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Differences reported in the lifespan and aging of male Wistar rats maintained on diets containing fat with different fatty acid profiles (virgin olive, sunflower or fish oils) are not reflected by histopathological lesions found at death in central nervous and endocrine systems

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ABSTRACT

The present study was designed to examine if dietary fat sources that have shown differences in lifespan and if some aging-related aspects can modulate the range of histopathologic changes in central nervous and endocrine systems that occur during the lifespan of Wistar rats. Moreover, it was attempted to gain insight into the relationship between longevity and the development of the different pathological changes, as well as possible interaction with diet. In order to achieve this, male Wistar rats were randomly assigned to three experimental groups fed semisynthetic and isoenergetic diets from weaning until death with different dietary fat sources, namely virgin olive, sunflower, or fish oil. An individual follow-up until death of each animal was performed. Incidence, severity, and burden of specific or group (i.e., neoplastic or non-neoplastic proliferative and non-proliferative) of lesions was calculated along with individual's disease and individual organ lesion burden. Most of the histopathological lesions found have been described in previous studies. Neoplasms, and in particular pituitary adenomas followed by brain tumors, were the most prevalent lesions found in the rats and the main cause of death involving both systems. Incidence of brain lesions was associated with age-at-death. Assayed dietary fats did not present differential effects on pathological changes occurring in endocrine and central nervous systems throughout rat lifespan.

1. Introduction

The rapid aging of the human population has increased interest in gerontological research (Li et al., 2014; Sousa-Victor et al., 2014). Rodent studies have shown that different nutritional interventions, such as caloric restriction (Anderson et al., 2009; Mercken et al., 2012; Pamplona and Barja, 2006; Speakman et al., 2016; Swindell, 2012) and protein or methionine restriction (Orentreich et al., 1993; Pamplona and Barja, 2006a,b; Sanchez-Roman and Barja, 2013; Zimmerman et al., 2003), may contribute to extend life expectancy. Replacement of saturated fat with n-6 polyunsaturated fatty acids (PUFA) as main dietary fat can reduce mortality because of its effects on blood lipoproteins and triglycerides, which are known cardiovascular risk factors. Moreover, higher levels of oxidative damage markers were found in different tissues (Varela-Lopez et al., 2019; 2017a; Roche et al., 2014; Bullon et al.,

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Abbreviations

AIN-93G	AIN-93 diet for growth diet
AIN-93M	AIN-93 for maintenance diet
ANOVA	analysis of variance
BN	Brown Norway
COD	cause of death
F344	Fisher 344
FO	fish oil
INHAND	International Harmonization of Nomenclature and Diagnostic Criteria
MUFA	monounsaturated fatty acids
NNLA	NTP's Noneoplastic Lesions Atlas
NTP	National Toxicology Program
PUFA	polyunsaturated fatty acids
SO	sunflower oil
VO	virgin olive oil

2013) of old rats fed diets rich in sunflower oil compared to those found in rats fed diets with virgin olive oil as the unique dietary fat. Considering that a proper adaptation of fatty acid profiles of plasma, but also of mitochondrial membranes, to the dietary fat have been widely verified in rats (Varela-López et al., 2017a, 2017b; Varela-López et al., 2019; Villalba et al., 2015; Jové et al., 2014; Valencak et al., 2011; Roche et al., 2014), these results support the proposed relationship between the degree of unsaturation of biological membranes, resistance to endogenous damage and aging (Pamplona and Barja, 2006; Hulbert et al., 2014). In fact, it was reflected by the differences in median lifespan between rats fed similar diets (Ramirez-Tortosa et al., 2020). On the other hand, n-3 PUFA may contribute to a lower production of proinflammatory prostanoids compared to n-6 PUFA, which would have beneficial effects on pathologies and conditions associated with inflammation (Bustos et al., 2017). Studies comparing fish oil–based diets with n-6 PUFA-rich diets have reported that reactive oxygen species production (Varela-Lopez et al., 2018) and detrimental changes associated with aging are prevented or delayed by fish oil-based diets (Varela-Lopez et al., 2018). The relevance of n-3 PUFA content of the diet for longevity has also been reported in mice (Strong et al., 2016; Ueda et al., 2011). Importantly, the mentioned effects correlated with the effects in age-related changes found in biochemical and physiological indicators (Bullon et al., 2013; Jové et al., 2014; Navarro-Hortal et al., 2019a, 2019b; Valencak et al., 2011; Roche et al., 2014; Varela-López et al., 2017, 2018; Varela-López et al., 2019; Villalba et al., 2015). In this sense, consumption of a n-6 PUFA-rich diet was associated with higher alveolar bone loss at periodontium (Bullon et al., 2013), number of β-cells and insulin content in the pancreas (Roche et al., 2014), and fibrosis levels in the liver (Varela-Lopez et al., 2018) and lower femur bone mass density (Varela-López et al., 2017). Likewise, compared with n-6, n-3 PUFA prevented age-related alveolar bone loss (Bullon et al., 2013) and liver fibrosis (Varela-Lopez et al., 2018) and reduced cardiac inflammation (Navarro-Hortal et al., 2019a), although it could promote the development of acinar fibrosis and macrophage infiltrates in peri-insular regions (Roche et al., 2014) and alveolar bone loss (Bullon et al., 2013).

However, the contribution of the mentioned age-related changes to overall aging has been underappreciated from the organismal perspective. Moreover, it has not been confirmed if the effects of the mentioned diets on aging and age-related changes led to pathological and function alterations or occurred in additional tissues. The age-related cognitive decline which along mobility limitations derived from the progressive decline of functional capabilities of central nervous system in older adults (Alexander et al., 2012; Dykiert et al., 2012; Glorioso et al., 2010; Levin et al., 2014) can contribute to overall aging. Likewise, there is an involution of endocrine functions leading to a cluster of phenomena typically associated with aging including altered body composition and insulin resistance (Björntorp, 1995). Therefore, organs that form part of the endocrine and nervous systems could be crucial for organismal aging. In fact, the role of nervous and endocrine systems in aging has been emphasized by the "neuroendocrine" theory of aging, developed by Vladimir Dilman, (Diggs, 2008). Considering the relevance of these systems for aging, necropsies of organs belonging to these systems at age-of-death have been performed in rats receiving isocaloric and normolipid diets rich in MUFA, n-6 PUFA or n-3 PUFA by using virgin olive, sunflower or fish oil as unique dietary fat sources throughout life with the following three objectives: (1) to characterize the pathologic changes which occur in endocrine and central nervous systems as a result of aging in the Wistar rat strain, (2) to determine the causes of death involving these systems in the studied animals and (3) to determine possible effects of the different dietary fats on both pathological changes and causes of death in the rats.

2. Material and methods

2.1. Chemicals

All the chemical products and solvents, of the highest grade available, were acquired from Sigma (St. Louis. MO) and Merck (Darmstadt. Germany).

2.2. Animals and diets

Seventy-five male Wistar rats (*Rattus norvegicus*) weighing 80–90 g were housed three to a cage and maintained at 20 °C in a 12-h light to 12-h dark cycle with free access to water. The rats were randomly assigned to three experimental groups with 25 rats per group, fed semisynthetic and isoenergetic diets formulated according to the AIN-93 rodent diet criteria (Reeves, 1997) except for the dietary fat source. The diets were composed of (in g/100g of diet): 14 casein, 46.57 starch, 10 sucrose, 15.5 Dextrose, 4 dietary fat, 5 cellulose, 0.25 choline, 0.18 L-cystine, 1.0 vitamin mixture and 3.5 mineral mixture. The dietary fats used were virgin olive oil (VO) provided by the agricultural research center "Venta del Llano" (Mengibar, Spain), sunflower oil (SO) acquired in a local supermarket, or pure fish oil (FO) ROPUFA 30 (DSM, Kaiseraugst, Switzerland), obtaining diets rich in MUFA, n-6 PUFA, or n-3 PUFA respectively as reported in previous studies (Bullon et al., 2013). The diets were provided *ad libitum* for the first 2 months and then at 25 g per rat per day for the rest of the experiment (in order to avoid being overweight). From weaning up to 2 months of age, the animals received diets formulated according to the AIN-93 diet for growth (AIN-93G) whereas the AIN-93 as maintenance diet (AIN-93M) was followed for the remaining period. An individual follow-up until death of each animal was performed. The animals were treated in accordance with the guidelines of the Spanish Society of Laboratory Animals and the study was approved by the Ethics Committee of the University of Granada (permit number 20-CEA-2004).

2.3. Pathological evaluation

After death, each animal underwent a routine necropsy conducted by three board-certified pathologists. Samples of all major organ systems and any grossly abnormal tissues were preserved by immersion in 10% neutral phosphate-buffered formalin. Fixed tissues were trimmed using a standard protocol developed by the University of Washington Veterinary Diagnostic Laboratory (Snyder et al., 2015) so that the same section of organ was examined from each animal. The tissues were routinely processed, and the paraffin-embedded samples were sectioned at 3–4 μm and stained with hematoxylin and eosin. Lesions found in each organ were described based on previous studies in rodents (D'Cruz et al., 2002; D'Cruz and Uckun, 2001; Hubert et al., 2000). All observed lesions were termed according to the International Harmonization of Nomenclature

and Diagnostic Criteria (INHAND) (Keenan et al., 2015). Nonneoplastic lesions were diagnosed and recorded following the strategy proposed by the National Toxicology Program (NTP) in the NTP Nonneoplastic Lesions Atlas (NNLA) (https://ntp.niehs.nih.gov/nnl/). According to it, certain lesions were not diagnosed if they were a consequence or a feature of another previous lesion or pathological process, although they were described in the pathology narrative. Lesions were grouped into the broad categories of neoplastic, non-neoplastic and non-neoplastic proliferative, and they were also collectively examined (Table 1).

2.4. Grading of histopathological lesions

Most lesions were assigned severity grades. An ascending four-level numerical scheme was followed for the gradation to both neoplastic and non-neoplastic lesions. All neoplastic processes were graded according to Ikeno and colleagues (Ikeno et al., 2003), with modifications. Neoplastic processes were morphologically diagnosed and categorized as hematolymphoid, epithelial, mesenchymal, neuroglial or miscellaneous and the severity of each primary tumor was assigned a grade based on the extent of tumor size and distribution (metastasis) within the tissues examined, as well as effects on surrounding parenchyma or adjacent tissues. Grade 1 tumors included small, focal benign tumors contained within the primary site and causing no observable changes to the surrounding parenchyma and, thus, they did not interfere with organ function in a mild to moderate way. Grade 2 tumors included malignant neoplasms localized to one organ but did not affect the organ or likely interfere with normal function, or benign tumors larger than grade 1 tumors that had observable effects on surrounding parenchyma (compression or necrosis). Grade 3 tumors had metastatic foci to one or two organs or were large, localized neoplasms that affected organs sufficiently to interfere with normal function or were associated with ulceration and hemorrhage. Grade 4 tumors were malignant neoplasms with metastatic spread to >2 organs or localized malignant neoplasms that affected and infiltrated sufficiently to interfere with adjacent tissue.

Non-neoplastic lesions were graded according to the NTP's four-level numerical scheme for gradable non-neoplastic lesions [1 = minimal, 2 = mild, 3 = moderate, and 4 = marked]. Non-neoplastic lesions were categorized as either incidental lesions (defined as those minor lesions that would not have contributed to the animal's moribund state) or contributing lesions (defined as those lesions that would affect the moribund state) and graded on the severity scale. Small or focal incidental lesions were assigned to grade 1. Grade 2 non-neoplastic lesions were multifocal or diffuse lesions that were incidental. Grade 3 was used for contributory lesions not thought to be the primary COD but which interfered with normal function. Grade 4 was used for contributory lesions severe enough to cause illness and death. The most common non-neoplastic contributory lesions (those with 10% prevalence in any cohort) were analyzed individually for severity and population lesion burden. According to recommendations of NTP, certain lesions were not graded when they were a consequence or a feature of another previous lesion or pathological process, although they were described in the pathology narrative. Likewise, some morphologic tissue changes (e.g., cysts) were generally not graded but were routinely recorded as "present". On the other hand, organisms (bacteria, fungi, protozoan and metazoan parasites) in tissues were always graded as "present" no matter what their number.

Table 1
Criteria for classification in grades of neoplastic and non-neoplastic lesions.

Grade	Neoplastic lesions	Non-neoplastic lesions
1	Small, focal benign tumors contained within the primary site and causing no observable changes to the surrounding parenchyma	Small or focal incidental lesions
2	malignant neoplasms localized to one organ but did not affect the organ or likely interfere with normal function or benign tumors larger than grade 1 tumors that had observable effect on surrounding parenchyma	multifocal or diffuse lesions that are incidental
3	Tumors had metastatic foci to one or two organs or were large, localized neoplasms that affected organs sufficiently to interfere with normal function or were associated with ulceration and hemorrhage	Contributory lesions not thought to be the primary COD but interfered with normal function
4	Malignant neoplasm with metastatic spread to >2 organs or localized malignant neoplasm that affected and infiltrated sufficiently to interfere with adjacent tissue	Contributory lesions severe enough to cause illness and death

2.5. Determination of cause of death

Cause-of-death (COD) assignment was based on a scheme proposed by a report from the National Center for Toxicological Research (Kodell et al., 1995). If a severe and likely fatal lesion was identified, then this was deemed the "probable'' COD. If no fatal lesion was identified, then additional "contributory'' causes of death were listed for lesions that were significant but not likely to be the singular COD. The term "equivocal" was also considered for possible cause of death. Finally, if no COD was apparent, then the COD was assigned as "unknown''.

2.6. Determination of lesion incidences

The rate of occurrence of individual lesions in each dietary group was calculated. Lesions occurring in at least 10% of either group were arbitrarily defined as common. In this study, common lesions were grouped into the broad categories of neoplastic, or non-neoplastic proliferative and non-proliferative; they were collectively examined to establish whether the groups were affected by diet or were related to age-at-death.

2.7. Determination of disease and lesion burden

Neoplastic and non-neoplastic lesion burdens were calculated for each rat. The neoplastic lesion burden was calculated as the sum of severity scores (grades) for each primary tumor within a rat. The non-neoplastic lesion burden was calculated as the sum of severity scores (grades) for each non-neoplastic lesion with grade 3 or 4 within a rat. The disease burden was calculated as the average sum of neoplastic and non-neoplastic severity scores for each organ within each cohort as already proposed (Ikeno et al., 2003). The overall lesion burden was calculated as the average sum of neoplastic and non-neoplastic severity scores for all rats within each cohort as already proposed (Ikeno et al., 2003). Only non-neoplastic lesions with Grade 3 or 4 (moderate to severe) were considered, with the rationale that Grade 1 and 2 lesions are too mild to contribute any significant burden. Neoplastic severity scores for all neoplastic lesions were used, because neoplasia of any severity could be burdensome.

2.8. Number of lesions

The number of distinct lesions was counted for each age-diet group to determine whether the variety of lesions can act as a biomarker of aging.

2.9. Statistical analysis

Statistical analysis was performed using SPSS 24.0 for Windows (IBM, Chicago, IL, USA). Incidences of specific lesions or kinds of pathologies and causes of death were compared between cohorts maintained on different diets using the Pearson's chi-square test. When the expected frequencies were too small for the chi-square test, the data

were analyzed using Fisher's exact test. A fully factorial one-way analysis of variance (ANOVA) was used to compare scores of severity for specific lesions, lesions and disease burdens per organ and per animal and number of lesions per animal between dietary groups. For multiple comparisons, the Bonferroni *post hoc* test was used. For non-normal variables, the non-parametric test, Kruskal-Wallis was used. To test for age-at-death effects on prevalence of common pathologic outcomes (with prevalence of 10% or higher), incidences and severity scores of specific lesions were compared between animals grouped in 6-month intervals by age-at-death within both each cohort and total population. In all analyses, significance was determined at a p-value of 0.05.

3. Results

3.1. Central nervous system

Almost all proliferative lesions found in the rats of this study were primary neoplasms except for a metastasis found in an individual of the FO group (Fig. 1), which was a cerebral metastasis from a thyroid carcinoma that was considered the COD (Fig. 2) and probably caused more alterations. The remaining neoplasms were three granular cell tumors, one of them infiltrating. This type is considered as a common meningeal neoplasm in the rat (Roth et al., 1993), but is not included in the human classification. Lastly, two glial tumors, one of them hemorrhagic, were also found. Glial tumors were established as the COD for the rats that had them. In most of the cases, proliferative lesions found in the present research were benign neoplasms, including the ganglioneuroma and the granular cell tumors, that were mostly associated with vascular and hemodynamic alterations in CNS of the rats and were also established as COD (Fig. 2). No differences for lesion severities (Fig. 3 and Table S3) and lesions burden per systems or per organs (Fig. 4 and Table S4) between dietary groups were found. Images of different Neoplastic lesions found in the central nervous system in the present study are presented in Fig. 5.

Non-proliferative lesions found in the brains of rats in the present study were mostly vascular or were related to vascular alterations. Hemorrhages were the most frequent lesions, leading to or related to death in all cases. These are manifested in several ways within the

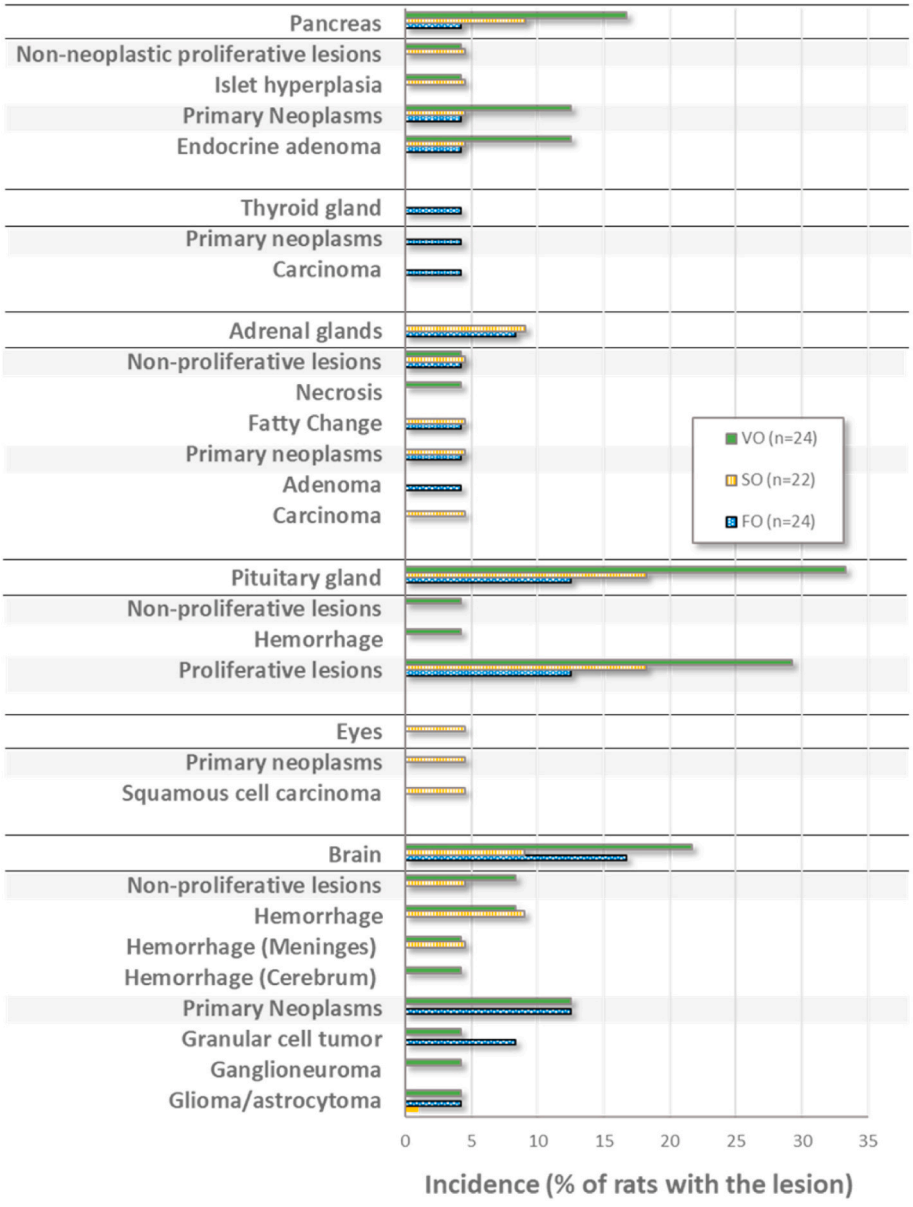

Fig. 1. Incidence of Central Nervous and Endocrine Lesions Diagnosed at End of Life in male Wistar rat cohorts Fed on Different Fat Sources. Abbreviations: VO: virgin olive oil, SO: sunflower oil; FO: fish oil; n: sample size.

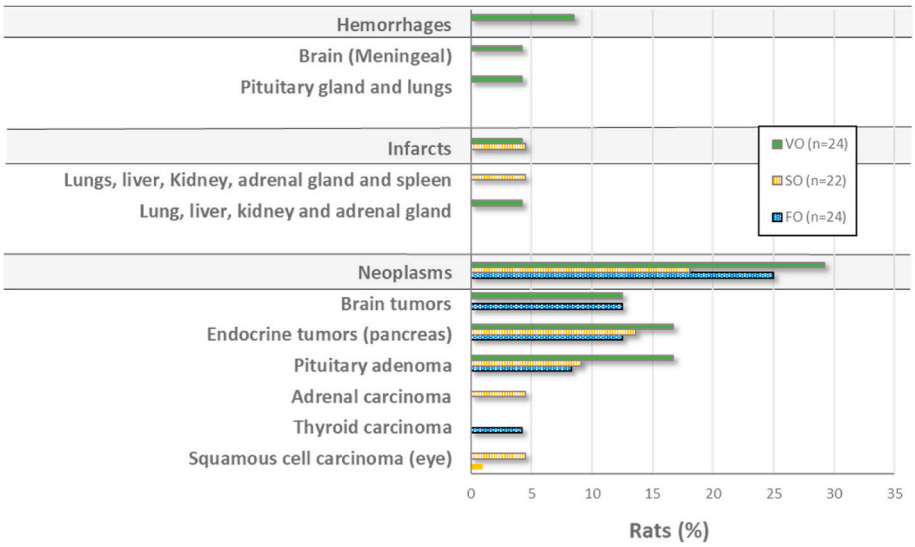

Fig. 2. Relative frequency of rats died from different Causes of Death (CODs) Involving Central Nervous and Endocrine Systems in Male Wistar rat Cohorts Fed on Different Fat Sources. Abbreviations: VO: virgin olive oil, SO: sunflower oil; FO: fish oil; n: sample size.

nervous system. In this case, it was possible to distinguish three meningeal (subarachnoid) hemorrhages and one intracerebral hemorrhage. In two cases, meningeal hemorrhages occurred in a context of vascular congestion in multiple organs related to prostatitis which was the cause of the death. Another rat presented sings of hemorrhage observed in the brain and the lungs accompanied by a certain degree of vascular congestion in the lungs and liver. As several foci of enzymatic necrosis were observed in the pancreas, the pathological process observed in this organ was considered responsible for the mentioned alterations and established as COD (Fig. 2). Moreover, it is known that some intracerebral neoplasms may cause disruption of the vasculature in rats that results in substantial perilesional hemorrhage, edema, and necrosis (Solleveld and Boorman, 1986). In this study, one subject died because of a cerebral hemorrhagic tumor. In addition, the disruption of the vasculature caused by a tumor of cerebral granular cells and a ganglioneuroma could also be responsible for ischemic changes observed in the brain from one rat of the FO group and two of the VO group. All hemorrhages were diagnosed and graded except for those within or secondary to a neoplasm as suggested in the NNLA (Little and Rao, 2014). Likewise, infarctions were not diagnosed as necrosis because they were secondary to neoplasms.

3.2. Special senses

3.2.1. Eyes

A squamous cell carcinoma with abscess was noted in the left eye of a rat of the SO group that was considered the COD of the rat (Fig. 2). Interestingly, this animal also presented two additional primary neoplasms, a retroperitoneal hemangioma and a fibroma.

3.3. Endocrine system

3.3.1. Pituitary gland

Proliferative lesions in pituitary gland were seen in 20.2% of animals in the present research (Table S1). In two animals pituitary adenomas were cystic or had areas of hemorrhage. In another two, they were highly vascular with dilated blood vessels. Furthermore, this pathology was established as the COD for 11.4% of animals (Table S2). Moreover, there was another animal with this neoplasm, but the COD came from hemorrhages in multiple organs. Lastly, one individual presented hemorrhages concomitantly in this organ and in the lungs, both contributing to COD of the animal. No differences were found for lesion severities (Fig. 3) and lesion burden per systems or per organs (Fig. 4 and Table S4) between dietary groups.

3.3.2. Adrenal glands

Six rats presented some lesions in the adrenal gland, but most of them were non-proliferative (Fig. 1). Vascular congestion in adrenal glands was observed in four animals, in one of them accompanied by a hemorrhage, but also in other organs. Anyway, these were considered acute sings of death secondary to other processes. Two rats died from multiple organ infarcts; adrenal glands were also affected. Lastly, fatty change (increased diffuse cortical vacuolization) (Fig. 6) was detected in three rats. In one rat, it was associated with congestion that also appeared along with necrosis in another rat. Regarding proliferative lesions, these were a subcapsular cell adenoma in a rat of the FO group and a metastatic carcinoma in a rat of the SO group that was responsible for the death of the animal that presented the metastases in the brain and hemorrhagic infarcts in the lungs and the liver (see Fig. 7).

3.3.3. Thyroid gland

One rat of FO group presented a thyroid gland metastatic follicular carcinoma to the brain that was determined as COD (Table S2). This was presented in association with hemorrhagic infarcts observed in lungs and liver from the same animal, which correlated with the presence of thrombi in cardiac cavity and vascular congestion in bone marrow.

3.3.4. Pancreatic islets

Lesions of the pancreatic islets were almost exclusively proliferative (except for one rat that had focal degeneration), and all but one of the proliferative lesions were neoplastic. Islet cell adenomas were observed in two animals. In one case, this was associated with necrotizing pancreatitis with enzymatic focal necrosis. Likewise, islet cell hyperplasia was diagnosed in two rats. No differences were found for lesion severities (Fig. 3 and Table S3) and lesion burden per systems or per organs (Fig. 4 and Table S4) between dietary groups.

4. Discussion

The rapid aging of the human population has increased the interest not only in unraveling the causes of aging, but also in discovering how we can manipulate potential causes of aging to decrease, stop, or even revert its rate of progression (Li et al., 2014; Sousa-Victor et al., 2014). However, this phenomenon is multidimensional, involving several

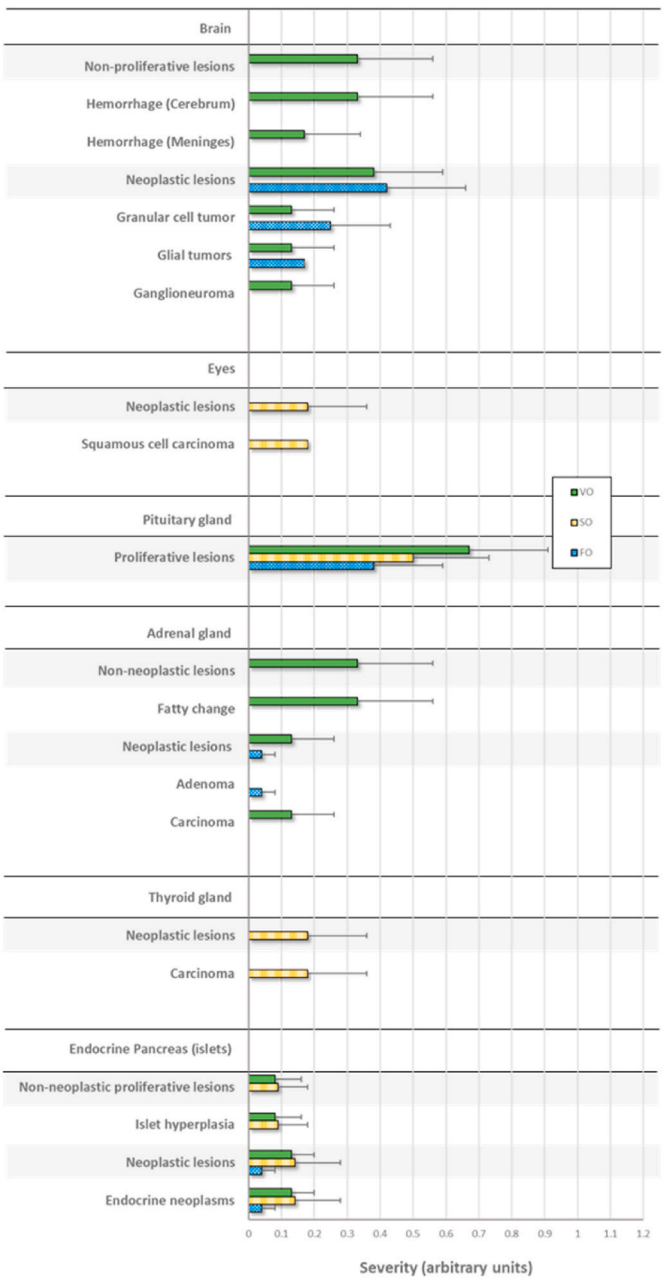

Fig. 3. Average Severity of Central Nervous and Endocrine Lesions Diagnosed at End of Life in male Wistar rat cohorts Fed on Different Fat Sources. Error bars represent standard error of the mean. Abbreviations: VO: virgin olive oil, SO: sunflower oil; FO: fish oil; n: sample size.

processes still not well understood. Therefore, continuing gerontological research (Li et al., 2014; Sousa-Victor et al., 2014) that involves the study of normal aging processes and age-related diseases, is mandatory. The difficulty for understanding of the aging process is in part due to the complexity of specialized organs and tissues that are highly customized to perform essential functions. Many gerontological studies focused on organismal aging or aging as it occurs in the whole organism. On the other hand, there are studies focused on how aging and age-related diseases affect individual organs but the contribution of each organ to overall aging is largely ignored or underappreciated from the organismal perspective. Establishing pathological changes and causes of death in preclinical research studies from necropsy data provides an optimal opportunity to obtain useful translational information when comparing outcomes with those of the human population death in aging studies investigating interventions to prolong life span (Ladiges, 2016, 2017). Unfortunately, the number of studies evaluating the effects of different nutritional interventions in aging with the mentioned approach is very small.

The size, temperament, and the wealth of published physiological and biochemical data on young rats make rat a good choice for studies of the changes that occur during normal aging to perform studies of basic mechanisms of aging and age-related diseases (Nadon, 2006). Anyway, there are many differences with respect to the incidence and types of lesions found in aged rats between strains, but also between different colonies or stocks of the same strain, particularly in outbred strains such as Wistar (Nadon, 2006). This diversity has been attributed to genetic differences but also to environmental factors such as nutrition that is known to have an important impact on overall mortality and morbidity. The present study was designed to examine if dietary fat sources that have shown to affect lifespan and some aging-related changes can modulate the range of histopathologic changes that occur during the lifespan of Wistar rats in central nervous and endocrine systems. We considered both a reduction of incidence and severity of pathologies or a possible harmful effect, even chronic toxicity features, since some of the studied fats at the assayed doses have shown lipotoxicity in some organs (Bullon et al., 2013; Navarro-Hortal et al., 2019a, 2019b; Roche et al., 2014; Varela-López et al., 2017; 2018). For this purpose, incidence, severity, and burden of specific or group (i.e., neoplastic, non-neoplastic proliferative, and non-neoplastic non-proliferative) of lesions was calculated along with animal's disease and animal organ lesion burden. Moreover, it was attempted to gain insight into the relationship between longevity and the development of the different pathological changes, as well as possible interaction with diet. With this objective, possible differences were evaluated in the mentioned histopathologic parameters by grouping the animals in 6-month intervals according to their age-at-death.

A wide range of histopathological changes in both systems was observed in the cohort of male Wistar rats investigated in the present study. Most of the histopathological lesions have been previously described in literature. Still, there are some findings that have not been previously reported in both cross-sectional (Roth et al., 1993) and longitudinal studies (Kroes et al., 1981; Roth et al., 1993), including histological analyses in Wistar rats. Among the non-previously described events, the ones that should be considered are necrosis in adrenal glands and several neoplastic lesions, such as a lymphoma manifested in multiple organs, pancreatic neuroendocrine adenomas, a squamous cell carcinoma in one eye, and a ganglioneuroma as a cerebral tumor. On the other hand, different lesions previously described in rats were absent here. Concerning eyes, with some exceptions (Kroes et al., 1981), cataracts and histiocytic infiltration of the ciliary body were noted with relative frequency in the eyes of rats in previous studies (Lipman et al., 1996; Roth et al., 1993; Wolf et al., 2000), but here these lesions were absent and only a squamous cell carcinoma with abscess was noted.

Regarding the incidence of the different lesions, neoplasms were the most prevalent lesions found in the rats with 41.4% of animals presenting at least one tumor in the evaluated organs. In most of the cases, proliferative lesions were benign neoplasms, including the ganglioneuroma and the granular cell tumors, that were mostly associated with vascular and hemodynamic alterations in CNS of the rats and were established as COD. Notwithstanding, only 7% had any malignant tumor and only two animals showed metastases to other areas different from the area where the primary tumor was located. Still, 24.3% of the rats died because of some neoplasms, located in the organs of the endocrine system.

The organs most affected by primary neoplasms among those evaluated were, in this order, the pituitary gland (18.6%), all of them adenomas; the brain (10%); and the endocrine component of the pancreas (7.1%). Although many metastases (most of them in the lungs) were reported in the Wistar stock (Roth et al., 1993), in other stocks, as here, its incidence was also low (Kroes et al., 1981; Roth et al., 1993). Tumor

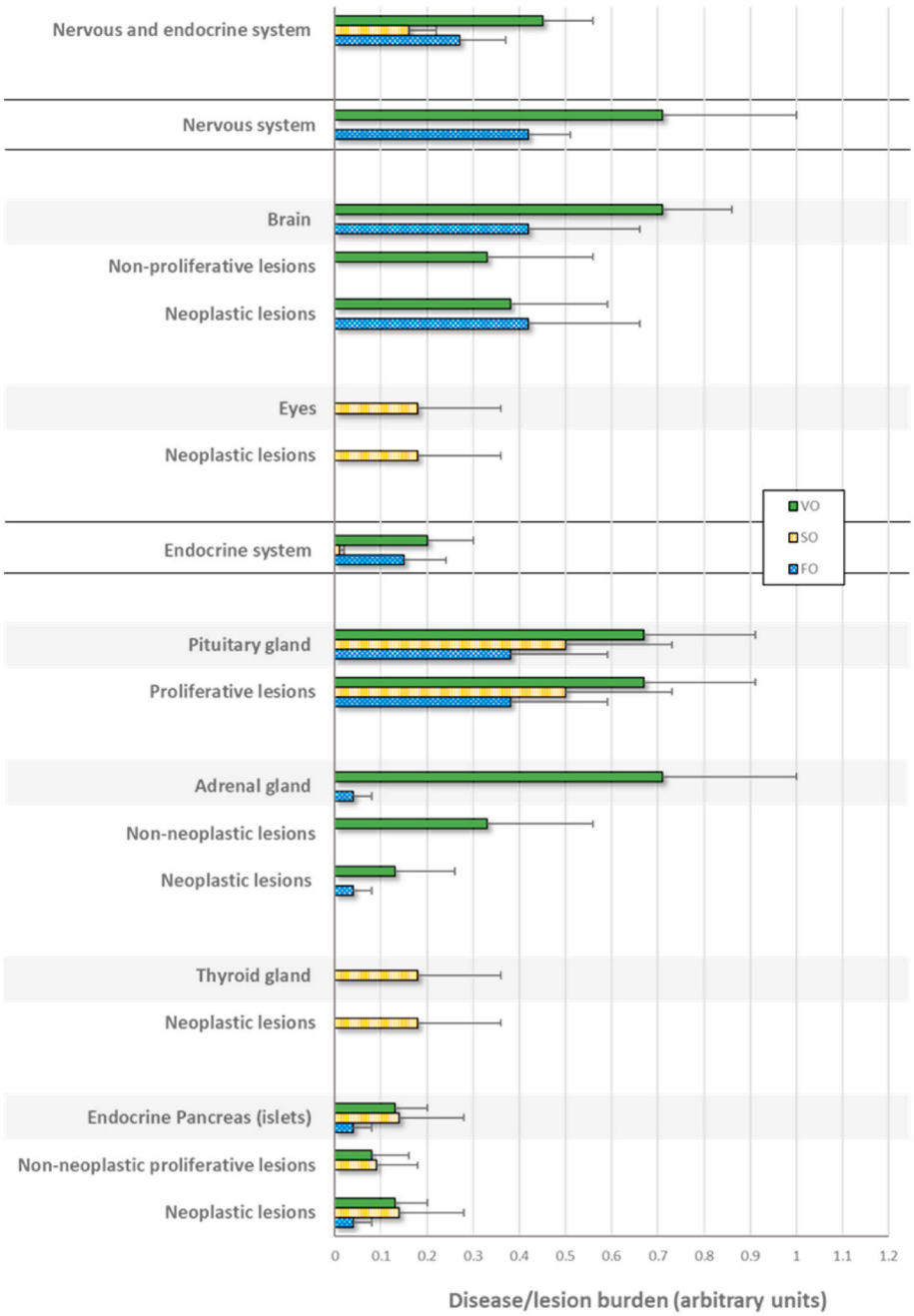

Fig. 4. Average Lesion and Disease Burdens of Central Nervous and Endocrine Lesions Diagnosed at End of Life in male Wistar rat cohorts Fed on Different Fat Sources. Error bars represent standard error of the mean. Abbreviations: VO: virgin olive oil, SO: sunflower oil; FO: fish oil; n: sample size.

incidences may vary considerably from strain to strain (Kroes et al., 1981; Lipman et al., 1996; Roth et al., 1993), but results found in the present research are consistent with the scientific literature that indicated that pituitary tumors are very common neoplasms in different rat strains (Kroes et al., 1981; Lipman et al., 1996; Roth et al., 1993), although there are some exceptions (Zöller et al., 1978; Zöller and Matzku, 1978).

Spontaneous pituitary adenomas have been recognized as common findings in old rats (Kovacs et al., 1977). Adenoma of the pituitary gland has been reported to have a high incidence in different Wistar rat stocks (Kroes et al., 1981) and to be the only notable neoplasm in a Wistar rat stock (Roth et al., 1993), but, importantly, incidences of these tumors, along with mononuclear cell leukemia, also had one of the highest values in the F344 strain (Coleman et al., 1977; Losco and Ward, 1984; Maeda et al., 1985). In the present research, proliferative lesions in the pituitary gland were seen in 20.2% of animals, a value which is intermediate to that seen in F344 rats (39%) and Brown Norway (BN) rats (10%) (Lipman et al., 1999), but within the rank of incidences of pituitary adenomas (10–47%) found between different colonies of Wistar rats (Kroes et al., 1981; Roth et al., 1993). It has been previously described that pituitary adenomas may be cystic or have areas of hemorrhage (Brändli-Baiocco et al., 2018) and this occurred in two animals of the present study. However, they could have been highly vascular with dilated blood vessels (Brändli-Baiocco et al., 2018) a finding that was observed in another two animals of the present study. Adenomas do not invade other organs but may have infiltrative growth within the gland including the pars intermedia and nervosa, and large adenomas may compress the brain (Brändli-Baiocco et al., 2018). Despite the fact that the contribution of this pathology to rat morbidity in previous studies was lower (Roth et al., 1993), the relevance of pituitary gland

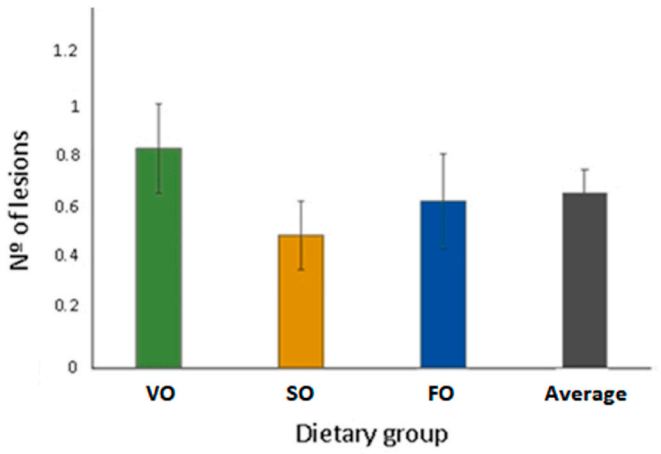

Fig. 5. Average Number of Central Nervous and Endocrine Lesions Diagnosed at End of Life in male Wistar rat cohorts Fed on Different Fat Sources. Error bars represent standard error of the mean. Abbreviations: VO: virgin olive oil, SO: sunflower oil; FO: fish oil; n: sample size.

proliferative lesions for the health of the animals of the present study was manifested by the fact that this pathology was responsible for 11.4% of the deaths. Moreover, there was another animals with this neoplasm, but the COD came from hemorrhages in multiple organs. Lastly, one individual presented hemorrhages concomitantly in this organ and in the lungs, both contributing to COD of the animal.

Brain tumors were also important because of their incidence, but also because they led to death in almost all cases. Among rodents, the rat presents the highest incidence of tumors of the nervous system, which reaches 11% (Swenberg, 1986). Almost all proliferative lesions found in CNS of the rats of this study were primary neoplasms except for a cerebral metastasis from a thyroid carcinoma. As here, several brain tumor types have been previously described, but most of them were granular cell tumors (Kroes et al., 1981). This type is considered common meningeal neoplasm in the rat (Roth et al., 1993), but is not included in the human classification. Glial tumors have also been previously found in Wistar rats although they were infrequent (Roth et al., 1993). Based on the expansive growth pattern in an enclosed cranial space and/or their infiltrative behavior, these have been considered a malignant neoplasm. In fact, they were responsible for the death of the two rats presenting them. Lastly, intracranial ganglioneuromas are very rare in rodents (Weber et al., 2011) despite the fact that one was found here. Notwithstanding, it has been described in B6C3F1 mice where it was established as possibly originating from the trigeminal ganglion (Yasui et al., 2009). Regardless of the malignancy of brain tumors, it is also known that some intracerebral neoplasms may cause disruption of the vasculature in rats that results in substantial perilesional hemorrhage, edema, and necrosis (Solleveld and Boorman, 1986) which can seriously affect health. In this study, one rat died because of a cerebral hemorrhagic tumor. In addition, the disruption of the vasculature caused by two tumors of cerebral granular cells and a ganglioneuroma could be also responsible for ischemic changes observed in the brain of one rat of the FO group and two of the VO group.

As in previous studies in Wistar rats (Roth et al., 1993), lesions of the pancreatic islets were almost exclusively proliferative (except for one rat that had focal degeneration), and all but one of the proliferative lesions were neoplastic. In previous studies in Wistar rats, islet cell adenomas were present in 1–7% of the animals, whereas incidence for islet cell carcinoma was more reduced, from 0 to 1.5% (Poteracki and Walsh, 1998; Roth et al., 1993). Similarly, an incidence of 3.7% has been reported in Sprague Dawley male rats (Chandra et al., 1992). In the present study, prevalence of islet cell adenomas was within the reported range. In one case, this was associated with necrotizing pancreatitis with enzymatic focal necrosis, but this association and possible implications of the endocrine neoplasm in the etiopathology of pancreatitis have not been reported before. In contrast, islet cell hyperplasia, one of the most reported age-related non-neoplastic lesions in the aged rat in other studies, especially in BN rats (Lipman et al., 1999), was diagnosed only in two rats in the present study.

Lastly, tumors in the thyroid gland and the adrenal cortex were rare, as reported in the literature (Kroes et al., 1981; Russfield, 1966; Tamaschke, 1955). Regarding proliferative lesions of adrenal cortex, a subcapsular cell adenoma and a carcinoma were found. Both, adenomas and carcinomas of adrenal glands, have been described in Wistar rats, but also at similar, very low frequencies (0–2%) (Kroes et al., 1981; Poteracki and Walsh, 1998; Roth et al., 1993). However, pheochromocytomas, reported to be one of the most frequent neoplasms in some Wistar rat stocks (5–20%) (Kroes et al., 1981; Poteracki and Walsh, 1998; Roth et al., 1993), were not found here. In Sprague Dawley male rats, proliferative lesions found in this organ are also rare with 3.6% of the individuals presenting thyroid adenomas (Chandra et al., 1992). Despite their low prevalence, tumors in these organs have dramatical consequences for the health of the animals because of their invasiveness. Carcinoma of the adrenal gland was responsible for the death of the animal that presented metastases in the brain and hemorrhagic infarcts in the lungs and liver. Likewise, thyroid gland follicular carcinoma was also determined as COD and metastatic, reaching the brain. This was presented in association with hemorrhagic infarcts observed in lungs and liver from the same animal, which correlated with the presence of thrombi in cardiac cavity and vascular congestion in bone marrow. Several studies indicate that high levels of thyroid hormone lead to more coagulation and less fibrinolysis (Akinci et al., 2011; Demir et al., 2009; Engelmann et al., 2015; Hooper et al., 2012; Horacek et al., 2015; Liu et al., 1993; Myrup et al., 1995; Stuijver et al., 2012; Verkleij et al., 2013) increasing the risk of venous thromboembolism (Danescu et al., 2009; Dekkers et al., 2017; Lin et al., 2010; Ramagopalan et al., 2011). Maybe, transient hyperthyroidism occurred during carcinoma development in this rat causing the mentioned hemodynamic alterations, but this has not been described in scientific literature in rats.

Non-neoplastic non-proliferative lesions affecting the central nervous system and endocrine system were much reduced and, moreover, they constituted a common COD as in previous studies. Hemorrhages and infarcts were the most lethal, but they also occurred concomitantly in other systems and in many cases the primary cause was not established, suggesting some alteration with systemic consequences, although the cause was not clear.

Non-proliferative lesions of the adrenal gland have been reported to be very common in F344 rats and both F344BN F1 and BNF344 F1 hybrids (Lipman et al., 1996) whereas the estimated incidence in a cohort of Sprague-Dawley male rats was 1.2% (Chandra et al., 1992). Here, only four rats presented some non-proliferative lesions in the adrenal gland. Blood-filled sinuses (telangiectasis) and cysts (peliosis) have been reported as common findings in rats (Yarrington and O'Neal Johnston, 1992). In BN/Bi and WAG/Rij strains a 5% incidence has been reported for telangiectasia (Burek, 2017). Here, vascular congestion was observed in the adrenal gland of the four animals, in one of them accompanied by a hemorrhage, but also in other organs. Anyway, these were considered acute sings of death secondary to other processes. Lastly, fatty change was detected in three rats. In one rat, it was associated with congestion that also appeared along with necrosis in another rat. Another two rats died from multiple organ infarcts; adrenal glands were also affected.

Concerning the brain, only a few spontaneous non-neoplastic lesions were described in the brains of Wistar rats from other colonies. These included hydrocephalus and mineralization (Roth et al., 1993). Notwithstanding, atherosclerosis, hemorrhage and necrosis have been described in brains of Fisher 344 (F344) rats older than 18 months (Coleman et al., 1977). Similarly, non-proliferative lesions found in the brains of the rats in the present study were mostly vascular or were related to vascular alterations. Among them, hemorrhages were the

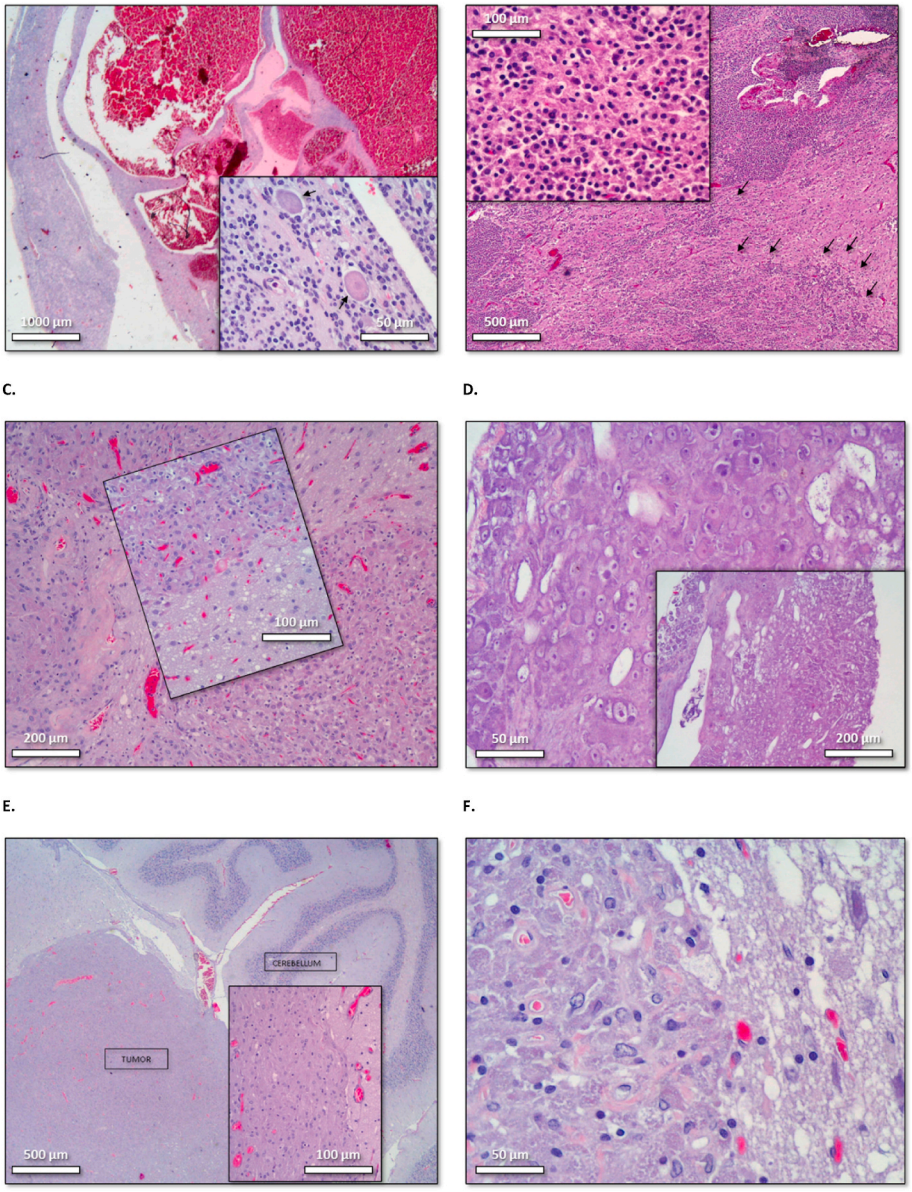

Fig. 6. Neoplastic lesions found in the central nervous system (CNS) after hematoxylin and eosin staining. Image captures were obtained using a DP27 camera Olympus (Tokyo, Japan) from a Olympus BX41 microscope**. A.** Hemorrhagic glioma NOS (not otherwise specified, the presence or absence of mutation is not specified) (note the large vascular spaces filled with red blood cells). **B.** Glial malignant cells infiltrate in the brain parenchyma. **C.** Solid nests of cells of epithelial lineage (brain metastasis) within the brain parenchyma. **D.** Ganglioneuroma. **E.** Granular cell tumor. **F.** Granular cell tumor. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

most frequent lesions, leading or relating to death in all cases. These manifest in several ways within the nervous system. In this case, it was possible to distinguish three meningeal (subarachnoid) hemorrhages and one intracerebral hemorrhage. Isolated hematomas are quite rare in rodents (Jubb and Huxtable, 1992). In fact, erosion or rupture of vascular walls in the meninges or parenchyma commonly arises from pathologic processes (Jubb and Huxtable, 1992; Kaufmann et al., 2012) or trauma. In two cases, meningeal hemorrhages occurred in a context of vascular congestion in multiple organs related to prostatitis that was the cause of death. Another rat presented sings of hemorrhage in the brain and lungs accompanied by a certain degree of vascular congestion in the lungs and liver. As several foci of enzymatic necrosis were observed in the pancreas, the pathological process observed in this organ was considered responsible for the above mentioned alterations and established as COD. As mentioned, in four cases, vascular alterations would be a consequence of vasculature disruption by brain tumors. In one subject, these consisted of hemorrhages that were enough severe to cause death. In the other three, the disruption of the vasculature was caused by two tumors of cerebral granular cells and a ganglioneuroma that would be also responsible for ischemic changes observed in the brain.

Contrary to other rat stocks, non-neoplastic proliferative lesions were absent in the thyroid gland. Similarly, cataracts and histiocytic infiltration of the ciliary body previously reported in Wistar rats (Roth et al., 1993) were unobservable here.

Lastly, non-neoplastic proliferative lesions were rare. In a previous study (Roth et al., 1993) hyperplasia had been reported in several sites and organs, including bile duct, parathyroids, and adrenal medulla (Roth et al., 1993). Here, it was mainly observed in pancreas, and also in the pituitary gland. Another important issue for aging research is to focus on the so-called age-related pathologies. It is not feasible to study aging without considering age-related lesions. Some authors reported an age-dependent increase in the tumor percentage (Coleman et al., 1977; Gilbert et al., 1958; Prejean et al., 1973; Ross and Bras, 1971; Schardein et al., 1968). Furthermore, in animals dying at an early age from other pathological conditions, the incidence of tumors located in both system organs is low. Neoplasms affecting the organs were evaluated in this study and it was found that two gliomas were the cause of death in all

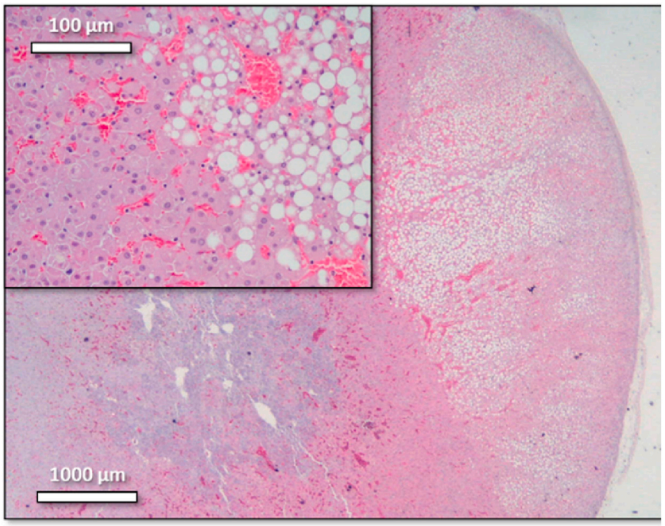

Fig. 7. Lesions found in adrenal glands after hematoxylin and eosin staining. Image captures were obtained using a DP27 camera Olympus (Tokyo, Japan) from a Olympus BX41 microscope. Fatty Change (vacuolization cortical increased diffuse).

animals that died early (before 24 months). Other tumors, for example, granular cell tumors, insulomas (pancreatic endocrine adenoma), and phaeochromocytomas have been reported to appear only later (after 24 months) in life (Dunning, 1963; Pollard and Luckert, 1975; Shain et al., 1975). Moreover, here, as in previous studies, metastases were usually not seen before 22 months (Kroes et al., 1981). Nevertheless, despite, the above mentioned coincidences, no statistically significant associations were found between age-at-death and tumor incidences in central nervous and endocrine systems and individual organs in this study. Moreover, previous studies, have concluded that many tumors did not cause illness or death and were therefore discovered only at the time of autopsy since most of them appeared only after 24 months (Kroes et al., 1981). However, except for tumors affecting the pancreas, in the present study most of the neoplasms were responsible for death. Thus, in the case of pancreatic neoplasms, establishing age-related associations as above could be quite arbitrary. Regarding non-neoplastic lesions, results from different studies supported an age-associated increasing incidence of cataracts and histiocytic infiltration of the ciliary body (Roth et al., 1993), and focal hyperplasia of adrenal gland (Roth et al., 1993), at least in the stocks where these lesions were frequently found. In any case, it seems that the incidences of many other lesions were too small to establish an age-related relationship. In the present study, it was found that the incidence of brain lesions in rats that died at age of 6–12 months was lower than those observed in rats that died at age of 24–36 months, but only when data of three dietary groups were combined, which again emphasizes the importance of sample size when studying the associations with age-at-death. Despite relationships found in this and other studies, it is also not appropriate to consider lesions as mere epiphenomena of aging, because aging cannot be measured as an entity apart from the lesions that are found associated with it (Lipman et al., 1999).

Regarding aging, a general trend for laboratory rodents is that lesions develop with increasing frequency as a function of age, which is considered endogenous to the process of aging (Lipman et al., 1996, 1999). For this reason, lesion incidence itself has been suggested as a biomarker of aging (Lipman, 1997). Considered its utility, the number of lesions affecting the endocrine system, nervous system or both were compared to clarify if there were different rates of aging between dietary groups. However, no differences were found between dietary groups or age-at-death groups (data not shown). This was expected because of the longitudinal design of the study. On the other hand, the fact of only considering lesions affecting both of systems is an important limitation for the power of this parameter.

Because the genotype and environment were kept constant and because sampling artifacts did not play a significant role, the variability in prevalence of most lesions comes from chance alone. Moreover, since the prevalence of most lesions increased with age, at least when results from different studies were taken into account, it can be argued that the effect of aging on lesion development is to increase the *odds* that an animal will develop any of the lesions to which it is genetically predisposed (Lipman et al., 1999). Given the stochastic nature of lesion development, one rat will develop one set of lesions and another identical rat will develop a different set, though both will be subsets of the total repertoire of lesions that the genotype can develop. Rats of another genotype will also differ from one another for the same reasons. Some of their lesions will be the same as those of the other rat genotype, because both genotypes share some common age-related lesion genes. The more similar two genotypes are, the more similar will be the set of lesions that they can develop. However, if two genotypes are genetically quite different, as are the F344 and BN strains, then their sets of age-related lesions will be distinct. Future studies on aging should focus on those things that change in individuals as they age which make them more likely to develop specific lesions. Results from previous studies (Lipman et al., 1999) strongly suggest that the set of lesions that any rat genotype develops is regulated by both genes and diet.

Importantly, it has been widely demonstrated that manipulation of environmental factors (e.g diet, specific-pathogen-free status, lighting) could impact on the pattern of pathology noted in lab animals. In this sense, dietary or caloric restriction led to a decrease in the prevalence of most age-related lesions, at least of those evaluated, whether neoplastic, non-neoplastoic proliferative, or non-proliferative, in different rat strains including F344, BN, BNF344Fl hybrids andLobund-Wistar rat (Snyder et al., 1990), but also Wistar (Goodrick et al., 1982). Some examples are nephropathy (Goodrick et al., 1982; Maeda et al., 1985) and tumors (Goodrick et al., 1982), In turn, this has been related to a reduction of mortality and increased median lifespan values (Goodrick et al., 1982; Maeda et al., 1985). One exception is retinal degeneration (Lipman et al., 1999). Consequently, caloric restricted rats nearly always appear younger than *ad libitum* rats of the same chronological age because their lesion burdens are smaller (Lipman et al., 1999). A similar effect could be attributed to other dietary interventions. In this sense, Masoro et al. (1989) noted a marked influence on the incidence of nephropathy in Fischer 344 rats associated with the amount and type of dietary protein, a dietary factor also associated with longevity in rodents (Orentreich et al., 1993; Zimmerman et al., 2003).

Regarding dietary fat, it has been reported that administration of corn oil by gavage increased the incidence of acinar hyperplasia, adenomas and adenocarcinomas in rats (Eustis and Boorman, 1985; Haseman et al., 1985). Moreover, it has been reported that n-6 PUFA of sunflower oil could impact favorably upon mortality, which in turn might unveil other pathologies emerging at later ages (Ferrucci et al., 2019). However, median lifespan has been reported to be higher in rats fed diets with virgin olive oil as dietary source compared to those receiving a diet with sunflower oil as dietary fat (Ramirez-Tortosa et al., 2020). Despite the fact that the effects of diets like those used in this study have been scantly evaluated in organs of the nervous and endocrine system, several aspects of the pancreas have been reported to be differentially affected (Fig. 3 and Table S3). Compared with animals fed on a virgin olive oil–based diet, 24-month-old rats receiving a sunflower oil–based diet showed a higher number of β-cells and insulin content at pancreas level (Roche et al., 2014). However, no differences between dietary groups were observed here concerning pancreatic neoplasms prevalence or severity. Anyway, new publications are expected to be published evaluating other organs and systems with a similar approach. Moreover, it would be interesting to perform new studies comparing different age groups to clearly test if onset and progression of certain pathologies could be modulated by dietary fat despite their prevalence were similar.

5. Conclusions

Most of the histopathological lesions found here have been described in literature. Neoplasms and in particular pituitary adenomas followed by brain tumors were the most prevalent lesions found in the rats. In addition, they were also the main COD in most rats that died from any pathology affecting the neuroendocrine system. Non-proliferative lesions, mainly hemorrhages and infarcts, affecting CNS and endocrine system were greatly reduced, and, moreover, they constituted an important COD. Regarding associations with age, it seems that the incidences of many other lesions were too small to establish an age-related relationship. Dietary fats assayed in the present research have shown interesting effects on longevity and aging related changes in different organs, which would probably indicate a delay of the aging process. However, these previously described effects did not seem to have differential effects on pathological changes occurring in central nervous and endocrine systems throughout rat lifespan, at least with the cohort size investigated in the present study.

CRediT authorship contribution statement

Alfonso Varela-López: Investigation, Methodology, Writing – original draft, Writing – review & editing. **César L. Ramírez-Tortosa:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Francisco M. Ramos-Pleguezuelos:** Methodology, Investigation. **Bélgica Márquez-Lobo:** Methodology, Investigation. **Maurizio Battino:** Resources, Conceptualization, Supervision, Writing – review & editing. **José L. Quiles:** Resources, Conceptualization, Supervision, Writing – review & editing, Project administration, Resources, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2022.113357.

References

Akinci, B., Demir, T., Comlekci, A., Yener, S., Ozcan, M.A., Karaoglu, O., Yuksel, F., Secil, M., Yesil, S., 2011. Effect of levothyroxine suppression therapy on plasma thrombin activatable fibrinolysis inhibitor antigen levels in benign thyroid nodules. Med. Princ. Pract. 20, 23–28.

Alexander, G.E., Ryan, L., Bowers, D., Foster, T.C., Bizon, J.L., Geldmacher, D.S., Glisky, E.L., 2012. Characterizing cognitive aging in humans with links to animal models. Front. Aging Neurosci. 4, 21.

Anderson, R.M., Shanmuganayagam, D., Weindruch, R., 2009. Caloric restriction and aging: studies in mice and monkeys. Toxicol. Pathol. 37, 47–51. https://doi.org/10.1177/0192623308329476.

Björntorp, P., 1995. Neuroendocrine ageing. J. Intern. Med. 238, 401–404. https://doi.org/10.1111/j.1365-2796.1995.tb01217.x.

Brändli-Baiocco, A., Balme, E., Bruder, M., Chandra, S., Hellmann, J., Hoenerhoff, M.J., Kambara, T., Landes, C., Lenz, B., Mense, M., Rittinghausen, S., Satoh, H., Schorsch, F., Seeliger, F., Tanaka, T., Tsuchitani, M., Wojcinski, Z., Rosol, T.J., 2018. Nonproliferative and proliferative lesions of the rat and mouse endocrine system. J. Toxicol. Pathol. 31, 1S–95S. https://doi.org/10.1293/tox.31.1S.

Bullon, P., Battino, M., Varela-Lopez, A., Perez-Lopez, P., Granados-Principal, S., Ramirez-Tortosa, M.C., Ochoa, J.J., Cordero, M.D., Gonzalez-Alonso, Adrian, Ramirez-Tortosa, C.L., Rubini, C., Zizzi, A., Quiles, José L., 2013. Diets based on virgin olive oil or fish oil but not on sunflower oil prevent age-related alveolar bone resorption by mitochondrial-related mechanisms. PLoS One 8, e74234.

Burek, J.D. (Ed.), 2017. Pathology of Aging Rats: A Morphological and Experimental Study of the Age-Associated Lesions in Aging BN/Bi, WAG/Rij and (WAG × BN) F1 Rats. CRC Press, Boca Raton. https://doi.org/10.1201/9781351075435.

Bustos, V., Partridge, L., 2017. Good Ol' Fat: links between lipid signaling and longevity. Trends Biochem. Sci. 42, 812–823.

Chandra, M., Riley, M.G., Johnson, D.E., 1992. Spontaneous neoplasms in aged Sprague-Dawley rats. Arch. Toxicol. 66, 496–502. https://doi.org/10.1007/BF01970675.

Coleman, G.L., Barthold, S.W., Osbaldiston, G.W., Foster, S.J., Jonas, A.M., 1977. Pathological changes during aging in, barrier-Reared Fischer 344 male Rats1. J. Gerontol. 32, 258–278. https://doi.org/10.1093/geronj/32.3.258.

Danescu, L.G., Badshah, A., Danescu, S.C., Janjua, M., Marandici, A.M., Matta, F., Yaekoub, A.Y., Malloy, D.J., Stein, P.D., 2009. Venous thromboembolism in patients hospitalized with thyroid dysfunction. Clin. Appl. Thromb. Hemost. 15, 676–680.

Dekkers, O.M., Horvath-Puho, E., Cannegieter, S.C., Vandenbroucke, J.P., Sørensen, H.T., Jørgensen, J.O.L., 2017. Acute cardiovascular events and all-cause mortality in patients with hyperthyroidism: a population-based cohort study. Eur. J. Endocrinol. 176, 1–9.

Demir, T., Akinci, B., Comlekci, A., Karaoglu, O., Ozcan, M.A., Yener, S., Yuksel, F., Secil, M., Yesil, S., 2009. Levothyroxine (LT4) suppression treatment for benign thyroid nodules alters coagulation. Clin. Endocrinol. 71, 446–450.

Diggs, J., 2008. Neuroendocrine (aging clock) theory of aging. In: Loue, S.J., Sajatovic, M. (Eds.), Encyclopedia of Aging and Public Health. Springer US, Boston, MA, pp. 584–586. https://doi.org/10.1007/978-0-387-33754-8_313.

Dunning, W.F., 1963. Hosts and in the rats bearing the subcutaneously transplanted tumors. Skeletal metastases are an unusual finding in rats bearing subcutaneously. Biology of the Prostate and Related Tissues: Proceedings 12, 351.

Dykiert, D., Der, G., Starr, J.M., Deary, I.J., 2012. Age differences in intra-individual variability in simple and choice reaction time: systematic review and meta-analysis. PLoS One 7, e45759.

D'Cruz, O.J., Uckun, F.M., 2001. Short-term (13-week) toxicity study of 5-Bromo-6-methoxy-5,6-dihydro-3′-azidothymidine-5′-(p-bromophenyl) methoxyalaninyl phosphate (WHI-07), a novel anti-HIV and contraceptive agent, in B6C3F1 mice. Toxicol. Sci. 60, 373–378. https://doi.org/10.1093/toxsci/60.2.373.

D'Cruz, O.J., Erbeck, D., Waurzyniak, B., Uckun, F.M., 2002. Two-year toxicity and carcinogenicity studies in B6C3F1 mice with 5-bromo-6-methoxy-5,6-dihydro-3′-azidothymidine-5′-(p-bromophenyl) methoxyalaninyl phosphate (WHI-07), a novel anti-HIV and contraceptive agent. Toxicology 179, 61–77. https://doi.org/10.1016/S0300-483X(02)00319-0.

Engelmann, B., Bischof, J., Dirk, A.-L., Friedrich, N., Hammer, E., Thiele, T., Führer, D., Homuth, G., Brabant, G., Völker, U., 2015. Effect of experimental thyrotoxicosis onto blood coagulation: a proteomics study. European thyroid journal 4, 119–124.

Eustis, S.L., Boorman, G.A., 1985. Proliferative lesions of the exocrine pancreas: relationship to corn oil gavage in the National Toxicology Program. J. Natl. Cancer Inst. 75, 1067–1073.

Ferrucci, D., Silva, S.P., Rocha, A., Nascimento, L., Vieira, A.S., Taboga, S.R., Mori, M., Lenz-Cesar, C., Carvalho, H.F., 2019. Dietary fatty acid quality affects systemic parameters and promotes prostatitis and pre-neoplastic lesions. Sci. Rep. 9, 19233 https://doi.org/10.1038/s41598-019-55882-5.

Gilbert, C., Gillman, J., Loustalot, P., Lutz, W., 1958. The modifying influence of diet and the physical environment on spontaneous tumour frequency in rats. Br. J. Cancer 12, 565.

Glorioso, C.A., Oh, S., Sibille, E., 2010. Brain molecular aging, promotion of neurological disease and modulation by sirtuin5 longevity gene polymorphism. Neurobiol. Dis. 41, 279–290.

Goodrick, C.L., Ingram, D.K., Reynolds, M.A., Freeman, J.R., Cider, N.L., 1982. Effects of intermittent feeding upon growth and life span in rats. Gerontology 28, 233–241.

Haseman, J.K., Huff, J.E., Rao, G.N., Arnold, J.E., Boorman, G.A., McConnell, E.E., 1985. Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N × C3H/HeN)F1 (B6C3F1) mice. JNCI: J. Natl. Cancer Inst. 75, 975–984. https://doi.org/10.1093/jnci/75.5.975.

Hooper, J.M., Stuijver, D.J., Orme, S.M., van Zaane, B., Hess, K., Gerdes, V.E., Phoenix, F., Rice, P., Smith, K.A., Alzahrani, S.H., 2012. Thyroid dysfunction and fibrin network structure: a mechanism for increased thrombotic risk in hyperthyroid individuals. J. Clin. Endocrinol. 97, 1463–1473.

Horacek, J., Maly, J., Svilias, I., Smolej, L., Cepkova, J., Vizda, J., Sadilek, P., Fatorova, I., Zak, P., 2015. Prothrombotic changes due to an increase in thyroid hormone levels. Eur. J. Endocrinol. 172, 537–542.

Hubert, M.-F., Laroque, P., Gillet, J.-P., Keenan, K.P., 2000. The effects of diet, ad libitum feeding, and moderate and severe dietary restriction on body weight, survival, clinical pathology parameters, and cause of death in control sprague-Dawley rats. Toxicol. Sci. 58, 195–207. https://doi.org/10.1093/toxsci/58.1.195.

Hulbert, A.J., Kelly, M.A., Abbott, S.K., 2014. Polyunsaturated fats, membrane lipids and animal longevity. J. Comp. Physiol. B 184, 149–166.

Ikeno, Y., Bronson, R.T., Hubbard, G.B., Lee, S., Bartke, A., 2003. Delayed occurrence of fatal neoplastic diseases in Ames Dwarf mice: correlation to extended longevity. J. Gerontol.: Series A 58, B291–B296.

Jové, M., Naudí, A., Ramírez-Núñez, O., Portero-Otín, M., Selman, C., Withers, D.J., Pamplona, R., 2014. Caloric restriction reveals a metabolomic and lipidomic signature in liver of male mice. Aging Cell 13, 828–837.

Jubb, K.V.F., Huxtable, C.R., 1992. Chapter 3. Nervous system. In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, vol. 1. Academic Press, San Diego, CA, pp. 267–439.

Kaufmann, W., Bolon, B., Bradley, A., Butt, M., Czasch, S., Garman, R.H., George, C., Gröters, S., Krinke, G., Little, P., McKay, J., Narama, I., Rao, D., Shibutani, M., Sills, R., 2012. Proliferative and nonproliferative lesions of the rat and mouse central and peripheral nervous systems. Toxicol. Pathol. 40, 87S–157S. https://doi.org/10.1177/0192623312439125.

Keenan, C.M., Baker, J., Bradley, A., Goodman, D.G., Harada, T., Herbert, R., Kaufmann, W., Kellner, R., Mahler, B., Meseck, E., Nolte, T., Rittinghausen, S., Vahle, J., Yoshizawa, K., 2015. International harmonization of nomenclature and Diagnostic criteria (INHAND): progress to date and future plans. Toxicol. Pathol. 43 (5), 730–732. https://doi.org/10.1177/0192623314560031.

Kodell, R.L., Blackwell, B.-N., Bucci, T.J., Greenman, D.L., 1995. Cause-of-Death assignment at the national center for toxicological research. Toxicol. Pathol. 23, 241–247. https://doi.org/10.1177/019262339502300301.

Kovacs, K., Horvath, E., Ilse, R.G., Ezrin, C., Ilse, D., 1977. Spontaneous pituitary adenomas in aging rats. A light microscopic, immunocytological and fine structural study. Beitr. Pathol. 161, 1–16. https://doi.org/10.1016/s0005-8165(77)80106-6.

Kroes, R., Garbis-Berkvens, J.M., de Vries, T., van Nesselrooy, H.J., 1981. Histopathological profile of a Wistar rat stock including a survey of the literature. J. Gerontol. 36, 259–279. https://doi.org/10.1093/geronj/36.3.259.

Ladiges, W., 2016. Pathology assessment is necessary to validate translational endpoints in preclinical aging studies. Pathobiol Aging Age Relat Dis 6, 31478. https://doi.org/10.3402/pba.v6.31478.

Ladiges, W., 2017. The emerging role of geropathology in preclinical aging studies. Pathobiol Aging Age Relat Dis 7, 1304005. https://doi.org/10.1080/20010001.2017.1304005.

Levin, O., Fujiyama, H., Boisgontier, M.P., Swinnen, S.P., Summers, J.J., 2014. Aging and motor inhibition: a converging perspective provided by brain stimulation and imaging approaches. Neurosci. Biobehav. Rev. 43, 100–117.

Li, C.-I., Lin, C.-H., Lin, W.-Y., Liu, C.-S., Chang, C.-K., Meng, N.-H., Lee, Y.-D., Li, T.-C., Lin, C.-C., 2014. Successful aging defined by health-related quality of life and its determinants in community-dwelling elders. BMC Publ. Health 14, 1013. https://doi.org/10.1186/1471-2458-14-1013.

Lin, H.-C., Yang, L.-Y., Kang, J.-H., 2010. Increased risk of pulmonary embolism among patients with hyperthyroidism: a 5-year follow-up study. J. Thromb. Haemostasis 8, 2176–2181.

Lipman, R.D., 1997. Pathobiology of aging rodents: inbred and hybrid models. Exp. Gerontol. 32, 215–228. https://doi.org/10.1016/s0531-5565(96)00037-x.

Lipman, R.D., Chrisp, C.E., Hazzard, D.G., Bronson, R.T., 1996. Pathologic characterization of brown Norway, brown Norway x Fischer 344, and Fischer 344 x brown Norway rats with relation to age. J Gerontol A Biol Sci Med Sci 51, B54–B59. https://doi.org/10.1093/gerona/51a.1.b54.

Lipman, R.D., Dallal, G.E., Bronson, R.T., 1999. Effects of genotype and diet on age-related lesions in ad libitum fed and calorie-restricted F344, BN, and BNF3F1 rats. J Gerontol A Biol Sci Med Sci 54, B478–B491. https://doi.org/10.1093/gerona/54.11.b478.

Little, P., Rao, D.B., 2014. Brain. In: Cesta, M., Herbert, R., Brix, A., Malarkey, D., Sills, R. (Eds.), National Toxicology Program Nonneoplastic Lesion Atlas.

Liu, L., Wang, X., Lin, Z., Wu, H., 1993. Elevated plasma levels of VWF: Ag in hyperthyroidism are mediated through beta-adrenergic receptors. Endocr. Res. 19, 123–133.

Losco, P.E., Ward, J.M., 1984. The early stage of large granular lymphocyte leukemia in the F344 rat. Veterinary pathology 21, 286–291.

Maeda, H., Gleiser, C.A., Masoro, E.J., Murata, I., McMahan, C.A., Yu, B.P., 1985. Nutritional influences on aging of fischer 344 rats: II. Pathology. J. Gerontol. 40, 671–688. https://doi.org/10.1093/geronj/40.6.671.

Masoro, E.J., Iwasaki, K., Gleiser, C.A., McMahan, C.A., Seo, E.J., Yu, B.P., 1989. Dietary modulation of the progression of nephropathy in aging rats: an evaluation of the importance of protein. Am. J. Clin. Nutr. 49, 1217–1227. https://doi.org/10.1093/ajcn/49.6.1217.

Mercken, E.M., Carboneau, B.A., Krzysik-Walker, S.M., de Cabo, R., 2012. Of mice and men: the benefits of caloric restriction, exercise, and mimetics. Ageing Research Reviews, Special Issue: Exercise and Ageing 11, 390–398. https://doi.org/10.1016/j.arr.2011.11.005.

Myrup, B., Bregengård, C., Faber, J., 1995. Primary haemostasis in thyroid disease. J. Intern. Med. 238, 59–63.

Nadon, N.L., 2006. Chapter 25 - gerontology and age-associated lesions. In: Suckow, M. A., Weisbroth, S.H., Franklin, C.L. (Eds.), The Laboratory Rat, second ed., American College of Laboratory Animal Medicine. Academic Press, Burlington, pp. 761–772. https://doi.org/10.1016/B978-012074903-4/50028-5.

National Toxicology Program (NTP). The NTP's nonneoplastic lesions Atlas (NNLA). https://ntp.niehs.nih.gov/nnl/.

Navarro-Hortal, M.D., Ramírez-Tortosa, C.L., Varela-López, A., Romero-Márquez, J.M., Ochoa, J.J., Forbes-Hernández, T.Y., Granados-Principal, S., Battino, M., Quiles, J.L., 2019a. Heart histopathology and mitochondrial ultrastructure in aged rats fed for 24 Months on different unsaturated fats (virgin olive oil, sunflower oil or fish oil) and affected by different longevity. Nutrients 11, 2390.

Navarro-Hortal, M.D., Varela-López, A., Romero-Márquez, J.M., Piquer-Martinez, C., Bullón, P., Forbes-Hernández, T.Y., Quiles, J.L., 2019b. Twenty-four months feeding on unsaturated dietary fats (virgin olive, sunflower, or fish oil) differentially modulate gingival mitochondria in the rat. eFood 1.

Orentreich, N., Matias, J.R., DeFelice, A., Zimmerman, J.A., 1993. Low methionine ingestion by rats extends life span. J. Nutr. 123, 269–274. https://doi.org/10.1093/jn/123.2.269.

Pamplona, R., Barja, G., 2006a. Mitochondrial oxidative stress, aging and caloric restriction: the protein and methionine connection. Biochim. Biophys. Acta Bioenerg. 1757, 496–508. https://doi.org/10.1016/j.bbabio.2006.01.009.

Pamplona, R., Barja, G., 2006b. Mitochondrial oxidative stress, aging and caloric restriction: the protein and methionine connection. Biochim. Biophys. Acta 1757, 496–508.

Pollard, M., Luckert, P.H., 1975. Transplantable metastasizing prostate adenocarcinomas in rats. J. Natl. Cancer Inst. 54, 643–649.

Poteracki, J., Walsh, K.M., 1998. Spontaneous neoplasms in control Wistar rats: a comparison of reviews. Toxicol. Sci. 45, 1–8. https://doi.org/10.1093/toxsci/45.1.1.

Prejean, J.D., Peckham, J.C., Casey, A.E., Griswold, D.P., Weisburger, E.K., Weisburger, J.H., 1973. Spontaneous tumors in sprague-Dawley rats and Swiss mice. Cancer Res. 33, 2768–2773.

Ramagopalan, S.V., Wotton, C.J., Handel, A.E., Yeates, D., Goldacre, M.J., 2011. Risk of venous thromboembolism in people admitted to hospital with selected immune-mediated diseases: record-linkage study. BMC Med. 9, 1–8.

Ramirez-Tortosa, C.L., Varela-López, A., Navarro-Hortal, M.D., Ramos-Pleguezuelos, F. M., Márquez-Lobo, B., Ramirez-Tortosa, Mc, Ochoa, J.J., Battino, M., Quiles, J.L., 2020. Longevity and cause of death in male Wistar rats fed lifelong diets based on virgin olive oil, sunflower oil, or fish oil. J. Gerontol.: Series A 75, 442–451. https://doi.org/10.1093/gerona/glz091.

Reeves, P.G., 1997. Components of the AIN-93 diets as improvements in the AIN-76A diet. J. Nutr. 127, 838S–841S.

Roche, E., Ramírez-Tortosa, C.L., Arribas, M.I., Ochoa, J.J., Sirvent-Belando, J.E., Battino, M., Ramírez-Tortosa, M.C., Adrián González-Alonso, A., Pérez-López, M.P., Quiles, J.L., 2014. Comparative analysis of pancreatic changes in aged rats fed life long with sunflower, fish, or olive oils. J Gerontol A Biol Sci Med Sci 69, 934–944.

Ross, M.H., Bras, G., 1971. Lasting influence of early caloric restriction on prevalence of neoplasms in the rat. J. Natl. Cancer Inst. 47, 1095–1114.

Roth, G.S., Brennecke, L.H., French, A.W., Williams, N.G., Waggie, K.S., Spurgeon, H.A., Ingram, D.K., 1993. Pathological characterization of male Wistar rats from the gerontology research center. J. Gerontol. 48, B213–B230. https://doi.org/10.1093/geronj/48.6.B213.

Russfield, A.B., 1966. Tumors of Endocrine Glands and Secondary Sex Organs. U.S. Public Health Service.

Sanchez-Roman, I., Barja, G., 2013. Regulation of longevity and oxidative stress by nutritional interventions: role of methionine restriction. Exp. Gerontol. 48, 1030–1042. https://doi.org/10.1016/j.exger.2013.02.021.

Schardein, J.L., Fitzgerald, J.E., Kaump, D.H., 1968. Spontaneous tumors in Holtzman-source rats of various ages. Pathol. Vet. 5, 238–252.

Shain, S.A., Boesel, R.W., Axelrod, L.R., 1975. Aging in the rat prostate: reduction in detectable ventral prostate androgen receptor content. Arch. Biochem. Biophys. 167, 247–263. https://doi.org/10.1016/0003-9861(75)90461-0.

Snyder, D.L., Pollard, M., Wostmann, B.S., Luckert, P., 1990. Life span, morphology, and pathology of diet-restricted germ-free and conventional Lobund-Wistar rats. J. Gerontol. 45, B52–B58. https://doi.org/10.1093/geronj/45.2.b52.

Snyder, J.M., Ward, J.M., Treuting, P.M., 2015. Cause-of-Death Analysis in Rodent Aging Studies. https://doi.org/10.1177/0300985815610391.

Solleveld, H.A., Boorman, G.A., 1986. Spontaneous renal lesions in five rat strains. Toxicol. Pathol. 14, 168–174. https://doi.org/10.1177/019262338601400204.

Sousa-Victor, P., Perdiguero, E., Muñoz-Cánoves, P., 2014. Geroconversion of aged muscle stem cells under regenerative pressure. Cell Cycle 13, 3183–3190.

Speakman, J.R., Mitchell, S.E., Mazidi, M., 2016. Calories or protein? The effect of dietary restriction on lifespan in rodents is explained by calories alone. Exp. Gerontol. 86, 28–38. https://doi.org/10.1016/j.exger.2016.03.011.

Strong, R., Miller, R.A., Antebi, A., Astle, C.M., Bogue, M., Denzel, M.S., Fernandez, E., Flurkey, K., Hamilton, K.L., Lamming, D.W., Javors, M.A., de Magalhães, J.P., Martinez, P.A., McCord, J.M., Miller, B.F., Müller, M., Nelson, J.F., Ndukum, J., Rainger, G.E., Richardson, A., Sabatini, D.M., Salmon, A.B., Simpkins, J.W., Steegenga, W.T., Nadon, N.L., Harrison, D.E., 2016. Longer lifespan in male mice treated with a weakly estrogenic agonist, an antioxidant, an α-glucosidase inhibitor or a Nrf2-inducer. Aging Cell 15, 872–884. https://doi.org/10.1111/acel.12496.

Stuijver, D.J., Hooper, J.M., Orme, S.M., Van Zaane, B., Squizzato, A., Piantanida, E., Hess, K., Alzahrani, S., Ajjan, R.A., 2012. Fibrin clot structure and fibrinolysis in hypothyroid individuals: the effects of normalising thyroid hormone levels. J. Thromb. Haemostasis 10, 1708–1710.

Swenberg, J.A., 1986. Brain tumours–problems and perspectives. Food Chem. Toxicol. 24, 155–158. https://doi.org/10.1016/0278-6915(86)90350-9.

Swindell, W.R., 2012. Dietary restriction in rats and mice: a meta-analysis and review of the evidence for genotype-dependent effects on lifespan. Ageing Res. Rev. 11, 254–270. https://doi.org/10.1016/j.arr.2011.12.006.

Tamaschke, C., 1955. Spontaneous Tumors of Small Laboratory Animals and Their Importance for Experimental Oncology. Strahlentherapie (West Germany) 96.

Ueda, Y., Wang, M.-F., Irei, A.V., Sarukura, N., Sakai, T., Hsu, T.-F., 2011. Effect of dietary lipids on longevity and memory in the SAMP8 mice. J Nutr Sci Vitaminol, Journal of Nutritional Science and Vitaminology 57, 36–41.

Valencak, T.G., Ruf, T., 2011. Feeding into old age: long-term effects of dietary fatty acid supplementation on tissue composition and life span in mice. J. Comp. Physiol. B 181, 289–298.

Varela-López, A., Ochoa, J.J., Llamas-Elvira, J.M., López-Frías, M., Elena Planells, E., Ramirez-Tortosa, M.C., Ramirez-Tortosa, C.L., Giampieri, F., Battino, M., Quiles, J. L., 2017. Age-related loss in bone mineral density of rats fed lifelong on a fish oil-based diet is avoided by coenzyme Q10 addition. Nutrients 9, 176.

Varela-López, A., Ochoa, J.J., Llamas-Elvira, J.M., López-Frías, M., Elena Planells, E., Speranza, L., Battino, M., Quiles, J.L., 2017. Loss of bone mineral density associated with age in male rats fed on sunflower oil is avoided by virgin olive oil intake or coenzyme Q supplementation. Int. J. Mol. Sci. 18, 1397.

Varela-Lopez, A., Pérez-López, M.P., Ramirez-Tortosa, C.L., Maurizio Battino, M., Granados-Principal, S., Ramirez-Tortosa, M.C., Ochoa, J.J., Vera-Ramirez, L., Giampieri, F., Quiles, J.L., 2018. Gene pathways associated with mitochondrial function, oxidative stress and telomere length are differentially expressed in the liver of rats fed lifelong on virgin olive, sunflower or fish oils. J. Nutr. Biochem. 52, 36–44.

Verkleij, C.J., Stuijver, D.J., van Zaane, B., Squizzato, A., Brandjes, D.P., Büller, H.R., Meijers, J.C., Gerdes, V.E., 2013. Thrombin-activatable fibrinolysis inhibitor in hypothyroidism and hyperthyroxinaemia. Thromb. Haemostasis 109, 214–220.

Villalba, J.M., López-Domínguez, J.A., Chen, Y., Khraiwesh, H., González-Reyes, J.A., Fernández del Río, L., Gutiérrez-Casado, E., del Río, M., Calvo-Rubio, M., Ariza, J., de Cabo, R., López-Lluch, G., Navas, P., Hagopian, K., Burón, M.I., Ramsey, J.J., 2015. The influence of dietary fat source on liver and skeletal muscle mitochondrial modifications and lifespan changes in calorie-restricted mice. Biogerontology 16, 655–670.

Weber, K., Garman, R.H., Germann, P.-G., Hardisty, J.F., Krinke, G., Millar, P., Pardo, I. D., 2011. Classification of neural tumors in laboratory rodents, emphasizing the rat. Toxicol. Pathol. 39, 129–151.

Wolf, N.S., Li, Y., Pendergrass, W., Schmeider, C., Turturro, A., 2000. Normal mouse and rat strains as models for age-related cataract and the effect of caloric restriction on its development. Exp. Eye Res. 70, 683–692.

Yarrington, J., O'Neal Johnston, J., 1992. Aging in the adrenal cortex. In: Mohr, U., Dungworth, D., Capen, C. (Eds.), Pathobiology of the Aging Rat. International Life Sciences Institute, Washington, DC, pp. 227–244.

Yasui, Y., Ohta, Y., Ueda, Y., Hasegawa, K., Kihara, T., Hosoi, M., Miyajima, R., Shiga, A., Imai, K., Toyoda, K., 2009. Spontaneous ganglioneuroma possibly originating from the trigeminal ganglion in a B6C3F1 mouse. Toxicol. Pathol. 37, 343–347.

Zimmerman, J.A., Malloy, V., Krajcik, R., Orentreich, N., 2003. Nutritional control of aging. Exp. Gerontol. 38, 47–52.

Zöller, M., Matzku, S., 1978. Spontaneous tumours in BDX rats. Br. J. Cancer 38, 355.

Zöller, M., Matzku, S., Goerttler, K., 1978. High incidence of spontaneous transplantable tumours in BDX rats. Br. J. Cancer 37, 61–66.