Energy metabolism and carcinogenesis

Report of a workshop held on 9–10 March 1999

MRC Institute for Environment and Health

FORA 2

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Preface

This report is based on a workshop which was convened as part of the Ministry of Agriculture, Fisheries and Food/Food Standards Agency-sponsored Food Risk Assessment (FORA) project — a collaborative research programme between the MRC Toxicology Unit and the MRC Institute for Environment and Health in Leicester and the MRC Dunn Clinical Nutrition Unit in Cambridge. The programme aims to consider and conduct research into fundamental and applied approaches to food risk assessment, most specifically in the context of diet and cancer.

Workshop participants are listed at the end of the report. Their affiliations are given as well as an indication of any special role: speaker, rapporteur or chairman. Many thanks are due to all the workshop participants, and especially to the chairman and speakers for their contributions.

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Executive summary

One of the most fundamental aspects of diet is provision of energy to the body. It is well known that the biochemical process of converting energy-providing nutrients in food into energy implies risk of damage to cellular macromolecules by chemical intermediates such as reactive oxygen species. Thus, one of the basic requirements for life carries with it risk of oxidative damage to DNA and, therefore, potentially of cancer. There is a substantial amount of research showing that life-long restriction of dietary intake in many species of animal, and most notably in laboratory rats and mice, confers protection against tumours and many degenerative diseases and lengthens life span. This report, based on a workshop convened as part of the Food Risk Assessment (FORA) project, discusses whether there is any evidence to suggest an analogous effect in humans.

The workshop examined the current state of knowledge about the links between energy metabolism and carcinogenesis. Two particular topics were addressed: how two-year rodent bioassays used to predict toxicity, and specifically carcinogenicity, of chemicals in humans should be interpreted, in view of the fact that energy intake affects the results; and whether energy metabolism in humans affects their risk of cancer.

Several aspects of energy metabolism and carcinogenesis are reviewed in this report: the influence of caloric intake on bioassays and on xenobiotic pathways in animals; the epidemiology of energy metabolism and human cancers; mechanisms to protect against oxidative damage; the role of growth-promoting and stress-induced hormonal systems in the promotion and progression of tumours; and the relevance of animal models to understanding energy metabolism and carcinogenesis in humans.

The report of the workshop reaches the following conclusions.

The appropriateness of extrapolating from observations linking energy metabolism and carcinogenesis in rodents in order to predict events in humans is limited, despite many genetic and phenotypic similarities. Quantitative effects of dietary energy restriction in rodents cannot be expected to occur in the same way as in humans although there may be some conservation of the phenomenon across these species.

Arguably the most important facet of using energy restriction in rodent toxicological studies, including the two-year bioassay used to predict toxicity in humans, is the improved statistical sensitivity it confers, with improved chances of detecting toxic effects of the test compounds. This is especially important for testing the carcinogenicity of chemicals in humans. Ongoing research into the effects of energy restriction in animals will also continue to produce much information on possible mechanisms of carcinogenesis and of ageing, which may be of relevance to and stimulate investigations in humans.

Evaluating the influence of energy metabolism is difficult, in part because changes in body size or composition are bidirectionally linked to changes in energy status. In addition, all aspects of energy metabolism, except long-term energy balance, are very difficult to measure with confidence in population studies, so that links with cancer incidence in humans are difficult to establish.

Despite the lack of direct data, there is circumstantial evidence that one or more aspects of energy dynamics play a role in human carcinogenesis. Higher body mass index (BMI) is the most clear-cut indicator of energy status to be established as a risk factor for some cancers in humans. Increases in body weight, adiposity and BMI are manifestations of long-term positive energy balance. In terms of contribution to prevention of weight gain, the balance of opinion

tends towards a consensus that increased physical activity rather than decreased total food intake may be a primary protective factor in carcinogenesis. It is not known to what extent this interpretation of the available information is due to the difficulties of measuring energy intake in free-living humans.

Oxidative stress may be less important in cancer initiation than previously thought, but its effect has not been ruled out. Evidence is emerging that the lack of controlled amounts of oxidative stress, for example due to lack of regular exercise or to the consumption of mega-doses of antioxidant supplements, may paradoxically be a risk factor for cancer because endogenous defence mechanisms that have evolved to manage habitual amounts of oxidative stress are either not stimulated or overridden.

From a pragmatic policy-making point of view, rather than decrease energy intake to protect against cancer, advice to the population to increase physical activity would be more consistent with other public health issues.

Ongoing and future work on the mechanisms and indicators of energy metabolism, DNA damage and repair, chromosomal damage and carcinogenesis can be expected to help clarify the relative importance of the metabolism of energy and of xenobiotic and endogenous chemicals in the development of cancer in humans.

1 General introduction

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1.1 Background

One of the most fundamental aspects of diet is provision of energy to the body. It is well known that the biochemical process of converting energy-providing nutrients in food into energy implies risk of damage to cellular macromolecules by chemical intermediates, such as reactive oxygen species. Thus, one of the basic requirements for life carries with it risk of oxidative damage to DNA and, therefore, potentially of cancer. There is a substantial amount of research showing that life-long restriction of dietary intake in many species of animal, and most notably in laboratory rats and mice, confers protection against tumours and many degenerative diseases and lengthens life span. This report examines whether there is any evidence to suggest an analogous effect in humans. The report is based on a workshop convened as part of the Ministry of Agriculture Fisheries and Food/Food Standards Agency-sponsored Food Risk Assessment (FORA) project — a collaborative research programme based at the MRC Toxicology Unit and the MRC Institute for Environment and Health in Leicester and the MRC Dunn Clinical Nutrition Unit in Cambridge.

The workshop brought together scientists with expertise primarily in the fields of nutrition and toxicology and also in several other relevant disciplines. Participants in the workshop were identified and topics for presentations were designed with the aim of representing key aspects of the different issues that might contribute to the discussion. The workshop examined the current state of knowledge about the links between energy metabolism and carcinogenesis with the expectation that advances in understanding in this area will help to improve food risk assessment.

This general introduction and short summary papers prepared for the workshop by invited speakers (presented in Sections 2 to 4), were pre-circulated to workshop participants and provided the basis for the discussions at the workshop.

Papers in Section 2 review the influence of caloric intake on animal bioassays and on xenobiotic pathways in animals, and the epidemiology of energy metabolism and human cancers. Section 3 describes some of the mechanisms that protect against damage that arises from reactive oxygen species, which are associated with early biochemical events related to energy production. Such mechanisms include protective antioxidant systems and DNA repair. There is an accelerating interest in the role of growth-promoting and stress-induced hormonal systems in promotion and progression of tumours, and these are also described in Section 3. In Section 4, the relevance of animal models to an understanding of energy metabolism and carcinogenesis in humans is discussed. Finally a record of the discussions at the workshop and overall conclusions are provided in Sections 5 and 6.

1.2 Introducing the role of energy metabolism

McCay, Crowell and Maynard published a paper in the 1930s on the relationships between food intake, body size and longevity in rats (McCay *et al.*, 1935), reporting that food restriction, starting at weaning and continuing intermittently throughout most of life, resulted in a population of smaller rats with longer life span than those fed *ad libitum* throughout their lives. Since then a substantial amount of research has been conducted on the links between energy intake and longevity, especially in rodents. An extensive literature, including many reviews (e.g. Weindruch & Walford, 1988; Kritchevsky, 1993; Hart *et al.*, 1995; Keenan *et al.*, 1996; Masoro, 1996; Sohal & Weindruch,

1996), covers investigations into the extent, mechanisms and applications of the phenomena that energy restriction protects against many of the diseases associated with ageing, including spontaneous tumour formation in rats and mice, and promotes longevity.

Although these effects of energy intake in rodents are interesting in themselves they give rise to two sets of questions about relevance to humans. The first concerns the use of the two-year rodent bioassay to predict toxicity, and specifically carcinogenicity, of chemicals in humans and how such studies should be interpreted, in view of the fact that energy intake affects the results. The second focuses on whether energy metabolism in humans affects their risk of cancer.

Although most of the rodent data refer to differences in energy intake, the more general term 'metabolism' is used here to include energy intake, energy expenditure and also the balance between the two, since it is not known which, if any, of these aspects of metabolism is important for carcinogenicity in humans.

In this introduction the main features of the phenomena in rodents and the reasons for posing questions about relevance to humans are presented. More detailed discussions of specific issues are presented in Sections 2 to 4.

1.3 Energy metabolism and body composition

Energy metabolism encompasses a number of dynamic concepts. Energy intake in the context of the whole body is that which is eaten as biochemical fuels called macronutrients — fat, carbohydrate, protein and alcohol — in the form of food and drink. At the level of organs, tissues or cells, availability of energy is affected by many factors, such as physical and chemical availability and rates of uptake of fuels, and the picture may be complicated by the use of energy stores from within the body.

Energy expenditure, which is fundamentally the oxidative release of energy from biochemical fuels in foods, is required for life, since it is a necessary and integral part of cellular maintenance and function. Part of an animal's energy expenditure is related to the rates of turnover and metabolism of cellular and intra-cellular systems and to the size and number of metabolically active cells, hence the importance of body size and composition in the amount of energy expended for basal (i.e. minimal, resting, fasting and unstimulated) metabolic states. Exercise or physical activity also requires oxidative energy expenditure, and this is usually regarded as a separate component of total energy use, although it is directly related to the weight of body-parts that are being moved in the process. Energy expenditure is also required for growth, which entails net synthesis of biochemical body constituents. In all of these processes any energy not stored within chemical structures is ultimately released from the body as heat. Heat is itself a requirement for life and when body temperature drops below a critical level the enzymatic processes necessary for life slow down or stop.

Energy balance is the difference between intake and expenditure, and is commonly viewed in terms of fuel stores. Carbohydrate, protein and fat, in increasing order of their potential storage capacity, may be deposited, withdrawn from storage and, to a limited extent, inter-converted. Alcohol is also metabolised to provide energy and may be stored only as fat.

Body size and composition are indices of medium- to long-term energy balance. Body composition is most simplistically expressed as the relative proportions by weight of 'lean' — including internal organs and skeletal components — and 'fat' tissue. It is not yet feasible to measure short-term and immediate energy balance. The distinction between long- and short-term energy balance is illustrated by the fact that it is possible to be overweight and in short-term negative energy balance, and *vice versa*. Furthermore, energy balance at one level of intake and expenditure could have different metabolic implications from that at a different level.

Body composition and energy intake and expenditure are clearly inextricably linked, as the effects of dietary restriction or *ad libitum* overfeeding described below demonstrate. The larger size and increased fatness (proportion of total body weight as fat) of laboratory rodents which overeat on *ad libitum* diets creates many complications, both in interpretation of results from studies using this regime and also in investigations into mechanisms whereby energy metabolism affects longevity.

Doses of test compounds and requirements for energy and other nutrients are usually expressed on a body-weight basis, yet metabolic energy expenditure is usually considered to relate only to lean body weight, since the metabolic activity of fat tissue is negligible. Thus amounts of glucose used as fuel or basal metabolic rates in animals of

different sizes are often expressed per kilogram of lean body mass. In comparisons between animals or species about aspects of metabolic fuel utilisation, which may be affected by the animal's surface-to-volume ratio, an allometric scaling factor such as body weight$^{0.75}$, is often used for denominators. This variety of expression can be confusing. However, it is probably too simplistic to confine expression of comparisons to only one denominator since this may impede understanding of the mechanisms.

1.4 Energy intake and carcinogenesis in rodents

1.4.1 Phenomenology

Compared with rats fed *ad libitum*, those given restricted amounts of food (energy) on a life-long basis survive longer, provided that intake of essential micronutrients such as vitamins and minerals is maintained. It is widely held that food restriction, also called dietary, caloric or energy restriction, slows the ageing process, since rodents under such dietary control maintain a wide spectrum of physiological processes and systems in a state compatible with a younger age, for example enzyme activities, immune responses, DNA repair capacities, hormonal action, gene expression and female reproductive function (Sohal & Weindruch, 1996).

Energy restriction to between 60 and 80 percent of the *ad libitum* intake produces animals that are much smaller and leaner than their *ad libitum*-fed counterparts. Described in Section 2.1 as 'moderate dietary restriction', it delays the onset and slows the progression of many diseases associated with age, such as chronic nephropathy in rats, and it retards the incidence of most spontaneous tumours in rats and mice (Masoro, 1996), although it has been reported not to have any significant effect on tumour growth rate or volume (Keenan *et al*., 1996). To date, caloric restriction has been the only intervention found consistently and reproducibly to produce such a range of anti-ageing effects in mammals. Pituitary tumours are the most common neoplastic cause of death in both sexes of Sprague-Dawley (SD) rats. As pituitary tumours secrete prolactin, mammary gland tumours are the second most common cause of death in female rats (Keenan *et al*., 1996).

It is not only in the context of the ageing process that there is interest in the protective effect of energy restriction. The rodent bioassay, usually a two-year exposure study, is a mainstay of toxicological testing for the carcinogenicity of chemical compounds. Rats and mice used for these assays are usually housed in groups and fed *ad libitum*, and there is wide variation between animals in their food intake and resulting body weights (Turturro *et al*., 1995), presumably partly due to variations in physical activity. Some animals become grossly obese under such conditions, and over the past two decades the mean food consumption and adult body weight in these assays have drifted upwards and survival has concomitantly decreased, so that many (often over 50%) of the animals do not survive the full two years required for the lifetime assay. Sometimes, because the compound being tested depresses food intake and induces weight loss by other toxicological mechanisms, the survival of the 'test' animals is better than that of the controls. This problem, the so-called 'fat-rat syndrome', has led to calls for better standardisation of assays (Festing, 1997), in particular standardisation of dietary restriction, in the rodent bioassay for toxicity testing (Allaben *et al*., 1996; Keenan *et al*., 1996; Newberne & Sotnikov, 1996).

In addition to effects on health and spontaneous or 'baseline' neoplasms, energy restriction has also been found to affect rodents' response to test compounds in bioassays, in accordance with the widely observed pattern of protective effects against stresses of many kinds. In studies on rats fed either *ad libitum* or energy-restricted diets, most of the treatment-related morphological, toxicological and pathological effects expected in standard toxicity tests of pharmaceutical compounds were found in both groups (Keenan *et al*., 1996). However, in the same experiments, a shift was noted in the dose–response curve, with the rats on energy-restricted diets able to tolerate larger doses, resulting in 2–4 fold increases in maximum tolerated doses and no-effect levels, relative to rats fed *ad libitum*. This was despite similar or greater plasma levels of the drugs in energy-restricted compared with *ad libitum*-fed animals, which suggested that the results were not due to altered drug-metabolising enzymes or systemic exposure (Keenan & Soper, 1995). These effects are discussed further in Sections 2.1 and 2.2.

Owing to their use in carcinogenicity testing, most studies on the effects of energy restriction have been carried out in rodents. The decline in survival corresponding with increased food consumption and body weight has been observed in both sexes of most rodent strains examined, and the phenomenon has been investigated particularly in the outbred Wistar and Sprague-Dawley stocks as well as the inbred Fischer-344 rat strain and several strains of

mice. Species-specific life span extension has also been observed in several other species, for example fish, spiders, and water-fleas. There is currently much interest in the outcome of experiments on non-human primates, which, having longer species-specific life span and slower rates of growth, are closer to the human state (Weindruch, 1996).

The most commonly investigated level of energy restriction in rodents is 40%, but protection against tumorigenesis has been observed with restriction as moderate as 20%. The restricted experimental regime is usually imposed from weaning age, but energy restriction started in mice at 12 months (Weindruch & Walford, 1982) and Fischer-344 rats at 6 months of age (Yu *et al*., 1985) has also been found to increase their maximum life span.

Numerous studies have been conducted in Fischer-344, Wistar or Sprague Dawley rats suggesting that it is the total energy intake per animal rather than an effect of individual macronutrients such as protein or fat that has the effect on longevity (Keenan *et al*., 1996). For example, *ad libitum*-fed Fischer-344 rats developed more severe nephropathy than rats on an energy-restricted diet, although the latter animals were given 1.7 times the amount of protein intake per unit body mass (Masoro *et al*., 1989). Even the use of a diet with lower energy-density due to a higher fibre content did not significantly improve survival of Sprague Dawley rats if *ad libitum* access to the diet was allowed (Keenan *et al*., 1996).

1.4.2 Mechanisms

The most well-studied of the numerous mechanisms that have been postulated for the effect of energy intake on longevity in rodents (e.g. Keenan *et al*., 1996; Masoro, 1996; Leakey *et al*., 1998) are described below. While some of these have now been dismissed, most are still under investigation and discussion. There is evidence that most of these proposed mechanisms also apply to some extent in humans, and it is likely that in reality several may operate concurrently.

Retardation of growth and development

One hypothesis attributes increased longevity to the general retardation of growth and development that is observed in energy-restricted rodents and humans alike. It has largely been discarded on the grounds that the protective effects of energy restriction have been shown even when the restriction is started after the growth phase of rodents, as described above.

Growth rate

Animals fed more energy, especially during their growth phase, grow more quickly and tend to attain a larger adult size, within genetic limits. Studies have shown that body weight at 12 months, rather than initial body weight, in Fischer-344 (Turturro *et al*., 1995) rats, is negatively correlated with survival rates. Many factors associated with faster growth rates are also associated with the later stages of carcinogenesis and neoplastic tissue growth, for example higher circulating levels of growth-promoting hormones (see Section 3.3) and higher rates of cell division and of turnover of macromolecules such as proteins and DNA.

Body fatness

The proportion of body weight as fat is greater with overfeeding, although absolute amounts of both lean and fat tissue are increased. Despite the fact that obesity is causally associated with several degenerative diseases, the proportion of body fat has been dismissed as being causally linked with longevity on the basis of some experimental findings in rats and mice. For example, among *ad libitum*-fed rats no correlation was found between body fat and longevity and among energy-restricted rats a positive relationship was found (Bertrand *et al*., 1980), which has been taken to suggest that the degree of fatness of an animal is not sufficient to explain the inferior survival of *ad libitum*-fed animals. Also, a study by Harrison *et al.* (1984) showed that energy-restricted *ob*/*ob* mice (a strain genetically prone to obesity) lived much longer although they were fatter than *ad libitum*-fed C57BL/6J mice.

Body temperature and metabolic rate

Restricted food intake lowers body temperature, especially in smaller mammals, and slows metabolic rate, enzymatic reactions and most processes of the body. A drop in metabolic rate would conceivably decrease the generation of reactive oxygen species (free radicals) such as hydrogen peroxide and superoxide and hydroxyl radicals, which have been widely implicated in the ageing process and in carcinogenesis (Yu, 1993). However, the metabolic rate hypothesis for ageing has been dismissed (Masoro, 1996) on the grounds of a similar oxygen consumption and energy intake per unit *lean body mass* of *ad libitum*-fed and energy-restricted male Fischer-344 rats. This is one example of confusion arising from different ways of expressing energy metabolism, since it is generally agreed that it is the energy intake *per animal* which is the important dietary factor in the longevity phenomenon.

Oxidative stress and free radical production

Related to body temperature and metabolic rate, excess energy intake results in transitory increased oxidative energy metabolism and also in storage as increased body weight. This in turn tends to increase the perceived need for energy intake, and the larger animal uses more oxygen than one that has not stored excess energy. Increased oxidative metabolism raises the probability of free radical production (Cadenas, 1989) and resulting damage to DNA, proteins and membranes. In the absence of degradation or repair of damaged macromolecules or both it is thought that such damage is implicated in cancer induction and progression (Cerutti, 1991; Clayson *et al*., 1994).

Protective mechanisms against oxidative damage

Rodents on energy-restricted diets have been found to have preserved or increased activities of enzyme systems, such as tissue catalase and glutathione peroxidase, that are responsible for protection against oxidative damage (Yu, 1993; Feuers *et al*., 1995), as well as preserved or enhanced DNA repair activity (Lee & Yu, 1990). These protective systems are described further in Section 3.1.

Altered fuel use and glycation of macromolecules

Blood glucose levels have been found to be higher throughout the day in *ad libitum*-fed than in energy-restricted Fischer-344 rats (Masoro, 1996). It has been suggested that non-enzymatic glycation by Maillard reactions of proteins and other macromolecules, including DNA, may contribute to the ageing process and increase the probability of carcinogenesis.

Hormonal involvement

Increased energy intake results in raised levels of growth-promoting hormones such as growth hormone, insulin, insulin-like growth factor (IGF)-1, prolactin and other mammotrophic hormones, and decreased levels of growth-inhibiting adrenocorticoids (e.g. Leakey *et al*., 1998). These are not only signals for growth of normal tissue but also for neoplastic development. Hormones are recognised to be implicated in the development of cancers at several sites of the body (e.g. Shephard & Shek, 1998; Stoll, 1998), and are discussed in Sections 2.3 and 3.3.

Intake of toxic contaminants

Speculation that energy restriction delays tumorigenesis in control animals by exposing the animal to lower intakes of (unintentional) toxic contaminants has been dismissed (Keenan *et al*., 1996), as intakes of food, and therefore also of contaminants, are the same, per unit body weight, in energy restricted and *ad libitum*-fed rodents. This argument assumes that the contaminant-to-food ratio remains the same, which may be true in rodent bioassays but would not necessarily be the case for free-living humans. Attention has been drawn to the importance of controlling intakes of nutrients, micronutrients and contaminants in toxicity testing (Newberne & Sotnikov, 1996).

Other mechanisms

The mechanisms described above are not comprehensive, and ongoing research is still producing and investigating evidence for other ways by which energy metabolism might influence carcinogenesis and survival. For example, overfeeding might result in increased expression of tumour virus genes or proto-oncogenes, decreased apoptosis of normal, aged and preneoplastic cells, depressed immune and enhanced auto-immune responses, and inhibition of other general protective mechanisms, such as the heat shock protein system.

1.5 Energy intake and carcinogenesis in humans

Does energy intake affect carcinogenesis in humans? In stark contrast to the well-established case in rodents, there is no direct experimental evidence to answer this question, and relatively few attempts have been made to assess it (e.g. Albanes, 1987; Willett, 1987; Velthuis-te Wierik & van den Berg, 1994; Kritchevsky, 1995). Some of the epidemiological evidence is discussed in Section 2.3. The reasons for the paucity of data are easy to understand. They include the long lifetime of humans in relation to rats, and the long lag times thought to be involved in human carcinogenesis. Humans are highly variable in their biological, behavioural and environmental characteristics, all of which influence energy intake, and the degree of control required for definitive experiments is virtually impossible to achieve on a long-term basis. Measurements of both intake and expenditure of energy in free-living individuals are difficult; methods that are available to overcome technical difficulties and variations caused by behavioural differences are intensive and expensive.

Despite this, it is recognised that, given the strength of the evidence in rodents, the phenomenon warrants investigation in humans. Experiments with dietary restriction in non-human primates have been started (Weindruch, 1996). One team of

investigators has published reports on a 10-week restriction of habitual energy intake among normal-weight middle-aged men (Velthuis-te Wierik *et al*., 1995b). No effects were reported on the indices of oxidative stress or antioxidant systems measured, despite a modest loss of body weight which continued throughout the study, and despite many other physiological changes beneficial to long-term health (Velthuis-te Wierik *et al*., 1994; Velthuis-te Wierik *et al*., 1995a). The authors speculated that several factors could have prevented an effect being seen on the toxicological variables, the most cogent of these being the short experimental period.

Another potential source of experimental information is the Biosphere 2 project in the USA, in which eight adults lived for two years in a 3.15 acre space, which was energetically open but materially closed to the rest of the environment. The subjects' diet was low in energy (mean 1780 kcal/d), low in fat (10% of energy) and nutrient dense, and resulted in the expected losses of body weight (from an initial average of 67 kg to 56 kg after the first year) and several physiological changes conducive to prevention of degenerative disease, similar to the changes seen in animals on an energy-restricted diet (Walford *et al*., 1992). Also in the USA, a study of dietary restriction in humans has been started to complement the animal data (Hass *et al*., 1996).

Indirect evidence for or against an effect of energy in carcinogenesis comes largely from epidemiological observations. Ecological analyses of cancer incidence support the idea that a low-energy diet may represent a lower risk for cancer; low incidences of most types of cancer are reported in countries with the low average energy intakes typical of developing countries. However, the information gained from such analyses is known to be lacking in specificity and, in general, case–control or longitudinal studies do not lend much support for the effect (Kritchevsky, 1995). One major review of the epidemiological evidence has concluded that higher energy intake is associated with a consistently demonstrable increased risk of cancer of the colon, pancreas and prostate, despite the fact that estimates of energy intake *per se* may be somewhat misleading for several reasons (World Cancer Research Fund, 1997). Another review concludes similarly that 'reducing caloric intake and relative body weight may lead to a considerable decrease in cancer risk in humans' (Albanes, 1987). However, a third major review found consistent evidence for a positive effect of energy intake only in endometrial cancer (Department of Health, 1998).

Energy intake, as measured in many epidemiological studies, often displays an inconsistent relationship with cancer risk because low intakes are disproportionately reported by subjects of higher body mass index (BMI; Price *et al*., 1997; Macdiarmid & Blundell, 1998), a paradox that is thought to be due largely to under-reporting of food intake. Low intakes may also be reported by inactive lean smokers. High energy intakes, on the other hand, may be associated with lower cancer risk due mostly to the apparently beneficial effect of higher physical activity: thin people are much more likely than obese ones to engage in vigorous exercise. A high energy intake is thus more likely to be reported by a lean individual with a very high energy expenditure driven by exercise than by an obese individual reporting accurately their true intake, which is high due to a higher body mass and possible hyperphagia (ingestion of greater than optimal quantities of food).

Notwithstanding the many confusing facets of energy intake as an epidemiological variable, it is recognised by some experts that the total amount of food (energy) eaten has a significant influence, either in itself or as an indicator of other variables, on analyses of dietary constituents (nutrients or foods) and cancer risk, so that it has become standard practice by some groups to 'adjust' for energy intake in analyses of individual nutrients or foods in relation to cancer risk (Willett & Stampfer, 1986). The rationale given is that body size, including height, and exercise level play important roles in the amount of any nutrient eaten, simply because size and activity affect the total amount of food consumed, and adjusting for the total energy intake should neutralise this influence. However, this adjustment is often made without due discretion, and may mask any primary effects of energy intake or related variables on cancer or other diseases.

1.6 Energy expenditure and carcinogenesis in humans

As explained above (Section 1.3), energy expenditure and intake are related in a complex manner, and expenditure is attributable only partly and to a varying degree to physical activity (exercise). Exercise tends to be viewed as optional in contrast to expenditure for basal metabolism which is required for maintenance of the organism.

Comment on exercise levels and their variability in the rodent bioassay is rare in the toxicological literature. However, there is mounting epidemiological evidence that in humans increased

levels of long-term physical activity provide protection against cancer at all sites, most consistently cancer of the colon and possibly also cancer of the lung, breast and reproductive tract (World Cancer Research Fund, 1997; Shephard & Shek, 1998). People who have been physically active life-long, either occupationally or recreationally (World Cancer Research Fund, 1997; Shephard & Shek, 1998), and even those who were athletes during their college years have been reported to be at lower risk of some cancers than their less-active peers (Frisch *et al.*, 1985).

It has been suggested that the mechanism for the protective effect of exercise on breast cancer is related to a lower lifetime exposure to circulating oestrogens, due to longer and less regular ovulatory cycles in athletes. Lower body fat levels in young females are an important factor both in later menarche and in amenorrhoea, which in turn contribute to lower exposure to the oestrogen, oestradiol, the single most potent known risk factor for breast cancer. Exercise tends to promote a higher lean to fat ratio and lower body weight, and a more favourable milieu of growth factors and insulin, proposed as another mechanism for protection against cancer (Giovannucci, 1995). All of these mechanisms are consistent with the long-term energy-restriction hypothesis.

The so-called 'oxygen paradox' presents a challenge in integrating hypotheses for energy metabolism and carcinogenesis. Physical activity increases the use of oxygen and would conceivably raise the risk of lipid peroxidation and tissue damage (Kanter, 1998), in the absence of sufficient endogenous antioxidant systems. Such free radical production has been linked particularly to strenuous exercise which induces tissue hypoxia, and has given rise to suggestions by some that the endogenous antioxidant systems should be assisted in combating the damaging effects of heavy bouts of exercise by the ingestion of antioxidant supplements. The apparent inconsistency between exercise having a protective overall role against cancer and constituting an increased risk for oxidative damage illustrates the complexity of the issue: it may be that the various metabolic effects of exercise have opposing influences on cancer risk (Kazakoff *et al.*, 1996). This paradox is addressed in Section 3.2.

The UK Working Group on Diet and Cancer of the Committee on Medical Aspects of Food and Nutrition Policy did not consider exercise to be part of its remit in its recent report on nutrition and cancer (Department of Health, 1998). However, it is clearly important to consider energy expenditure along with intake and balance, since it is unlikely that mechanisms can be understood for these elements in isolation.

1.7 Energy balance and carcinogenesis in humans

Despite being a somewhat blunt tool to estimate body fatness, BMI is the most commonly used index of long-term energy balance because it is easily and reproducibly measured.

Higher BMI has consistently been found to be associated with increased risks of colon cancer, particularly in men, and of breast cancer in post-menopausal women, with some evidence for a similar effect on endometrial cancer (World Cancer Research Fund, 1997; Department of Health, 1998). The first of the summary overall recommendations of the UK Working Group on Diet and Cancer of the Committee on Medical Aspects of Food and Nutrition Policy is to maintain a BMI within the range 20–25 kg/m^2, commonly regarded as 'normal', and not to increase it during adult life (Department of Health, 1998).

The mechanisms by which increased body fatness may contribute to cancer risk, especially colon cancer risk, are debatable. Cancers of the reproductive tract are expected to be hormonally mediated, and hormone status is affected by body fatness, as discussed above (Section 1.6) and in Sections 2.3 and 3.3.

The idea that avoiding being overweight may assist cancer prevention has recently been made more complex by a preliminary report on analyses after the Biosphere 2 experiment. It is suggested that repeated gain and loss of weight could exacerbate cancer risk because environmental lipophilic toxins, which tend to be stored within adipose tissues, may be released into the bloodstream when fat is withdrawn from storage. In contrast, it has been speculated that a single slow weight loss, even though releasing toxins, may not be so harmful (Cohen, 1999).

1.8 Of mice and men...

Very little is known about how applicable knowledge of energy metabolism and carcinogenesis in rodents is to humans. There are many similarities and also differences between humans and rodents to take into account. The uses and limitation of rodents as a model for humans are discussed in Section 4.

References

Albanes D (1987) Caloric intake, body weight, and cancer: A review. *Nutr Cancer, 9,* 199–217

Allaben WT, Turturro A, Leakey JEA, Seng JE & Hart RW (1996) FDA points-to-consider documents: The need for dietary control for the reduction of experimental variability within animal assays and the use of dietary restriction to achieve dietary control. *Toxicol Pathol, 24,* 776–781

Bertrand HA, Lynd FT, Masoro EJ & Yu BP (1980) Changes in adipose mass and cellularity through the adult life of rats fed *ad libitum* or a life prolonging restricted diet. *J Gerontol, 35,* 827–835

Cadenas E (1989) Biochemistry of oxygen toxicity. *Annu Rev Biochem, 58,* 79–110

Cerutti PA (1991) Oxidant stress and carcinogenesis. *J Clin Invest, 21,* 1–5

Clayson DB, Mehta R & Iverson F (1994) Oxidative DNA damage — the effects of certain genotoxic and operationally nongenotoxic carcinogens. *Mutat Res, 317,* 25–42

Cohen P (1999) Poisonous fat. *New Sci, 161,* 13

DH (1998) *Nutritional Aspects of the Development of Cancer* (Report on Health and Social Subjects 48), London, UK, The Stationery Office

Festing MFW (1997) Fat rats and carcinogenesis testing. *Nature, 388,* 321–322

Feuers RJ, Duffy PH, Chen F, Desai V, Oriaku E, Shaddock JG, Pipkin JW, Weindruch R & Hart RW (1995) Intermediary metabolism and antioxidant systems. In: Hart RW, Neumann DA & Robertson RW, eds, *Dietary Restriction: Implications for the Design and Interpretation of Toxicity and Carcinogenicity Studies*, Washington DC, USA, ILSI Press, pp 181–195

Frisch RE, Wyshak G, Albright NL, Albright TE, Schiff I, Jones KP, Witschi J, Shiang E, Koff E & Marguglio M (1985) Lower prevalence of breast-cancer and cancers of the reproductive system among former college athletes compared to non-athletes. *Brit J Cancer, 52,* 885–891

Giovannucci E (1995) Insulin and colon cancer. *Cancer Causes Control, 6,* 164–179

Harrison DE, Archer JR & Astle CM (1984) Effects of food restriction on aging: Separation of food intakes and adiposity. *Proc Natl Acad Sci USA, 81,* 1835–1838

Hass BS, Lewis SM, Duffy PH, Ershler W, Feuers RJ, Good RA, Ingram DK, Lane MA, Leakey JEA, Lipschitz D, Poehlman ET, Roth GS, Sprott RL, Sullivan DH, Turturro A, Verdery RB, Walford RL, Weindruch R, Yu BP & Hart RW (1996) Dietary restriction in humans: Report on the Little Rock Conference on the value, feasibility, and parameters of a proposed study. *Mech Aging Dev, 91,* 79–94

Kanter M (1998) Free radicals, exercise and antioxidant supplementation. *Proc Nutr Soc, 57,* 9–13

Kazakoff K, Cardesa T, Liu J, Adrian TE, Bagchi D, Bagchi M, Birt DF & Pour PM (1996) Effects of voluntary physical exercise on high-fat diet-promoted pancreatic carcinogenesis in the hamster model. *Nutr Cancer, 26,* 265–279

Keenan KP, Laroque P, Ballam GC, Soper KA, Dixit R, Mattson BA, Adams SP & Coleman JB (1996) The effects of diet, *Ad libitum* overfeeding, and moderate dietary restriction on the rodent bioassay: The uncontrolled variable in safety assessment. *Toxicol Pathol, 24,* 757–768

Keenan KP & Soper KA (1995) The effects of *ad libitum* overfeeding and moderate dietary restriction on Sprague-Dawley rat survival, spontaneous carcinogenesis, chronic disease and the toxicologic response to pharmaceuticals. In: Hart RW, Neumann DA & Robertson RW, eds, *Dietary Restriction: Implications for the Design and Interpretation of Toxicity and Carcinogenicity Studies*, Washington DC, USA, ILSI Press, pp 99–126

Kritchevsky D (1993) Energy restriction and carcinogenesis. *Food Res Int, 26,* 289–295

Kritchevsky D (1995) The effect of overnutrition and undernutrition on cancer. *Eur J Cancer Prev, 4,* 445–451

Leakey JEA, Seng JE, Barnas CR, Baker VM & Hart RW (1998) A mechanistic basis for the beneficial effects of caloric restriction on longevity and disease: Consequences for the interpretation of rodent toxicity studies. *Int J Toxicol, 17 (suppl 2),* 5–56

Lee DW & Yu BP (1990) Modulation of free radicals and superoxide dismutases by age and dietary restriction. *Aging, 2,* 357–362

Macdiarmid J & Blundell J (1998) Assessing dietary intake: Who, what and why of under-reporting. *Nutr Res Rev, 11,* 231–253

Masoro EJ (1996) Possible mechanisms underlying the antiaging actions of caloric restriction. *Toxicol Pathol, 24,* 738–741

Masoro EJ, Iwasaki K, Gleiser CA, McMahan CA, Seo EJ & Yu BP (1989) Dietary modulation of the progression of nephrology in aging rats: An evaluation of the importance of protein. *Am J Clin Nutr, 49,* 1217–1227

McCay CM, Crowell MF & Maynard LA (1935) The effect of retarded growth upon the length of life and upon the ultimate body size. *J Nutr, 10,* 63–79

Newberne PM & Sotnikov AV (1996) Diet: The neglected variable in chemical safety evaluations. *Toxicol Pathol, 24,* 746–756

Price GM, Paul AA, Cole TJ & Wadsworth MEJ (1997) Characteristics of the low-energy reporters in a longitudinal national dietary survey. *Brit J Nutr, 77,* 833–851

Shephard RJ & Shek PN (1998) Associations between physical activity and susceptibility to cancer — possible mechanisms. *Sports Med, 26,* 293–315

Sohal RS & Weindruch R (1996) Oxidative stress, caloric restriction and aging. *Science, 273,* 59–63

Stoll BA (1998) Association between breast and colorectal cancers. *Brit J Surg, 85,* 1468–1472

Turturro A, Duffy P & Hart RW (1995) The effect of caloric modulation on toxicity studies. In: Hart RW, Neumann DA & Robertson RT, eds, *Dietary Restriction: Implications for the Design and Interpretation of Toxicity and Carcinogenicity Studies*, Washington DC, USA, ILSI Press, pp 79–97

Velthuis-te Wierik EJM, Meijer P, Kluft C & van den Berg H (1995a) Beneficial effect of moderately energy-restricted diet in fibrinolytic factors in non-obese men. *Metab Clin Exper, 44,* 1548–1552

Velthuis-te Wierik EJM, van Leeuwen REW, Hendriks HFJ, Verhagen H, Loft S, Poulsen HE & van den Berg H (1995b) Short-term moderate energy restriction does not affect indicators of oxidative stress and genotoxicity. *J Nutr, 125,* 2631–2639

Velthuis-te Wierik EJM & van den Berg H (1994) Energy restriction, the basis for successful aging in man? *Nutr Res, 14,* 1113–1134

Velthuis-te Wierik EJM, van den Berg H, Schaafsma G, Hendriks HFJ & Brouwer A (1994) Energy restriction, a useful intervention to retard human ageing? Results of a feasibility study. *Eur J Clin Nutr, 48,* 138–148

Walford RL, Harris SB & Gunion MW (1992) The calorically restricted low-fat, nutrient dense diet in Biosphere 2 significantly lowers blood glucose, total leukocyte count, cholesterol, and blood pressure in humans. *Proc Natl Acad Sci USA, 89,* 11533–11537

Weindruch R (1996) The retardation of aging by caloric restriction: Studies in rodents and primates. *Toxicol Pathol, 24,* 742–745

Weindruch R & Walford RL (1982) Dietary restriction in mice beginning at 1 year of age: Effect on life-span and spontaneous cancer incidence. *Science, 215,* 1415–1418

Weindruch R & Walford RL (1988) *The Retardation of Aging and Disease by Dietary Restriction*, Springfield IL, USA, Charles C. Thomas Publisher

Willett WC (1987) Implications of total energy intake for epidemiologic studies of breast and large-bowel cancer. *Am J Clin Nutr, 45 (suppl),* 354–360

Willett W & Stampfer MJ (1986) Total energy-intake — implications for epidemiologic analyses. *Am J Epidemiol, 124,* 17–27

World Cancer Research Fund (1997) *Food, Nutrition and Cancer: A Global Perspective*, Washington DC, USA, World Cancer Research Fund/American Institute for Cancer Research

Yu BP, Masoro EJ & McMahan CA (1985) Nutritional influences on aging of Fischer 344 rats: Physical, metabolic and longevity characteristics. *J Gerontol, 40,* 657–670

2 Influence of caloric intake

2.1 Effects of overfeeding and dietary restriction on the rodent bioassay

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Rodent bioassays used for human safety assessment must attempt to control as many potentially confounding experimental variables as possible in order to define precisely dose-related and treatment changes. Over the past decades, the early onset and increased severity of certain spontaneous degenerative diseases and tumours in rodents have resulted in decreased animal survival and a loss of bioassay sensitivity to detect true treatment effects (Keenan *et al.,* 1992; Turturro *et al.,* 1995; Allaben *et al.,* 1996). These adverse events have been the result of overnutrition leading to increased growth and body weight in most rodent strains and stocks currently used in these bioassays.

Caloric intake is the most poorly controlled variable in these bioassays and adversely affects almost every physiological process and anatomical structure to the molecular level. The total amount of food (calories) per animal per day is the critical factor affecting their physiology, health and longevity (Laroque *et al.,* 1997). Caloric overfeeding can best be controlled by moderate dietary restriction (DR). Moderate DR has been defined as the level of food intake that improves the 2-year rodent survival by decreasing the incidence and severity of the spontaneous degenerative diseases without preventing the occurrence of spontaneous tumours. Moderate DR corresponds to 65–75% of the maximum unrestricted *ad libitum* food intake in adult rats. Moderate DR does not require addition of essential micronutrient supplements, as the amounts of nutrients given per gram of body weight are equal or slightly greater in DR-fed animals than in *ad libitum*-fed animals. In addition, no adverse effects on physiology, metabolism, clinical biochemistry or pathology have been seen in animals under moderate DR in 2-year bioassays.

The use of moderate DR provides a better-controlled animal model (Keenan *et al.,* 1995a, b; Roe *et al.,* 1995; Keenan *et al.,* 1996a). Moderate DR has the great advantage of reducing and delaying age-related spontaneous renal, cardiac and endocrine diseases. By improving survival, DR allows more time for treatment-related tumours to become detectable and increases exposure times to the test substances. In addition, DR-fed animals are more resistant to long-term metabolic injury and maintain their antioxidant and other protective mechanisms for a longer period than their *ad libitum*-fed counterparts. Therefore, they can withstand higher xenobiotic loads and thus frequently achieve higher maximum tolerated doses of test substances than *ad libitum*-overfed animals. Using the DR-fed model not only reduces variability and background diseases, but also allows higher doses of test substances to be tested, thus increasing drug and dose exposure and bioassay sensitivity (Keenan *et al.,* 1996b; Keenan *et al.,* 1998).

Overall, moderate DR increases the biological and statistical sensitivity of the 2-year rodent bioassay to detect true treatment-related changes; it decreases the occurrence of interfering factors such as degenerative cardiac and renal diseases, decreases the variability within and among bioassays, increases the 2-year survival of animals, allows testing of high levels of compounds for long periods of time in 2-year rodent bioassays and allows detection of late-onset tumours.

In conclusion, moderate DR is the appropriate model for well-controlled toxicity and carcinogenicity studies. It reduces the individual and study-to-study variability and, therefore, provides a better model to determine true treatment-related effects and dose-responses for risk assessment.

References

Allaben WT, Turturro A, Leakey JEA, Seng JE & Hart RW (1996) FDA points-to-consider documents: The need for dietary control for the reduction of experimental variability within animal assays and the use of dietary restriction to achieve dietary control. *Toxicol Pathol, 24,* 776–781

Keenan KP, Smith PF, Ballam GC, Soper KA & Bokelman DL (1992) The effects of diet and dietary optimization (caloric restriction) on rat survival in carcinogenicity studies — an industrial view point. In: McAuslane JAN, Lumley CF & Walker SR, eds, *Centre for Medicines Research Workshop — The Carcinogenicity Debate*, London, UK, Butler and Tanner Ltd, pp 77–102

Keenan KP, Soper KA, Smith PF, Ballam GC & Clark RL (1995a) Diet, overfeeding and moderate dietary restriction in control Sprague-Dawley rats: I. Effects on spontaneous neoplasms. *Toxicol Pathol, 23,* 269–286

Keenan KP, Soper KA, Hertzog PR, Gumprecht LA, Smith PF, Mattson BA, Ballam GC & Clark RL (1995b) Diet, overfeeding and moderate dietary restriction in control Sprague-Dawley rats. II. Effects of age-related proliferative and degenerative lesions. *Toxicol Pathol, 23,* 287–302

Keenan KP, Laroque P, Ballam GC, Soper KA, Dixit R, Mattson BA, Adams SP & Coleman JB (1996a) The effects of diet, *Ad libitum* overfeeding, and moderate dietary restriction on the rodent bioassay: The uncontrolled variable in safety assessment. *Toxicol Pathol, 24,* 757–768

Keenan KP, Laroque P, Soper KA, Morrissey RE & Dixit R (1996b) The effects of overfeeding and moderate dietary restriction on Sprague-Dawley rat survival, pathology, carcinogenicity, and the toxicity of pharmaceutical agents. *Exper Toxicol Pathol, 48,* 139–144

Keenan KP, Laroque P & Dixit R (1998) Need for dietary control by caloric restriction in rodent toxicology and carcinogenicity studies. *J Toxicol Environ Health, 1B,* 101–114

Laroque P, Keenan KP, Soper KA, Dorian C, Gerin G, Hoe C-M & Duprat P (1997) Effect of initial body weight and moderate dietary restriction on survival in Sprague-Dawley rats. *Exper Toxicol Pathol, 49,* 459–465

Roe FJC, Lee PN, Conybeare G, Kelly D, Matter B, Prentice D & Tobin G (1995) The Biosure Study: Influence of composition of diet and food consumption on longevity, degenerative disease and neoplasia in Wistar rats studied for up to 30 months post weaning. *Food Chem Toxicol, 33 (suppl 1),* 1–100

Turturro A, Duffy P & Hart RW (1995) The effect of caloric modulation on toxicity studies. In: Hart RW, Neumann DA & Robertson RT, eds, *Dietary Restriction: Implications for the Design and Interpretation of Toxicity and Carcinogenicity Studies*, Washington DC, USA, ILSI Press, pp 79–97

2.2 The interaction of energy metabolism with xenobiotic pathways

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A decrease in caloric intake without a deficiency in essential nutrients extends maximum life span (McCay *et al*., 1939) and retards the rate of onset of both spontaneous and induced carcinogenesis (Hart & Turturro, 1997). Interestingly, the impact of decreased caloric intake appears to be consistent across sex, strain and species within the placental mammals, regardless of level of biological organisation — molecular, biochemical and metabolic or physiological (Hart *et al*., 1995).

Since body weight is reduced proportionally with decreased caloric intake it is not surprising that metabolic output (oxygen consumption) per gram of lean body mass (LBM) is not affected by reduced caloric intake in either rats (Duffy *et al*., 1989) or mice (Duffy *et al*., 1990). However, body temperature is significantly reduced by a prolonged decrease in caloric intake (Duffy *et al*., 1989, 1990), with the greatest impact occurring in species with the least body mass. Studies found the daily variations in respiratory quotient (RQ) were increased by decreased caloric intake, indicating rapid substrate-dependent shifts in metabolic pathways from carbohydrate metabolism (immediately after feeding) to fatty acid metabolism (several hours following feeding). Similar results were found in other studies in which the level of key liver enzymes associated with intermediary metabolism (Feuers *et al*., 1989) and drug metabolism (Leakey *et al*., 1989) were up-regulated with a decrease in caloric intake.

Thus decreased caloric intake appears to be associated with a spectrum of biochemical and physiological changes that characterise the organism's adaptation to reduced caloric intake and which could provide the mechanistic basis for the effect of caloric restriction on longevity and health. There is evidence suggesting that the primary adaptation appears to be a rhythmic hypercorticism in the absence of elevated adrenocorticotropin (ACTH). This characteristic hypercorticism evokes a spectrum of responses including reduced body temperature, increased metabolic efficiency, decreased mitogenic response coupled with an increased rate of apoptosis, reduced inflammatory response, reduced oxidative damage to macromolecules, reduced reproductive capacity and altered drug-metabolising enzyme expression. These same features are observed as a function of decreased caloric intake. In both cases there is an increase in the ability of the organism to withstand chemical, physical or biological stress and a concurrent decrease in growth and metabolism in peripheral tissues with a sparing of central function. The impact of these changes on chemical toxicity is described below using three classes of chemical agents (Leakey *et al*., 1998).

The effect of reduced caloric intake on chemical carcinogenesis is striking (Hart *et al*., 1995). Even moderate levels of reduction in caloric intake can significantly reduce the expression of various agent-induced cancers by altering key physiological functions. For example, as caloric intake decreases urinary output increases by up to four-fold (Hart *et al*., 1995), thereby enhancing compound conjugation and elimination (Chou *et al*., 1993). Specific cytochrome P450 isoenzyme activities are decreased by reduced caloric intake whereas the activities of certain glucuronidases are increased, with a result of improved agent detoxification (Hart *et al*., 1995). Even when the total amount of agent that combines with the genome is not affected by caloric intake the formation of specific adducts correlated with carcinogenicity can be inhibited (Xiao *et al*., 1993). Thus, by a myriad of mechanisms, changes in caloric intake can reduce the amount of carcinogenic insult that results from interaction of the organism with a chemical carcinogen.

Cardiotoxicity of classic agents such as isoproterenol can also be altered by changes in caloric intake (Hart *et al*., 1995). Physiologic performance was measured by heart rate, electrocardiogram (ECG) and body temperature as a function of diet (high versus low caloric intake), and age (12 months- versus 36 months-old animals), following exposure of rodents to various doses of isoproterenol given by a single intramuscular injection over a range of 0.005 mg/kg LBM to 300 mg/kg LBM. It was observed that a strong age- and caloric-intake dependency existed, with a four-order-of-magnitude difference being observed in the dose required for 50% mortality between restricted and non-restricted 36 months-old animals. Interestingly, isoproterenol elevated the heart rate in both high- and low-caloric intake rodents to a similar extent. Since inversion of the T complex (ventricular repolarisation) of the ECG waveform occurred within minutes after injection of the agent, it is reasonable to conclude that severe ischaemia resulted in both groups. The difference

appears to be that as caloric intake is reduced the threshold for fibrillation is increased, since in the animals on reduced caloric intake long periods of drug-induced ischaemia did not cause arrhythmias and cardiac arrest. Since isoproterenol-related cardiac arrhythmias are linked to calcium (Ca^{++}) overload and free radical damage to the sarcolemma and mitochondria it is possible that reducing caloric intake may help protect membranes by blocking Ca^{++} channels, thereby preventing cardiac arrest.

Ganciclovir sodium or 9-(1,3-dihydroxy-2-propoxymethyl)guanine monosodium salt (DHPG) is an acyclic nucleoside analogue of guanine. DHPG is an effective antiviral agent, which appears to act by inhibiting DNA polymerase activity. The degree of inhibition is dependent on phosphorylation of DHPG by cellular kinases to the triphosphate form. The antiviral activity of DHPG appears to be due to competitive inhibition of viral DNA polymerases and the direct incorporation of DHPG triphosphate into viral DNA (Berg *et al*., 1994). An unwanted side effect of ganciclovir is its haematopoietic toxicity, with approximately 40% of the patients receiving it developing granulocytopenia and 20% thrombocytopenia (Berg *et al*., 1994). Studies have demonstrated that caloric restriction reduced drug-induced toxicity by up to 25-fold in both young and middle-aged mice, as with DHPG both an age and a nutritional effect was observed.

Thus, reduced caloric intake may activate a survival mechanism that provides rodents and other mammals with an adaptive advantage to cope with fluctuations in food supply. During periods of abundance, body growth and fecundity are favoured over endurance and longevity. Conversely, during periods of food scarcity, reproductive performance and growth are sacrificed to endure survival of individuals to breed in better times.

References

Berg T, Breen P, Feuers R, Orisku E, Chen F & Hart RW (1994) Acute toxicity of glanciclovir: Effect of dietary restriction and chronobiology. *Food Chem Toxicol, 32,* 45–50

Chou MW, Kong J, Chung K & Hart RW (1993) Effect of caloric restriction on the metabolic activation of xenobiotics. *Mutat Res, 295,* 223–235

Duffy PH, Feuers RJ, Leakey JA, Nakamura KD, Turturro A & Hart RW (1989) Effect of chronic restriction on physiological variables related to energy metabolism in the male Fischer 344 rat. *Mech Aging Dev, 48,* 117–133

Duffy PH, Feuers R & Hart RW (1990) Effect of chronic caloric restriction on the circadian regulation of physiological and behavioural variables in old male B6C3F1 mice. *Chronobiol Int, 7,* 291–303

Feuers R, Duffy PH, Leakey JEA & Hart RW (1989) Effect of chronic caloric restriction on hepatic enzymes of intermediary metabolism in the male Fischer 344 rat. *Mech Aging Dev, 48,* 179–189

Hart RW, Neumann DA & Robertson RT, eds (1995) *Dietary Restriction: Implications for the Design and Interpretation of Toxicity and Carcinogenicity Studies*, Washington DC, USA, ILSI Press

Hart RW & Turturro A (1997) Dietary restriction and cancer. *Environ Health Perspect, 105,* 989–992

Leakey JEA, Cunny H, Bazare J, Hart RW, Webb PJ, Feuers RJ & Duffy PH (1989) Effects of aging and caloric restriction on hepatic drug metabolizing enzymes in the Fischer 344 rat. I. The cytochrome P-450 dependent monooxygenase system. *Mech Aging Dev, 48,* 145–155

Leakey JEA, Seng JE, Barnas CR, Baker VM & Hart RW (1998) A mechanistic basis for the beneficial effects of caloric restriction on longevity and disease: Consequences for the interpretation of rodent toxicity studies. *Int J Toxicol, 17 (suppl 2),* 5–56

McCay C, Maynard L, Sperling G & Barnes L (1939) Retarded growth, life span, ultimate body size and age changes in the albino rat after feeding diets restricted in calories. *J Nutr, 18,* 15–25

Xiao Y, von Tungeln L, Chouo M, Hart RW & Fu P (1993) Effect of caloric restriction on the metabolism of 7-bromobenz(alpha)anthracene by male B6C3F1 mouse liver microsomes: Reduction of metabolic activation pathways. *Age, 16,* 160–165

2.3 The epidemiology of cancer and energy intake

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Incidence and mortality rates of cancers of the colon, rectum, pancreas, gallbladder, breast, endometrium, ovary, prostate, and kidney are high in Western societies, where lifestyle is characterised by low physical activity levels, and by energy-dense diets rich in total and saturated fats and rapidly digestible carbohydrates.

Earlier individual-based studies of diet and cancer, mostly of a case–control design, focused especially on risks associated with high intake of total or saturated fats, and often showed high-fat diets to be associated with increased risk of cancers of the colon, breast, pancreas, or prostate. Other studies have shown increased risks of cancers of the colon, rectum and pancreas in subjects with high consumption of sugar or refined carbohydrates. However, these results have not always been confirmed by prospective cohort studies and, in addition, are often difficult to interpret because of lack of proper adjustment for total energy intake. Besides the macronutrients, many studies have shown decreased risks of cancer in subjects with diets rich in dietary fibre, vegetables and fruit. Physical activity has also been intensively studied in relation to cancer risk, and overall has especially been shown to protect against cancers of the colon and breast.

Low levels of physical activity and the macronutrient composition of diet typical of Western societies are both thought to be major causes of obesity, which in these societies is becoming increasingly prevalent. There is considerable controversy, however, about what type of macronutrient excess may be related to development of obesity. According to a theoretical model by Flatt, based on controlled calorimetric experiments in animals and humans, food intake is mainly regulated to maintain carbohydrate balance, so that more or less food is consumed depending on the carbohydrate composition of the diet. When the dietary fat/carbohydrate ratio rises, the oxidation of fats does not rise acutely but only after weight gain and an increase in plasma concentration of free fatty acids which are released from adipose tissue. The prediction from Flatt's model that high-fat diets would cause obesity finds some support in results from human dietary intervention studies, as well as from cross-sectional epidemiological studies. Nevertheless, methodological criticisms can be raised against some of the human intervention studies, and opposing recent time-trends in the USA in the prevalence of obesity and in the average fat content of diet would seem to argue against Flatt's predictions.

A positive balance between energy intake and energy expenditure in the long term causes obesity. A high body mass index (BMI; weight/height^2) has been found to be positively associated with increased risks of cancers of endometrium, breast (in postmenopausal women only), pancreas, gallbladder, and kidney. Risks of several of these cancers appear to be related particularly to abdominal adiposity, as indicated by a increased waist-to-hip ratio of body circumference measurements. A high BMI, however, does not seem to have any positive association with risk of breast cancer before menopause, or cancers of the ovary and prostate. Associations of cancer risk with obesity reflect relationships with positive energy balance especially during adult life. Risks of several forms of cancer, however — including cancers of breast, colon and rectum — also appear associated with available energy and body growth in infancy and childhood, as reflected by associations of higher risk with greater adult body stature, and with early menarche.

Major mechanisms through which a Western lifestyle and overnutrition may lead to cancer include alterations in the metabolism of hormones and growth factors, increased endogenous generation of reactive oxygen species or other radical compounds, or changes in immune response.

A large number of epidemiological studies have been conducted to examine relations of endogenous (plasma) hormone levels with cancer risk, particularly in relation to cancers of the breast, endometrium, and prostate. So far, however, these studies have focused mostly on sex steroids, and have often been of a case–control design. A promising new line of epidemiological research, more often now within prospective cohorts with banks of blood specimens, focuses on alterations in the metabolism of insulin and insulin-like growth factors as a possible physiological link to increased tumour formation.

Insulin is a key hormone in the regulation of energy metabolism. It increases the bio-activity of insulin-like growth factor-1 (IGF-1) by enhancing its synthesis, and by downregulating several of its binding proteins (IGFBP-1 and IGFBP-2). Overnutrition and/or obesity tend to increase plasma insulin and bio-active IGF-1. Insulin and IGF-1 both stimulate anabolic (growth) processes,

as a function of available energy and elementary substrates (e.g. amino acids). In excess, the anabolic signals by insulin or IGF-1 can promote tumour development by inhibiting apoptosis and by stimulating cell proliferation. In addition, insulin and IGF-1 can increase cancer risk by inhibiting the hepatic synthesis of sex-hormone binding globulin (SHBG) and by enhancing sex steroid synthesis, thus increasing the bio-availability of plasma sex steroids.

Preliminary data from recent case–control studies and a few cohort studies suggest that risk of obesity-related cancers (postmenopausal breast cancer, endometrial cancer, colon cancer) may be increased in subjects with high plasma levels of insulin, and low levels of IGFBP-1, but normal levels of total IGF-1. In contrast, risks of cancers unrelated to BMI (premenopausal breast cancer, prostate cancer) appear increased especially in subjects with high total plasma IGF-1 levels.

In the near future, large prospective cohort studies with banks of blood samples, such as the European Prospective Investigation into Cancer and Nutrition (EPIC), may help to identify further metabolic and hormonal alterations through which a Western type of lifestyle and nutrition may cause cancer.

Selected bibliography

Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, Hennekens CH & Pollak M (1998) Plasma insulin-like growth factor-1 and prostate cancer risk: A prospective study. *Science, 279,* 563–566

Flatt JP (1993) Dietary fat, carbohydrate balance and weight maintenance. *Ann N Y Acad Sci, 683,* 122–140

Hankinson SE, Willett WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, Rosner B, Speizer FE & Pollak M (1998) Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. *Lancet, 351,* 1393–1396

Kaaks R (1996) Nutrition, hormones and breast cancer: Is insulin the missing link? *Cancer Causes Control, 7,* 605–625

McTiernan A, Ulrich C, Slate S & Potter J (1998) Physical activity and cancer etiology: Associations and mechanisms. *Cancer Causes Control, 9,* 487–509

Riboli E & Kaaks R (1997) The EPIC project: Rationale and study design. *Int J Epidemiol, 26 (suppl 1),* 6–14

Willett WC (1998) Is dietary fat a major determinant of body fat. *Am J Clin Nutr, 67 (suppl 3),* 556–562

World Cancer Research Fund (1997) *Food, Nutrition and Cancer: A Global Perspective*, Washington DC, USA, World Cancer Research Fund/American Institute for Cancer Research

3 Mechanisms

3.1 Oxidative DNA damage and DNA repair

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DNA oxidation *in vivo* probably derives mainly from oxygen free radicals released as a by-product of normal respiration. The DNA damage measured in cells such as lymphocytes represents a steady state between input of damage and its cellular repair. These are effectively in balance. The input of damage is kept in check by intracellular antioxidants - glutathione, superoxide dismutase, catalase etc. Antioxidants of dietary origin, such as vitamin C, carotenoids and flavonoids, also decrease the level of damage detected.

Oxidative damage includes strand breaks and oxidised bases. 8-Oxoguanine (8-oxogua) is the best known and most commonly measured product, but many other species have been identified. Gas chromatography–mass spectrometry (GC–MS) and high-performance liquid chromatography (HPLC) with electrochemical detection are widely used to measure 8-oxogua or 8-oxodeoxyguanosine (8-oxodG), respectively and equivalently, but care must be taken to avoid the serious artefact of oxidation of guanine during sample preparation. An alternative approach employs lesion-specific endonucleases to create DNA breaks at sites of damage, which are then measured by a technique such as the comet assay (single cell gel electrophoresis). Endonuclease III detects oxidised pyrimidines; formamidopyrimidine glycosylase (fpg) breaks DNA at 8-oxogua. According to the enzymic assays and the more precise HPLC determinations, the background level of oxidation in normal lymphocytes is probably no more than 10 000 8-oxogua per cell — much less than previously thought (see Table 3.1).

Table 3.1 Estimates of baseline levels of 8-oxoguanine (or equivalently 8-oxo-deoxyguanosine) in human cells

Method	Cell type	8-Oxogua/10^5 gua	Reference
HPLC	Monocytes	**3.6**	Dandona *et al.,* 1996
	Lymphocytes	**2.9**	Inoue *et al.,* 1993
	Leukocytes	**0.43**	Collins *et al.,* 1997
	Monocytes	**0.24**	Nakajima *et al.,* 1996
GC-MS	Lymphocytes	**30**	Podmore *et al.,* 1998
	Lymphocytes (cancer patients)	**33**	Olinski *et al.,* 1996
Enzymic	Lymphocytes	**0.06**	Pflaum *et al.,* 1997
	Lymphocytes	**0.04**	Collins *et al.,* 1997

Mean (or median) values are shown, for groups of normal individuals (except for the determination on lymphocytes of cancer patients, as indicated). 1 8-oxogua per 10^5 gua is equivalent to about 30 000 per cell

The modulation of oxidative damage by dietary antioxidants is demonstrated by:

- the decrease in *in-vivo* damage in lymphocyte DNA after several weeks of supplementation of the diet with vitamin C, vitamin E and β-carotene (Duthie *et al.,* 1996);

- the increased resistance of lymphocytes to DNA oxidation *in vitro* following dietary supplementation with these antioxidants (an effect that can be seen after a single large dose; Duthie *et al.,* 1996; Panayiotidis & Collins, 1997);

- the increased resistance of lymphocytes to DNA oxidation *in vitro* that we have found following ingestion of food rich in antioxidants, such as kiwifruit; and

- the negative correlation between endogenous oxidative base damage and serum carotenoid concentrations (Collins *et al.,* 1998a).

However, the biological significance of dietary antioxidant protection is open to question. Several-fold differences in the level of 8-oxodG were seen in lymphocytes from men in five different countries of Europe, even though blood antioxidant levels did not vary (Collins *et al.,* 1998b). Women had similar, low levels of damage in all five countries. At the population level, 8-oxodG levels correlated with risk of coronary heart disease but not with cancer. Large-scale beta-carotene supplementation trials have shown an increase in lung cancer incidence with supplementation in smokers (The Alpha Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994; Omenn *et al.,* 1996).

Another marker of oxidative stress in humans is the appearance of 8-oxodG in urine. This may be a product of repair (though not from the major pathway, base excision repair, which removes the base rather than the nucleoside), or it may reflect oxidation of deoxyguanine (dG) during the breakdown of DNA from dead cells. We have found strong correlations between 8-oxodG in urine and 8-oxodG in lymphocyte DNA measured either by HPLC or by the comet assay with fpg. All three markers are thus validated as indicators of oxidative stress.

Measurement of DNA repair products would be a valuable indicator of the amount of damage incurred, but so far this is elusive. Another, related, challenge is the estimation of repair rates; there is a long-standing belief that DNA repair capacity varies significantly between individuals, and declines with age, and that these changes contribute to ageing and to relative cancer risk. It is possible to measure the removal of DNA damage by repair after treatment of lymphocytes *in vitro* with hydrogen peroxide. Repair of both strand breaks and oxidised bases seems to be very slow (compared with rates observed in cultured cells), but this may be because the lymphocytes are suffering additional oxidation during incubation in equilibrium with atmospheric oxidation (Fillion *et al.,* 1998). This extra oxidation is decreased if the lymphocytes are isolated from blood following one week of carotenoid supplementation. An alternative approach is to measure the ability of a cell extract to repair a damaged DNA substrate — a completely *in vitro* assay system.

References

Collins AR, Duthie SJ, Fillion L, Gedik CM, Vaughan N & Wood SG (1997) Oxidative DNA damage in human cells: The influence of antioxidants and DNA repair. *Biochem Soc Trans, 25,* 326–331

Collins AR, Olmedilla B, Southon S, Granado F & Duthie SJ (1998a) Serum carotenoids and oxidative DNA damage in human lymphocytes. *Carcinogenesis, 19,* 2159–2162

Collins AR, Gedik CM, Olme, Southon S & Bellizzi M (1998b) Oxidative DNA damage measured in human lymphocytes: Large differences between the sexes and between countries, and correlations with heart disease mortality rates. *FASEB J, 12,* 1397–1400

Dandona P, Thusu K, Cook S, Snyder B, Makowski J, Armstrong D & Nicotera T (1996) Oxidative damage to DNA in diabetes mellitus. *Lancet, 347,* 444–445

Duthie SJ, Ma A, Ross MA & Collins AR (1996) Antioxidant supplementation decreases oxidative damage in human lymphocytes. *Cancer Res, 56,* 1291–1295

Fillion L, Collins A & Southon S (1998) β-Carotene enhances the recovery of lymphocytes from oxidative DNA damage. *Acta Biochim Pol, 45,* 183–190

Inoue T, Mu Z, Sumikawa K, Adachi K & Okochi T (1993) Effect of physical exercise on the content of 8-hydroxydeoxyguanosine in nuclear DNA prepared from human lymphocytes. *J Cancer Res, 84,* 720–725

Nakajima M, Takeuchi T, Takeshita T & Morimoto K (1996) 8-Hydroxydeoxyguanosine in human leukocyte DNA and daily health practice factors: Effects of individual alcohol sensitivity. *Environ Health Perspect, 104,* 1336–1338

Olinski R, Zastawny TH, Foksinski M, Windorbska W, Jaruga P & Dizdaroglu M (1996) DNA base damage in lymphocytes of cancer patients undergoing radiation therapy. *Cancer Letts, 106,* 207–215

Omenn GS, Goodman GE, Thornquist MD Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL, Valanis B, Williams JH, Barnhart S & Hammar S (1996) Effects of a combination of β-carotene and vitamin A on lung cancer and cardiovascular disease. *New Engl J Med, 334,* 1150–1155

Panayiotidis M & Collins AR (1997) *Ex vivo* assessment of lymphocytic antioxidant status using the comet assay. *Free Rad Res, 27,* 533–537

Pflaum M, Will O & Epe B (1997) Determination of steady-state levels of oxidative DNA base modifications in mammalian cells by means of repair endonucleases. *Carcinogenesis, 18,* 2225–2231

Podmore ID, Griffiths HR, Herbert KE, Mistry N, Mistry P & Lunec J (1998) Vitamin C exhibits pro-oxidant properties. *Nature, 392,* 559

The Alpha-Tocopherol BCCPG (1994) The effect of vitamin E and β-carotene on the incidence of lung cancers in male smokers. *New Engl J Med, 330,* 1029–1035

3.2 Exercise, oxidative stress and cancer: Risks and benefits

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Evidence indicates that regular exercise provides protection against the occurrence of a number of chronic diseases, including coronary heart disease, hypertension, non-insulin-dependent diabetes and certain cancers (such as colon cancer, Patte *et al.,* 1995). The mechanisms underlying these apparent beneficial effects are likely to be mainly non-specific, relating to improved cardiovascular performance etc., and in subjects undertaking many endurance training regimens the benefits appear to persist despite a general increase in dietary energy intake.

In recent years there has been increasing interest in the possibility that exercising skeletal muscle generates an increased amount of free radicals and other reactive oxygen species. Most work in this area has concentrated on the mitochondria as the most likely source of this increase. Oxidative skeletal muscle contains substantial numbers of mitochondria and is subjected to large changes in oxygen flux during exercise. As part of the process of delivery of energy supplies for muscle activity, molecular oxygen generally undergoes four-electron reduction catalysed by cytochrome oxidase. This process has been claimed to account for 95–98% of the total oxygen consumption by muscle, but the remainder (i.e. 2–5% of the total) may undergo one-electron reduction with the production of the superoxide radical. Further one-electron reduction of superoxide produces hydrogen peroxide, and isolated mitochondria have been shown to release hydrogen peroxide (Boveris *et al.,* 1972). It appears that in young organisms this proportion of molecular oxygen converted to superoxide by mitochondria does not vary, thus during dynamic exercise an increased flux of molecular oxygen of up to 50-fold will lead to a proportionate increase in superoxide production (see Jackson, 1996 for a review).

There has been considerable interest in whether this rise in free radical activity leads to oxidative damage to skeletal muscle and other tissues. Initial suggestions indicated that free radical-mediated processes, such as lipid peroxidation, were elevated during whole body exercise in humans and rats (Davies *et al.*, 1982), although whether this has any functional significance has become the subject of considerable controversy (Jackson, 1996).

The lack of evidence for a chronic increase in oxidative damage in subjects who habitually take extensive exercise regimens may be explained by recent findings that muscle cells adapt to increased free radical activity during contraction to reduce the risk of free radical damage to the tissue. Thus exercise training has been shown to increase the activity in muscle of several antioxidant enzymes, such as superoxide dismutase and catalase, and recent data indicate that there is an increase in heat shock proteins (HSP) in muscle following exercise (Salo *et al.*, 1991). It is now recognised that these adaptations can protect skeletal muscle against further bouts of contractile activity (McArdle *et al.*, 1997). In these latter studies mice underwent a short period of non-damaging exercise and 4 hours later (when tissue HSP content was elevated) the pre-exercised muscle was subjected to a normally damaging exercise protocol. This procedure induced a substantial resistance to contraction-induced damage in the pre-exercised muscles.

There is therefore increasing evidence that exercise-induced oxidative stress induces an adaptive response in skeletal muscle which serves to protect the tissue against further stress. The impact of antioxidant supplementation on this process is not clear.

One mechanism by which this adaptation to increased free radical activity might occur is by influencing the activity of various transcription factors. In essence free radicals can influence the ability of transcription factors, such as nuclear factor-κB (NF-κB), activation protein-1 (AP-1) and heat shock factor-1 (HSF-1), to bind to their respective sites within the promoter region of specific genes. This effect appears to be mediated by oxidation of redox-sensitive cysteines within the binding region (Stortz & Polla, 1996; Jackson *et al.*, 1998).

These changes are part of a general process by which cells respond to increased oxidative stress by adaptive changes in the expression of a variety of proteins involved in maintenance of cellular integrity. This has been extensively studied in model systems by Davies and co-workers, who have shown that mammalian cells respond to varying levels of oxidative stress by variations in the rate of cell growth, changes in the length of the cell cycle and marked adaptive responses in resistance to oxidative stress (Wiese *et al.*, 1995). The process has not been fully explored in other systems although analogous changes must occur and responses will

inevitably differ in other cell types, such as those that are unable to divide and enter the cell cycle. These responses include an up-regulation of the activity of antioxidant enzymes such as catalase and superoxide dismutase, increased activity of DNA repair enzymes and expression of cytoprotective proteins such as HSP.

Thus, although contractile activity of skeletal muscle increases the generation of free radical species, the ability of these cells to respond to oxidative stress is considerable. Such changes may explain the apparent lack of chronic oxidative damage in subjects who habitually take extensive exercise programmes.

References

Boveris A, Oshire N & Chance B (1972) Cellular production of hydrogen peroxide. *Biochemistry, 128,* 617–630

Davies KJA, Quintanilla AT, Brooks GA & Packer L (1982) Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Commun, 107,* 1198–1205

Jackson MJ (1996) Oxygen-radical production and muscle damage during running exercise. In: Marconnet P, Saltin B, Komi P & Poortmans J, eds, *Human Muscular Function During Dynamic Exercise*, Basel, Germany, Karger Publishing, pp 121–133

Jackson MJ, McArdle A & McArdle F (1998) Antioxidant micronutrients and gene expression. *Proc Nutr Soc, 57,* 301–305

McArdle A, McArdle C & Jackson MJ (1997) Stress proteins and protection of skeletal muscle against contraction-induced skeletal damage in anaesthetised mice. [Abstract] *J Physiol, 499P,* 9–10

Patte RR, Prott M & Blair SN (1995) Physical activity and public health. *J Am Med Assoc, 273,* 402–407

Salo DC, Donovan CM & Davies KJA (1991) HSP70 and other possible heat shock or oxidative proteins are induced in skeletal muscle, heart and liver during exercise. *Free Rad Biol Med, 11,* 239–246

Storz G & Polla BS (1996) Transcriptional regulators of oxidative stress-inducible genes in prokaryotes and eukaryotes. In: Fiege U, Morimoto RI, Yahara I & Polla BS, eds, *Stress-Inducible Cellular Responses*, Basel, Germany, Birkhauser Verlag, pp 239–254

Wiese AG, Pacifici RE & Davies KG (1995) Transient adaptation to oxidative stress in mammalian cells. *Arch Biochem Biophys, 318,* 231–240

3.3 Hormonal responses to energy intake and balance and possible relationships with carcinogenesis

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Most endocrine systems are influenced by energy intake to a greater or lesser extent; responses may be widespread, multifunctional and interdependent. This means that it is possible to generate plausible links between energy metabolism and any endocrine system known to influence carcinogenesis, although the links may be more tenuous in some cases than others. Current knowledge derives from both animal and human studies and because the distinction between these sources is not always made, care must be taken in deciding how applicable to humans some commonly assumed responses actually are.

Human physiological responses to energy intake

Much is known about human physiological responses to undernutrition, experimental starvation or semi-starvation, and about chronically undernourished populations (see Shetty, 1990). Whilst reductions in the resting metabolic rate (RMR) are induced by starvation/undernutrition, the extent to which these reflect changes in cellular energy expenditure is not entirely resolved, because of the simultaneous changes in body composition. Thus it is widely accepted that the major part of the fall in the RMR (e.g. 36% after 12 weeks semi-starvation) reflects a loss of metabolically active lean body mass (LBM) tissue; the fall in RMR/kg lean tissue is, therefore, less (14%). Furthermore, in chronically undernourished Indian populations, the RMR/kg LBM may actually be increased due to selective loss of skeletal muscle compared with more active visceral organs, and the high metabolic activity of the brain is well preserved. Since there are no data on the actual metabolic rate of organs, the extent of true cellular changes in energy expenditure, if they occur at all, is unknown. Studies of rates of processes which contribute to the metabolic rate, such as protein turnover in undernourished populations, are also confounded by changes in body composition; there are currently no firm data to demonstrate clear reductions in protein turnover.

Human responses to over-nutrition are more complex. Distinctions need to be made between an *increased energy flux* and *positive energy balance*. Increased energy flux is associated with increased energy expenditure (e.g. increased physical activity), and it would appear that appetite regulation works effectively to match intake to expenditure, so that energy balance may be zero or even negative (as with elite cyclists who are very lean).

Positive energy balance may occur in sedentary people even while on quite low dietary intakes, and in this case appetite regulation does not work effectively. As there is little evidence of dietary induced thermogenesis, a positive energy balance may lead to obesity and insulin resistance. This is in contrast to observations in rodents and pigs, which can increase energy intake and exhibit an increased energy expenditure through dietary induced thermogenesis in brown fat (as with rats fed a highly palatable very varied 'cafeteria' diet and with low protein-fed pigs).

Endocrine responses to energy intake

Four categories of endocrine responses are discussed below: the first and second are directly related to energy intake, while the third and especially, the fourth, are indirectly related.

- Responses that relate to the overall rate of energy turnover and influence the metabolic rate
- Responses that mediate food energy disposal and relate to substrate balance
- Responses relating to growth and development
- Androgens and oestrogens

Energy turnover and the metabolic rate: Catecholamines and thyroid hormones

The catecholamines, adrenaline and noradrenaline, which are regulated through the sympathetic–adrenal axis and thyroid hormones (T4 and especially T3), influence the metabolic rates of tissues by controlling both ATP-dependent energy requiring processes, such as ion transport, turnover of protein and other substrates, and muscle contraction (heart rate, shivering etc.), and direct non ATP-dependent thermogenic processes, such as those occurring in the mitochondria of brown fat. Catecholamines are assumed to be the major sensors of energy intake, independently of energy balance; they vary directly with energy intake, and generally regulate adaptive thermogenesis (e.g. by mitochondrial uncoupling

in brown fat). Thyroid hormones (the active T3 and its precursor, T4) are more responsive to energy balance, and regulate the metabolic rate and cellular energy expenditure. These two systems are synergic: thyroid status influences sympathetic nervous system activity and noradrenaline production and turnover (inversely), as well as tissue sensitivity to catecholamines (directly). Catecholamines, in turn, directly influence cellular T3 production from T4.

Food energy disposal and substrate balance: Insulin, glucagon, glucocorticoids and growth hormone (GH) and insulin-like growth factor -1 (IGF-1)

Insulin is the major controller of energy balance. Postprandial increases in insulin mediate carbohydrate, fat and protein deposition. Postabsorptive (fasting) decreases in blood insulin concentration directly mobilise stored energy and allow the action of the directly catabolic hormones (GH, which mobilises fat; glucagon, which mediates gluconeogenesis, and glucocorticoids, which mobilise protein) to become dominant. Postabsortive fasting is associated with increases in GH and glucagon levels, but cortisol levels do not rise except in severe starvation.

In addition to this short-term role, insulin exerts a longer-term influence on other endocrine systems. Insulin is thermogenic through its central stimulation of the sympathetic nervous system and consequent noradrenaline levels. It is also anabolic in terms of growth and development through its peripheral direct regulation of tissue T3 production from T4, and GH induced expression of IGF-1 and IGF-1 binding protein.

There are at least six IGF-1 binding proteins (Jones & Clemmons, 1995); IGFBP-1 and IGFBP-3 are the best understood. IGFBP-1 is insulin dependent (insulin inhibits); in the short term its concentration varies inversely with insulin. Its action is to inhibit IGF-1 binding with the IGF-1 receptor. It is argued that the variation in IGFBP-1 with food intake allows short-term regulation of glucose disposal by IGF-1, preventing unwanted hypoglycaemia in the postabsorptive state, even though IGF-1 concentration does not vary with food intake. The importance of IGF-1 is further indicated by the fact that it is stimulated by cortisol (Katz *et al.*, 1998). IGFBP-3 is GH dependent and is thought to act as the main binding protein for IGF-1 in the circulation, in effect acting as a store and regulator of the amount of IGF-1 available. Measurements of free IGF-1, thought to be a better indicator of IGF-1 biological status, are now being reported (Attia *et al.,* 1998). This is important since, as discussed below, free and total IGF-1 do not always change together.

The hyperinsulinaemia associated with the insulin resistance syndrome is attracting attention in the present context. In this case the relationship with energy intake is complex; hyperinsulinaemia is not related to the absolute energy intake but rather to an excess of intake over requirements, especially when requirements are low, as a result of physical inactivity, and there is obesity.

The situation is also complex in that the relationship between insulin levels and insulin action at target sites is altered (i.e. there is insulin resistance) so that it cannot be assumed that insulin action varies directly with insulin levels in these hyperinsulinaemic-insulin resistant states.

Overall levels of IGF-1 reflect energy intake, although the responses are slow and not a function of acute energy intake. In contrast, as indicated above, IGFBP-1, the main regulator of IGF-1 action, is acutely inhibited by insulin. Thus energy intake may be expected to influence both overall level of IGF-1 and its activity via levels of IGFBP-1. Thus many authors suggest that hyperinsulinaemia will enhance IGF-1 'bioactivity', assuming that IGFBP-1 levels will be lowered and, as discussed below (under insulin and overnutrition), evidence is emerging that this is the case.

Growth and development: GH and IGF-1

It is clear from the above that the GH–IGF-1 axis is sensitive to energy intake, mainly at the level of peripheral mediation by GH of IGF-1 and IGF-1 binding protein expression. Thus although GH is needed to initiate IGF-1 expression in tissues, expression does not occur without insulin and adequate food intake. Indeed, GH will rise in starvation, and this is important in mediating fatty acid mobilisation, but IGF-1 levels will fall markedly, in part due to falls in insulin levels.

Androgens and oestrogens

In the case of androgens and oestrogens, the link with energy intake is much more complex and indirect, but it may nevertheless be important.

Undernutrition (e.g. during war or famine) and anorexia nervosa are associated with amenorrhea and low levels of blood oestrogens, without any compensating increase in gonadotropin secretion. It is widely believed that undernutrition of girls,

either in malnourished populations or self-imposed (such as in eating disorders or in some athletes), delays menarche and may well induce amenorrhea in postpubertal women

Energy intake may also influence sex hormone levels during hyperinsulinaemia by depressing levels of sex-hormone binding globulins, thus increasing levels of free oestradiol (Stoll, 1999).

Endocrine aspects of carcinogenesis

Thyroid hormones

Thyroid hormones, like steroid hormones, have direct influences on cellular differentiation, and they are likely to be potent inducers of differentiation in neoplastic cells, given that steroid and thyroid hormones (and the retinoids) all act through a nuclear receptor superfamily of transcriptional activators. (De Luca, 1991). Thyroid hormones are commonly reported to enhance oxidative processes and act as a co-transforming factor in carcinogenesis (e.g. Borek, 1993).

Insulin and hyperinsulinaemia

Insulin is an important cell growth factor (e.g. of colonic epithelial cells) and is a mitogen of tumour cell growth *in vitro*. The insulin receptor (IR) is a potential oncogene for mammary epithelial cells: it is overexpressed in mammary gland tumours initiated by other oncogenes, and is therefore a candidate for mediating the growth of breast cancers (Frittitta *et al.,* 1997).

Insulin and undernutrition

Kritchevsky (1995) argues that the way in which caloric restriction reduces carcinogenesis may well include reduction in insulin levels and consequent reduced oncogene expression. Also Rogers *et al.* (1993) suggest that reduced energy intake is linked to reduced carcinogenesis through reduced insulin and IGF-1; in 30% feed-restricted mice, reduced IGF-1 and insulin correlate with reduction in diethylnitrosamine (DENA)-induced preneoplastic hepatic foci and tumours.

Insulin and overnutrition

Several authors suggest there is epidemiological evidence linking the insulin resistance and hyperinsulinaemia of central obesity and physical inactivity with elevated colon cancer risk (Giovannucci, 1995; Stoll, 1999). Several lines of experimental evidence also indicate a role for hyperinsulinaemia in carcinogenesis.

Rats treated with insulin become hyperphagic and obese and exhibit changes in colon histology associated with carcinogenesis (Corpet *et al.,* 1997). Also insulin injections, in rats with colon tumours (initiated by azoxymethane) that did not become hyperphagic and gain weight, influenced tumour promotion, with an increased average number of colon tumours and number of large tumours (Tran *et al.,* 1996).

Another proposed mechanism of action of insulin proposed by Stoll & Secreto (1992) is that hyperinsulinaemia can increase the ovarian production of androgen, and this abnormal hormonal profile may stimulate proliferative activity in mammary epithelium. However, direct experimental evidence for this is sparse.

More recently Stoll (1999) has suggested that hyperinsulinaemia and its concomitant effects can increase the promotion of mammary carcinogenesis through increasing IGF-1. This suggestion is based on the premise of (a) an increased breast cancer risk associated with higher IGF-1 levels (not always found), (b) increased IGF-1 levels associated with insulin resistance simply on the basis that energy intake is a strong determinant of IGF-1 levels in the blood, and (c) an assumed lowering of IGFBP-1 by the hyperinsulinemia (not often measured). In fact, evidence is emerging that this view may be correct, with the hyperinsulinaemia of insulin resistance being associated with increased IGF-1 bioactivity, although the literature has been confusing. Thus it is well known that hyperinsulinaemic insulin-resistant non-insulin dependent diabetes mellitus patients and obese subjects have decreased serum IGF-1 concentrations, often because their GH levels are low, or because the usual action of insulin to stimulate IGF-1 levels does not occur (Bang *et al.,* 1994). It now appears that total IGF-1 may be a poor index of free IGF-1 and when the latter is measured the picture is clarified. Thus, in a study of insulin resistance and regulation in adolescent obesity, Attia *et al.* (1998) have shown that insulin resistance is associated with lowered total IGF-1 but increased free levels of the hormone. Furthermore, the compensatory hyperinsulinaemia that characterises adolescent obesity was shown to suppress levels of IGFBP-1 chronically, and these authors suggest that low IGFBP-1 concentrations may serve to increase the bioavailability of free IGF-1, which may, in turn, contribute to lower circulating GH, total IGF-1, and IGFBP-3 concentrations. Travers *et al. (*1998) have also investigated such relationships between insulin sensitivity, obesity, IGF-1 and IGFBP-I levels in a group of pubertal children, and have shown that insulin sensitivity, obesity, and IGF-1 are important

predictors of IGFBP-I levels. In this case, the conclusions were that insulin-mediated suppression of IGFBP-I in obese children may increase free IGF-1 levels and thus contribute to somatic growth.

For the adult, the obvious implication of such data is that the same interrelations could lead to increased free IGF-1 levels and increased cancer risk.

Epidemiological data suggest that IGF-1 and its binding proteins are very strongly related to cancer, especially breast and prostate cancer. The association between circulating IGF-1 concentrations and risk of breast cancer has been examined by Hankinson *et al. (*1998) in the Nurses' Study; they reported a striking relationship between circulating IGF-1 concentration and risk among premenopausal but not postmenopausal women. The relative risk among premenopausal women for the top versus bottom tertile IGF-1 concentration was 2.33, rising to 4 among premenopausal women less than 50 years old. Furthermore, after adjustment for plasma IGFBP-3 concentrations, the relative risks increased to 2.88 and 7.28, a remarkable finding.

In the Physicians' Health Study (Chan *et al.,* 1998) an association between plasma IGF-1 levels and prostate cancer risk has been identified in a nested case–control study. A strong positive association was observed between IGF-1 levels and prostate cancer risk, in that men in the highest quartile of IGF-1 levels had a relative risk of 4.3 (95 percent confidence interval 1.8 to 10.6) compared with men in the lowest quartile; this was independent of baseline prostate-specific antigen levels.

Undernutrition and glucocorticoids

Rogers *et al.* (1993) suggest that endocrine links between reduced energy intake and reduced carcinogenesis could include elevated glucocorticoids, which would increase hepatic enzymes associated with xenobiotic and steroid hormone metabolism. Certainly, adrenalectomy reverses the inhibition of skin tumours in food restricted mice.

IGF-1

IGF-1 is a potent inducer of cellular proliferation in normal growth (e.g. chondrogenesis within the growth plate of bones) and is tumorigenic. Thus overexpression of IGF-1 in transgenic mice results in hyperplasia, dermal abnormalities, and spontaneous tumour formation (Bol *et al.,* 1997). This may occur either through IGF-1 acting simply to increase cell proliferation or through an inhibition of apoptosis (Dunn *et al.,* 1997).

Stoll (1999) suggests that the proliferative activity of breast cancer cells *in vitro* is stimulated either by increased production of IGF-1, overexpression of IGF-1 receptors or changes in the level of IGF-1BPs. Also there is two-way synergy between IGF-1 and oestradiol in stimulating growth of human mammary cancer cell lines. Oestradiol may sensitise cells to the proliferative effect of IGF-1, and the growth stimulation by oestradiol of normal breast tissue is associated with upregulation of the IGF-1 receptors. Tamoxifen and some other anti-oestrogen drugs are associated with a lowering of blood IGF-1 levels (Hankinson *et al.,* 1998).

As described above, Rogers *et al. (*1993) have also suggested an IGF-1 (and insulin) mediated endocrine link between reduced energy intake and carcinogenesis.

Androgens and oestrogens

The risks of breast, prostate and endometrial cancers appear to be related to overall (lifetime) oestrogen/androgen exposure, and an influence of energy intake on this would be important. There is good evidence from the Dutch Famine that a delay in menarche due to prepubertal undernutrition reduces subsequent breast cancer risk, as does repeated pregnancy and sustained lactation. Further, an analysis of childhood energy intake in the Boyd Orr cohort study of diet (1937–1939), and subsequent adult mortality from cancer, shows a significant association between childhood energy intake and cancer mortality (Frankel *et al.,* 1998). However evidence for a direct relationship between energy intake and sex hormone levels is complex and difficult to disentangle. Studies of variation in energy intake, in the normal mature female population, and levels of oestrogens and androgens do not indicate any obvious relationship which can be easily related to hormone-sensitive cancer risk (Kurzer & Calloway, 1986; Dorgan *et al.,* 1996). It has also been argued that the age of menarche is not a simple function of energy intake but rather of other outcome factors, mainly height and fatness (Maclure *et al.,* 1991), and after correcting for these positive determinants of early menarche, the effect of energy intake *per se* disappears. In contrast, in the Boyd Orr cohort study, among the two thirds of the sample for whom anthropometric data were available, and adjusting for childhood height and BMI, the energy intake cancer mortality relationship is unchanged (Frankel *et al.,* 1998). However, the adult height of the cohort is not reported.

Studies of the neuroendocrine changes associated with ageing of the female reproductive system in C57BL/6J mice show that ageing is normally associated with oestrous cycle lengthening and reduced plasma oestradiol levels, and that chronic calorie restriction retards these changes. This is the opposite of what would be expected if energy restriction reduces hormone-sensitive cancer risk, since the undernutrition appears to increase oestrogen exposure. In fact, Nelson *et al.* (1995) suggest that the hyperadrenocortical state of energy restriction potentiates cellular and organismic homeostasis throughout life, in a manner similar to that achieved during acute stress, thereby retarding ageing processes and extending life span and presumably lowering cancer risk.

A further suggestion is that hyperinsulinaemia, which may be associated with increased risk of breast cancer (see above), acts through decreasing levels of sex-hormone binding globulins, thus increasing levels of free oestradiol (Stoll, 1999).

Animal models and man

As indicated above, there are species differences in the responses to increased energy intakes. The increased sympathetic, adrenergic, and thyroid status of hyperphagia in rodents is not seen in humans. Similarly the hyperinsulinaemic insulin resistance syndrome is not easily reproduced in animals.

As far as the responses to undernutrition are concerned, in general terms in rodent models hormone responses are much more extreme. Mild undernutrition, which allows some growth, is associated with marked falls in insulin, IGF-1 and thyroid hormones. It is not surprising, therefore, that tumorigenesis is lowered in energy restricted models. In humans such reductions are only seen in severe malnutrition.

Another notable difference relates to insulin resistance and sensitivity. In humans, insulin levels (and possibly those of IGF-1) vary much more with physical activity and energy balance than with energy intake *per se*. Thus the lowest levels of insulin may occur in subjects with the highest intakes of energy, when such subjects are active and fit, and the converse may occur (high insulin-low energy intakes) among the sedentary population. However, since there are marked changes in hormone sensitivity in these varying circumstances, it is difficult to interpret the significance of hormone levels, and as far as insulin/IGF-1 mediated mechanisms of carcinogenesis are concerned, direct human evidence for a relationship is very limited.

A third difference between species relates to the hormone-sensitive cancers. Although there is much experimental work *in vitro* and in cell culture, there are no real animal models for the hypothesis relating energy restriction in humans with delayed menarche and/or prolonged menstrual cycle.

References

Attia N, Tamborlane WV, Heptulla R, Maggs D, Grozman A, Sherwin RS & Caprio S (1998) The metabolic syndrome and insulin-like growth factor I regulation in adolescent obesity. *J Clin Endocrinol Metab, 83,* 1467–1474

Bang P, Brismar K, Rosenfeld RG & Hall K (1994) Fasting affects insulin-like growth factors (IGFs) IGF-binding proteins differently in patients with noninsulin diabetes mellitus versus healthy nonobese and obese subjects. *J Clin Endocrinol Metab, 78,* 960–967

Bol DK, Kiguchi K, Gimenez-Conti I, Rupp T & DiGiovanni J (1997) Overexpression of insulin-like growth factor I induces hyperplasia, dermal abnormalities, and spontaneous tumor formation in transgenic mice. *Oncogene, 10,* 1725–1734

Borek C (1993) Molecular mechanisms in cancer induction and prevention. *Environ Health Perspect, 101 (suppl 3),* 237–245

Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, Hennekens CH & Pollak M (1998) Plasma insulin-like growth factor-1 and prostate cancer risk: A prospective study. *Science, 279,* 563–566

Corpet DE, Jacquinet C, Peiffer G & Tache S (1997) Insulin injections promote the growth of aberrant crypt foci in the colon of rats. *Nutr Cancer, 27,* 316–320

De Cree C (1998) Sex steroids metabolism and menstrual irregularities in the exercising female: A review. *Sports Med, 25,* 369–406

De Luca LM (1991) Retinoids and their receptors in differentiation, embryogenesis and neoplasia. *FASEB J, 5,* 2924–2933

Dorgan JF, Reichman ME, Judd JT, Brown C, Longscope C, Schatzkin A, Forman M, Campbell WS, Franz C, Kahle L & Taylor PR (1996) Relation of energy, fat, and fiber intakes to plasma concentrations of estrogens and androgens in premenopausal women. *Am J Clin Nutr, 64,* 25–31

Dunn SE, Hardman RA, Kari FW & Barrett JC (1997) Insulin-like growth factor 1 (IGF-1) alters drug sensitivity of HBL100 human breast cancer cells by inhibition of apoptosis induced by diverse anticancer drugs. *Cancer Res, 57,* 2687–2693

Frankel S, Gunnell DJ, Peters TJ, Maynard M & Davey-Smith G (1998) Childhood energy intake and adult mortality from cancer: The Boyd Orr Cohort study. *Brit Med J, 316,* 499–504

Frittitta L, Cerrato A, Sacco MG, Weidener N, Goldfine ID & Vigneri R (1997) The insulin receptor content is increased in breast cancers initiated by three different oncogenes in transgenic mice. *Breast Cancer Res Treat, 45,* 141–147

Giovannucci E (1995) Insulin and colon cancer. *Cancer Causes Control, 6,* 164–179

Hankinson SE, Willett WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, Rosner B, Speizer FE & Pollak M (1998) Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. *Lancet, 351,* 1393–1396

Jones JI & Clemmons DR (1995) Insulin-like growth factors and their binding proteins: biological actions. *Endocrine Reviews, 16,* 3–34

Katz LEL, Satin-Smith MS, Colett-Solberg P, Baker L, Stanley CA & Cohen P (1998) Dual regulation of insulin-like growth factor binding protein-1 levels by insulin and cortisol during fasting. *J Clin Endocrinol Metab, 83,* 4426–4430

Kritchevsky D (1995) The effect of overnutrition and undernutrition on cancer. *Eur J Cancer Prev, 4,* 445–451

Kurzer MS & Calloway DH (1986) Effects of energy deprivation on sex hormone patterns in healthy menstruating women. *Am J Physiol, 251,* 14/4

McClure M, Travis LB, Willett W & MacMahon BA (1991) A prospective cohort study of nutrient intake and age at menarche. *Am J Clin Nutr, 54,* 649–656

Nelson JF, Karelus K, Bergman MD & Felicio LS (1995) Neuroendocrine involvement in aging: Evidence from studies of reproductive aging and caloric restriction. *Neurobiol Aging, 16,* 837–843

Rogers AE, Zeisel SH & Groopman J (1993) Diet and carcinogenesis. *Carcinogenesis, 14,* 2205–2217

Shetty, PS (1990) Physiological mechanisms in the adaptive response of metabolic rates to energy restriction. *Nutrition Research Reviews 3,* 49–74

Stoll BA (1999) Western nutrition and the insulin resistance syndrome: A link to breast cancer. *J Clin Nutr, 53,* 83–87

Stoll BA & Secreto G (1992) New hormone-related markers of high risk to breast cancer. *Ann Oncol, 3,* 435–438

Tran TT, Medline A & Bruce WR (1996) Insulin promotion of colon tumors in rats. *Cancer Epidemiol, Biomarkers Prev, 5,* 1013–1015

Travers SH, Labarta JI, Gargosky SE, Rosenfeld RG, Jeffers BW & Eckel RH (1998) Insulin-like growth factor binding protein-I levels are strongly associated with insulin sensitivity and obesity in early pubertal children. *J Clin Endocrinol Metab, 83,* 1935–1939

4 The usefulness and limitations of rodents as models for humans

4.1 Contribution of rodent genetics to an understanding of obesity, energy metabolism and carcinogenesis

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Introduction

Obesity results from an imbalance between energy intake and energy expenditure. It is commonly associated with hyperglycaemia, hyperinsulinaemia and insulin resistance, and can lead to a wide range of pathological conditions including circulatory disorders and cancer. Already one in three Americans is clinically obese, and problems associated with this condition are likely to become increasingly important in the developed world in the next decade. In practice, it has proved to be almost impossible to cure obesity in humans by any technique which is socially acceptable, such as dieting, and no drugs of proven safety have yet been developed.

The control of energy intake and expenditure is extremely complex. It depends both on environmental/social factors such as the availability of palatable food, exercise, and the social environment and on complex genetic susceptibility. Whether an obese person goes on to develop severe pathological conditions such as heart disease or cancer probably also depends on both genetic susceptibility to these conditions and environmental influences such as exposure to carcinogens. Unfortunately, the level of complexity is such that it is extremely difficult to study obesity and its consequences in humans. Animal models are, therefore, of great value in fundamental studies even though many different models will be needed to cover even a proportion of the complexity found in humans.

The place of models in biomedical research

Laboratory animals are widely used as models of human biology and disease, so it is important to have some understanding about the theory of models in research. According to Wartofsky (1979)

> Theories, hypotheses, models and analogies I take all to be species of a genus, and my thesis is best stated directly by characterising this genus as *representation* (though 'imaging' or 'mirroring' will do quite as well).

He goes on to suggest that anything can be a model of anything because there is always some property that two things share. All that is necessary for something to be a representation of something else is that it is taken to be so. It is even possible to have models of things, such as a winged Pegasus, that do not exist.

In biomedical research there are three main types of model: predictive, exploratory, and explanatory.

- Predictive models are widely used in toxicology where the response of an animal such as a rat to a xenobiotic is used to predict toxicity in humans. In considering such models it is useful to consider their *fidelity*, that is the extent to which they are like the object being modelled in every respect (Russel & Burch, 1959). A rat has higher fidelity than an insect as a model of humans, but it has lower fidelity than a primate. High fidelity is usually a desirable, but not an essential property of a predictive model. Of at least equal importance is the power of the

model to *discriminate* between treatments. Thus, in toxicity testing an *in vitro* test which has good ability to discriminate between genotoxic and non-genotoxic chemicals would be useful even if it has low fidelity. Indeed, the use of *in vitro* methods in toxicology is entirely dependent on their discriminating power, which is often good for highly specific purposes. Finally, the choice between a high fidelity or a potentially high discrimination model may depend on the extent to which the model has been *validated*. Validation is the formal testing of a predictive model to determine how well it performs. In toxicology, a model will usually be validated using chemicals of known toxicity in humans or in high fidelity models. Often animal models are assumed to have such high fidelity that they are not formally validated, though reliance on high fidelity may sometimes lead to incorrect predictions.

- Exploratory models are used to gain an understanding of a biological situation or response without undue initial concern about whether it predicts anything. The obese mutant mouse is a good example. There have been thousands of papers published on the biology of this mutant, although until recently there were no known examples of it occurring in humans. The argument was that unless we can understand obesity in some relatively simple, well defined and controlled strains of rodents, we have very little chance of understanding it in humans. Low fidelity models such as tissue culture, insects, nematodes and mathematical models are commonly used as exploratory models. A large part of biomedical research involves exploratory models, and it is only after the models themselves are understood that their implications for human health need to be assessed. Possibly the best example of an exploratory model has been the discovery of the laws of genetics by Gregor Mendel using garden peas. It could not have been predicted at the time that the same laws would apply to human inheritance.

- Explanatory models are usually inanimate, and are used as a tool to gain an understanding of a biological situation and to formulate hypotheses for further study. These models can range all the way from a set of mathematical equations, through drawings, to a physical model such as a representation of a molecule using wire and wood. Most statistical analysis of a set of data, for example, involves fitting mathematical models to the actual observations. Explanatory models are clearly of great importance in the development of a science.

Genetically defined rodents

Twenty years on from a symposium on Animal Models of Obesity (Festing, 1979a), it appears that the most, possibly the only, significant advance has been as a result of molecular genetic analysis of mouse and rat models of obesity.

Genetically defined rodents provide one of the most important tools for gaining an understanding of the control of energy balance. In addition to a number of single gene mutations causing obesity in mice and rats, there are many inbred strains which can be regarded as immortal clones of genetically identical individuals, each with its own unique characteristics. More than 400 such strains of mice and 200 strains of rats have been described (Festing, 1999*). Transgenic techniques are already providing additional tools for studying the genetic control of obesity.

Single-locus rodent models of obesity

Single locus models of obesity have led to exciting advances in understanding of some of the factors controlling energy balance during the last five or six years. There are seven single-gene models of obesity in mice, and most of these have been mapped and cloned, and their human homologues identified (Naggert *et al.*, 1995).

The obese (*ob*) mutation causes obesity, hyperglycaemia, hyperinsulinaemia, insulin resistance, hyperphagia, and immunological and hormonal defects. In a classical series of experiments, the obese (*ob*) gene was backcrossed to the C57BL/6 inbred background and the resulting mice were parabiosed (i.e. surgically joined) to normal C57BL/6 mice. This led to the *ob*/*ob* mice eventually becoming cured of the obesity. In contrast, when diabetic *(db/db)* mice, which are phenotypically indistinguishable from *ob*/*ob* mice, were parabiosed to normal mice, the latter became emaciated and eventually died from hypophagia. As a result of these studies, it was suggested that the *ob* mutation causes a defect in a circulating substance which, as part of a feedback loop, normally controls the levels of fat stores. In contrast, the *db* mutation, which results in the same phenotype, controls a receptor for this circulating substance. The *ob*/*ob* mutation (now re-designated Lep^{ob}/Lep^{ob}) has now been positionally cloned, and has been shown to code for a defect in a hormone named leptin which is synthesised in adipose tissue

* Festing, MFW (1999) *Inbred strains of mice and rats* available (at May 00) from http://www.informatics.jax.org/external/festing/search_form.cgi

and normally controls appetite and thermogenesis via the hypothalamus (Zhang *et al.,* 1994). The *db* locus (now designated *Lepr*^{db}) codes for the leptin receptor, and the fatty-Zucker rat appears to have a defect in the same gene. Humans with the *ob*/*ob* mutation have recently been found.

Extensive work has also been done on the yellow (A^y) and viable yellow (A^{vy}) mutant mice, which in addition to the alteration in pigmentation also become obese, develop mature onset diabetes and have a higher incidence of both spontaneous and induced cancer. The viable yellow mouse is particularly interesting in that even within an isogenic strain there is enormous phenotypic variation ranging from a pseudo-agouti to a yellow colour, but only the yellow mice become obese and more susceptible to cancer. The obesity is apparently caused by ectopic over-expression of the agouti protein which blocks the action of α-melanocyte stimulating hormone on a melanocortin receptor, MC4-R (Miltenburger *et al.,* 1997; Wolff, 1997), but it is not known why these animals are more susceptible to cancer. Two other obese mouse mutants, tubby and fat, have also been studied in some detail, with the mode of action now becoming quite well understood (Naggert *et al.,* 1995).

Transgenic models

Transgenic techniques are already contributing to an understanding of the causes of obesity. For many years it has been known that obese animals, and probably also humans, have defects in thermoregulatory thermogenesis, which may be associated with the brown adipose tissue. The situation is complicated by scale effects. Obese mice, for example, have a higher metabolic rate per animal than their lean litter mates, but this is because they are larger. If expressed relative to surface area, their metabolic rate is lower (York, 1979). Transgenic methods have been used to ablate the brown adipose tissue by expressing a toxin under the control of a brown adipose-tissue specific promoter, leading to obesity (Klaus *et al.,* 1998) and increased susceptibility to dietary-induced obesity (Hamann *et al.,* 1996). In contrast, targeting a toxin gene to white adipose tissue makes mice resistant to obesity (Ross *et al.,* 1993). Transgenic techniques are destined to play an important role in dissecting out the multiple causes of obesity and its associated pathology.

Inbred strains

Inbred strains, produced by many generations of brother–sister mating, provide another useful research model which is not available in humans (Festing, 1997). An inbred strain may be regarded as an immortal clone of genetically identical animals. Such animals tend to be phenotypically uniform, with each strain having its own unique phenotypic characteristics, some of which are of great value in obesity research. Thus, there are some mouse strains such as PBB, BRSUNT, NZO and FL/1Re which become obese on a normal laboratory diet (Festing, 1979b). Such obesity is due to multiple loci, in contrast with the single locus models such as the obese mutant.

Some strains become obese on high fat diets whereas others are resistant, yet the exact response depends on dietary constituents (West *et al.,* 1992; Eberhart *et al.,* 1994; West *et al.,* 1995). In some mouse strains, such as C57BL/6, a high fat diet induces athersclerosis, whereas in others, such as strain A/J, it does not do so (Mills *et al.,* 1993). Although members of an inbred strain are genetically identical, they are not phenotypically absolutely identical because individuals can differ due to accidents of development, different environments *in utero* and after birth, and as a result of social hierarchy. Nevertheless, inbred strains are usually much more uniform than genetically heterogeneous stocks, and as a result fewer of them are needed in controlled experiments to achieve a given level of statistical power.

There are also strains with a high incidence of various types of spontaneous cancer, such as strain AKR which develops lymphatic leukaemia, C3H which is susceptible to the induction of mammary tumours by the mammary tumour virus and also develops hepatomas, and SJL which develops reticulum cell sarcomas. These strains might be used to study the relationship between diet and spontaneous or virus induced cancer. Other strains are highly susceptible to carcinogens, though the target organ is strain-dependent, and can be used to study the interrelationships between metabolism of xenobiotics, diet and cancer.

Limitations of rodent genetic models of obesity

Human obesity is primarily due to social causes superimposed on the genetic susceptibility of some individuals. The ready availability of extremely palatable food from an early age is probably the main factor, but lack of exercise and of environmentally-induced thermogenesis due to central heating probably also play a role. Some of these factors can be modelled to a limited extent with laboratory animals. For example, giving rats access to a highly palatable mixed and varied 'cafeteria diet' can induce obesity which, over the short-term, is reversible (Stock *et al.,* 1979). The fundamental studies based on mouse mutants and transgenic strains referred to earlier will almost

certainly result in the development of new drugs to combat obesity and its associated pathology. However, controlling obesity by the consumption of drugs is not ideal, as very few of such drugs are likely to be entirely without side effects. What is really needed is the development of methods to control appetite without medical or pharmacological intervention. Whether this will ever be possible is debatable, and it is unlikely that animal models could play a significant part in understanding and controlling the human social conditions which lead to obesity.

References

Eberhart GP, West DB, Boozer CN & Atkinson RL (1994) Insulin sensitivity of adipocytes from inbred mouse strains resistant or sensitive to diet-induced obesity. *Am J Physiol, 266,* R1423–R1428

Festing MFW, ed (1979a) *Animal Models of Obesity*, Basingstoke, UK, Macmillan Press

Festing MFW (1979b) Discovery and mode of inheritance of genetic models of obesity. In: Festing MFW, ed, *Animal Models of Obesity*, London, UK, Macmillan Press, pp 15–37

Festing MFW (1997) Inbred strains of mice: A vital resource for biomedical research. *Mouse Genome, 95,* 845–855

Hamann A, Flier JS & Lowell BB (1996) Decreased brown fat markedly enhances susceptibility to diet-induced obesity, diabetes and hyperlipidemia. *Endocrinology, 137,* 21–29

Klaus S, Munzberg H, Truloff C & Heldmaier G (1998) Physiology of transgenic mice with brown fat ablation: Obesity is due to lowered body temperature. *Am J Physiol, 274,* R287–R293

Mills E, Kuhn CM, Feinglos MN & Surwit R (1993) Hypertension in CB57BL/6J mouse model of non-insulin dependent diabetes mellitus. *Am J Physiol, 264,* R73–R78

Miltenburger RJ, Mynat RL, Wilkinson JE & Woychik RP (1997) The role of the agouti gene in the yellow obese syndrome. *J Nutr, 127 (suppl),* 1902–1907

Naggert JK, Fricker LD, Varlamov O, Nishima PM, Rouille Y, Steiner DF, Carroll RJ, Paigen BJ & Leiter EH (1995) Hyperproinsulinaemia in obese fat/fat mice associated with a carboxypeptidase-e mutation which reduces enzyme-activity. *Nature Genet, 10,* 135–142

Ross SR, Graves RA & Spiegelman BM (1993) Targeted expression of a toxin gene exposed to adipose tissue: Transgenic mice resistant to obesity. *Genes Dev, 7,* 1318–1324

Russell WMS & Burch RL (1959) *The Principles of Humane Experimental Technique*, Potters Bar, UK, Universities Federation for Animal Welfare

Stock MJ & Rothwell NJ (1979) Energy balance in reversible obesity. In: Festing MFW, ed, *Animal Models of Obesity*, London, UK, Macmillan Press, pp 141–151

Wartofsky MW (1979) *Models: Representation and the Scientific Understanding*, Dordrecht, Holland, D Reidel Publishing Company

West DB, Boozer CN, Moody DL & Atkinson RL (1992) Dietary obesity in nine inbred mouse strains. *Am J Physiol, 262,* R1025–R1032

West DB, Waguespack J & McCollister S (1995) Dietary obesity in the mouse: Interaction of strain with diet composition. *Am J Physiol, 268,* R658–R665

Wolff GL (1997) Obesity as a pleitropic effect of gene action. *J Nutr, 127 (suppl),* 1897–1901

York DA (1979) The characteristics of genetically obese rodents. In: Festing MFW, ed, *Animal Models of Obesity*, London, UK, Macmillan Press, pp 39–64

Zhang YY, Proenca R, Maffei M, Barone M, Leopold L & Friedman JM (1994) Positional cloning of the mouse obese gene and its human homolog. *Nature, 372,* 425–432

4.2 Physiological differences between rodents and humans

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The comparison of rodent to human physiology is difficult because, firstly, there is an enormous amount of information available, and secondly, because rodents and humans are not equivalent taxonomic units. Rodents make up the largest order of the class Mammalia, comprising over 3000 species, broadly grouped into three main super-families which had their origins at the dawn of the Tertiary some 60 million years ago. By contrast humans are a single species in the primate genus *Homo* which may have its origins as recently a 120 000 years ago. Rodent physiology is extremely diverse and includes, for example, groups which hibernate (such as ground squirrels, *Ammospermophilus*), and others which are incapable of hibernation (e.g. microtine voles, *Microtus*), groups in which reproductive and other events are strongly triggered by photoperiodic changes (e.g. Hamsters, *Phodopus*) and other groups which are less sensitive to photoperiod (e.g. Rats, *Rattus*). Before any meaningful comparison can be made therefore it is necessary to choose an appropriate 'model' rodent and restrict the scope of the physiology that will be addressed. Accordingly the scope of this discussion is limited to aspects of energy metabolism, and the comparison is confined to domesticated mice. This choice is in part driven by the availability of information; however, there are other similarities between domesticated mice and humans which make them ideal for comparison. In particular, both species have largely been released from the constraints of natural selection over the latter part of their evolutionary history.

An immediately apparent difference between mice and humans is the enormous difference in body size. Mature mice weigh on average around 30g while mature humans weigh on average around 80 kg. Thus there is more than a 2000-fold difference in size. A key question therefore is to what extent differences in physiology are reflections only of this profound body size difference. A direct consequence of the difference in body size is a large contrast in the surface to volume ratio. On average, for each gram of body mass a human has 0.25 cm^2 of surface, but a mouse has over ten times this amount at 3.2 cm^2/g. Because heat loss is a surface phenomenon and both mice and humans generally live in environments where ambient temperature is lower than body temperature, the different surface to volume ratio means that each gram of mouse tissue must expend much more energy than each gram of human tissue to balance the flow of heat from the surface. Measurements of humans using the doubly-labelled water method suggest they expend on average 11.8 MJ/day which is roughly equivalent to an oxygen consumption of 0.35 ml/g/hour. Mice in captivity, however, expend about 55 kJ each day, which is equivalent to 4.5 ml oxygen/g/hour — about 12 times the metabolic activity level of the human tissue.

Both mice and men need to transfer oxygen from the outside atmosphere to their cells to allow respiratory activity to proceed. The sizes of the organs they have to facilitate this transfer are remarkably similar. The lungs comprise 5.2–5.3% of body mass and the heart 0.6% of body mass in both species. To transfer adequate oxygen to the cells, therefore, mice must have mechanisms other than larger organs. Anatomically these include smaller alveoli diameters and greater capillary packing densities to facilitate oxygen diffusion. Physiologically the animals have greater heart and ventilation rates and differences in haemoglobin structure which allow more efficient oxygen uptake and offloading capacity.

The different rates at which mice and humans consume oxygen also relate to the differences in the size, quantity and structure of their mitochondria. Mice have more mitochondria per cell with greater membrane surface area than humans. Moreover, the enzymes of the Krebs cycle typically have activities an order of magnitude higher in mice. Surprisingly, however, the reverse is true of glycolytic enzymes, such as lactate dehydrogenase and pyruvate kinase. It has been suggested that this difference reflects the very short duration over which glycolysis can support activity in the much smaller mouse.

In addition to these differences in the mitochondria, mice also have several adaptations which facilitate heat production, including the retention of brown adipose tissue into adult life, and higher expression rates for the uncoupling proteins (UCP-1, 2 and 3), at least one of which is used entirely for heat production.

The very high rate at which mouse tissue consumes oxygen might be expected to result in high rates of oxygen free radical production. Superficially then one might anticipate the levels of the primary free radical scavenging enzymes to be greater in the mouse. However, this is not the case. Activity of superoxide dismutase (SOD) in humans is almost twice that of mice and the ratio of manganese SOD

(located in the mitochondria) to total SOD is about 0.4 in humans but only 0.1 in mice. However glutathione peroxidase and catalase levels are greater in the mouse, perhaps suggesting that the free radical scavenging strategy differs between the two species. Circulating levels of dietary and metabolic antioxidants are also generally higher in humans. Levels of urate are four times greater, carotenoids over 50 times, and vitamins A and E 1.5 times greater in humans than in the mouse. However, levels of vitamin C, which mice can manufacture endogenously, are about twice those found in humans.

The consequences of consuming oxygen at a rate over ten times higher while having a poorer free radical defence system is that mice sustain much greater levels of DNA damage than humans. Urinary markers of damage (thymidine glycol and 8-hydroxydeoxyguanosine; 8-OHdG) are approximately 30 times higher in the mouse, and unscheduled DNA repair rates in response to UV damage are only a fifth of the human level. It might be argued that the observed differences between mice and men in the ages at which first onset of cancer is observed are a direct consequence of these physiological differences in energy metabolism and protective physiology. A 2% prevalence is observed in mice at 1 year and in humans at 30 years, with a 30% prevalence of cancer at 2.5 and 85 years, respectively.

An outstanding question is why mice and humans differ in this manner. A major difference between wild mice and 'wild humans' is the ecological niches they occupy. Small rodents suffer high levels of mortality in the wild, and their life expectancies are seldom greater than a year. Reconstructions of life in Africa when humans were evolving indicate that ancestral humans would most certainly have lived much longer than this and probably had an ecological life span of 25–30 years. It would appear that both mice and men are engineered to resist cancer for the duration of their natural life span. In other words natural selection has led to the evolution of a protection system which results in a low risk of cancer in their life expectancy in nature. For mice this would be about a year, and for ancestral humans living in Africa this was probably around 25 years. It is interesting that in both humans and mice, when they are released from constraints operating in their natural habitats, the system appears to be over-engineered so that the resultant life span (3 years in mouse and 75–90 years in humans) is about three times the ecological life span, and the absolute maximum life span is about four times the ecological life span (i.e. 3.5–4 and 110–120 years respectively). These figures appear to be consequences of evolving a system which protects completely against cancer development over the ecological life span.

5 Discussion

The following discussion on energy metabolism and carcinogenesis, arising from the workshop deliberations and the background papers presented in Sections 1 to 4, is divided into five main categories.

- The influence of energy restriction on the results of toxicity tests in laboratory animals, and the implications this may have on the applicability to humans of results from such studies.
- The possible effects of energy metabolism in humans on the health effects associated with xenobiotic compounds.
- The importance, in human carcinogenesis, of chemicals in food relative to the total energy content of the diet.
- Whether any aspect of energy metabolism can be held to be a risk factor in human carcinogenesis, as is clearly the case in rodents and other small animals.
- How the difficulties of measuring the processes involved point to areas for future research.

5.1 Interpretation of toxicological studies in experimental animals

5.1.1 Assay sensitivity versus biological sensitivity of animals

It is easy to confuse the sensitivity of a toxicological assay with the biological sensitivity of the test animals, when discussing the influence of dietary restriction (energy restriction) of animals used for toxicological testing. It is important, when assaying the toxicity of xenobiotic compounds, to be sure that the assay measures the effect of the compound in question and not the effect of the food intake of the test animals.

Energy restriction affords a greater chance of detecting a small effect of a test compound if it results in the background level of tumours being lower and the exposure time being longer, due to improved survival of test animals. Thus, although energy restriction can affect the biological sensitivity of animals to the test compound, in ways that can vary according to the specific nature of the treatment, it is the increased reproducibility between assays and statistical sensitivity to detect dose–response relationships within assays that are the main benefits of energy restriction compared with *ad libitum* feeding.

Similarly it is important that the assay result does not reflect, primarily, the genetic make-up of the test animals. There are different opinions on how best to ensure that genetic variability is taken into account, two main alternatives being to conduct the assay with several inbred strains separately, or to use outbred strains with some degree of inherent genetic heterogeneity.

5.1.2 Effect of nutritional status on toxicology results

The level of energy intake employed in studies in rats or mice can affect the size of the maximum tolerated dose (MTD), the no-effect level and the derived acceptable daily intake (ADI). The MTD is usually raised by energy restriction, meaning that a larger quantity of the test compound can be used as the top dose in the study, but the effect on the value of the ADI cannot be predicted universally, as this depends on the metabolism of individual compounds.

Laboratories using different strains of rat may achieve similar percentage body weight reductions

with energy restriction, but at widely differing absolute body weights of the animals. This could have a bearing on any metabolic effects seen with energy restriction, since at higher absolute body weights, such as 1200g, a proportional reduction of, for example, 30%, is likely to be mainly in adipose tissue, while at lower absolute body weights, such as 600g, there might be some loss of lean body mass, with a greater effect on metabolism and tumorigenesis.

5.1.3 Are 'fat rats' good models for 'fat humans'?

It has been suggested that, in chemical testing, *ad libitum*-fed rats might be a more appropriate model for humans living modern Western lifestyles, whom many perceive as being generally relatively overfed and having too little exercise. However, although this may be the case on average, or at least for some humans, it can not be said to apply to all individuals, even in the West.

As noted in the earlier discussion about models (Section 4.1), such questions are not as important as the consideration of assay sensitivity and reproducibility discussed above. These toxicological tests can be seen as forming the hazard evaluation part of risk assessment and, while energy restriction may assist identification or classification of carcinogens by improving the sensitivity of assays, the energy-restricted rat should not be regarded as a direct model of the human situation. Rat models can also provide some information about possible biological mechanisms, and comparisons of phenotypic measurements and genetic conservation between species can suggest the extent to which results obtained in rats may be used for humans. However, the quantitative dose–response element of risk assessment requires more appropriate models, and this implies the use of data obtained in humans, however imperfect such data might be.

5.2 The influence of nutritional status of humans on the metabolism of xenobiotics

A prime concern, in seeking to protect individuals from food-borne hazards, is how to balance beneficial with hazardous components of foods, taking into account the influence other aspects of food and nutrition may have on the overall effect. The question remains whether, in humans, total food intake or any other aspect of energy metabolism can modulate the health effects of chemicals, whether xenobiotics or chemicals such as phytoestrogens that are naturally present in many foods. There is considerable evidence, some of which has been discussed earlier (Sections 2.1 and 2.2), that energy restriction affects the metabolism of xenobiotics in rodents and other animals, but direct evidence in humans is sparse.

One relevant piece of data in humans is the observation from the Biosphere 2 study (Walford, 1999), referred to previously (Section 1), that levels of circulating xenobiotic lipophilic chemicals fluctuate with changes in body weight. As lipophilic xenobiotics may be stored in adipose tissue and subsequently released into the bloodstream when fat stores are broken down, it seems more desirable to prevent obesity than to attempt to reverse the condition later.

Another way, rarely discussed, in which nutrition can interact with toxicology in humans, is the influence the macronutrient composition of the diet (e.g. carbohydrate content and type) can have on the growth of microflora in the gut (Cummings & Macfarlane, 1997). These microflora, in turn, are important determinants of xenobiotic transformation and cancer development.

5.3 The importance in cancer causation of food chemicals in relation to energy content of the diet

One reason for considering the role of energy metabolism in human carcinogenesis is the need to determine how great any influence of chemicals in food may be in the context of the total diet and the environment as a whole. Furthermore, is the risk presented by food-borne xenobiotics measurable or worth measuring? Some have argued that it is not, and that other dietary factors, which include total food (or energy) intake and the way in which energy is used by the body, as well as exposure to greater numbers of natural toxicants in food, are more important for human cancer risk than trace contaminants (Lutz & Schlatter, 1992; Ames & Gold, 1997).

One of the ways in which the Joint Food Safety and Standards Group* has addressed risk assessment is to consider specific chemicals in food. This is partly due to public concerns about the adverse effects of specific chemicals, and is done despite the uncertainty about the relative roles of macro- and micro- constituents of food in carcinogenesis.

* Food Standards Agency as of April, 2000

Regulators and advisors need an approach to ensure that consumers are protected, and to demonstrate that every reasonable effort is being made to achieve this.

Whether exposure to individual xenobiotics in food leads to cancer is as hard to evaluate as the role of energy metabolism in cancer. Exposures to chemicals are usually at doses too low to detect any effects, especially in relation to the numerous other exposures that occur simultaneously.

Alcohol should not be forgotten although in many respects it is a chemical with paradoxical characteristics and effects. It is usually regarded as a macro-component of diet, since it is metabolised to energy, contributing to problems of weight gain in some individuals. Although many people do not consume any alcohol, for those that do it can form a considerable proportion of their total energy intake. Median values for adult alcohol consumers in a UK population sample in 1986–1987 were 6.9% of energy from alcohol (ethanol) in men and 3.0% in women (Gregory *et al*., 1990). Alcohol is metabolised in pathways common to many xenobiotics and is a recognised risk factor for cancers of the oesophagus, stomach and breast, although it is not a mutagen in rat studies.

There is increasing recognition that macro-constituents of the diet are likely to be more important to cancer causation than the micro-additives, for example; but this belief is difficult to substantiate unequivocally, and is not the view of the general population. There is undoubtedly risk attached to macro — as well as micro-constituents of food, and a rational system is required for evaluating them together.

5.4 The role of energy metabolism as a primary factor in cancer causation in humans

5.4.1 Is there evidence for any component of energy metabolism being a primary risk factor?

Direct evidence is very sparse to support the notion that the protective effect of reduced energy intake, in particular, and the consequent reduced body weight seen in rodents and other species also applies in humans. Indeed, the only type of cancer in humans for which a higher energy intake has so far consistently been found to be a risk factor is endometrial cancer (Department of Health, 1998). There is some epidemiological evidence that energy balance, as reflected by higher BMI or obesity, is a risk factor in hormone-dependent cancers, particularly post-menopausal breast cancer, endometrial cancer and colon cancer in men (Department of Health, 1998). There is also mounting evidence for a protective effect of physical activity against cancers of the breast, endometrium and colon. However, since higher BMI tends also to be a risk factor for these cancers, it is very difficult to establish independence of effects, as physical activity and adiposity are inter-dependent.

Although there is very little unequivocal evidence for any effect of energy metabolism on cancer risk in humans, two main pieces of information suggest than such an effect cannot be excluded.

- Epidemiological evidence from studies of migrant populations (especially the Japanese, whose traditional diet is low in energy and in fat) suggests that adoption of a Western lifestyle and diet can lead to dramatic increases, both in body size and in incidence of key types of cancers, within one or two generations.

- The effects of caloric restriction (i.e. a delay in onset of tumours and prolongation of life span) are consistent in a wide variety of other animal species.

5.4.2 The role of oxidative stress in energy metabolism and carcinogenesis

Questions are being raised about the biological importance in the process of carcinogenesis of oxidative DNA damage arising from respiration, both because the extent of such damage may be less than previously thought and also because there are endogenous mechanisms designed to counteract or repair such damage. These natural protective mechanisms are wide-ranging and appear to function well when the levels of stress and damage are low, or comparable with levels previously encountered. They include endogenous antioxidant enzymes, DNA repair mechanisms and transcription factors such as so-called heat shock proteins, NFK-B and activated protein-1.

Signals for secondary adaptive mechanisms, which may protect the cell against future challenges, are thought to be triggered and transmitted by changes in redox potential caused by the original source of stress, for example in exercising muscle. There seems to be a 'training effect' of stress of this kind as well as of many other types of stress already recognised; small amounts evidently prime the protective mechanisms against future challenges. Thus low levels of oxidative stress may be seen as beneficial rather than damaging. Furthermore,

over-supplementation with exogenous antioxidants may interfere with the feedback mechanisms involved in the adaptive processes. Antioxidant protective systems need to be optimised rather than supplemented in excess.

5.4.3 Time windows

In rodents, energy restriction must be imposed post-weaning in order to have a beneficial effect; there is a detrimental effect on longevity if food intake is restricted before this stage of development. Similarly, in humans, low birthweight babies are at higher risk than higher birthweight babies of many types of chronic diseases in later life (Barker, 1994). It appears that organs, systems and protective mechanisms must be given time to develop before they can function well in protecting the animal or human against environmental challenges.

5.4.4 The energy balance see-saw: energy expenditure or energy intake?

It is not known whether intake, expenditure or balance of energy may be the driving factor, if any of these factors is important in carcinogenesis. The strongest evidence for any of these is for higher BMI as a risk factor for some types of cancer, as mentioned above. BMI is commonly used as a measure of adiposity, and obesity is an extreme case (BMI>30kg/m^2) of higher-than-normal BMI. High BMI and obesity are indicators of long-term (usually several years') energy imbalance and are by far the easiest facet of energy metabolism to measure with accuracy and precision, especially in epidemiological studies. The daily energy imbalance required for long-term increases in body weight is very small in relation to total energy intake or energy expenditure — of the order of one or two percent. This renders the measurement of short-term energy balance virtually impossible.

It is not known whether the fact that there is a stronger body of evidence for a causal relationship of cancer with BMI than with energy expenditure or energy intake is related to the relative ease of measurement of BMI. Obesity may in fact be an indicator of something else that causes cancer — for example physical inactivity or type or amount of food eaten.

Even among the multi-disciplinary group of biological scientists that contributed to the workshop on which this report is based, there was general uncertainty as to the primary causes of obesity on an individual basis. It is not clear whether it is primarily due to energy intake being too high or energy expenditure too low that positive imbalance occurs. There are several levels of possible causes of obesity — from cellular to behavioural and sociological. Further, obesity can develop at different levels of requirement for energy, and it is not known whether the overall level of intake and output has a physiological effect separate from the size of the imbalance itself.

There is similarly uncertainty about the reasons for the observed increases in prevalence of obesity, and whether on a population basis this trend is caused by too much food or too little exercise. Thus it is not clear which side of the energy equation should be changed first — energy expenditure or energy intake. Overall it seems most appropriate to advise first an increase in energy expenditure by increased physical activity. There is some evidence for health benefits of increasing physical activity but very little for benefits of decreasing energy intake in isolation. Also, it is thought that, on an individual basis, changes in physical activity may have more effect than changes in energy intake on the activity of hormone systems such as IGF-1 or insulin. Furthermore, there is a risk of nutrient deficiencies in some population subgroups if energy intake is decreased much more than at present.

Advice to the public about energy balance should be tailored to individuals and not generalised. For example, whether or not weight loss is advised, and if so, whether to attempt this by cutting energy intake or increasing energy expenditure or both, should be a matter for individuals. The recent history of widespread dieting and simultaneous inexorable increases in average population BMI and in percentage prevalence of obesity in Western society suggests that advice simply to reduce energy intake has limited effect.

5.4.5 Macronutrient composition of dietary energy

Investigators into the effects of dietary restriction in rodents have concluded that it is the total amount of energy intake per animal that has the prime effect on longevity and that different macronutrient compositions of the diet are much less important. In contrast, most studies of energy-providing components of the diet in relation to human carcinogenesis have focused on dietary composition rather than on the total amount eaten.

The most well-researched macronutrient, in the context of diet and cancer, is dietary fat. Some consider that fat should be a candidate for attention in terms of food safety and that it presents more of a health risk than food contaminants or pathogens. Much research has pointed to fat as being a risk

factor in breast cancer, but epidemiological results are equivocal. International ecological studies and time-trend analyses suggest associations between the proportion of energy intake as fat and some cancers, most notably those of the colon and breast. However, other components of the diets of populations, such as refined sugars and proportions of fat derived from animal sources, have changed simultaneously and cannot be excluded. Most case–control studies have concluded that higher fat intake is a risk factor for certain cancers, but cohort studies have failed to confirm this hypothesis. Adjustment of individuals' fat intakes for their energy intake in such studies usually abolishes any effect on cancer. Despite these problems and controversies, many epidemiologists believe that there is an effect of fat intake on cancer incidence, while acknowledging that it is difficult to separate the relative effects of fat (which is the most energy-dense nutrient) and energy.

Despite this continuing uncertainty, advice to eat less fat is often more effective in achieving overall lower energy intake in individuals than advice to decrease energy intake *per se.* Also, overall diet composition (e.g. proportions of fat, carbohydrate, protein) can play a role in appetite regulation, which in turn can help prevent obesity. This is an example of the pragmatism that is required in transferring information from experimental or theoretical models to the real situation. Despite a need to understand causes, mechanisms and effects, even if these are known, reductionist approaches such as the use of animal or *in vitro* models cannot necessarily be used directly to provide information for policy and advice, especially where behavioural aspects are involved. Policy may be aimed at realistically achievable targets rather than the ideal situation.

In the case of energy metabolism and carcinogenesis, the ideal situation is not even known, but there is some evidence suggesting that the ideal may be a higher level of physical activity and therefore energy expenditure, and a slightly lower energy intake relative to energy expenditure than is the current norm, throughout life after early childhood. This may delay the onset of some of the most common cancers in the West and would have many other health benefits. This statement assumes that the ideal situation includes a varied diet providing a good supply of essential and other micronutrients and non-nutrients.

5.5 Future directions: The difficult process of measurement

Aspects of energy metabolism present an even greater challenge to quantify than other characteristics of diet related to cancer risk. In most of the issues addressed in this report measurement of the processes involved is extremely difficult. For example, effects of low-level exposures to xenobiotics, tissue free radical activity, DNA repair and short-term energy balance are characterised by very small quantities, which are rapidly changing in nature or amount, and also by the insensitivity of currently available methods to quantify them. Indirect methods have to be used. In addition, although it is possible to measure human energy intake and expenditure on an experimental scale, these are widely variable quantities in free-living humans and cannot be measured precisely and without bias in populations.

There is a recognised ongoing need for intermediate markers of cancer risk. Investigators could use such a marker, or a panel of markers, to study how dietary factors influence them and then how they in turn predict risk. Such intermediate indicators would greatly shorten the time required to assess outcome in interventions aimed at reducing cancer incidence. It is not necessary to know the causal pathway between primary factors and an intermediate marker or between the marker and the final outcome for it to be useful, but the quantitative characteristics of the association must be well defined. The example of hypertension as an intermediate indicator of risk for cardiovascular disease illustrates this point.

Scientific and technical advances will increase the likelihood of improved measurements in the future, at least of some of the factors discussed above. For example, chromosomal damage in lymphocytes is easily measured in large samples, and high levels (the upper third of the population) of chromosomal aberrations measured in a Scandinavian cohort study were found to predict a cancer incidence of about twice that of the general population (Hagmar *et al.*, 1998). Technical developments, such as those in fluorescence-hybridisation, promise to allow easier determination of chromosomal damage. Unfortunately, DNA damage is probably not predictive on its own of cancer incidence for individuals, and there is some evidence that DNA damage may be tissue-specific and that lymphocytes may therefore not give a perfect indication of events in other organs, especially those in which

cancer occurs. Further research will clarify this issue in the future. Given the importance of protective mechanisms it is also necessary to know more about events subsequent to initiation of carcinogenesis.

There is a further need for physiological and biochemical indicators of energy metabolism that are more sensitive than weight gain. Hormones such as insulin and IGF-1 are candidates, but the activity of the hormones is dependent not only on systemic concentration but also on other factors, such as binding proteins and receptor number and activity, so that there is not yet a simple solution. This is an area of rapidly intensifying research.

References

Ames BN & Gold LS (1997) The causes and prevention of cancer: Gaining perspective. *Environ Health Perspect, 105 (suppl 4),* 865–873

Barker BJP (1994) *Mothers, babies and disease in later life*, London, UK, BMJ Publishing Group

Cummings JH & Macfarlane GT (1997) Role of intestinal bacteria in nutrient metabolism. *Clin Nutr, 16,* 3–11

DH (1998) *Nutritional Aspects of the Development of Cancer* (Report on Health and Social Subjects 48), London, UK, The Stationery Office

Gregory J, Foster K, Tyler H & Wiseman M (1990) *The Dietary and Nutritional Survey of British Adults*, London, UK, HMSO

Hagmar L, Bonassi S, Stromberg U, Brogger A, Knudsen LE, Norppa H, Reuterwall C & European Study Group on Cytogenic Biomarkers and Health (1998) Chromosomal aberrations in lymphocytes predict human cancer: A report from the European study group on cytogenetic biomarkers and health (ESCH). *Cancer Res, 58,* 4117–1421

Lutz WK & Schlatter J (1992) Chemical carcinogens and overnutrition in diet-related cancer. *Carcinogenesis, 13,* 2211–2216

Walford RL (1999) Carbon sink: A clue from Biosphere 2? *Science, 283,* 330

6 Overall conclusions

Various opinions are reflected in this report on certain aspects of energy metabolism and carcinogenesis, including the primary causes of obesity; however, there is agreement on several points.

A new understanding is that the appropriateness of extrapolating from observations linking energy metabolism and carcinogenesis in rodents in order to predict events in humans is limited, despite many genetic and phenotypic similarities.

An important difference between rodents and humans is the very much greater metabolic intensity of energy metabolism in the former, which is linked to their smaller size and shorter life span. Energy metabolism in rodents is dominated much more than in humans by the need to produce heat for temperature maintenance and, paradoxically, in rodents the endogenous protective mechanisms against oxidative damage are less active per unit body weight. Thus rats appear to be more at risk from oxidative damage arising from normal respiration than humans and also less able to counteract such damage. The quantitative effects of dietary energy restriction in rodents can therefore not be expected to occur in the same way in humans although there may be some conservation of the phenomenon across these species.

Arguably the most important facet of using energy restriction in rodent toxicological studies, including the two-year bioassay to predict toxicity in humans, is the improved statistical sensitivity it confers, with improved chances of detecting toxic effects of the test compounds. This is especially important for testing the carcinogenicity of chemicals in humans. Ongoing research into the effects of energy restriction in animals will also continue to produce much information on possible mechanisms of carcinogenesis and of ageing which may be of relevance to and stimulate investigations in humans.

Evaluating the influence of energy metabolism is difficult, in part because changes in body size or composition are bidirectionally linked to changes in energy status. In addition, all aspects of energy metabolism, except long-term energy balance, are very difficult to measure with confidence in population studies, so that links with cancer incidence in humans are difficult to establish.

Despite the lack of direct data, there is circumstantial evidence that one or more aspects of energy dynamics play a role in human carcinogenesis. Higher body mass index (BMI) is the most clear-cut indicator of energy status to be established as a risk factor for some cancers in humans. Increases in body weight, adiposity and BMI are manifestations of long-term positive energy balance. In terms of contribution to prevention of weight gain, the balance of opinion tends towards a consensus that increased physical activity rather than decreased total food intake may be a primary protective factor in carcinogenesis. It is not known to what extent this interpretation of the available information is due to the difficulties of measuring energy intake in free-living humans.

Oxidative stress may be less important in cancer initiation than previously thought, but its effect has not been ruled out. Evidence is emerging that the lack of controlled amounts of oxidative stress, for example due to lack of regular exercise or to the consumption of mega-doses of antioxidant supplements, may paradoxically be a risk factor for cancer, because endogenous defence mechanisms that have evolved to manage habitual amounts of oxidative stress are either not stimulated or overridden.

From a pragmatic policy-making point of view, rather than decrease energy intake to protect against cancer, advice to the population to increase physical activity would be more consistent with

other public health issues such as avoidance of the risk of nutritional deficiencies by preventing further reductions in energy intake in some population subgroups, greater likelihood of choice and consumption of a wider variety of foods (conducive to optimal nutritional balance), improvement of appetite regulation for prevention of obesity, improvement of dietary fat metabolism and lipid profile for cardiovascular health, increased insulin sensitivity and prevention of non-insulin dependent diabetes, and maintenance of lean body mass, functional independence and bone density throughout life.

Ongoing and future work on the mechanisms and indicators of energy metabolism, DNA damage and repair, chromosomal damage and carcinogenesis can be expected to help clarify the relative importance of the metabolism of energy and of xenobiotic and endogenous chemicals in the development of cancer in humans.

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