: 10% at 14 wk, 25% at 15 wk, and 40% at age 16 wk	Hepatocellular tumours, mostly adenoma or carcinoma (%): 0–27 mo, 28–40 mo	*$P < 0.05$, **$P < 0.001$ vs controls, Fisher exact test	Data of necropsied mice from the SS group and the lifespan study group were combined AL: 31 hepatocellular neoplasms, 13 of them carcinomas; 7 metastasized to the lungs 40% DR: 18 hepatocellular neoplasms, 9 of them carcinomas; 1 metastasized to the lungs
		Control (AL)	**F:** 7/56 (*12.5%*), 14/131 (*10.7%*) **M:** 13/39 (*33.3%*), 48/118 (*40.7%*)		
		40% DR	**F:** 0/10 (*0%*), 1/72 (*1.4%*)* **M:** 0/7 (*0%*), 1/45 (*2.2%*)**		
$B6D2F_1$ (F, M) 4 wk 56/group Lifelong Sheldon et al. (1996)	Spontaneous neoplasms	NIH-31 diet, fed AL or 40% DR	Benign and malignant liver tumours:	*[NS]; **[$P < 0.001$]	
		Control (AL)	**F:** 13%, **M:** 24%		
		40% DR	**F:** 4%*, **M:** 2%**		
$B6C3F_1$ (M) 6 wk 50 or 52/group 104 wk NTP (1997)	SASP, 2700 mg/kg bw in corn oil by gavage, once a day, 5 d/wk	NIH-07 diet, fed AL or (on average) 15% DR (weight-matched to SASP group), or 40% DR	Hepatocellular adenoma; carcinoma; adenoma or carcinoma (combined):		
		Untreated, AL	13/50, 13/50, 24/50	*$P < 0.05$ vs untreated AL group, χ^2 test	
		Untreated, 15% DR	8/50, 6/50, 14/50*		
		Untreated, 40% DR	13/52, 7/52, 18/52	*$P < 0.001$, **$P < 0.05$ vs SASP AL group, χ^2 test or Fisher exact test	
		SASP, AL	42/50, 8/50, 44/50		
		SASP, 40% DR	9/50*, 1/50**, 9/50*		

Table 3.12 (continued)

Strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration, duration of study	Type of diet, dosing regimen, and duration of intervention	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
$B6C3F_1$ (M) 6 wk 70/group 104 wk NTP (1997)	SHT, 25 mg/kg bw in corn oil by gavage, once a day, 5 d/wk	NIH-07 diet, fed AL or (on average) 15% DR (weight-matched to SHT group), or 40% DR	Hepatocellular adenoma; carcinoma; adenoma or carcinoma (combined):		Untreated weight-matched group had 20% lower bw compared with AL group
		Untreated, AL	26/50, 6/50, 30/50	*$P < 0.001$ vs untreated AL group, χ^2 test	
		Untreated, 15% DR	5/50*, 5/50, 10/50*		
		Untreated, DR	3/50*, 2/50, 5/50*	*$P < 0.001$ vs untreated AL group, χ^2 test or Fisher exact test	
		SHT, AL	8/50*, 7/50, 15/50**	*$P < 0.001$, **$P < 0.01$ vs untreated AL group, χ^2 test	
		SHT, 40% DR	0/50*, 1/50*, 1/50**	*$P < 0.05$, **$P < 0.001$ vs SHT AL group, Fisher exact test	

AL, ad libitum; bw, body weight; d, day or days; DR, dietary restriction; F, female; G6Pd, glucose-6-phosphatase-deficient; i.p., intraperitoneal; M, male; mo, month or months; NDEA, *N*-nitrosodiethylamine; NS, not significant; NTP, National Toxicology Program; SASP, salicylazosulfapyridine; SHT, scopolamine hydrobromide trihydrate; SS, scheduled-sacrificed; vs, versus; wk, week or weeks.

the SHT AL controls. [The protective effect on adenoma development may have been due to decreased food intake and was not a direct effect of administration of SHT.]

In male Swiss OF1 mice treated with *N*-nitrosodiethylamine, the incidence of glucose-6-phosphatase-deficient pre-neoplastic foci was significantly lower in the 30% DR group compared with the AL group at age 12 weeks. In the AL group, 80% of the mice had hepatocellular adenoma or carcinoma at 24 weeks, and 100% at 36 weeks and 48 weeks. The incidence of hepatocellular neoplasms (adenoma and carcinoma combined) was significantly lower in the 30% DR group than in the AL group at 24 weeks, 36 weeks, and 48 weeks (Lagopoulos et al., 1991).

3.3.4 Cancer of the pancreas

See Table 3.13.

The incidence of spontaneous pancreatic tumours is very low in mice, on both C57BL6 and B6C3F$_1$ backgrounds (Blackwell et al., 1995; Sheldon et al., 1995). LSL-*Kras*^{G12D}; Pdx-1/Cre mice, a genetic model, develop pancreatic precursor lesions such as PanIN, which progress to PDAC. In one study (Lanza-Jacoby et al., 2013), two regimens for 25% DR were used with this model. One was CDR at 25% less than the AL average intake; the other was IDR, i.e. 50% restriction for 1 week after 100% provision of AL intake for 1 week, and therefore IDR also reduced the calorie intake by 25% over the 2-week interval. The body weight of the IDR mice fluctuated according to calorie intake. In the 100% feeding week, body weights were similar to those in the AL group; in the 50% restriction week, body weights were lower than those in the CDR mice. The incidence of PanIN was lower in both the CDR and IDR groups than in the AL group, with a greater effect of DR in the IDR regimen than in the CDR regimen. PDAC was found in the AL group, whereas no PDAC was observed in the CDR and IDR groups at age 44 weeks.

Another genetic model uses FVB-Tg (BK5. COX-2) mice and calculates a composite score for pancreatic dysplasia. In the study of Lashinger et al. (2011), formation of severe dysplasia in pancreatic ducts was lower in the 30% DR group than in the AL group. When tumour cells were injected into wild-type mice, tumour weight at 4 weeks after injection was significantly lower in 30% DR mice. Lashinger et al. (2013) used *Kras*^{G12D}/*Ink4a*$^{+/-}$ male mice and observed longer median survival in 30% DR mice than in AL mice, and no PDAC in 30% DR mice compared with 3 PDAC in AL mice.

In Sprague-Dawley rats, 4–6% of the AL group rats spontaneously developed pancreatic islet adenomas or carcinomas (Keenan et al., 1995). The overall incidence of pancreatic islet neoplasms over a 2-year study seemed to be lower in the 35% DR group. [The baseline incidence was low, and therefore the effect of 35% DR on spontaneous islet neoplasms could not be evaluated.] In another study in Sprague-Dawley rats (Molon-Noblot et al., 2001), the incidence of adenomas was 24% and 18% in female and male AL groups, respectively. The inhibition of adenomas by three different levels of DR was significant in female rats, whereas the effect of DR was modest in male rats. In a third study in male Sprague-Dawley rats (Duffy et al., 2008), the incidence of islet adenomas was lower in the 31% DR group than in the AL group, but this difference was not statistically significant.

In male Lewis rats, the effect of DR on the post-initiation phase of pancreatic carcinoma induced by azaserine was assessed (Roebuck et al., 1993). Feeding AL for a limited time (5–6 hours per day), designated as a "meal-fed" regimen, reduced the food intake to an equivalent of 10–15% DR relative to AL. The meal-fed regimen significantly reduced the incidence of adenomas and carcinomas 14 months after azaserine initiation.

In male Syrian golden hamsters, the incidence of pancreatic carcinoma, induced by BOP, did not differ among control AL, 20% CR, and 40% CR groups (Birt et al., 1997). However, the

Table 3.13 Studies on the prevention of tumours of the pancreas by dietary/calorie restriction in experimental animals

Species, strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration, duration of study	Type of diet, dosing regimen, and duration of intervention	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
Mouse					
FVB-Tg (BK5.COX-2) (F, M) 6 wk 24/group 20 wk Lashinger et al. (2011)	Genetic model for dysplastic lesions	Control diet (Research Diets, Inc., New Brunswick, NJ, #D12450B), fed AL or 30% DR	Composite score for pancreatic dysplasia: AL: 10 ± 4 30% DR: 5 ± 4**	$^{**}P < 0.01$	
FVB-WT (F, M) 6 wk 24/group 20 wk Lashinger et al. (2011)	JC101 pancreatic tumour cell injection at age 13–15 wk	Control diet (Research Diets, Inc., New Brunswick, NJ, #D12450B), fed AL or 30% DR	Tumour weight 4 wk after s.c. transplantation: AL: 1.05 ± 0.38 g 30% DR: 0.47 ± 0.30 g*	$^{*}P < 0.05$	
LSL-*Kras*^{G12D}; Pdx-1/Cre (M) 6 wk 31/group 44 wk Lanza-Jacoby et al. (2013)	Genetic model for PDAC	Modified AIN-93 diets, fed AL or 25% DR (CDR) or IDR, 6–44 wk	PanIN (PanIN-2 or greater); PDAC: Control (AL): 70%, 27% (n = 11) CDR: 40%*, 0% (n = 15) IDR: 27%*/#, 0% (n = 16)	$^{*}P < 0.05$ vs AL control group, exact Poisson regression analysis $^{*}P < 0.05$ vs AL control group, $^{\#}P < 0.05$ vs CDR group, exact Poisson regression analysis	IDR: 50% DR for 1 wk, then 100% of AL intake for 1 wk
Kras^{G12D}/*Ink4a*$^{+/-}$ (M) 6 wk 42–43/group 10 wk or 56 wk Lashinger et al. (2013)	Genetic model for PanIN-2 or PDAC	Control diet (Research Diets, Inc., New Brunswick, NJ, #D12450B), fed AL or 30% DR, from age 6–9 wk	PanIN (PanIN-2 or greater); PDAC: 10 wk study: AL: 7/15 (46.7%), 3/15 (20%) 30% DR: 9/15 (60%), 0/15 (0%) 56 wk study, median survival: AL: 20.0 wk 30% DR: 30.0 wk*	NS $^{*}P < 0.001$, log-rank test	

Table 3.13 (continued)

Species, strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration, duration of study	Type of diet, dosing regimen, and duration of intervention	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
Rat					
Lewis (M) 2 wk 22–23/group 14 mo Roebuck et al. (1993)	Azaserine, 30 mg/kg bw, i.p., once, at age 2 wk	AIN-76A diet, fed AL or 10–15% DR by meal-feeding Control	Pancreatic adenoma (%); carcinoma (%); adenoma multiplicity; carcinoma multiplicity: AL: 23/23 (100%), 15/23 (65%), 8.04 ± 0.99, 1.60 ± 0.214 10–15% DR: 5/22 (23%)*, 0/22 (0%)*, 1.00 ± 0#, 0	*$P < 0.05$ vs control group, χ^2 test or Fisher exact test #$P < 0.05$ vs AL group, ANOVA with Bonferroni test	
Sprague-Dawley [Crl:CD® (SD) BR] (F, M) 36 d 70/group 106 wk Keenan et al. (1995)	Spontaneous neoplasms	Purina Certified Rodent Chow 5002 or 5002-9 at various regimens Chow 5002: Control (AL) DR 6.5 h (AL for 6.5 h) 35% DR Chow 5002-9: Control (AL) DR (fed at the same calorie intake as rats fed 35% DR chow 5002)	Pancreatic islet adenoma (%); islet carcinoma (%): **F:** 3/70 (*4.3%*), 0/70 (*0%*) **M:** 4/70 (*6.2%*), 4/70 (*6.2%*) **F:** 1/70 (*1.4%*), 1/70 (*1.4%*) **M:** 2/70 (*2.9%*), 7/70 (*10%*) **F:** 1/70 (*1.4%*), 0/70 (*0%*) **M:** 5/70 (*7.1%*), 1/70 (*1.4%*) **F:** 3/70 (*4.3%*), 0/70 (0%) **M:** 3/70 (*4.3%*), 5/70 (*7.1%*) **F:** 1/70 (*1.4%*), 2/70 (*2.9%*) **M:** 3/70 (*4.3%*), 0/70 (*0%*)	 — NS NS NS NS — NS NS	Chow 5002: 21.4% protein, 5.7% fat, 4.1% crude fibre, energy value 3.07 kcal/g; Chow 5002-9: 13.6% protein, 4.6% fat, 15.7% crude fibre, energy value 2.36 kcal/g Baseline incidence was low, so that the effect of DR could not be evaluated

Table 3.13 (continued)

Species, strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration, duration of study	Type of diet, dosing regimen, and duration of intervention	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
Sprague-Dawley [Crl:CD® (SD) IGS BR] (F, M) 7 wk 15/group 100 wk Molon-Noblot et al. (2001)	Spontaneous neoplasms	Purina Certified Rodent Diet, 21–28% DR, 28–32% DR, 52–53% DR	Pancreatic islet adenoma (%); islet carcinoma (%):	*$P < 0.05$ vs AL group, Fisher exact test	
		Control (AL)	**F:** 12/50 (24%), 2/50 (4%) **M:** 9/50 (18%), 3/50 (6%)		
		21–28% DR	**F:** 3/52* (5.8%), 0/52 (0%) **M:** 16/50 (32%), 5/50 (10%)		
		28–32% DR	**F:** 3/52* (5.8%), 0/52 (0%) **M:** 12/50 (24%), 0/50* (0%)		
		52–53% DR	**F:** 1/51* (2%), 0/51 (0%) **M:** 4/50 (8%), 3/50 (6%)		
Sprague-Dawley [Crl:CD® (SD) BR] (M) 6 wk 40 or 60/group 108 wk Duffy et al. (2008)	Spontaneous neoplasms	AIN-93M diet, fed AL or 31% DR from age 6–114 wk	Pancreatic islet adenoma: AL: 11.8% ($n = 57$) 31% DR: 6.7% ($n = 38$)	NS (poly-3 test)	
Hamster					
Syrian golden (M) 8 wk 25–35/group Up to 44 wk Birt et al. (1997)	BOP, 20 mg/kg bw, 3 weekly s.c. injections	Control diet, fed AL or 20% CR or 40% CR for 42–44 wk	Pancreatic carcinoma; multiplicity: AL: 17/29, 0.9 ± 0.2 20% CR: 19/29, 1.2 ± 0.2 40% CR: 17/26, 1.7 ± 0.3*	Incidence: NS Multiplicity: *$P < 0.02$ vs control	

AL, ad libitum; ANOVA, analysis of variance; BOP, *N*-nitrosobis(2-oxopropyl)amine; bw, body weight; CDR, chronic dietary restriction; CR, calorie restriction; d, day or days; DR, dietary restriction; F, female; h, hour or hours; IDR, intermittent dietary restriction; i.p., intraperitoneal; M, male; mo, month or months; NS, not significant; PanIN, pancreatic intraepithelial neoplasia; PDAC, pancreatic ductal adenocarcinoma; s.c., subcutaneous; vs, versus; wk, week or weeks; WT, wild-type.

multiplicity of pancreatic carcinoma was greater in the 40% CR group than in the AL group.

3.3.5 Cancer of the skin and subcutaneous tissue

See Table 3.14.

Lifespan studies using mice and rats have indicated the low incidence of spontaneous skin tumours (Blackwell et al., 1995; Sheldon et al., 1995). In one study, less than 2% of scheduled-sacrificed male B6C3F$_1$ mice had skin or subcutaneous tumours (Sheldon et al., 1995). In contrast, female mice had relatively high incidences of skin or subcutaneous tumours. However, there was no statistical difference between the AL and DR groups for either sex (Sheldon et al., 1995). [The occurrence of skin tumours could be delayed in the DR group.]

Topical application of benzo[*a*]pyrene is one of the models used to induce skin tumours in rodents. Boutwell et al. (1949) demonstrated that 50% DR reduced the incidence of skin carcinoma induced by benzo[*a*]pyrene.

Birt et al. (1991) reported the effect of DR on either the initiation or the promotion phase of a chemically induced skin carcinoma model in female SENCAR mice. They used two regimens for 40% DR: one was a total DR of the control diet (TDR), and the other involved CR using a diet that was low in fat and glucose but high in protein and fibre. Mice that were subjected to 40% TDR or CR at the initiation phase had reduced weight gain, and the subsequent AL regimen led to recovery of the weight gain within 4 weeks. Mice that were subjected to 40% TDR and CR starting at the promotion phase had significantly lower body weight according to the energy restriction levels. Neither 40% TDR nor CR at the initiation phase affected the incidence or multiplicity of skin papilloma or the incidence of skin carcinoma. Mice that were subjected to 40% TDR and CR starting at the promotion phase had significantly lower incidence of skin papilloma and skin carcinoma.

Birt et al. (1993) tested the effect of 35% CR from fat or carbohydrate in the chemically induced DMBA skin tumour model in female SENCAR mice. The incidence of skin carcinoma was significantly lower in the groups subjected to CR from either fat or carbohydrate, and there was no difference in incidence between the two CR regimens, although papilloma multiplicity was greater in the carbohydrate-restricted (and thus HFD) group, compared with the fat-restricted group [no statistics were reported]. A subsequent study indicated that moderate (i.e. 20%) CR of a HFD or a LFD was ineffective, suggesting the importance of the level of CR (Birt et al., 1996).

Birt et al. (1994) also tested the effect of 40% DR in the two-stage promotion protocol, which comprised early-stage treatment with DMBA at age 9 weeks, promotion by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) for 2 weeks, and subsequent late-stage promotion by mezerein for 15 weeks. The individual effect of DR on the promotion phase by either TPA or mezerein was evaluated. Mice subjected to DR at age 10–27 weeks recovered their weight loss once they returned to AL feeding with a control diet at age 28 weeks. However, those mice subjected to DR during the period of treatment with TPA and/or mezerein had a reduction of approximately 10% in body weight compared with the control AL mice at age 71 weeks. DR during the entire period of promotion, i.e. at age 10–27 weeks (DR/DR group), and DR during the period of treatment with mezerein, at age 12–27 weeks (AL/DR group), significantly reduced the incidence and multiplicity of skin papilloma at 28 weeks; the cumulative incidence of skin carcinoma was also reduced (not significantly) in the DR/DR group and the AL/DR group. However, 2-week DR feeding during TPA treatment was insufficient to produce an inhibitory effect of DR on skin tumorigenesis.

Table 3.14 Studies on the prevention of tumours of the skin and subcutaneous tissue by dietary/calorie restriction in mice

Strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention (if not until termination of study)	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
Rockland (F) ~2–3 mo 48/group 173 d Boutwell et al. (1949)	60 µg of benzo[*a*]pyrene (skin application), twice a wk for 19 wk from 26 d after beginning of DR	High-calorie, low-fat (AL), or low-calorie, low-fat (50% DR) Control (AL) 50% DR	Skin carcinoma: 32/39 8/44**	$^{**}P < 0.01$, χ^2 test	
C57BL/6 (M) 4.5 mo 7–10/group 5–6 wk Ershler et al. (1986)	10^5 B16 melanoma cells injected s.c., 2 wk after beginning of DR	Purina Laboratory Chow, fed AL or 40% DR Control (AL) 40% DR	Tumour volume (mm^3)/1000 (mean ± SE): 7.9 ± 1.5 2.3 ± 0.5*	$^{*}P < 0.05$, Student *t* test	
SENCAR (F) 6 wk or 10 wk 15–30/group Up to 56 wk Birt et al. (1991)	10 nmol of DMBA (skin application), once at age 9 wk; 3.2 nmol of TPA (skin application), twice a wk for 20 wk from age 10 wk	AIN; fed AL or 40% DR by TDR or CR, from 6–9 wk or 10–56 wk Control (AL) 40% TDR, 6–9 wk 40% TDR, 10–56 wk 40% CR, 6–9 wk 40% CR, 10–56 wk	Skin papilloma (%) at age 30 wk; papilloma multiplicity at age 34 wk (mean ± SE); skin carcinoma (%) until age 56 wk 27/30 (90%), 5.7 ± 0.4, 15/24 (71%) 24/30 (80%), 4.9 ± 0.9, 14/24 (58%) 11/20 (55%)***, 2.2 ± 0.4***, 7/17 (41%)* 25/29 (89%), 6.6 ± 0.9, 11/17 (69%) 13/23 (56%)***, 1.8 ± 0.4***, 5/15 (28%)*	— NS $^{***}P < 0.001$, $^{*}P < 0.05$ vs AL, χ^2 test or ANOVA NS $^{***}P < 0.001$, $^{*}P < 0.05$ vs AL, χ^2 test or ANOVA	Both TDR and CR from 10–56 wk lowered bw by 30% No significant difference in tumour incidence and multiplicity between TDR and CR groups [Multiplicity at age 34 wk read from figure]

Table 3.14 (continued)

Strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention (if not until termination of study)	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
CD-1 (F) ~6–7 wk 42–59/group 100 d Pashko & Schwartz (1992)	51.2 µg of DMBA (skin application), once at age 6 wk or 7 wk; 2 µg of TPA (skin application), twice a wk for 82 d from 1 wk after beginning of DR	Purina 5015 chow, fed AL or ~35% DR from age 8–9 wk Control (AL) DR	Number of skin papillomas/mouse: 5.5 0.9*	*$P < 0.01$, Wilcoxon Mann–Whitney sum test	
SENCAR (F) 6 wk 38–42/group Up to 68 wk Birt et al. (1993)	10 nmol of DMBA (skin application), once at age 9 wk; 2.0 µg of TPA (skin application), twice a wk for 20 wk from age 10 wk	AIN diet fed AL, balanced high fat (BHD) AL, 35% CR with high carbohydrate (HCD), 35% CR with high fat (HFD), from age 10 wk Control AL BHD AL 35% CR HCD 35% CR HFD	Number of papillomas/mouse at 28 wk after DMBA; time to carcinoma at 50% incidence 6.5, ~40 wk 6.2, ~44 wk 1.5*, > 59 wk# 3.2*, > 59 wk#	*$P < 0.05$ vs control and BHD AL #$P < 0.01$ vs control and BHD AL, log-rank test	Bw reduced in HCD and HFD vs control AL and BHD AL groups; no difference between HCD and HFD, or between control AL and BHD AL
SENCAR (F) 7 wk 30–52/group Up to 62 wk Birt et al. (1994)	10 nmol of DMBA (skin application), once at age 9 wk; 3.2 nmol of TPA (skin application), twice a wk at 10–11 wk, and 10 nmol of MEZ twice a wk at age 12–27 wk	Ingredient diet, fed AL or 40% DR at various regimens Control (AL), age 7–71 wk 40% DR, 10–27 wk; AL, 28–71 wk 40% DR, 10–11 wk; AL, 12–71 wk AL, 10–11 wk; DR, 12–27 wk; AL, 28–71 wk	Skin papilloma; papilloma multiplicity at age 28 wk; skin carcinoma (%) at age 71 wk: 42%, 0.9, 14/48 (29%) 17%*, 0.3*, 3/31# (10%) 54%, 0.9, 10/30 (33%) 17%*, 0.2*, 5/33 (15%)	— *$P < 0.05$, #NS vs AL, χ^2 test or Fisher exact test NS *$P < 0.05$ vs AL	Bw loss of ~10% in DR mice vs AL at age 71 wk Multiplicity was tested by a Poisson random variable, using the generalized estimating equation approach of Liang and Zeger, and the estimation of regression coefficients

Table 3.14 (continued)

Strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention (if not until termination of study)	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
$B6C3F_1$ (F, M) 4 wk 245–266/group SS at 12–42 mo, or lifelong Sheldon et al. (1995)	Spontaneous neoplasms	NIH-31 diet, fed AL or 40% DR; DR increased gradually: 10% at 14 wk, 25% at 15 wk, and 40% at age 16 wk; SS at age 12, 18, 24, 30, 36, 42 mo	Skin tumours (%): 0–27 mo, 28–33 mo, 34–39 mo, 40–51 mo:	NS	Incidence of spontaneous skin tumours was low even in the AL groups
		Control (AL)	**M:** 1/72 (1.4%), 1/83 (1.2%), 0/65 (0%), 0/15 (0%) **F:** 4/97 (4.1%), 10/110 (9.1%), 1/50 (2%), 0/1 (0%)		
		40% DR	**M:** 0/49 (0%), 0/27 (0%), 0/48 (0%), 0/138 (0%) **F:** 1/52 (1.9%), 2/38 (5.3%), 6/64 (9.4%), 3/111 (2.7%)		
SENCAR (F) 9 wk 35/group 45 wk Birt et al. (1996)	10 nmol of DMBA (skin application), once at age 9 wk; 3.2 nmol of TPA (skin application), twice a wk for 18 wk from age 10 wk	Fed AL, 20% or 40% CR from fat or carbohydrate using LFD (10% fat) or HFD (42% fat), from age 10 wk	Skin carcinoma (%):		
		Control: LFD AL 20% CR of LFD 40% CR of LFD HFD AL 20% CR of HFD 40% CR of HFD	35% 20% 15%* 35% 35% 0%*	*$P < 0.05$ vs LFD AL, HFD AL, and 20% CR of HFD	
ICR (M), *Nrf2* gene knockout 10 wk 10–15/group up to 42 wk Pearson et al. (2008)	25 µg of DMBA (skin application), once at age 15 wk or 16 wk; 4 µg of TPA (skin application), twice a wk, from 2 wk after DMBA until appearance of first papilloma	Teklad 2018 diet, fed AL or 20% DR, 30% DR, or 40% DR for 5–6 wk	Skin tumour: time at 25% and 50% incidence:		Kaplan–Meier survival analysis was performed to compare the 2 curves of papilloma occurrence Time of appearance of tumour was recorded when at least 1 papilloma with a radius > 1 mm was identified
		Control (AL) 20% DR 30% DR 40% DR	13 wk, 15 wk 18 wk, 25 wk (< 50% incidence) 18 wk, 20 wk 42 wk, 42 wk	— $P < 0.05$	

Table 3.14 (continued)

Strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention (if not until termination of study)	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
129S1/SvImJ (M) 3–5 mo 11–13/group 18 wk Minor et al. (2011)	25 µg of DMBA (skin application), once; 4 µg of TPA (skin application), twice a wk, from 2 wk after DMBA until appearance of first papilloma	AIN-93G diet, fed AL or 30% DR	Skin papilloma (%); multiplicity ± SD	*$P < 0.0083$ vs WT AL, Bonferroni *t* tests	[Values read from graph]
		Control (AL)	100%, 4.0 ± 2.7	—	
		30% DR	58%, 1.0 ± 1.1*		
ICR (F) 7 wk 30/group 58 wk Moore et al. (2012)	25 nmol of DMBA (skin application), once at age 7 wk; 3.4 nmol of TPA, twice a wk (skin application), from age 15 wk for 50 wk	AIN-76A diet, fed AL,15% DR, or 30% DR from age 11 wk	Skin papilloma (%) at age 30 wk; multiplicity at age 39 wk; skin carcinoma (%) at age 65 wk; multiplicity at age 65 wk:		Bw lower in both DR groups
		Control (AL)	81%, 8.2, 92%, 1.6	—	
		15% DR	81%, 6.2*, 69%#, 1.6	*$P < 0.05$ vs AL, Mann–Whitney *U* test #$P < 0.05$ vs AL, χ^2 test	
		30% DR	68%, 4.3*, 58%#, 1.0*	*$P < 0.05$ vs AL, Mann–Whitney *U* test #$P < 0.05$ vs AL, χ^2 test	

AL, ad libitum; ANOVA, analysis of variance; BHD, balanced high-fat diet; bw, body weight; CR, calorie restriction; d, day or days; DMBA, 7,12-dimethylbenz[*a*]anthracene; DR, dietary restriction; F, female; HCD, high-carbohydrate diet; HFD, high-fat diet; LFD, low-fat diet; M, male; MEZ, mezerein; mo, month or months; NS, not significant; s.c., subcutaneous; SD, standard deviation; SE, standard error; SS, scheduled-sacrificed; TDR, total dietary restriction; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; vs, versus; wk, week or weeks; WT, wild-type.

Moore et al. (2012) used female ICR mice to assess the effect of 15% DR or 30% DR on the promotion of skin tumours. At the end of the experiment, the body weights were 36% lower in the 30% DR group and 15% lower in the 15% DR group, compared with the control AL group. The cumulative incidences of skin papilloma in the experimental groups did not differ significantly among these groups. However, the multiplicity of skin papilloma was significantly lower in the 15% DR and 30% DR groups compared with the AL group. The incidences of skin carcinoma were also significantly lower in the 15% DR and 30% DR groups; the multiplicity of skin carcinoma was significantly lower only in the 30% DR group (Moore et al., 2012).

In the B16 melanoma cell injection model, the tumour volume was significantly lower in C57BL/6 mice subjected to 40% DR compared with AL mice (Ershler et al., 1986).

Using the two-stage skin tumorigenesis model in CD-1 mice treated with DMBA, Pashko & Schwartz (1992) reported that DR suppressed TPA promotion of skin papillomas.

The preventive effect of 40% DR on DMBA-TPA-induced skin tumours was diminished in ICR mice (Pearson et al., 2008) and in 129S mice (Minor et al., 2011).

3.3.6 Cancer of the pituitary gland

See Table 3.15.

In lifespan studies in mice, the incidence of pituitary tumours is very low in males (Blackwell et al., 1995; Sheldon et al., 1995, 1996).

The incidence in female scheduled-sacrificed C57BL6 control mice was reported to be 14% at 24 months and 64% at 30 months. In DR mice, no pituitary tumours were found at 24 months and 30 months. Necropsies of mice that died spontaneously or were killed when moribund also indicated a significant reduction in the incidence of pituitary tumours in the female DR group compared with the AL group (Blackwell et al., 1995). In female B6C3F$_1$ and B6D2F$_1$ mice, the incidence of spontaneously occurring pituitary tumours was also lower in the DR group than in the AL group (Sheldon et al., 1995, 1996).

In a 2-year study in Sprague-Dawley rats fed 35% DR with either a standard diet or a low-protein, high-fibre diet, incidences of spontaneous pituitary adenomas were very high in both male and female AL rats (Keenan et al., 1995). In male rats, both 35% DR groups had lower incidences of pituitary adenomas. In contrast, in female rats the preventive effect of 35% DR was observed only in the 35% DR group fed the low-protein, high-fibre diet and not in the 35% DR group fed the standard diet.

In a lifespan study in F344 rats (Thurman et al., 1994), the incidence of pituitary tumours in the 40% DR group was significantly lower than in the AL group in both female and male rats. The mean age at death of rats bearing pituitary tumours was also higher in the 40% DR group compared with the AL group in both male and female rats.

Estrogen stimulates the proliferation of prolactin-producing lactotrophs, and therefore continuous administration of estrogen promotes the development of prolactin-producing tumours in the rat. Pituitary weight can be measured as a quantitative indicator of estrogen-induced pituitary tumour development, because increased weight correlates with increases in pituitary cell number and DNA content. Several studies (Shull et al., 1998; Spady et al., 1998, 1999; Harvell et al., 2001) have used this model to study pituitary tumour development, and some have shown a reduction of tumour development with 40% DR. [In this model, response to DR for the inhibition of tumours depends on the strain of rat used. These results are confounded by the fact that body weight was reduced by estrogen administration in the AL group, whereas it was not significantly reduced in the DR group. In addition, there was no indication of histopathology or of tumour incidence. Therefore, these studies are regarded

Table 3.15 Studies on the prevention of tumours of the pituitary gland by dietary/calorie restriction in mice and rats

Species, strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention (if not until termination of study)	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
Mouse					
C57BL6 (F) Weanling 266/group SS at 12–36 mo, or lifelong Blackwell et al. (1995)	Spontaneous neoplasms	NIH-31 open formula diet, fed AL or 40% DR from age 4 wk; SS at 12, 18, 24, 30, 36 mo	Pituitary tumours (%): Mice SS at 0–24 mo, 30 mo:	[*$P < 0.05$, **$P < 0.0005$ vs controls, Fisher exact test]	
		Control (AL)	6/43 (14%), 9/14 (64%)		
		40% DR	0/44 (0%)*, 0/15 (0%)**		
			Pituitary tumours (%): Mice that died spontaneously or were killed when moribund in the SS and the lifespan study groups (0–27 mo, 28–33 mo):	*$P < 0.001$ vs controls, Fisher exact test	
		Control (AL)	17/75 (22.7%), 31/40 (77.5%)		
		40% DR	0/50 (0%)*, 2/50 (4%)*		
$B6C3F_1$ (F) Weanling 266/group SS at 12–36 mo, or lifelong Sheldon et al. (1995)	Spontaneous neoplasms	NIH-31 open formula diet, fed AL or 40% DR from age 4 wk; SS at 12, 18, 24, 30, 36 mo	Pituitary tumours: Mice SS at 24 mo, 30 mo, 36 mo:	NS	
		Control (AL)	0/41, 4/15, 3/14		
		40% DR	0/42, 1/15, 0/15		
			Pituitary tumours (%): Mice that died spontaneously or were killed when moribund in the SS and the lifespan study groups (0–27 mo, 28–33 mo, 34–39 mo):	*$P < 0.001$ vs controls, Fisher exact test	
		Control (AL)	3/56 (5.4%), 13/95 (13.7%), 15/36 (41.7%)		
		40% DR	0/10 (0%), 0/23 (0%)*, 1/49 (2.0%)*		

Table 3.15 (continued)

Species, strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention (if not until termination of study)	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
$B6D2F_1$ (F) Weanling 4 wk 56/group Lifelong Sheldon et al. (1996)	Spontaneous neoplasms	NIH-31 open formula diet, fed AL or 40% DR Control (AL) 40% DR	Pituitary tumours: 17% 0%	NR	
Rat					
F344 (F, M) 4 wk 54/group SS at 18–30 mo, or lifelong Thurman et al. (1994)	Spontaneous neoplasms	NIH-31 open formula diet, fed AL or 40% DR, from age 14 wk Control (AL) 40% DR Control (AL) 40% DR	Pituitary tumours: Mice SS at 18 mo, 24 mo, 30 mo: **F:** 3/10, 11/12, 11/12 **M:** 6/10, 9/11, 9/9 **F:** 0/10, 4/12*, 4/12* **M:** 2/12, 3/12**, 7/12 Pituitary tumours: Mice that died spontaneously or were killed when moribund: **F:** 118/160, **M:** 92/128 **F:** 60/137*, **M:** 55/152*	*$P < 0.01$, **$P < 0.05$ vs controls, Fisher exact test *$P < 0.01$ vs controls, χ^2 test	Mean age at death of 40% DR rats bearing pituitary tumours was higher compared with AL rats in both sexes

Table 3.15 (continued)

Species, strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention (if not until termination of study)	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
Sprague-Dawley [Crl:CD® (SD) BR] (F, M) 36 d 70/group 106 wk Keenan et al. (1995)	Spontaneous neoplasms	Purina Certified Rodent Chow 5002 (energy value: 3.07 kcal/g) or 5002-9 (energy value: 2.36 kcal/g), fed at various regimens Chow 5002: Control (AL) DR 6.5 h (AL for 6.5 h) 35% DR Chow 5002-9: Control (AL) DR (fed at the same calorie intake as rats fed 65% DR chow 5002)	Pituitary adenoma: **F:** 50/70, **M:** 40/70 **F:** 49/70, **M:** 32/70 **F:** 47/70, **M:** 28/70* **F:** 55/70, **M:** 37/70 **F:** 31/70*, **M:** 19/70**	 — NS *$P < 0.05$ vs controls, χ^2 test NS *$P < 0.001$, **$P < 0.01$ vs controls, χ^2 test	No difference in incidence of pituitary focal hyperplasia between groups
F344 (M) 32 d 6–8/group 9 wk Shull et al. (1998)	Silastic tubing implants containing 5 mg of DES, s.c. at age 39 d; animals killed 8 wk after DES	Ingredient diet, fed AL or 40% DR Control (AL) 40% DR	Prolactin-producing pituitary tumour: Fold increase of the weight vs DES-untreated counterparts; pituitary-to-bw ratio (bw ± SD): 11.2-fold, 55.8×10^{-5} (125 ± 7 g) 3.5-fold*, 18.4×10^{-5} (80 ± 3 g)	*$P < 0.05$ vs controls	Pituitary weight was measured as a quantitative indicator of estrogen-induced pituitary development DES treatment reduced the food intake and thus bw by ~50% in both AL and DR groups

Table 3.15 (continued)

Species, strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention (if not until termination of study)	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
Holtzman (M) 32 d 6–8/group 9 wk Shull et al. (1998)	Silastic tubing implants containing 5 mg of DES, s.c. at age 39 d; animals killed 8 wk after DES	Ingredient diet, fed AL or 40% DR Control (AL) 40% DR	Prolactin-producing pituitary tumour: Fold increase of the weight vs DES-untreated counterparts; pituitary-to-bw ratio (bw ± SD): 5.3-fold, 31.6×10^{-5} (263 ± 10 g) 4.1-fold, 30.2×10^{-5} (175 ± 5 g)	NS	Pituitary weight was measured as a quantitative indicator of estrogen-induced pituitary development DES treatment reduced the food intake and thus the bw by ~40% in both AL and DR groups
F344 (F-OVX) 57 d 5–8/group 11 wk Spady et al. (1998)	Silastic tubing implants containing 27.5 mg of E2, by s.c. injection at age 63 d; animals killed 10 wk after E2	Ingredient diet, fed AL, 25% DR, or 40% DR Control (AL) 25% DR 40% DR	Prolactin-producing pituitary tumour: Fold increase of the weight vs E2-untreated counterparts: 4.9-fold 4.1-fold 2.0-fold*	*$P < 0.05$ vs controls	Pituitary weight was measured as a quantitative indicator of estrogen-induced pituitary development
ACI (F-OVX) 35 d 12/group 22 wk Spady et al. (1999)	Silastic tubing implants containing 27.5 mg of E2, s.c. at age 45 d; animals killed 20 wk after E2	Ingredient diet, fed AL or 40% DR Control (AL) 40% DR	Prolactin-producing pituitary tumour: Fold increase of the weight vs E2-untreated counterparts; pituitary-to-bw ratio (bw): 5.3-fold, 31.6×10^{-5} (160 g) 4.1-fold, 30.2×10^{-5} (85 g)	NS	Pituitary weight was measured as a quantitative indicator of estrogen-induced pituitary development E2 treatment reduced the bw by 22% in AL rats and 21% in DR rats, vs respective untreated groups

Table 3.15 (continued)

Species, strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention (if not until termination of study)	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
COP (F-OVX) or ACI (F-OVX) 35 d 12/group 13 wk Harvell et al. (2001)	Silastic tubing implants containing 27.5 mg of E2, s.c. at age 45 d; animals killed 12 wk after E2	Ingredient diet, fed AL or 40% DR	Prolactin-producing pituitary tumour: Fold increase of the weight vs E2-untreated counterparts:	NR	Pituitary weight was measured as a quantitative indicator of estrogen-induced pituitary development
		COP			
		Control (AL)	2.4-fold		
		40% DR	1.5-fold		
		ACI			
		Control (AL)	3.4-fold		
		40% DR	4.5-fold		

AL, ad libitum; bw, body weight; d, day or days; DES, diethylstilbestrol; DR, dietary restriction; E2, 17β-estradiol; F, female; h, hour or hours; M, male; mo, month or months; NR, not reported; NS, not significant; OVX, ovariectomized; s.c.; subcutaneous; SD, standard deviation; SS, scheduled-sacrificed; vs, versus; wk, week or weeks.

as less informative and are considered to provide only supporting evidence.]

3.3.7 Cancer of the prostate

See Table 3.16.

Several rat models have been used to evaluate the effects of DR on prostate cancer development. Only one early study reported the effect of DR on spontaneous prostate cancer development in rats. Lobund-Wistar rats raised in conventional or germ-free conditions were followed up for up to 41 months. Rats subjected to 30% DR and raised in conventional conditions had reduced incidence of prostate adenocarcinoma compared with the AL rats (6% vs 26%), but among rats raised in germ-free conditions, the incidence was higher in the 30% DR rats than in the AL rats (10% vs 5%), although the overall incidence of prostate cancer was reduced for rats raised in germ-free conditions compared with those raised in conventional conditions (Pollard et al., 1989).

In a carcinogen-induced prostate cancer model, Wistar-Unilever rats were treated with the luteinizing hormone-releasing antagonist cyproterone, followed by treatment with testosterone, followed by administration of MNU to induce prostate cancer. Rats subjected to 20% DR had longer prostate cancer-free survival compared with AL rats, and this was accompanied by reduced body weight (Boileau et al., 2003). However, in a similar study, no effect of 15% DR or 30% DR on incidence of prostate cancer was reported, and DR did not reduce body weight gain (McCormick et al., 2007).

Transgenic models have also been used to determine the effect of DR on the development of prostate cancer in rodents. In the probasin/SV40 T antigen transgenic rat model, 30% DR had no effect on incidence of PIN or of adenocarcinoma but significantly reduced the percentage ratio of the epithelial area to the whole prostate area (which included PIN and tumour cells) (Kandori et al., 2005). There was reduced weight gain due to DR. [The Working Group noted the difficulty in performing morphometric measures in the prostate gland.]

Another model has been the TRAMP mouse. When TRAMP mice were subjected to 20% DR from age 7 weeks for 4 weeks or 13 weeks, lesions of a lower grade were reported compared with AL mice [no body weight information was provided] (Suttie et al., 2003). In a second study from the same group, 20% DR was not implemented until age 20 weeks and had no effect on survival or lesion severity [body weight data were not presented] (Suttie et al., 2005). In an additional study using TRAMP mice, two different modes of DR were used, with the same overall degree of restriction, i.e. 25% DR (Bonorden et al., 2009a, b). Mice that received IDR – 2 weeks of 50% DR with 2 weeks of AL feeding, for 11 cycles – had delayed time to prostate tumour detection, compared with both AL and 25% DR mice. Body weights were lower in the 25% DR mice than in the AL mice. [Although the findings are of interest, the Working Group noted the strong influence of the transgene as the mice age, thus possibly limiting the model's usefulness for evaluating the effect of DR on prostate cancer development.]

The Hi-Myc mouse model was also used to assess the effects of 30% DR or a HFD (60% fat) implemented at age 6 weeks compared with an AL group. At age 26 weeks, the incidence of prostate adenocarcinomas was 62% in the AL mice, compared with no tumours observed in the 30% DR group (Blando et al., 2011).

3.3.8 Cancers of the haematopoietic system

(a) Lymphoma

See Table 3.17.

Two lifespan studies in male B10C3F$_1$ (Weindruch & Walford, 1982) and C57BL/6 mice (Volk et al., 1994) started DR at age 45–50 weeks and assessed incidence of malignant lymphoma. DR (44% DR or 25% DR) significantly increased

Table 3.16 Studies on the prevention of tumours of the prostate by dietary/calorie restriction in rats and mice

Species, strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
Rat					
Lobund-Wistar (M) Weanling *n* = 100 up to 41 mo Pollard et al. (1989)	Spontaneous tumours in either conventional or germ-free housing	Cereal-based L-485 30% DR for 6, 18, 30 mo or > 30 mo	Prostate adenocarcinoma: Conventional AL, 26%; 30% DR, 6%* Germ-free AL, 5%; 30% DR, 10%	[*$P < 0.008$, Fisher exact test, 2-tailed]	A second study (Snyder et al., 1990), with the same design, reported similar results: 22%, 7% [NS], 7%, 6%
Wistar-Unilever (M) 6 wk *n* = 194 age 73 wk Boileau et al. (2003)	Cyproterone, 4 weekly i.p. injections; then testosterone, daily i.p. injection; then MNU, 50 mg/kg bw, at age 9 wk	AIN-93M for 4 wk; then half of all rats given 20% DR	Prostate adenocarcinoma: Cancer-free survival at 50 wk: AL, 35%; 20% DR, 52%	$P = 0.03$	Lower bw in DR rats than in AL rats Additional groups fed lycopene or tomato supplements AL or at 20% DR
Probasin/SV40 T antigen transgenic on Sprague-Dawley background (M) 6 wk *n* = 40 13 wk Kandori et al. (2005)	Probasin/SV40 T antigen	NIH-07 (soybean-free) 30% DR	Adenocarcinoma: Ratio of epithelial area to whole prostate area	NS $P < 0.01$ for ventral, lateral, or dorsal prostate	100% incidence of PIN in all groups Small *n* values; bw significantly lower in 30% DR group vs controls
Wistar-Unilever (M) 7–8 wk *n* = 159 52 wk McCormick et al. (2007)	Cyproterone, oral gavage for 21 d; then testosterone, daily s.c. for 3 d; then MNU, 30 mg/kg bw, at age ~12 wk	Purina 5001 laboratory chow 15% DR 30% DR	Prostate adenocarcinoma: Control, 74% 15% DR, 64% 30% DR, 72%	NS	30% DR had no effect on bw
Mouse					
TRAMP on C57BL6 background (M) 7 wk *n* = 10 13 wk Suttie et al. (2003)	TRAMP mice	NTP-2000 20% DR for 4 wk or 13 wk	Lower grade of lesions: 11 wk: ventral***, lateral***, dorsal***, and anterior** lobes 20 wk: ventral, lateral**, dorsal***, and anterior* lobes	*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, Mann–Whitney *U* test	

Table 3.16 (continued)

Species, strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
TRAMP on C57BL6 background (M) 20 wk *n* = 10 19 wk Suttie et al. (2005)	TRAMP mice	NTP-2000 20% DR for 4 wk, 12 wk, or 19 wk	No effect on grade of lesions Survival at 19 wk: AL, 75%; DR, 90%	NS NS	[Model not adequate for studying prostate cancer at later age]
TRAMP on C57BL6 background (M) 5 wk *n* = 130 48–50 wk Bonorden et al. (2009a)	TRAMP mice	Ain-93M fed AL, 25% DR, or 25% IDR (50% DR for 2 wk, then AL for 2 wk for 11 cycles)	Adenocarcinoma: Latency to detection: AL, 33 wk; 25% DR, 35 wk; IDR, 38 wk	$P < 0.006$, IDR vs AL $P = 0.39$, DR vs AL	Bw of DR mice fairly constant, whereas that of IDR mice fluctuated See also cross-sectional study by Bonorden et al. (2009b)
Hi-Myc FVB/N (M) 6–8 wk *n* = 36 age 26 wk Blando et al. (2011)	Transgenic Hi-Myc mice	AIN76A, fed AL, 30% DR, or HFD (60% fat)	Prostate invasive adenocarcinoma: AL, 62%; DR, 0%*; HFD, 97% Prostate in situ carcinoma: AL, 100%; DR, 38%*; HFD, 100%	*$P = 0.0001$, DR vs AL or HFD	[Values read from graph]

AL, ad libitum; bw, body weight; d, day or days; DR, dietary restriction; F, female; HFD, high-fat diet; IDR, intermittent dietary restriction; i.p., intraperitoneal; M, male; mo, month or months; MNU, *N*-methyl-*N*-nitrosourea; NR, not reported; NS, not significant; PIN, prostatic intraepithelial neoplasia; TRAMP, transgenic adenocarcinoma of the mouse prostate; vs, versus; wk, week or weeks.

Table 3.17 Studies on the prevention of lymphomas by dietary/calorie restriction in mice

Strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
B10C3F$_1$ (M) 45–50 wk 67–68/group Lifelong Weindruch & Walford (1982)	Spontaneous neoplasms	Semi-purified diet, fed AL or 44% DR with supplementation, from age 12–13 mo Control (160 kcal/wk) 44% DR (90 kcal/wk)	Lymphoma (%); mean lifespan with lymphoma: 47%, 31.9 mo 31%#, 36.2 mo*	#NS ($P < 0.08$) *$P < 0.01$, *t* test	Animals were killed when moribund
C57BL/6 (M) 45 wk 60–72/group Age 25 mo Volk et al. (1994)	Spontaneous neoplasms	AL or 25% DR, from age 12 mo Control (AL) 25% DR	Lymphoma (%): 14/72 (19%) 3/60 (5%)*	*$P < 0.05$, χ^2 test	
C57BL6 (F, M) 4 wk 266/group SS at 12–36 mo, or lifelong Blackwell et al. (1995)	Spontaneous neoplasms	NIH-31 open formula diet, fed AL or 40% DR. Mice were SS at 12, 18, 24, 30, and 36 mo, or necropsied when died spontaneously or were killed when moribund Control (AL) 40% DR	Lymphoma: Mice SS at 0–24 mo, 30 mo: **F:** 11/43, 5/14 **M:** 2/43, 2/14 **F:** 3/44*, 0/15 **M:** 2/44, 0/15	*$P < 0.05$ vs controls, Fisher exact test	SS mice were combined because of the low incidence of lymphoma
			Lymphoma (%): Mice that died spontaneously or were killed when moribund in the SS and the lifespan study groups (0–33 mo): Control (AL) 40% DR	*$P < 0.01$ vs controls, χ^2 test **F:** 35/115 (29.6%) **M:** 12/156 (7.7%) **F:** 13/100 (13%)* **M:** 9/106 (8.5%)	

Table 3.17 (continued)

Strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
Blackwell et al. (1995) (cont.)			Histiocytic sarcoma: SS at 0–24 mo, 30 mo:	NS	
		Control (AL)	**F:** 1/43, 5/14 **M:** 1/43, 4/14		
		40% DR	**F:** 3/44, 3/15 **M:** 1/44, 5/15		
			Histiocytic sarcoma (%): Mice that died spontaneously or were killed when moribund in the SS and the lifespan study groups (0–27 mo, 28–33 mo):	*$P < 0.001$ vs controls, Mantel–Haenszel χ^2 test	
		Control (AL)	**F:** 19/75 (25%), 18/40 (45%) **M:** 34/83 (41%), 54/73 (74%)		
		40% DR	**F:** 20/50 (40%), 27/50 (54%) **M:** 9/32 (28.1%), 31/74 (41.9%)*		
$B6C3F_1$ (F, M) 4 wk 266/group SS at 12–36 mo, or lifelong Sheldon et al. (1995)	Spontaneous neoplasms	NIH-31 open formula diet, fed AL or 40% DR. Mice were SS at 12, 18, 24, 30, and 36 mo, or necropsied when died spontaneously or were killed when moribund	Lymphoma (%): SS at 30 mo, 36 mo:	NS	Incidence of malignant lymphoma during the period 0–24 mo was < 3% in F and M mice of AL and DR groups
		Control (AL)	**F:** 4/15 (26.7%), 9/14 (64.3%) **M:** 3/15 (20.0%), 6/15 (40.0%)		
		40% DR	**F:** 0/15 (0%), 1/15 (6.7%) **M:** 0/15 (0%), 0/15 (0%)		
			Lymphoma (%): Mice that died spontaneously or were killed when moribund in the SS and the lifespan study groups (0–27 mo, 28–40 mo):	NS	
		Control (AL)	**F:** 24/56 (42.9%), 84/161 (52.2%) **M:** 8/39 (20.5%), 46/118 (39%)		

Table 3.17 (continued)

Strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
Sheldon et al. (1995) (cont.)		40% DR	**F:** 2/10 (20%), 28/72 (38.9%) **M:** 0/7 (0%), 13/45 (28.9%)		
			Histiocytic sarcoma (%): Mice that died spontaneously or were killed when moribund in the SS and the lifespan study groups (27–33 mo, 28–40 mo):	NS	
		Control (AL)	**F:** 7/95 (7.4%), 4/36 (11.1%) **M:** 9/68 (13.2%), 5/50 (10%)		
		40% DR	**F:** 3/23 (13%), 3/49 (6.1%) **M:** 4/12 (33%), 5/33 (15%)		
$p53^{-/-}$, $p53^{+/+}$ [94% C57BL/6, 6% 129/Sv] (M) 4–7 wk 28–30/group Lifelong Hursting et al. (1997)	Spontaneous neoplasms	AIN-76A diet, fed AL or 40% DR from 6–9 wk, lifelong	Lymphoma; mean time to death by lymphoma (*n*):	*$P < 0.05$ vs controls	Median time to death: 16 wk in $p53^{-/-}$ AL, 25 wk in $p53^{-/-}$ DR; 68 wk in $p53^{+/+}$ AL, 102 wk in $p53^{+/+}$ DR
		Control (AL)	$p53^{-/-}$: 17/30, 110 ± 50 d (16) $p53^{+/+}$: 4/30, 384 ± 187 d (4)		
		40% DR	$p53^{-/-}$: 19/28, 162 ± 59 d (16) $p53^{+/+}$: 6/30, 679 ± 198 d* (6)		
$p53^{+/-}$ C57BL6 (M) 10.5 mo 31–32/group 12.5 mo Berrigan et al. (2002)	Spontaneous neoplasms	AIN-76A diet, fed AL or 40% DR, or 1 d/wk fast (14% DR)	Number of mice that died from lymphoma/effective number of mice; mean lifespan:	*$P = 0.001$, **$P = 0.039$ vs AL group, Cox proportional hazards analysis (1-tailed)	Mean bw: ~50 g in AL, 27 g in 40% DR, 38 g in 1 d/wk fast
		Control (AL) 40% DR 1 d/wk fast (14% DR)	17/32, 313 ± 17 d 15/31, 388 ± 23 d* 15/31, 357 ± 23 d**		

AL, ad libitum; bw, body weight; d, day or days; DR, dietary restriction; F, female; M, male; mo, month or months; NS, not significant; SS, scheduled-sacrificed; vs, versus; wk, week or weeks.

the mean lifespan of mice with lymphoma or reduced the incidence of lymphoma.

In another study (Blackwell et al., 1995), the incidence of spontaneous malignant lymphoma was significantly lower in the female 40% DR group than the female AL group at 24 months and 30 months; however, there was no difference between the male 40% DR and AL groups. [Lymphoma incidence in male AL mice was substantially lower than that in female AL mice.]

In the same study, the incidence of histiocytic sarcoma [diffuse large B-cell lymphoma] in scheduled-sacrificed mice did not differ significantly between the 40% DR and AL groups in either male or female mice. However, the incidence of histiocytic sarcoma in mice that died spontaneously or were killed when moribund was significantly lower in the male DR group compared with the male AL group (Blackwell et al., 1995). [The Working Group noted that the study provided separate results for scheduled-sacrificed and moribund animals, which makes evaluation of the effect difficult.]

In another study in male and female $B6C3F_1$ mice, the incidence of malignant lymphoma was lower than 3% up to 24 months in both AL and 40% DR groups (Sheldon et al., 1995). At age 30 months and 36 months, the incidence was greater than 20% in both female and male AL mice, whereas it remained at mostly 0% in 40% DR mice. In mice that died spontaneously or were killed when moribund during the periods of 0–27 months and 28–40 months, the incidence was lower in the DR groups than in the AL groups of male and female mice, although the difference was not statistically significant. No difference was observed for histiocytic sarcomas.

Tp53-deleted mice display earlier occurrence of spontaneous neoplasms, including malignant lymphoma (Hursting et al., 1994). The incidence of malignant lymphoma in necropsied *Tp53*$^{-/-}$ mice subjected to 40% DR did not differ from that in their AL counterparts, but the mean time to death by lymphoma was longer in the 40% DR group than in the AL group (Hursting et al., 1997). In another study, p53$^{+/-}$ mice prone to malignant tumours including lymphoma (mostly histiocytic sarcoma) were subjected to adult-onset 40% DR (Berrigan et al., 2002). The mean lifespan was longer in the 40% DR group than in the AL group. Even with 1 day of fasting per week followed by AL feeding, the regimen reduced the body weight to 76% of that of the AL group and extended the lifespan, although the effect was modest ($P = 0.039$) compared with that observed in the 40% DR group.

(b) Leukaemia

See Table 3.18.

In long-term studies, F344 rats (particularly F344/N) often develop leukaemia, mostly mononuclear (large granular) cell leukaemia.

The incidence of leukaemia in rats killed at 24 months and 30 months did not differ between AL and 40% DR groups of male and female rats (Thurman et al., 1994). The proportion of rats bearing leukaemia that died spontaneously or were killed when moribund seemed to be greater in the 40% DR groups of male and female rats. However, the mean ages at death in rats found dead or killed when moribund were higher in the 40% DR groups of male and female rats than in the AL groups. [The number of animals may have been too small to allow relevant statistics; also, leukaemia incidence may have been increased in the DR rats because they lived longer.]

Peto et al. (1980) and Gart et al. (1986) have addressed the biases inherent to long-term animal studies in which lifespan is extended by an intervention such as DR, and have described statistical analyses to circumvent the problem. By following their statistical procedures, Shimokawa et al. (1996) estimated that the onset rate of leukaemia in F344 rats was reduced by 20% in the 40% DR rats compared with the AL animals.

Pathological data of F344/N rats generated in the National Toxicology Program study of butyl benzyl phthalate (NTP, 1997) indicated

Table 3.18 Studies on the prevention of leukaemia by dietary/calorie restriction in rats

Strain (sex) Age at start Number of animals Duration of study Reference	Route, dose, and duration of carcinogen administration	Type of diet, dosing regimen, and duration of intervention	Type of tumours: Tumour incidence (number of tumours/effective number of animals, and/or percentage), multiplicity, or other outcomes as specified	Statistical significance	Comments
F344 (F, M) 4 wk 54/group Lifelong Thurman et al. (1994)	Spontaneous neoplasms	NIH-31 open formula diet, fed AL or 40% DR gradually implemented over 2 wk from age 14 wk Control (AL) 40% DR	Leukaemia (%) at 24 mo, 30 mo: **F:** 1/12 (8.3%), 7/12 (58.3%) **M:** 6/12 (50%), 5/9 (55.6%) **F:** 4/12 (33.3%), 3/12 (25%) **M:** 7/12 (58.3%), 6/12 (50%)	NS	
F344 (M) 6 wk 153 or 155/group Shimokawa et al. (1996)	Spontaneous neoplasms	Semi-synthetic diet, fed AL or 40% DR Control (AL) 40% DR	Leukaemia (%); relative onset rate: 38/111 (34.2%), 1.00 39/89 (43.8%), 0.80*	*$P < 0.05$, Peto test	The relative onset rate, defined by Peto et al. (1980), is a descriptive index useful in determining whether a dietary modulation influences the occurrence of a neoplasm. The expected number of rats with leukaemia/lymphoma was calculated by analysing the death rate and the prevalence rate separately.
F344/N (F, M) 6 wk 60/group 104 wk NTP (1997)	Spontaneous neoplasms	NIH-07 open formula diet, fed AL; **M:** 20% DR between 14 wk and 52 wk and 7% DR between 53 wk and 101 wk; **F:** 25% DR between 14 wk and 52 wk and 30% DR between 53 wk and 104 wk Control (AL) Weight-matched control of a carcinogen testing protocol (7–30% DR) from 14 wk to 104 wk	Leukaemia (mostly mononuclear cell leukaemia); adjusted rate: **F:** 21/50, 51.7% **M:** 31/50, 71.8% **F:** 13/50, 28.6% **M:** 15/50*, 34.9%	*$P < 0.01$	Adjusted rate: Kaplan–Meier-estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

AL, ad libitum; DR, dietary restriction; F, female; F344, Fischer 344; M, male; mo, month or months; NS, not significant; NTP, National Toxicology Program; wk, week or weeks.

a reduction in the incidence of leukaemia in F344 rats in which daily feed allocations were restricted to 7–30% less than that of untreated AL rats, to weight-match the animals. When the rates were adjusted for intercurrent mortality, the Kaplan–Meier-estimated incidences of leukaemia were approximately 50% less than those in AL animals.

References

Algire C, Amrein L, Zakikhani M, Panasci L, Pollak M (2010). Metformin blocks the stimulative effect of a high-energy diet on colon carcinoma growth *in vivo* and is associated with reduced expression of fatty acid synthase. *Endocr Relat Cancer*, 17(2):351–60. doi:10.1677/ERC-09-0252 PMID:20228137

Anisimov VN, Berstein LM, Egormin PA, Piskunova TS, Popovich IG, Zabezhinski MA, et al. (2005). Effect of metformin on life span and on the development of spontaneous mammary tumors in HER-2/neu transgenic mice. *Exp Gerontol*, 40(8–9):685–93. doi:10.1016/j.exger.2005.07.007 PMID:16125352

Anisimov VN, Egormin PA, Piskunova TS, Popovich IG, Tyndyk ML, Yurova MN, et al. (2010). Metformin extends life span of HER-2/neu transgenic mice and in combination with melatonin inhibits growth of transplantable tumors in vivo. *Cell Cycle*, 9(1):188–97. doi:10.4161/cc.9.1.10407 PMID:20016287

Bergen HT, Mizuno TM, Taylor J, Mobbs CV (1998). Hyperphagia and weight gain after gold-thioglucose: relation to hypothalamic neuropeptide Y and pro-opiomelanocortin. *Endocrinology*, 139(11):4483–8. PMID:9794456

Berrigan D, Perkins SN, Haines DC, Hursting SD (2002). Adult-onset calorie restriction and fasting delay spontaneous tumorigenesis in p53-deficient mice. *Carcinogenesis*, 23(5):817–22. doi:10.1093/carcin/23.5.817 PMID:12016155

Birt DF, Barnett T, Pour PM, Copenhaver J (1996). High-fat diet blocks the inhibition of skin carcinogenesis and reductions in protein kinase C by moderate energy restriction. *Mol Carcinog*, 16(2):115–20. doi:10.1002/(SICI)1098-2744(199606)16:2<115::AID-MC8>3.0.CO;2-H PMID:8645426

Birt DF, Pelling JC, Anderson J, Barnett T (1994). Consumption of reduced-energy/low-fat diet or constant-energy/high-fat diet during mezerein treatment inhibited mouse skin tumour promotion. *Carcinogenesis*, 15(10):2341–5. doi:10.1093/carcin/15.10.2341 PMID:7955075

Birt DF, Pelling JC, White LT, Dimitroff K, Barnett T (1991). Influence of diet and calorie restriction on the initiation and promotion of skin carcinogenesis in the SENCAR mouse model. *Cancer Res*, 51(7):1851–4. PMID:1900738

Birt DF, Pinch HJ, Barnett T, Phan A, Dimitroff K (1993). Inhibition of skin tumor promotion by restriction of fat and carbohydrate calories in SENCAR mice. *Cancer Res*, 53(1):27–31. PMID:8416746

Birt DF, Pour PM, Nagel DL, Barnett T, Blackwood D, Duysen E (1997). Dietary energy restriction does not inhibit pancreatic carcinogenesis by *N*-nitrosobis(2-oxopropyl)amine in the Syrian hamster. *Carcinogenesis*, 18(11):2107–11. doi:10.1093/carcin/18.11.2107 PMID:9395209

Birt DF, Salmasi S, Pour PM (1981). Enhancement of experimental pancreatic cancer in Syrian golden hamsters by dietary fat. *J Natl Cancer Inst*, 67(6):1327–32. PMID:6273636

Blackwell BN, Bucci TJ, Hart RW, Turturro A (1995). Longevity, body weight, and neoplasia in ad libitum-fed and diet-restricted C57BL6 mice fed NIH-31 open formula diet. *Toxicol Pathol*, 23(5):570–82. doi:10.1177/019262339502300503 PMID:8578100

Blando J, Moore T, Hursting S, Jiang G, Saha A, Beltran L, et al. (2011). Dietary energy balance modulates prostate cancer progression in Hi-Myc mice. *Cancer Prev Res (Phila)*, 4(12):2002–14. doi:10.1158/1940-6207.CAPR-11-0182 PMID:21952584

Bobe G, Barrett KG, Mentor-Marcel RA, Saffiotti U, Young MR, Colburn NH, et al. (2008). Dietary cooked navy beans and their fractions attenuate colon carcinogenesis in azoxymethane-induced *ob*/*ob* mice. *Nutr Cancer*, 60(3):373–81. doi:10.1080/01635580701775142 PMID:18444172

Boileau TWM, Liao Z, Kim S, Lemeshow S, Erdman JW Jr, Clinton SK (2003). Prostate carcinogenesis in *N*-methyl-*N*-nitrosourea (NMU)-testosterone-treated rats fed tomato powder, lycopene, or energy-restricted diets. *J Natl Cancer Inst*, 95(21):1578–86. doi:10.1093/jnci/djg081 PMID:14600090

Boissonneault GA, Elson CE, Pariza MW (1986). Net energy effects of dietary fat on chemically induced mammary carcinogenesis in F344 rats. *J Natl Cancer Inst*, 76(2):335–8. PMID:3080637

Bonorden MJL, Grossmann ME, Ewing SA, Rogozina OP, Ray A, Nkhata KJ, et al. (2012). Growth and progression of TRAMP prostate tumors in relation to diet and obesity. *Prostate Cancer*, 2012:543970. doi:10.1155/2012/543970 PMID:23304522

Bonorden MJL, Rogozina OP, Kluczny CM, Grossmann ME, Grambsch PL, Grande JP, et al. (2009a). Intermittent calorie restriction delays prostate tumor detection and increases survival time in TRAMP mice. *Nutr Cancer*, 61(2):265–75. doi:10.1080/01635580802419798 PMID:19235043

Bonorden MJL, Rogozina OP, Kluczny CM, Grossmann ME, Grande JP, Lokshin A, et al. (2009b). Cross-sectional analysis of intermittent versus chronic caloric restriction in the TRAMP mouse. *Prostate*, 69(3):317–26. doi:10.1002/pros.20878 PMID:19016490

Boutwell RK, Brush MK, Rusch HP (1949). The stimulating effect of dietary fat on carcinogenesis. *Cancer Res*, 9(12):741–6. PMID:15395908

Brandon EL, Gu J-W, Cantwell L, He Z, Wallace G, Hall JE (2009). Obesity promotes melanoma tumor growth: role of leptin. *Cancer Biol Ther*, 8(19):1871–9. doi:10.4161/cbt.8.19.9650 PMID:19713740

Buison AM, Pellizzon MA, Brogan KE, Barnes MJ, Jen CK-L (2005). Weight cycling did not increase tumor incidence in high fat-fed rats treated with a low-dose 7,12-dimethylbenzyl(1)anthracene. *Nutr Res*, 25(12):1097–108. doi:10.1016/j.nutres.2005.09.003

Cleary MP (2013). Impact of obesity on development and progression of mammary tumors in preclinical models of breast cancer. *J Mammary Gland Biol Neoplasia*, 18(3–4):333–43. doi:10.1007/s10911-013-9300-x PMID:24122258

Cleary MP, Grande JP, Juneja SC, Maihle NJ (2004b). Diet-induced obesity and mammary tumor development in MMTV-neu female mice. *Nutr Cancer*, 50(2):174–80. doi:10.1207/s15327914nc5002_7 PMID:15623464

Cleary MP, Grande JP, Maihle NJ (2004a). Effect of high fat diet on body weight and mammary tumor latency in MMTV-TGF-α mice. *Int J Obes Relat Metab Disord*, 28(8):956–62. doi:10.1038/sj.ijo.0802664 PMID:15254485

Cleary MP, Grossmann ME (2011). The manner in which calories are restricted impacts mammary tumor cancer prevention. *J Carcinog*, 10(1):21. doi:10.4103/1477-3163.85181 PMID:22013391

Cleary MP, Hu X, Grossmann ME, Juneja SC, Dogan S, Grande JP, et al. (2007). Prevention of mammary tumorigenesis by intermittent caloric restriction: does caloric intake during refeeding modulate the response? *Exp Biol Med (Maywood)*, 232(1):70–80. PMID:17202587

Cleary MP, Jacobson MK, Phillips FC, Getzin SC, Grande JP, Maihle NJ (2002). Weight-cycling decreases incidence and increases latency of mammary tumor development to a greater extent than does chronic restriction in mouse mammary tumor virus-transforming growth factor-α female mice. *Cancer Epidemiol Biomarkers Prev*, 11:836–43. PMID:12223427

Cleary MP, Juneja SC, Phillips FC, Hu X, Grande JP, Maihle NJ (2004d). Leptin receptor deficient MMTV-TGF-α/*Lepr*^{db}*Lepr*^{db} female mice do not develop oncogene-induced mammary tumors. *Exp Biol Med*, 229:182–93. doi:10.1177/153537020422900207 PMID:14734797

Cleary MP, Phillips FC, Getzin SC, Jacobson TL, Jacobson MK, Christensen TA, et al. (2004c). Genetically obese MMTV-TGF-α/*Lep*^{ob}*Lep*^{ob} female mice do not develop mammary tumors. *Breast Cancer Res Treat*, 77(3):205–15. doi:10.1023/A:1021891825399 PMID:12602920

Cowey SL, Quast M, Belalcazar LM, Wei J, Deng X, Given R, et al. (2005). Abdominal obesity, insulin resistance, and colon carcinogenesis are increased in mutant mice lacking gastrin gene expression. *Cancer*, 103(12):2643–53. doi:10.1002/cncr.21094 PMID:15864814

Dawson DW, Hertzer K, Moro A, Donald G, Chang HH, Go VL, et al. (2013). High-fat, high-calorie diet promotes early pancreatic neoplasia in the conditional *Kras*^{G12D} mouse model. *Cancer Prev Res (Phila)*, 6(10):1064–73. doi:10.1158/1940-6207.CAPR-13-0065 PMID:23943783

Dinkova-Kostova AT, Fahey JW, Jenkins SN, Wehage SL, Talalay P (2008). Rapid body weight gain increases the risk of UV radiation-induced skin carcinogenesis in SKH-1 hairless mice. *Nutr Res*, 28(8):539–43. doi:10.1016/j.nutres.2008.05.009 PMID:19083457

Dogan S, Hu X, Zhang Y, Maihle NJ, Grande JP, Cleary MP (2007). Effects of high-fat diet and/or body weight on mammary tumor leptin and apoptosis signaling pathways in MMTV-TGF-α mice. *Breast Cancer Res*, 9(6):R91. doi:10.1186/bcr1840 PMID:18162139

Duffy PH, Lewis SM, Mayhugh MA, Trotter RW, Hass BS, Latendresse JR, et al. (2008). Neoplastic pathology in male Sprague-Dawley rats fed AIN-93M diet ad libitum or at restricted intakes. *Nutr Res*, 28(1):36–42. doi:10.1016/j.nutres.2007.09.018 PMID:19083386

Dunlap SM, Chiao LJ, Nogueira L, Usary J, Perou CM, Varticovski L, et al. (2012). Dietary energy balance modulates epithelial-to-mesenchymal transition and tumor progression in murine claudin-low and basal-like mammary tumor models. *Cancer Prev Res (Phila)*, 5(7):930–42. doi:10.1158/1940-6207.CAPR-12-0034 PMID:22588949

Ealey KN, Lu S, Archer MC (2008). Development of aberrant crypt foci in the colons of *ob*/*ob* and *db*/*db* mice: evidence that leptin is not a promoter. *Mol Carcinog*, 47(9):667–77. doi:10.1002/mc.20419 PMID:18240295

Engelman RW, Day NK, Chen RF, Tomita Y, Bauer-Sardiña I, Dao ML, et al. (1990). Calorie consumption level influences development of C3H/Ou breast adenocarcinoma with indifference to calorie source. *Proc Soc Exp Biol Med*, 193(1):23–30. doi:10.3181/00379727-193-42984 PMID:2294519

Engelman RW, Day NK, Good RA (1994). Calorie intake during mammary development influences cancer risk: lasting inhibition of C3H/HeOu mammary tumorigenesis by peripubertal calorie restriction. *Cancer Res*, 54(21):5724–30. PMID:7923222

Ershler WB, Berman E, Moore AL (1986). Slower B16 melanoma growth but greater pulmonary colonization in calorie-restricted mice. *J Natl Cancer Inst*, 76(1):81–5. PMID:3455745

Everitt JI, Alder R (2013). Issues in laboratory animal science: the role of diet in toxicology studies. In: Hascheck WM, Rousseaux CG, Wallig MA, Bolon B, Ochoa R, Mahler BW, editors. Haschek and Rousseaux's handbook of toxicologic pathology. 3rd edition. London, UK: Academic Press, Elsevier; pp. 634–40.

Frederich RC, Hamann A, Anderson S, Löllmann B, Lowell BB, Flier JS (1995). Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nat Med*, 1(12):1311–4. doi:10.1038/nm1295-1311 PMID:7489415

Gart JJ, Krewski D, Lee PN, Tarone RE, Wahrendorf J (1986). Statistical methods in cancer research. Volume III – The design and analysis of long-term animal experiments. *IARC Sci Publ*, 79:1–219. PMID:3301661

Gillette CA, Zhu Z, Westerlind KC, Melby CL, Wolfe P, Thompson HJ (1997). Energy availability and mammary carcinogenesis: effects of calorie restriction and exercise. *Carcinogenesis*, 18(6):1183–8. doi:10.1093/carcin/18.6.1183 PMID:9214601

Gravaghi C, Bo J, LaPerle KMD, Quimby F, Kucherlapati R, Edelmann W, et al. (2008). Obesity enhances gastrointestinal tumorigenesis in *Apc*-mutant mice *Int J Obes (London)*, 32(11):1716–9. doi:10.1038/ijo.2008.149 PMID:18725892

Hakkak R, Holley AW, Macleod SL, Simpson PM, Fuchs GJ, Jo CH, et al. (2005). Obesity promotes 7,12-dimethylbenz(*a*)anthracene-induced mammary tumor development in female Zucker rats. *Breast Cancer Res*, 7(5):R627–33. doi:10.1186/bcr1263 PMID:16168107

Hakkak R, MacLeod S, Shaaf S, Holley AW, Simpson P, Fuchs G, et al. (2007). Obesity increases the incidence of 7,12-dimethylbenz(*a*)anthracene-induced mammary tumors in an ovariectomized Zucker rat model. *Int J Oncol*, 30(3):557–63. PMID:17273756

Harvell DM, Spady TJ, Strecker TE, Lemus-Wilson AM, Pennington KL, Shen F, et al. (2001). Dietary energy restriction inhibits estrogen induced pituitary tumorigenesis in a rat strain specific manner. In: Li JJ, Li SA, Daling JR, editors. Hormonal carcinogenesis III: proceedings of the third international symposium. New York, USA: Springer; pp. 496–501.

Harvell DME, Strecker TE, Xie B, Pennington KL, McComb RD, Shull JD (2002). Dietary energy restriction inhibits estrogen-induced mammary, but not pituitary, tumorigenesis in the ACI rat. *Carcinogenesis*, 23(1):161–9. doi:10.1093/carcin/23.1.161 PMID:11756237

Harvey AE, Lashinger LM, Otto G, Nunez NP, Hursting SD (2013). Decreased systemic IGF-1 in response to calorie restriction modulates murine tumor cell growth, nuclear factor-κB activation, and inflammation-related gene expression. *Mol Carcinog*, 52(12):997–1006. doi:10.1002/mc.21940 PMID:22778026

Hayashi K, Suzuki R, Miyamoto S, Shin-Ichiroh Y, Kohno H, Sugie S, et al. (2007). *Citrus* auraptene suppresses azoxymethane-induced colonic preneoplastic lesions in C57BL/KsJ-*db*/*db* mice. *Nutr Cancer*, 58(1):75–84. doi:10.1080/01635580701308216 PMID:17571970

Heston WE, Vlahakis G (1961). Influence of the A^y gene on mammary-gland tumors, hepatomas, and normal growth in mice. *J Natl Cancer Inst*, 26:969–83. PMID:13713953

Heston WE, Vlahakis G (1962). Genetic obesity and neoplasia. *J Natl Cancer Inst*, 29:197–209. PMID:13907025

Hill-Baskin AE, Markiewski MM, Buchner DA, Shao H, DeSantis D, Hsiao G, et al. (2009). Diet-induced hepatocellular carcinoma in genetically predisposed mice. *Hum Mol Genet*, 18(16):2975–88. doi:10.1093/hmg/ddp236 PMID:19454484

Hirose Y, Hata K, Kuno T, Yoshida K, Sakata K, Yamada Y, et al. (2004). Enhancement of development of azoxymethane-induced colonic premalignant lesions in C57BL/KsJ-*db*/*db* mice. *Carcinogenesis*, 25(5):821–5. doi:10.1093/carcin/bgh059 PMID:14729596

Hursting SD, Dunlap SM, Ford NA, Hursting MJ, Lashinger LM (2013). Calorie restriction and cancer prevention: a mechanistic perspective. *Cancer Metab*, 1(1):10. doi:10.1186/2049-3002-1-10 PMID:24280167

Hursting SD, Lashinger LM, Brown PH, Perkins SN (2011). The utility of transgenic mouse models for cancer prevention research. In: Teicher BA, editor. Tumor models in cancer research (cancer drug discovery and development). 2nd edition. New York, USA: Springer Science+Business Media; pp. 423–43. doi:10.1007/978-1-60761-968-0_18

Hursting SD, Perkins SN, Brown CC, Haines DC, Phang JM (1997). Calorie restriction induces a p53-independent delay of spontaneous carcinogenesis in p53-deficient and wild-type mice. *Cancer Res*, 57(14):2843–6. PMID:9230186

Hursting SD, Perkins SN, Phang JM (1994). Calorie restriction delays spontaneous tumorigenesis in p53-knockout transgenic mice. *Proc Natl Acad Sci USA*, 91(15):7036–40. doi:10.1073/pnas.91.15.7036 PMID:8041741

Hursting SD, Smith SM, Lashinger LM, Harvey AE, Perkins SN (2010). Calories and carcinogenesis: lessons learned from 30 years of calorie restriction research. *Carcinogenesis*, 31(1):83–9. doi:10.1093/carcin/bgp280 PMID:19969554

IARC (2002). Weight control and physical activity. Lyon, France: IARC Press (IARC Handbooks of Cancer Prevention, Vol. 6). Available from: http://publications.iarc.fr/376.

Jiang W, Zhu Z, Thompson HJ (2008a). Modulation of the activities of AMP-activated protein kinase, protein kinase B, and mammalian target of rapamycin by limiting energy availability with 2-deoxyglucose.

Mol Carcinog, 47(8):616–28. doi:10.1002/mc.20425 PMID:18247380

Jiang W, Zhu Z, Thompson HJ (2008b). Dietary energy restriction modulates the activity of AMP-activated protein kinase, Akt, and mammalian target of rapamycin in mammary carcinomas, mammary gland, and liver. *Cancer Res*, 68(13):5492–9. doi:10.1158/0008-5472.CAN-07-6721 PMID:18593953

Kanasaki K, Koya D (2011). Biology of obesity: lessons from animal models of obesity. *J Biomed Biotechnol*, 2011:197636. doi:10.1155/2011/197636 PMID:21274264

Kandori H, Suzuki S, Asamoto M, Murasaki T, Mingxi T, Ogawa K, et al. (2005). Influence of atrazine administration and reduction of calorie intake on prostate carcinogenesis in probasin/SV40 T antigen transgenic rats. *Cancer Sci*, 96(4):221–6. doi:10.1111/j.1349-7006.2005.00041.x PMID:15819720

Keenan KP, Soper KA, Smith PF, Ballam GC, Clark RL (1995). Diet, overfeeding, and moderate dietary restriction in control Sprague-Dawley rats: I. Effects on spontaneous neoplasms. *Toxicol Pathol*, 23(3):269–86. doi:10.1177/019262339502300305 PMID:7659952

Keenan KP, Wallig MA, Haschek WM (2013). Nature via nurture: effect of diet on health, obesity, and safety assessment. *Toxicol Pathol*, 41(2):190–209. doi:10.1177/0192623312469857 PMID:23334694

Khalid S, Hwang D, Babichev Y, Kolli R, Altamentova S, Koren S, et al. (2010). Evidence for a tumor promoting effect of high-fat diet independent of insulin resistance in HER2/Neu mammary carcinogenesis. *Breast Cancer Res Treat*, 122(3):647–59. doi:10.1007/s10549-009-0586-8 PMID:19851863

Klurfeld DM, Lloyd LM, Welch CB, Davis MJ, Tulp OL, Kritchevsky D (1991). Reduction of enhanced mammary carcinogenesis in LA/N-cp (corpulent) rats by energy restriction. *Proc Soc Exp Biol Med*, 196(4):381–4. doi:10.3181/00379727-196-43202 PMID:1901169

Klurfeld DM, Welch CB, Davis MJ, Kritchevsky D (1989a). Determination of degree of energy restriction necessary to reduce DMBA-induced mammary tumorigenesis in rats during the promotion phase. *J Nutr*, 119(2):286–91. PMID:2493082

Klurfeld DM, Welch CB, Lloyd LM, Kritchevsky D (1989b). Inhibition of DMBA-induced mammary tumorigenesis by caloric restriction in rats fed high-fat diets. *Int J Cancer*, 43(5):922–5. doi:10.1002/ijc.2910430532 PMID:2497075

Kritchevsky D (1999). Caloric restriction and experimental carcinogenesis. *Toxicol Sci*, 52(2 Suppl):13–6. doi:10.1093/toxsci/52.2.13 PMID:10630585

Kritchevsky D, Welch CB, Klurfeld DM (1989). Response of mammary tumors to caloric restriction for different time periods during the promotion phase. *Nutr Cancer*, 12(3):259–69. doi:10.1080/01635588909514025 PMID:2505241

Lagopoulos L, Sunahara GI, Würzner H, Dombrowsky I, Stalder R (1991). The effects of alternating dietary restriction and ad libitum feeding of mice on the development of diethylnitrosamine-induced liver tumours and its correlation to insulinaemia. *Carcinogenesis*, 12(2):311–5. doi:10.1093/carcin/12.2.311 PMID:1847320

Lanza-Jacoby S, Yan G, Radice G, LePhong C, Baliff J, Hess R (2013). Calorie restriction delays the progression of lesions to pancreatic cancer in the LSL-*Kras*^{G12D}; Pdx-1/Cre mouse model of pancreatic cancer. *Exp Biol Med (Maywood)*, 238(7):787–97. doi:10.1177/1535370213493727 PMID:23828595

Lashinger LM, Harrison LM, Rasmussen AJ, Logsdon CD, Fischer SM, McArthur MJ, et al. (2013). Dietary energy balance modulation of Kras- and Ink4a/Arf$^{+/-}$-driven pancreatic cancer: the role of insulin-like growth factor-I. *Cancer Prev Res (Phila)*, 6(10):1046–55. doi:10.1158/1940-6207.CAPR-13-0185 PMID:23980075

Lashinger LM, Malone LM, Brown GW, Daniels EA, Goldberg JA, Otto G, et al. (2011). Rapamycin partially mimics the anticancer effects of calorie restriction in a murine model of pancreatic cancer. *Cancer Prev Res (Phila)*, 4(7):1041–51. doi:10.1158/1940-6207.CAPR-11-0023 PMID:21593197

Lee WM, Lu S, Medline A, Archer MC (2001). Susceptibility of lean and obese Zucker rats to tumorigenesis induced by *N*-methyl-*N*-nitrosourea. *Cancer Lett*, 162(2):155–60. doi:10.1016/S0304-3835(00)00635-2 PMID:11146220

Lemke LB, Rogers AB, Nambiar PR, Fox JG (2008). Obesity and non-insulin-dependent diabetes mellitus in Swiss-Webster mice associated with late-onset hepatocellular carcinoma. *J Endocrinol*, 199(1):21–32. doi:10.1677/JOE-07-0588 PMID:18667586

Lipman NS, Leary SL (2015). Design and management of research facilities. In: Anderson LC, Otto GM, Pritchett-Corning KR, Whary MT, Fox JG, editors. Laboratory animal medicine. 3rd edition. Elsevier; pp. 1543–97. doi:10.1016/B978-0-12-409527-4.00036-5

Llaverias G, Danilo C, Wang Y, Witkiewicz AK, Daumer K, Lisanti MP, et al. (2010). A Western-type diet accelerates tumor progression in an autochthonous mouse model of prostate cancer. *Am J Pathol*, 177(6):3180–91. doi:10.2353/ajpath.2010.100568 PMID:21088217

Maeda H, Gleiser CA, Masoro EJ, Murata I, McMahan CA, Yu BP (1985). Nutritional influences on aging of Fischer 344 rats: II. Pathology. *J Gerontol*, 40(6):671–88. doi:10.1093/geronj/40.6.671 PMID:4056322

Mai V, Colbert LH, Berrigan D, Perkins SN, Pfeiffer R, Lavigne JA, et al. (2003). Calorie restriction and diet composition modulate spontaneous intestinal tumorigenesis in *Apc*^{Min} mice through different mechanisms. *Cancer Res*, 63(8):1752–5. PMID:12702556

Matthews SB, Zhu Z, Jiang W, McGinley JN, Neil ES, Thompson HJ (2014). Excess weight gain accelerates 1-methyl-1-nitrosourea-induced mammary carcinogenesis in a rat model of premenopausal breast cancer.

Cancer Prev Res (Phila), 7(3):310–8. doi:10.1158/1940-6207.CAPR-13-0297 PMID:24441676

McCormick DL, Johnson WD, Haryu TM, Bosland MC, Lubet RA, Steele VE (2007). Null effect of dietary restriction on prostate carcinogenesis in the Wistar-Unilever rat. *Nutr Cancer*, 57(2):194–200. doi:10.1080/01635580701277494 PMID:17571953

Minor RK, López M, Younts CM, Jones B, Pearson KJ, Anson RM, et al. (2011). The arcuate nucleus and neuropeptide Y contribute to the antitumorigenic effect of calorie restriction. *Aging Cell*, 10(3):483–92. doi:10.1111/j.1474-9726.2011.00693.x PMID:21385308

Mizuno NK, Rogozina OP, Seppanen CM, Liao DJ, Cleary MP, Grossmann ME (2013). Combination of intermittent calorie restriction and eicosapentaenoic acid for inhibition of mammary tumors. *Cancer Prev Res (Phila)*, 6(6):540–7. doi:10.1158/1940-6207.CAPR-13-0033 PMID:23550153

Molon-Noblot S, Keenan KP, Coleman JB, Hoe CM, Laroque P (2001). The effects of ad libitum overfeeding and moderate and marked dietary restriction on age-related spontaneous pancreatic islet pathology in Sprague-Dawley rats. *Toxicol Pathol*, 29(3):353–62. doi:10.1080/019262301316905318 PMID:11442021

Moore T, Beltran L, Carbajal S, Hursting SD, DiGiovanni J (2012). Energy balance modulates mouse skin tumour promotion through altered IGF-1R and EGFR crosstalk. *Cancer Prev Res (Phila)*, 5(10):1236–46. doi:10.1158/1940-6207.CAPR-12-0234 PMID:22896210

Mori A, Sakurai H, Choo MK, Obi R, Koizumi K, Yoshida C, et al. (2006). Severe pulmonary metastasis in obese and diabetic mice. *Int J Cancer*, 119(12):2760–7. doi:10.1002/ijc.22248 PMID:16998795

Newberne PM, Bueche D, Suphiphat V, Schrager TF, Sahaphong S (1990). The influence of pre- and postnatal caloric intake on colon carcinogenesis. *Nutr Cancer*, 13(3):165–73. doi:10.1080/01635589009514057 PMID:2308872

Nkhata KJ, Ray A, Dogan S, Grande JP, Cleary MP (2009). Mammary tumor development from T47-D human breast cancer cells in obese ovariectomized mice with and without estradiol supplements. *Breast Cancer Res Treat*, 114(1):71–83. doi:10.1007/s10549-008-9991-7 PMID:18392696

Nogueira LM, Dunlap SM, Ford NA, Hursting SD (2012). Calorie restriction and rapamycin inhibit MMTV-Wnt-1 mammary tumor growth in a mouse model of postmenopausal obesity. *Endocr Relat Cancer*, 19(1):57–68. doi:10.1530/ERC-11-0213 PMID:22143497

NTP (1997). Effect of dietary restriction on toxicology and carcinogenesis studies in F344/N rats and B6C3F$_1$ mice. *Natl Toxicol Program Tech Rep Ser*, 460:1–414. PMID:12587016

Nuñez NP, Perkins SN, Smith NC, Berrigan D, Berendes DM, Varticovski L, et al. (2008). Obesity accelerates mouse mammary tumor growth in the absence of ovarian hormones. *Nutr Cancer*, 60(4):534–41. doi:10.1080/01635580801966195 PMID:18584488

Olivo-Marston SE, Hursting SD, Perkins SN, Schetter A, Khan M, Croce C, et al. (2014). Effects of calorie restriction and diet-induced obesity on murine colon carcinogenesis, growth and inflammatory factors, and microRNA expression. *PLoS One*, 9(4):e94765. doi:10.1371/journal.pone.0094765 PMID:24732966

Pandey V, Vijayakumar MV, Ajay AK, Malvi P, Bhat MK (2012). Diet-induced obesity increases melanoma progression: involvement of Cav-1 and FASN. *Int J Cancer*, 130(3):497–508. doi:10.1002/ijc.26048 PMID:21387314

Pape-Ansorge KA, Grande JP, Christensen TA, Maihle NJ, Cleary MP (2002). Effect of moderate caloric restriction and/or weight cycling on mammary tumor incidence and latency in MMTV-Neu female mice. *Nutr Cancer*, 44(2):162–8. doi:10.1207/S15327914NC4402_07 PMID:12734063

Parisotto M, Metzger D (2013). Genetically engineered mouse models of prostate cancer. *Mol Oncol*, 7(2):190–205. doi:10.1016/j.molonc.2013.02.005 PMID:23481269

Park EJ, Lee JH, Yu GY, He G, Ali SR, Holzer RG, et al. (2010). Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell*, 140(2):197–208. doi:10.1016/j.cell.2009.12.052 PMID:20141834

Park H, Kim M, Kwon GT, Yu R, Sung MK, Lee KW, et al. (2012). A high-fat diet increases angiogenesis, solid tumor growth, and lung metastasis of CT26 colon cancer cells in obesity-resistant BALB/c mice. *Mol Carcinog*, 51(11):869–80. doi:10.1002/mc.20856 PMID:21919080

Pashko LL, Schwartz AG (1992). Reversal of food restriction-induced inhibition of mouse skin tumour promotion by adrenalectomy. *Carcinogenesis*, 13(10):1925–8. doi:10.1093/carcin/13.10.1925 PMID:1423856

Pearson KJ, Lewis KN, Price NL, Chang JW, Perez E, Cascajo MV, et al. (2008). Nrf2 mediates cancer protection but not prolongevity induced by caloric restriction. *Proc Natl Acad Sci USA*, 105(7):2325–30. doi:10.1073/pnas.0712162105 PMID:18287083

Peto R, Pike MC, Day NE, Gray RG, Lee PN, Parish S, et al. (1980). Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments. *IARC Monogr Eval Carcinog Risk Chem Hum Suppl*, (Suppl 2):311–426. PMID:6935185

Philip B, Roland CL, Daniluk J, Liu Y, Chatterjee D, Gomez SB, et al. (2013). A high-fat diet activates oncogenic Kras and COX2 to induce development of pancreatic ductal adenocarcinoma in mice. *Gastroenterology*, 145(6):1449–58. doi:10.1053/j.gastro.2013.08.018 PMID:23958541

Pollard M, Luckert PH, Pan G-Y (1984). Inhibition of intestinal tumorigenesis in methylazoxymethanol-treated rats by dietary restriction. *Cancer Treat Rep*, 68(2):405–8. PMID:6697329

Pollard M, Luckert PH, Snyder D (1989). Prevention of prostate cancer and liver tumors in L-W rats by moderate dietary restriction. *Cancer*, 64(3):686–90. doi:10.1002/1097-0142(19890801)64:3<686::AID-CNCR2820640320>3.0.CO;2-Q PMID:2472862

Pour PM, Permert J, Mogaki M, Fujii H, Kazakoff K (1993). Endocrine aspects of exocrine cancer of the pancreas. Their patterns and suggested biologic significance. *Am J Clin Pathol*, 100(3):223–30. doi:10.1093/ajcp/100.3.223 PMID:8379530

Raju J, Bird RP (2003). Energy restriction reduces the number of advanced aberrant crypt foci and attenuates the expression of colonic transforming growth factor beta and cyclooxygenase isoforms in Zucker obese (fa/fa) rats. *Cancer Res*, 63(20):6595–601. PMID:14583451

Ray A, Cleary MP (2013). Animal models to study the interplay between cancer and obesity. In: Kolomin MG, editor. Adipose tissue and cancer. New York, USA: Springer; pp. 99–119. doi:10.1007/978-1-4614-7660-3_6

Reddy BS, Wang C-X, Maruyama H (1987). Effect of restricted caloric intake on azoxymethane-induced colon tumor incidence in male F344 rats. *Cancer Res*, 47(5):1226–8. PMID:3815332

Ribeiro AM, Andrade S, Pinho F, Monteiro JD, Costa M, Lopes C, et al. (2010). Prostate cancer cell proliferation and angiogenesis in different obese mice models. *Int J Exp Pathol*, 91(4):374–86. doi:10.1111/j.1365-2613.2010.00726.x PMID:20666851

Roebuck BD, Baumgartner KJ, MacMillan DL (1993). Caloric restriction and intervention in pancreatic carcinogenesis in the rat. *Cancer Res*, 53(1):46–52. PMID:8416749

Rogozina OP, Bonorden MJL, Grande JP, Cleary MP (2009). Serum insulin-like growth factor-I and mammary tumor development in ad libitum-fed, chronic calorie-restricted, and intermittent calorie-restricted MMTV-TGF-α mice. *Cancer Prev Res (Phila)*, 2(8):712–9. doi:10.1158/1940-6207.CAPR-09-0028 PMID:19654106

Rogozina OP, Nkhata KJ, Nagle EJ, Grande JP, Cleary MP (2013). The protective effect of intermittent calorie restriction on mammary tumorigenesis is not compromised by consumption of a high fat diet during refeeding. *Breast Cancer Res Treat*, 138(2):395–406. doi:10.1007/s10549-013-2464-7 PMID:23446811

Ross MH, Bras G (1971). Lasting influence of early caloric restriction on prevalence of neoplasms in the rat. *J Natl Cancer Inst*, 47(5):1095–113. PMID:4330798

Rossi EL, de Angel RE, Bowers LW, Khatib SA, Smith LA, Van Buren E, et al. (2016). Obesity-associated alterations in inflammation, epigenetics and mammary tumor growth persist in formerly obese mice *Cancer Prev Res (Phila)*, 9(5):339–48. doi:10.1158/1940-6207.CAPR-15-0348 PMID:26869351

Ruggeri BA, Klurfeld DM, Kritchevsky D, Furlanetto RW (1989a). Caloric restriction and 7,12-dimethylbenz(*a*)anthracene-induced mammary tumor growth in rats: alterations in circulating insulin, insulin-like growth factors I and II, and epidermal growth factor. *Cancer Res*, 49(15):4130–4. PMID:2501021

Ruggeri BA, Klurfeld DM, Kritchevsky D, Furlanetto RW (1989b). Growth factor binding to 7,12-dimethylbenz(*a*)anthracene-induced mammary tumors from rats subject to chronic caloric restriction. *Cancer Res*, 49(15):4135–41. PMID:2501022

Sheldon WG, Bucci TJ, Blackwell B, Turturro A (1996). Effect of ad libitum feeding and 40% feed restriction on body weight, longevity, and neoplasms in $B6C3F_1$, C57BL6, and $B6D2F_1$ mice. In: Mohr U, Dungworth D, Capen C, Carlton W, Sundberg J, Ward J, editors. Pathobiology of the aging mouse. Washington (DC), USA: ILSI Press; pp. 21–6.

Sheldon WG, Bucci TJ, Hart RW, Turturro A (1995). Age-related neoplasia in a lifetime study of *ad libitum*-fed and food-restricted B6C3F1 mice. *Toxicol Pathol*, 23(4):458–76. doi:10.1177/019262339502300403 PMID:7501958

Shimokawa I, Higami Y, Yu BP, Masoro EJ, Ikeda T (1996). Influence of dietary components on occurrence of and mortality due to neoplasms in male F344 rats. *Aging (Milano)*, 8(4):254–62. PMID:8904955

Shull JD, Birt DF, McComb RD, Spady TJ, Pennington KL, Shaw-Bruha CM (1998). Estrogen induction of prolactin-producing pituitary tumours in the Fischer 344 rat: modulation by dietary-energy but not protein consumption. *Mol Carcinog*, 23(2):96–105. doi:10.1002/(SICI)1098-2744(199810)23:2<96::AID-MC6>3.0.CO;2-# PMID:9808163

Shull JD, Spady TJ, Snyder MC, Johansson SL, Pennington KL (1997). Ovary-intact, but not ovariectomized female ACI rats treated with 17β-estradiol rapidly develop mammary carcinoma. *Carcinogenesis*, 18(8):1595–601. doi:10.1093/carcin/18.8.1595 PMID:9276635

Snyder DL, Pollard M, Wostmann BS, Luckert P (1990). Life span, morphology, and pathology of diet-restricted germ-free and conventional Lobund-Wistar rats. *J Gerontol*, 45(2):B52–8. doi:10.1093/geronj/45.2.B52 PMID:2313040

Soga M, Hashimoto S, Kishimoto Y, Hirasawa T, Makino S, Inagaki S (2010). Insulin resistance, steatohepatitis, and hepatocellular carcinoma in a new congenic strain of Fatty Liver Shionogi (FLS) mice with the *Lep*^{ob} gene. *Exp Anim*, 59(4):407–19. doi:10.1538/expanim.59.407 PMID:20660987

Spady TJ, Lemus-Wilson AM, Pennington KL, Blackwood DJ, Paschall TM, Birt DF, et al. (1998). Dietary energy restriction abolishes development of prolactin-producing pituitary tumours in Fischer 344 rats treated

with 17β-estradiol. *Mol Carcinog*, 23(2):86–95. doi:10.1002/(SICI)1098-2744(199810)23:2<86::AID-MC5>3.0.CO;2-5 PMID:9808162

Spady TJ, Pennington KL, McComb RD, Birt DF, Shull JD (1999). Estrogen-induced pituitary tumour development in the ACI rat not inhibited by dietary energy restriction. *Mol Carcinog*, 26(4):239–53. doi:10.1002/(SICI)1098-2744(199912)26:4<239::AID-MC3>3.0.CO;2-H PMID:10569801

Sundaram S, Le TL, Essaid L, Freemerman AJ, Huang MJ, Galanko JA, et al. (2014). Weight loss reversed obesity-induced HGF/c-Met pathway and basal-like breast cancer progression *Front Oncol*, 4:175. doi:10.3389/fonc.2014.00175 PMID:25072025

Suttie A, Nyska A, Haseman JK, Moser GJ, Hackett TR, Goldsworthy TL (2003). A grading scheme for the assessment of proliferative lesions of the mouse prostate in the TRAMP model. *Toxicol Pathol*, 31(1):31–8. doi:10.1080/01926230390173842 PMID:12597447

Suttie AW, Dinse GE, Nyska A, Moser GJ, Goldsworthy TL, Maronpot RR (2005). An investigation of the effects of late-onset dietary restriction on prostate cancer development in the TRAMP mouse. *Toxicol Pathol*, 33(3):386–97. doi:10.1080/01926230590930272 PMID:15805078

Sylvester PW, Aylsworth CF, Meites J (1981). Relationship of hormones to inhibition of mammary tumor development by underfeeding during the "critical period" after carcinogen administration. *Cancer Res*, 41(4):1384–8. PMID:6783295

Sylvester PW, Aylsworth CF, Van Vugt DA, Meites J (1982). Influence of underfeeding during the "critical period" or thereafter on carcinogen-induced mammary tumors in rats. *Cancer Res*, 42(12):4943–7. PMID:6814746

Tannenbaum A (1945a). The dependence of tumor formation on the composition of the calorie-restricted diet as well as on the degree of restriction. *Cancer Res*, 5:616–25.

Tannenbaum A (1945b). The dependence of tumor formation on the degree of caloric restriction. *Cancer Res*, 5:609–15.

Teraoka N, Mutoh M, Takasu S, Ueno T, Nakano K, Takahashi M, et al. (2011). High susceptibility to azoxymethane-induced colorectal carcinogenesis in obese KK-A^y mice. *Int J Cancer*, 129(3):528–35. doi:10.1002/ijc.25711 PMID:20886595

Thompson HJ, McGinley JN, Spoelstra NS, Jiang W, Zhu Z, Wolfe P (2004b). Effect of dietary energy restriction on vascular density during mammary carcinogenesis. *Cancer Res*, 64(16):5643–50. doi:10.1158/0008-5472.CAN-04-0787 PMID:15313902

Thompson HJ, Zhu Z, Jiang W (2002). Protection against cancer by energy restriction: all experimental approaches are not equal. *J Nutr*, 132(5):1047–9. PMID:11983835

Thompson HJ, Zhu Z, Jiang W (2003). Dietary energy restriction in breast cancer prevention. *J Mammary Gland Biol Neoplasia*, 8(1):133–42. doi:10.1023/A:1025743607445 PMID:14587868

Thompson HJ, Zhu Z, Jiang W (2004a). Identification of the apoptosis activation cascade induced in mammary carcinomas by energy restriction. *Cancer Res*, 64(4):1541–5. doi:10.1158/0008-5472.CAN-03-3108 PMID:14973070

Thompson JH (2015). Impact of energy balance on chemically induced mammary carcinogenesis in a rat. In: Berger AN, editor. Murine models, energy balance, and cancer. Springer International Publishing; pp. 175–96. doi:10.1007/978-3-319-16733-6_9

Thompson MD, Grubbs CJ, Bode AM, Reid JM, McGovern R, Bernard PS, et al. (2015). Lack of effect of metformin on mammary carcinogenesis in nondiabetic rat and mouse models. *Cancer Prev Res (Phila)*, 8(3):231–9. doi:10.1158/1940-6207.CAPR-14-0181-T PMID:25681088

Thurman JD, Bucci TJ, Hart RW, Turturro A (1994). Survival, body weight, and spontaneous neoplasms in ad libitum-fed and food-restricted Fischer-344 rats. *Toxicol Pathol*, 22(1):1–9. doi:10.1177/019262339402200101 PMID:8073218

Tucker MJ (1979). The effect of long-term food restriction on tumours in rodents. *Int J Cancer*, 23(6):803–7. doi:10.1002/ijc.2910230611 PMID:468413

Volk MJ, Pugh TD, Kim M, Frith CH, Daynes RA, Ershler WB, et al. (1994). Dietary restriction from middle age attenuates age-associated lymphoma development and interleukin 6 dysregulation in C57BL/6 mice. *Cancer Res*, 54(11):3054–61. PMID:8187095

Waxler SH, Tabar P, Melcher LR (1953). Obesity and the time of appearance of spontaneous mammary carcinoma in C3H mice. *Cancer Res*, 13(3):276–8. PMID:13042818

Weber RV, Stein DE, Scholes J, Kral JG (2000). Obesity potentiates AOM-induced colon cancer. *Dig Dis Sci*, 45(5):890–5. doi:10.1023/A:1005560621722 PMID:10795750

Weindruch R, Walford RL (1982). Dietary restriction in mice beginning at 1 year of age: effect on lifespan and spontaneous cancer incidence. *Science*, 215(4538):1415–8. doi:10.1126/science.7063854 PMID:7063854

Wheatley KE, Williams EA, Smith NCP, Dillard A, Park EY, Nunez NP, et al. (2008). Low-carbohydrate diet versus caloric restriction: effects on weight loss, hormones, and colon tumor growth in obese mice. *Nutr Cancer*, 60(1):61–8. doi:10.1080/01635580701510150 PMID:18444137

White PB, True EM, Ziegler KM, Wang SS, Swartz-Basile DA, Pitt HA, et al. (2010). Insulin, leptin, and tumoral adipocytes promote murine pancreatic cancer growth.

J Gastrointest Surg, 14(12):1888–94. doi:10.1007/s11605-010-1349-x PMID:20859700

Wolff GL, Kodell RL, Cameron AM, Medina D (1982). Accelerated appearance of chemically induced mammary carcinomas in obese yellow (A^{vy}/A) (BALB/c × VY) F_1 hybrid mice. *J Toxicol Environ Health*, 10(1):131–42. doi:10.1080/15287398209530237 PMID:6290678

Wolff GL, Medina D, Umholtz RL (1979). Manifestation of hyperplastic alveolar nodules and mammary tumors in "viable yellow" and non-yellow mice. *J Natl Cancer Inst*, 63(3):781–5. PMID:288935

Wolff GL, Roberts DW, Mountjoy KG (1999). Physiological consequences of ectopic *agouti* gene expression: the yellow obese mouse syndrome. *Physiol Genomics*, 1(3):151–63. PMID:11015573

Yakar S, Nunez NP, Pennisi P, Brodt P, Sun H, Fallavollita L, et al. (2006). Increased tumor growth in mice with diet-induced obesity: impact of ovarian hormones. *Endocrinology*, 147(12):5826–34. doi:10.1210/en.2006-0311 PMID:16959846

Yang S, Lin HZ, Hwang J, Chacko VP, Diehl AM (2001). Hepatic hyperplasia in noncirrhotic fatty livers: is obesity-related hepatic steatosis a premalignant condition? *Cancer Res*, 61(13):5016–23. PMID:11431335

Yu BP, Masoro EJ, McMahan CA (1985). Nutritional influences on aging of Fischer 344 rats: I. Physical, metabolic, and longevity characteristics. *J Gerontol*, 40(6):657–70. doi:10.1093/geronj/40.6.657 PMID:4056321

Yu W, Cline M, Maxwell LG, Berrigan D, Rodriguez G, Warri A, et al. (2010). Dietary vitamin D exposure prevents obesity-induced increase in endometrial cancer in *Pten*$^{+/-}$ mice. *Cancer Prev Res (Phila)*, 3(10):1246–58. doi:10.1158/1940-6207.CAPR-10-0088 PMID:20858763

Yun JP, Behan JW, Heisterkamp N, Butturini A, Klemm L, Ji L, et al. (2010). Diet-induced obesity accelerates acute lymphoblastic leukemia progression in two murine models. *Cancer Prev Res (Phila)*, 3(10):1259–64. doi:10.1158/1940-6207.CAPR-10-0087 PMID:20823291

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature*, 372(6505):425–32. doi:10.1038/372425a0 PMID:7984236

Zhu P, Frei E, Bunk B, Berger MR, Schmähl D (1991). Effect of dietary calorie and fat restriction on mammary tumor growth and hepatic as well as tumor glutathione in rats. *Cancer Lett*, 57(2):145–52. doi:10.1016/0304-3835(91)90208-Y PMID:2025887

Zhu Z, Haegele AD, Thompson HJ (1997). Effect of caloric restriction on pre-malignant and malignant stages of mammary carcinogenesis. *Carcinogenesis*, 18(5):1007–12. doi:10.1093/carcin/18.5.1007 PMID:9163688

Zhu Z, Jiang W, McGinley J, Wolfe P, Thompson HJ (2005). Effects of dietary energy repletion and IGF-1 infusion on the inhibition of mammary carcinogenesis by dietary energy restriction. *Mol Carcinog*, 42(3):170–6. doi:10.1002/mc.20071 PMID:15599926

Zhu Z, Jiang W, Thompson HJ (1999a). Effect of energy restriction on tissue size regulation during chemically induced mammary carcinogenesis. *Carcinogenesis*, 20(9):1721–6.doi:10.1093/carcin/20.9.1721PMID:10469616

Zhu Z, Jiang W, Thompson HJ (1999b). Effect of energy restriction on the expression of cyclin D1 and p27 during premalignant and malignant stages of chemically induced mammary carcinogenesis. *Mol Carcinog*, 24(4):241–5. doi:10.1002/(SICI)1098-2744(199904)24:4<241::AID-MC1>3.0.CO;2-P PMID:10326860

Zhu Z, Jiang W, Thompson HJ (2002). An experimental paradigm for studying the cellular and molecular mechanisms of cancer inhibition by energy restriction. *Mol Carcinog*, 35(2):51–6. doi:10.1002/mc.10073 PMID:12325034

Zhu Z, Jiang W, Thompson MD, Echeverria D, McGinley JN, Thompson HJ (2015). Effects of metformin, buformin, and phenformin on the post-initiation stage of chemically induced mammary carcinogenesis in the rat. *Cancer Prev Res (Phila)*, 8(6):518–27. doi:10.1158/1940-6207.CAPR-14-0121 PMID:25804611

Zucker LM, Zucker TF (1961). Fatty, a new mutation in the rat. *J Hered*, 52:275–8.

Zyromski NJ, Mathur A, Pitt HA, Wade TE, Wang S, Nakshatri P, et al. (2009). Obesity potentiates the growth and dissemination of pancreatic cancer. *Surgery*, 146(2):258–63. doi:10.1016/j.surg.2009.02.024 PMID:19628082