Reduced tumour incidence in mice with inherited seborrhoeic dermatitis

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Summary

121 mice homozygous for the gene *seb* (inherited seborrhoeic dermatitis) and their 142 unaffected heterozygous littermates were kept for their natural lifespan. Heterozygotes showed 84.1% total tumour incidence in males and 95.9% in females. The most common neoplasms were lymphomas, osteomas, lung tumours and neoplasms of the female genital tract. Homozygotes showed a tumour incidence of 36.1% in males and 45.0% in females. The reduction in incidence included all types of neoplasms except epithelial tumours of the skin: skin tumours were detected in 11 homozygous but only in one heterozygous animal. Life expectancy was not affected significantly by genotype. Homozygous mice showed rough and greasy fur and became alopecic with age. Energy intake was increased but growth and depository fat was reduced compared with heterozygous mice. Higher heat loss may incompletely be compensated by higher metabolic rate and thus 'dietary restriction' results in decreased tumour rates. As females show small gonads and a higher increase in food consumption hormonal factors may also be involved.

Keywords Mice; tumour incidence; inherited seborrhoeic dermatitis; aging; energy intake; dietary restriction

Inherited seborrhoeic dermatitis (gene symbol *seb*) occurred spontaneously as a recessive autosomal mutation in a colony of Him:OF1 mice. Homozygous animals show greasy fur and lower viability and fertility. Histological examination of skin reveals hypertrophy of sebaceous glands, hyperkeratosis, parakeratosis, akanthosis and signs of inflammation (Höger *et al.* 1987). For further characterization of this trait and to investigate possible effects on longevity and tumour incidences homozygous seborrhoeic mice and their heterozygous normal littermates were kept for their natural lifespan.

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Materials and methods

The stock Him:OF1 was developed at Carworth Inc., New City, New York, USA, as CF1 and brought to Iffa-Credo, L'Arbresle, France, in 1966. The colony at the University of Vienna was developed from mice obtained from Iffa-Credo in 1980 and is maintained under SPF conditions. The subcolony segregating the gene *seb* was kept in the experimental unit of the same facility with identical environmental conditions but a minimal barrier system. Mice were bred and maintained in cages made of Makrolon and autoclaved woodchips were used as bedding. A standard rodent diet (Altromin 1314ff and 1324ff, Marek, Vienna, autoclaved at

115°C for 15 min) and water acidified to pH3 (GV-SOLAS 1978) from automatic valves were available *ad libitum*. Room temperature was 23 ± 1°C and relative humidity was 50 ± 10%. Ventilation with 100% fresh air had an air change rate of 15 times per hour. The room was illuminated with artificial light at an intensity of about 200 lx in 2 m from 7 am to 5 pm. Homozygous males were mated monogamously to heterozygous females.

Body weight data were collected from 50 animals of each sex and genotype except at 78 weeks of age when the group size was only 15 to 30. Food consumption was measured in mice 5 to 7 months old using a meal form of the standard diet. Six males and 8 females each of homozygous and heterozygous mice were sacrificed at 6 months of age to compare organ weights. Abdominal fat weight was taken as a parameter for total body fat content.

As homozygous mice have higher pre- and postweaning losses (Höger *et al.* 1987) mice for lifespan study were chosen as adults. Losses before one year of age are not included in this data.

A total of 121 virgin homozygous animals (61 males and 60 females) and their heterozygous littermates (69 males and 73 females) were maintained until severe clinical signs, large palpable tumours or natural death occurred. The mice were killed by cervical dislocation. All mice were examined carefully during necropsy. Organs were fixed in 10% buffered formalin. Bones and bone tumours were decalcified in 5% nitric acid prior to further preparation. After embedding in 'Paraplast plus' 4–5 μm thick slices were prepared and stained with haematoxylin and eosin and according to other methods if appropriate.

Comparison of mean life expectancy, body and organ weight and food consumption data were made using Student's *t*-test and χ^2 test and Fisher's exact test were used for comparison of tumour incidences.

Results

Clinical appearance

Homozygous mice were smaller throughout their whole life than the heterozygous controls (Fig 1). The coat was rough and

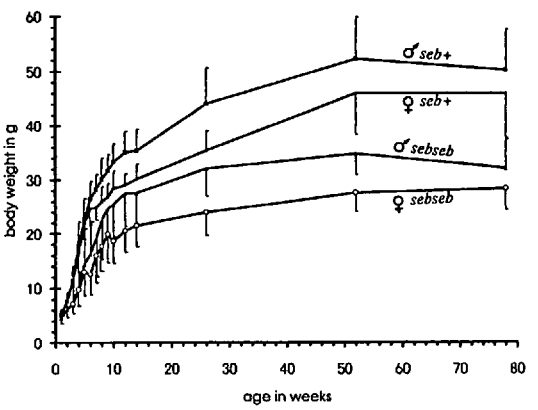

Fig 1 Growth in Him:OF1 mice. Vertical bars represent standard deviations. Differences are significant between mice of same sex and different genotype from week 1 through whole life ($P<0.001$) and between males and females of same genotype from week 6 through whole life ($P<0.05$, 0.01 or 0.001)

Table 1 Food consumption in 5 to 7 months old Him:OF1

	seb+ male	*sebseb* male	*seb*+ female	*sebseb* female
n	6	6	10	8
Mean body weight g ± SD	47.1 ± 4.1	31.9 ± 3.2[a]	35.8 ± 3.1	23.8 ± 3.1[a]
Food consumption g/d ± SD	5.64 ± 0.67	5.19 ± 0.22	4.93 ± 0.52	4.99 ± 0.95
Food consumption g/d/g BW ± SD	0.12 ± 0.02	0.16 ± 0.01[b]	0.14 ± 0.01	0.21 ± 0.03[a]
Food consumption g/d/g metabolic BW ± SD	0.31 ± 0.04	0.39 ± 0.02[b]	0.34 ± 0.02	0.46 ± 0.06[b]
Increase in energy intake per g metabolic BW		23.3%		37.3%

[a]Significantly different from *seb*+ mice of same sex ($P<0.001$)
[b]Significantly different from *seb*+ mice of same sex ($P<0.01$)

Table 2 Mean absolute organ weights in mg (±SD) in 6 month old Him:OF1

	seb+ male	*sebseb* male	*seb+* female	*sebseb* female
n	6	6	8	8
Body weight in g	46.6±6.6	27.4±2.6[a]	38.5±5.1	26.7±3.7[a]
Heart	203±23	141±10[a]	167±9	128±11[a]
Lung	247±31	195±27[c]	234±35	173±20[b]
Liver	2606±369	1847±200[b]	2397±313	1882±364[c]
Spleen	112±56	84±20	101±16	84±27
Kidney (both)	781±114	495±75[a]	547±60	416±49[b]
Brain	482±20	416±16[a]	484±17	427±25[b]
Testes/ovaries (both)	286±34	248±21[c]	17.0±1.4	9.6±3.2[b]
Accessory glands/uterine+cervix	627±156	280±36[a]	481±310	89±73[b]
Abdominal fat	2224±845	108±27[a]	1502±918	57±49[b]
Abdominal fat in mg/100 g BW	4664±1405	400±113[a]	3711±1890	202±178[a]

[a]Significantly different from *seb+* mice of same sex ($P<0.001$)
[b]Significantly different from *seb+* mice of same sex ($P<0.01$)
[c]Significantly different from *seb+* mice of same sex ($P<0.05$)

greasy and they underwent periods of partial alopecia. With increasing age fur became sparse and mice older than one year of age often appeared totally alopecic. Heterozygous controls had extensive body fat accumulation, some males weighed more than 70 g. Homozygotes often developed cysts in the throat region. Sometimes these perforated and subsequent healing occurred.

Food consumption

Food consumption was similar in homo- and heterozygous mice but calculated as values relative to body weight or metabolic bodyweight ($BW^{0.75}$) homozygous mice had a significant higher food intake (Table 1).

Organ weights

Organ weights of homozygous mice were significantly lower in all organs except the spleen (Table 2). Calculated as relative values to body weights most organs of homozygotes were significantly heavier (data not shown) but female gonads and abdominal fat tissue (Table 2) were still significantly lighter.

Lifespan

Mortality was very similar between all groups of mice (Fig 2). The longer mean lifespan in heterozygous females was statistically not significant (Table 3).

Tumour incidence

Heterozygous mice showed an overall tumour incidence of 84.1% in males and 95.9% in females. In homozygous mice the tumour incidence was 36.1 and 45.0 respectively, and the difference between homozygotes and heterozygotes was

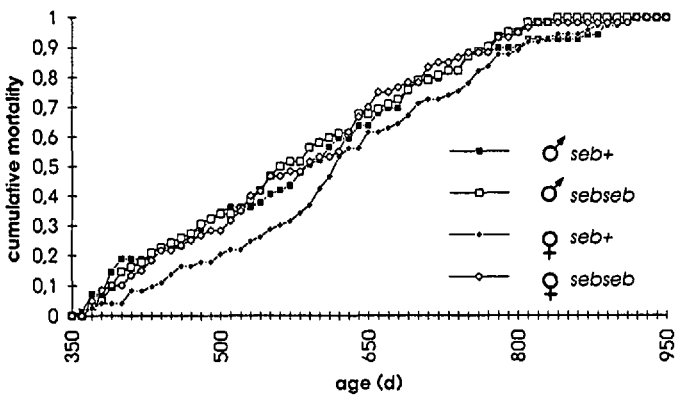

Fig 2 Cumulative mortality in Him:OF1 mice

Table 3 Tumour frequencies in Him:OF1 mice (numbers in parentheses: % of total number of mice)

	seb+ male	*sebseb* male	*seb*+ female	*sebseb* female
Total number of mice	69	61	73	60
Mean lifespan in d±SD	591±151	579±137	630±137	585±136
Number of mice with tumours	58 (84.1)	22 (36.1)[a]	70 (95.9)	27 (45.0)[a]
Haematopoietic system	30 (43.5)	9 (14.8)[a]	21 (28.8)	6 (10.0)[b]
Osteomas	22 (31.9)	4 (6.6)[a]	17 (23.3)	11 (18.3)
Lung tumours	13 (18.8)	2 (3.3)[b]	12 (16.4)	4 (6.7)
Skin tumours	0	7 (11.5)[b]	1 (1.4)	4 (6.7)
Liver tumours	5 (7.2)	0	5 (6.8)	0
Circulatory system	3 (4.3)	0	4 (5.5)	1 (1.7)
Soft tissue tumours	3 (4.3)	0	5 (6.8)	0
Stomach tumours	2 (2.9)	0	4 (5.5)	0
Reproductive system				
ovary			29 (39.7)	3 (5.0)[a]
uterus			41 (56.2)	2 (3.3)[a]
cervix			12 (16.4)	0[a]
Other tumours	1 (1.4)	1 (1.6)	3 (4.1)	1 (1.7)

[a]Significantly different from *seb*+ mice of same sex ($P<0.001$)
[b]Significantly different from *seb*+ mice of same sex ($P<0.01$)
[c]Significantly different from *seb*+ mice of same sex ($P<0.05$)

significant in both sexes (Table 3). The average reduction to less than half of the incidence in heterozygotes varied for the different types of tumours. The largest effect was seen in the neoplasms of the female reproductive system. Exceptions were the neoplasms of skin: they appeared more often in homozygous seborrhoeic mice than in phenotypically unaffected littermates (Table 3).

Neoplasms

Most neoplasms of the haematopoietic system (55 out of 66) were classified as lymphocytic lymphomas with or without involvement of the thymus. Only a few plasmacytomas, myeloid leukaemias and reticulum cell sarcomas were found. Most of the earlier deaths in heterozygous animals were caused by these life-limiting neoplastic processes. Second in tumour frequency were osteomas. Prevalence sites were the skull and the long bones of the limbs. Only large nodules within the cranial cavity were life-threatening. In most mice osteomas appeared on multiple sites of the skeleton. Affected homozygous seborrhoeic mice usually had fewer and smaller osteomas than osteoma-bearing heterozygotes. All lung tumours of homozygous mice were diagnosed as adenomas, 8 out of 26 found in 25 heterozygous animals were classified as malignant. In livers only one tumour per sex was benign. All except one tumour of the stomach was situated in the glandular part, most of them were classified as carcinomas and one was metastasizing to the lung. Haemangiomas and soft tissue tumours including one lipoma and different types of sarcomas were detected in various parts of the body without any prevalence site. Among the 'other tumours' in Table 1 2 neoplasms of the kidney, one thymoma, one Leydig cell tumour, one carcinoma of the small intestines and one luteinoma of the vagina are included. Six out of the 12 skin tumours were keratoakanthomas, 4 others cornified squamous cell carcinomas. One carcinoma, metastasizing to the regional lymph nodes, developed from the sebaceous glands. The only skin tumour detected in heterozygous mice was classified as a trichoepithelioma. Granulosa cell tumours were the most common neoplasms of the ovary (20/35 found in

32 females), but also adenomas, cystadenomas and others were found. The organ mainly affected in heterozygous females was the uterus. Usually more than one type of tumour were found in the uteri, mainly adenocarcinomas (in 29 females) and endometrial polyps (in 12 females, often multiple), but also cystadenomas, leiomyomas and leiomyosarcomas. Only one adenocarcinoma in the uterus was metastasizing to the lung. One teratoma was found in the uterus of a seborrhoeic female. In cervix uteri leiomyomas (6/14 in 12 females) and squamous cell carcinomas (4/14) were predominant.

Non-neoplastic lesions

About two-thirds of the heterozygous females had large uteri often with visible cysts diagnosed histologically as hyperplasia and cystic degeneration of the endometrium. All of the homozygous females had very small ovaries and uteri showing differing degrees of atrophy. Many homozygous mice of both sexes showed hyperplasia of the peripheral lymph nodes, often cystic enlargement and sometimes lymphadenitis, predominantly in the cervical region. All other non-neoplastic pathological findings (mainly degenerative processes in kidney, heart and other organs) revealed only negligible differences between the 4 groups of mice.

Discussion

A total tumour incidence of 84.1% in Him:OF1 males and 95.9% in females is very high. Reports on other outbred stocks like CD-1 or NMRI, kept for two years or for lifespan, show incidences of 9.5 to 68.1% in males and 13.2 to 75% in females (Kaspareit & Deerberg 1987, Rehm *et al.* 1985, Sher *et al.* 1982, Homburger *et al.* 1975, Weisse *et al.* 1975). Even under nearly identical conditions biological variability was observed by comparing results from different studies (Bomhard & Mohr 1989). As we observed interlitter variation in the most common neoplasms in Him:OF1 mice, in lymphomas and osteomas, this may be influenced by the outbred background. But even values in genetically uniform B6C3F1-Hybrids vary between 13 and 89% in males and 10 and 70% in females respectively (Chandra & Frith 1992, Tamano *et al.* 1988, Sher *et al.* 1982).

Whereas neoplasms of the haematopoietic system, the lung and the ovary occur very frequently in many strains of mice, Him:OF1 mice belong to the few osteoma prone strains and stocks (Luz *et al.* 1991, Höger *et al.* 1994). The number of tumours in the uterus of heterozygous OF1 females is also unusually high. This may be promoted by the high incidence and degree of cystic endometrial hyperplasia, which affects most of the *seb*+ females, but we consider this to be independent of pathological processes as it is indicated from studies in inbred mice (Frith *et al.* 1983). Spontaneous epithelial tumours of skin are rare in mice in general not exceeding 1% (Bogovski 1979). This is in accordance with data from heterozygous mice of this study. A spontaneous incidence of 11/121 *sebseb* mice is outstanding. Permanent irritation of skin by the inborn disease may function as a tumour promoter.

Differences in the total tumours incidence may depend on many factors: Source of animals, sex, genetic variation, diet, age at death, environmental conditions and even the histological criteria used by the pathologists. Most of these factors were excluded in this study as heterozygous littermates served as controls to homozygous mice. The low tumour incidence in homozygous mice is obviously an indirect effect of the gene *seb*. Reduction of tumour incidence is a well known phenomenon from many studies with quantitative dietary restriction. It is most effective when started early in life at about 50% of the unrestricted intake level. Beside reduced tumour rate it results in retarded growth, reduced body fat content, decreased rate of change for most age-sensitive biological indexes and prolonged life expectancy (Weindruch 1989). Selection for less body fat has no effect on lifespan and tumour incidence if food consumption is not restricted (Rehm *et al.* 1985).

In addition to their inherited skin disease *sebseb* mice show retarded growth and reduced size through their whole life compared with their heterozygous littermates (Fig. 1). Organ weights are also reduced but the largest differences in adult mice are seen in the abdominal fat (Table 2) taken as parameter for total body fat content. Animals under dietary restriction are slim but energy intake per unit of metabolic body mass does not differ from *ad libitum* fed animals (Bucci 1992). Food consumption of our seborrhoeic mice was at the same level expressed in absolute terms as in heterozygous animals but significantly higher if expressed in relation to body weight (Table 1). This additional energy intake can be explained by increased heat loss resulting from poor insulation of skin. Nearly complete loss of fur is seen in most *sebseb* mice over 1 year of age and intermittent during the first hair cycles. Mice used in the food consumption study had sparse fur. Homozygous animals of the hairless mutations *nu* and *sha* were reported to have a higher metabolic rate than their normal coated controls. The increase in energy intake was 24 to 34% per g metabolic body weight at 25°C environmental temperature (Flisch 1976). Our own results in *sebseb* mice showed a comparable increase of about 23% in males and 37% in females at 24°C. As this is adjusted for body mass, other factors may be responsible for the higher increase in females, perhaps hormonal influences. Abnormal hormonal status would also be supported by the small female gonads. As young homozygous mice are not alopecic the lower weight gain and high pre- and postweaning losses cannot be explained by suboptimal energy intake alone. But even alopecia starting in adults may influence tumour incidence, in the same way as dietary restriction beginning at one year of age (Weindruch & Walford 1982) and light restriction to just 80% of normal intake (Rehm *et al.* 1985) is effective.

Resulting from these data the low tumour incidence in mice with seborrhoeic dermatitis is explained—at least partly—as the effect of an endogenous restriction in energy. A general reduction in vitality may stand in competition with the life prolonging effects of energy restriction. This may lead to the same life expectancy of homozygous mice despite their low tumour incidence.

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