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High Protein Intake Increases Sirtuin 1 mRNA Expression in Rat Liver

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Abstract: Background: The relationship between aging or disease and diets, as in the relationship between the intake of calories and disease, has been studied. Appropriate protein intake is important for disease, frailty, and the life span. However, little is knew regarding to protein diet on any disease and lifespan. So we analyze the effects of high protein diets on rat liver oxidative stress and tumor marker. **Methodology**: Rats were separated into three groups: LPD with 5% protein, SPD with 20% protein, and HPD with 40% protein, administered over 4 weeks. The qRT-PCR method was used for measuring mRNA expression levels. as the expression of the anti-aging-related enzyme Sirtuin 1, superoxide dismutase (SOD), CuZn-SOD, EC-SOD, endothelial nitric oxide synthase (eNOS), tumor suppressor protein p53, and nuclear respiratory factor 1 (NRF-1). **Results:** A significant difference (p = 0.03853) was observed between the LPD and SPD groups for Sirtuin 1 and also between the LPD and HPD groups (p = 0.004914). There were significant differences in CuZn-SOD and EC-SOD between the LPD and SPD groups (CuZn-SOD: p = 0.02062, EC-SOD: p = 0.01906). A significant difference (p = 0.01359) between the LPD and SPD groups was also observed for eNOS. **Conclusions**: A significant difference in Sirtuin 1 expression was observed between the LPD and SPD, and between the LPD and HPD groups, indicates that high protein intake promotes Sirtuin 1 and prevents aging. As for CuZn-SOD, EC-SOD, and eNOS, the significant difference between the SPD and LPD groups indicates that low protein intake lowers the production of SOD and increases reactive oxygen species (ROS). An appropriate amount of protein is necessary for the prevention of aging, lifestyle-related diseases, and arteriosclerotic diseases caused by oxidative stress and to extend the life span.

Keywords: Sirtuin 1; SOD; CuZn-SOD; EC-SOD; EC-SOD; eNOS; p53; NRF-1; qRT-PCR

Introduction

Proteins, carbohydrates, and lipids are three major nutrients critical for mammals. The relationship between food and the prevention of aging and disease, examined as the relationship between calorie intake and disease occurrence, has been discussed over many years [1]. However, studies on the relationship between protein intake and aging, oncogenesis, and the development of other diseases are not available.

It is well known that the generation of reactive oxygen species (ROS) is involved in aging and lifestyle-related disease. Both of CuZn-SOD and extracellular SOD (EC-SOD), are the main antioxidant enzymes observed in the aerobic cell metabolism mechanism. SOD contributes towards physiological protection against oxidative stress. Several studies have demonstrated that an adequate balance of antioxidant enzymes, including SOD, is required to minimize the toxic effects of ROS. The generation of ROS has a causal relationship with the aging process and lifestyle-related diseases. When the generation of ROS cannot be suppressed by SOD, oxidative stress increases, ROS accumulate in the tissue, and there is an increase in aging, lifestyle-related disease, and the possibility of carcinogenesis [2].

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The antiaging-related enzyme NAD^+-dependent deacetylase silent information regulator 1 (Sirtuin 1) and cancer suppressor protein p53 are known to play important roles in the prevention of aging and cancer. In addition, nitrogen monoxide (NO) is an in vivo free radical that attacks bacteria and viruses, has a vasodilating action, and functions as a neurotransmitter.

A recent report shows that Sirtuin 1 and p53 are closely related in the mechanism of carcinogenesis, and their anticancer effects under dietary restrictions and their ability to improve type 2 diabetic nephropathy have been reported [3,4]. For our results, Sirtuin 1 and p53 are important for prevention of the lifestyle-related disease and carcinogenesis.

Adequate protein intake improves skeletal muscle composition and metabolism and has many beneficial effects in terms of obesity, metabolic syndrome, osteoporosis, and cardiovascular diseases [5]. However, it is not clear how differences in protein intake under equal energy intake influence aging and carcinogenesis.

Accordingly, as one of the few studies, an experiment using rat liver was carried out to verify that the differences in protein intake under diets containing identical calorie intakes has an effect on aging, lifestyle-related diseases, and carcinogenesis. The mRNA expression levels of the antiaging-related enzyme Sirtuin 1, CuZn-SOD, EC-SOD, endothelial nitric oxide synthase (eNOS), tumor suppressor protein p53, and nuclear respiratory factor 1 (NRF-1) were measured using the quantitative reverse-transcription polymerase chain reaction (qRT-PCR) method in liver tissues extracted after 4 weeks of rearing.

2. Materials and Methods

2.1. Rat

Approval (Approval No. 2950) was granted by the Rector of Saitama Medical University (38 Moro Hongo, Moroyama Town, Iruma County, Saitama, 350-0045, Japan) following a review conducted by the Animal Experiment Committee under the Saitama Medical University Regulations on Animal Experiments. This study was conducted at the Department of Social Medicine, Faculty of Medicine, Saitama Medical University. Five-week-old male Sprague Dawley (SD) rats were obtained from CLEA Japan Inc., Tokyo, Japan. After delivery, they were housed in individual cages for 1 week and adapted to the environment. The rats were then divided into three groups according to the protein level of the diet administered: a 5% low-protein diet (LPD) (n = 10), a 20% standard protein diet (SPD) (n = 9), and a 40% high-protein diet (HPD) (n = 10). The rats were housed in individual cages for 4 weeks. A summary of the composition of the LPD, SPD, and HPD is presented in Table 1. Approximately 16 g of food was administered per day, and water was always available.

Table 1. Composition of diets.

material	LPD	SPD	HPD
protein (casein)	5	20	40
hydrocarbon	76.2	62.9	41.4
lipid (soybean oil)	7	7	7
Cellulose	5	5	5
Minerals	3.5	3.5	3.5
Others	3.3	1.6	3.1
total (%)	100	100	100

2.2. Sample Collection

After 4 weeks of the experiment, the rats' organs were extracted under anesthesia (2% isoflurane) and blood was collected from the abdominal aorta. All organs and blood were immediately frozen in

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liquid nitrogen and stored in a refrigerator at −80 °C. Approximately 50 mg of the internal left lobe tissue of the extracted organ was used for analysis.

2.3. Determination of Serum Total Protein and Serum Albumin Levels

After thawing the frozen blood, a spectrophotometer was used to measure the serum total protein and albumin levels. The spectrophotometer used was an Ultramark microplate reader made by BIO-RAD, and the analysis software was Microplate Manager PC version 5.1. In terms of the parameters of the spectrophotometer, the serum total protein was measured at a wavelength of 540 nm and serum albumin was measured at a wavelength of 630 nm. The A/G B-Test Wako (Fujifilm Wako Pure Chemical Industries, Ltd. Japan), a commercially available kit whose measurement principle includes the Biuret and BCG methods, was used as a chromogenic reagent. An accompanying standard serum was used for calibration.

2.4. Quantitative Reverse-Transcription Polymerase Chain Reaction (qRT-PCR)

2.4.1. Extraction of Total RNA and cDNA Production

As a pre-treatment of the qRT-PCR method, 50 ng of total RNA was extracted from the frozen hepatic tissue. ISOGEN (Nippon Gene Co., Ltd.) was used as a RNA extraction agent. Then, 1 μg of liver tissue was homogenized with 500 μL of ISOGEN reagent, followed by the addition of 20% chloroform (50:10). After centrifugation at 4 °C for 15 min, only the aqueous layer separated into three layers was extracted, followed by the addition of isopropanol and further centrifugation. The total RNA was isolated by the addition of 70% ethanol. A 20 μL volume of complementary deoxyribose nucleic acid (cDNA) synthesis reagent (iScriptTM cDNA Synthesis Kit) was added to 1 μg of RNA sample, reacted at 25 °C for 5 min, and then at 42 °C for 30 min to obtain reverse-transcribed cDNA.

2.4.2. mRNA Measurement

A PCR reaction reagent (iQTM SYBR Green Supermix), F-primer of the target gene, and R-primer were added to 5 μL (50ng) of the obtained cDNA samples to prepare 25 μL, and the mRNA level was measured. Then, 45 cycles of denaturation were conducted at 94 °C for 30 s, annealing at 60 °C for 30 s, and elongation at 72 °C for 30 s. As a control, glycer-aldehyde-3-phosphate dehydrogenase (GAPDH) was used to calculate the relative expression of cDNA. The relative expression of genes was calculated using the $2^{-\Delta\Delta Ct}$ method. A base sequence of the primer used in this study is shown in Table 2.

Table 2. Oligonucleotide sequences of primers used for qRT-PCR analysis.

Gene name	Accession number	Sequence (5'-3')				position and length (bp)	total bp
Sirtuin-1	NM_001372090	F	5'-	GGAAC CTCTG CCTCA TCTAC AT	3'	734-755 (20)	97
		R	5'-	GCATACTCGCCACCTAA CC	3'	811-830 (20）	
CuZnSOD	NM_017050	F	5'-	GGTCCAGCGGATGAAGA G	3'	313-330 (18)	77
		R	5'-	GGACA CATTG GCCAC ACC	3'	373-390 (18)	
ECSOD	NM_012880	F	5'-	CTTGG GAGAG CTTGT CAGGT	3'	99-118 (20)	70
		R	5'	CACCA GTAGC AGGTT	3	149-168 (20)	

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			-	GCAGA	'		
eNOS	NM_021838	F	5'-	TGACC CTCAC CGATAC AACA	3'	1136-1155 (20)	452
		R	5'-	CGGGT GTCTA GATCC ATGC	3'	1148-1167 (20)	
p53	HM_030989	F	5'-	AGAGAGCACTGCCCACC A	3'	1038-1055 (18)	110
		R	5'-	AACATCTCGAAGCGCTC A	3'	1130-1147 (18)	
NRF-1	NM_001100708	F	5'-	TTATT CTGCT GTGGC TGATG G	3'	1286-1306	91
		R	5'-	CCTCT GATGC TTGCG TCGTCT	3'	1357-1376	
GAPDH	NM_017008	F	5'-	ACCAC AGTCC ATGCC ATCAC	3'	595-614 (20)	61
		R	5'-	TCCAC CACCC TGTTG CTGTA	3'	1027-1046 (20)	

2.5. Statistical Analysis

A one-way analysis of variance was used for the obtained results to test whether there was a significant difference between the concentrations and expression levels. It was found that the variance of each concentration group was not equal, and a Brown-Forsythe test and Welch's ANOVA were carried out. Dunnett's T3 multiple comparison test was subsequently performed as a post hoc test to confirm significant differences between each concentration group. The statistical graph preparation software GraphPad Prism 9.3.1.471 was used for these statistical processes.

3. Results

3.1. Effects of High Protein Intake on Body Weight

As a whole, body weight tended to increase with advancing age, and difference in body weight between the LPD, SPD, and HPD groups was remarkable. A significant difference in body weight was not observed between the SPD and HPD groups (Figure 1, Table 3). The diet consumption decreased with each week in both protein-fed groups. A lower diet intake was observed in the LPD group compared to the other groups for all 4 weeks (Figure 2).

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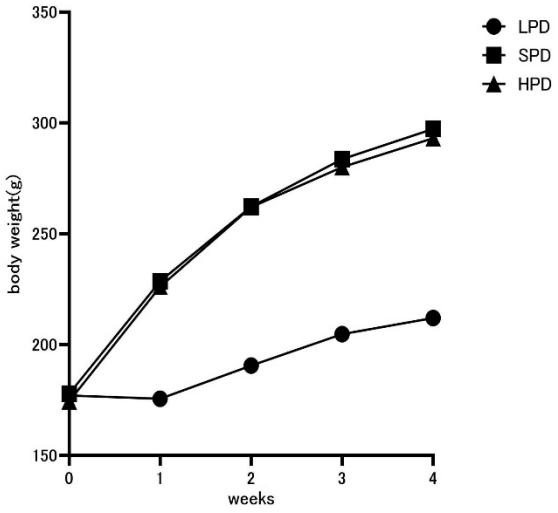

Figure 1. Change in rats' body weight over experimental period. No significant weight difference was observed between HPD and SPD groups, while LPD group exhibited less weight gain than HPD and SPD groups.

Table 3. Body weight (g) of rats over experimental period[1].

	LP	STD	HP
start	177.1±2.47	177.9±1.56	174.5±2.46
7 days	175.6±2.18	228.6±2.12	226.1±2.82
14 days	190.6±2.21	262.3±2.03	262.0±2.15
21 days	204.8±2.54	283.7±2.38	280.1±2.17
28 days	212.0±2.59	297.3±2.83	293.1±2.16

[1] Values are means ± SEM.

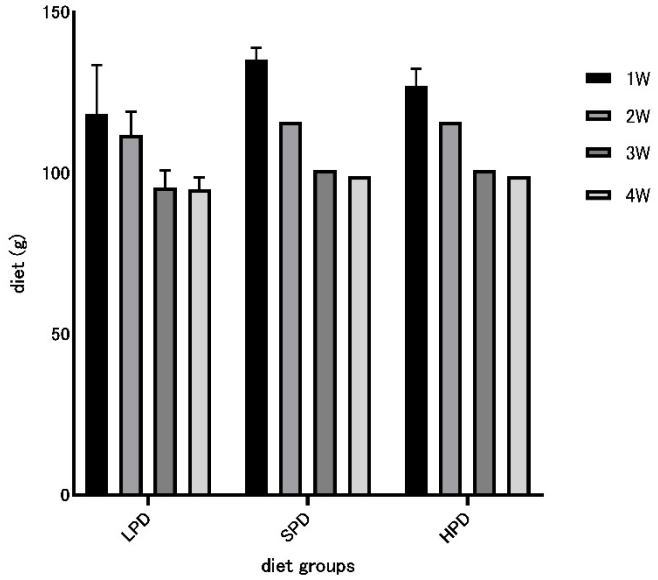

Figure 2. Changes in diet intake for experimental period. In each protein concentration group, the diet intake decreased over the weeks of the experiment. Each groups are same pattern, no significant difference was observed in the total intake amount between the HPD and SPD groups, but the total intake for the LPD group was lower than for the HPD group and SPD group. Bars represent the standard deviation (SD) of the mean.

3.2. Effect of High Protein Intake on Serum Protein Levels

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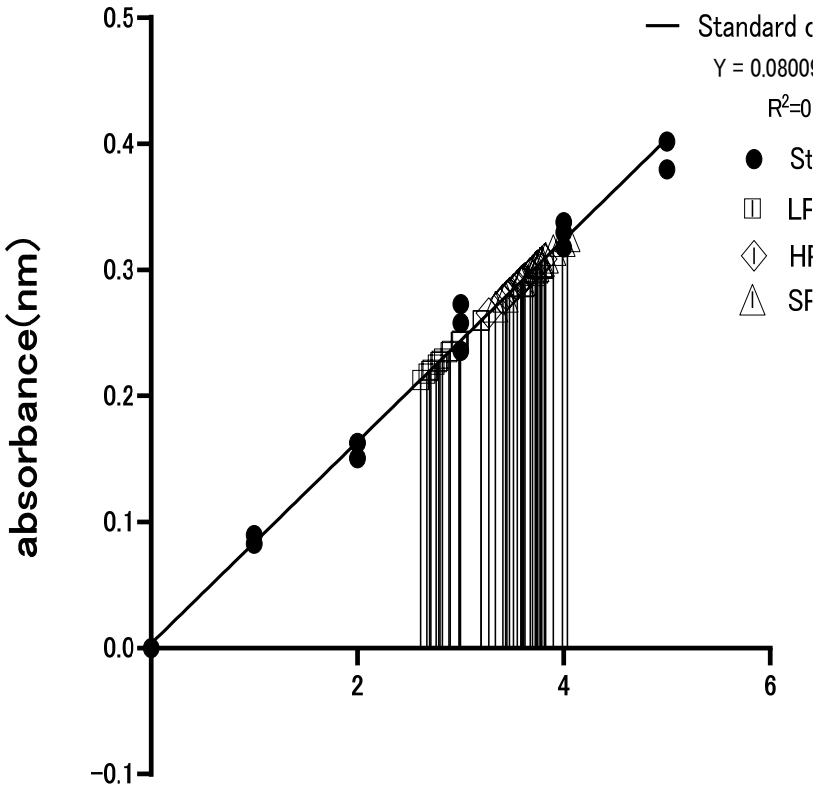
The distribution of serum total protein in each group is shown in Table 4. No significant difference was observed between the groups. As shown in Figure 3a, b, the serum albumin levels were significantly different between the LPD and SPD groups and between the LPD and HPD groups ($p < 0001$), and between the SPD and HPD groups ($p < 0.0435$).

Table 4. Comparison of serum total protein concentration (g/dL). Data were analyzed using Brown–Forsythe test, Welch's ANOVA, and Dunnett's T3 multiple comparison test, revealing no significant differences.

	LPD	SPD	HPD
Mean(g/dL)	4.86	5.26	5.00
SD	0.856	0.331	0.334
SE	0.2210	0.0855	0.0863



(**a**)

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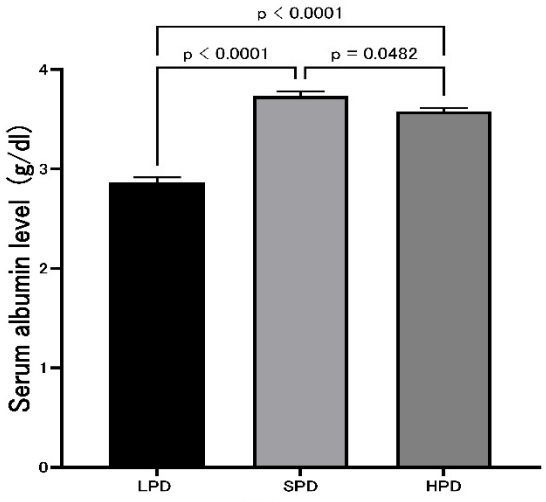

	LPD	SPD	HPD
MEAN	2.87	3.73	3.58
SD	0.178	0.186	0.140
SE	0.0461	0.0480	0.0362

(**b**)

Figure 3. (**a**) Correlation between absorbance and serum albumin concentration. (**b**) Comparison of serum albumin concentration. Data was analyzed using Brown-Forsythe test, Welch's ANOVA, and Dunnett's T3 multiple comparison test. Values without a common letter differ, $p < 0.05$. HPD vs. LPD: $p < 0.0001$, SPD vs. LPD: $p < 0.0001$, HPD vs. SPD: $p = 0.0482$. Bars represent the standard error (SE) of the mean.

3.3. Sirtuin 1

A significant difference between the LPD and SPD groups ($p = 0.03853$) was observed, with an additional significant difference between the LPD and HPD groups ($p = 0.004914$) (Figure 4).

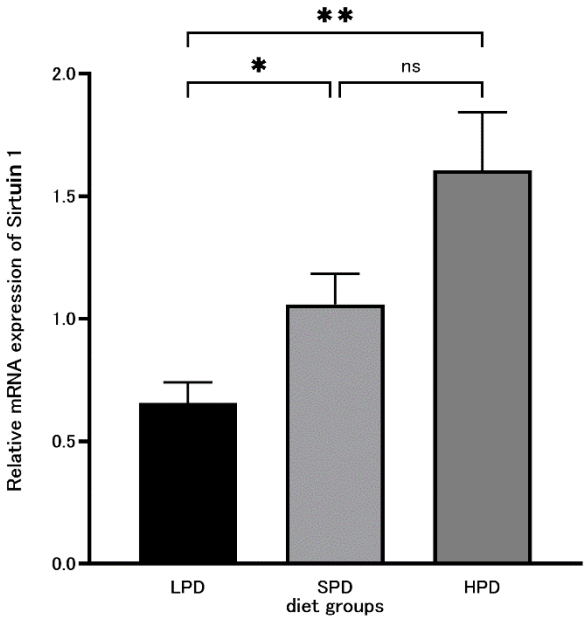

Figure 4. Comparison of mRNA Sirtuin 1 expression in the three rat groups. qRT-PCR of Sirtuin 1 expressed as a comparison of GAPDH. Data was analyzed using Brown–Forsythe test, Welch's ANOVA, and Dunnet's T3 multiple comparison test. Values without a common letter differ, $p < 0.05$. LPD vs. SPD * $p = 0.03853$, LPD vs. HPD ** $p = 0.004914$, SPD vs. HPD n.s. ($p = 0.1502$). Bars represent the standard error (SE) of the mean.

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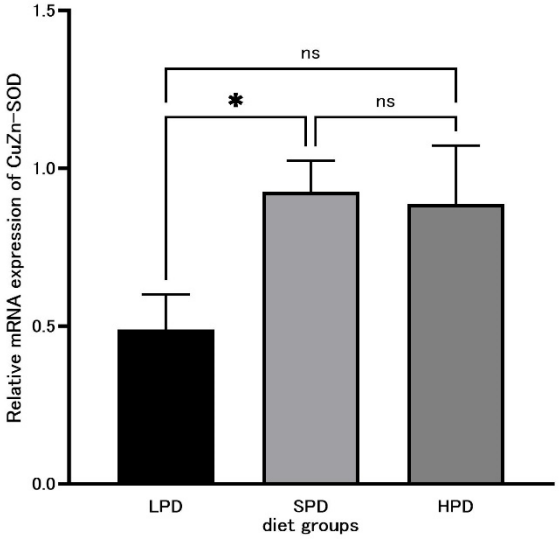
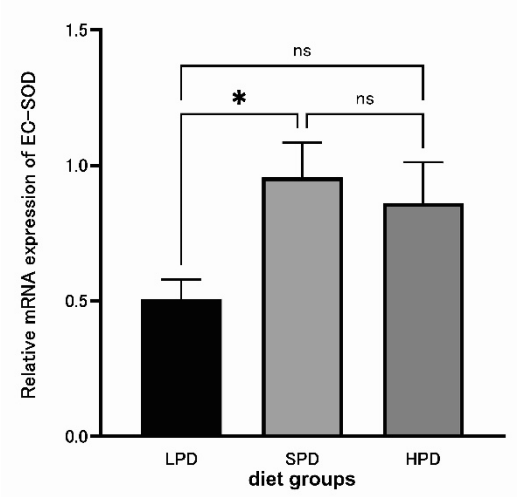
3.4. SOD

In terms of CuZn-SOD, a significant difference (p = 0.02062) between the LPD and SPD groups was observed (Figure 5a). In terms of EC-SOD, a significant difference (p = 0.01906) between the LPD and SPD groups was also observed (Figure 5b). A similar significant difference was observed between the LPD and SPD groups for eNOS (p = 0.01359) (Figure 5c).



(**a**)



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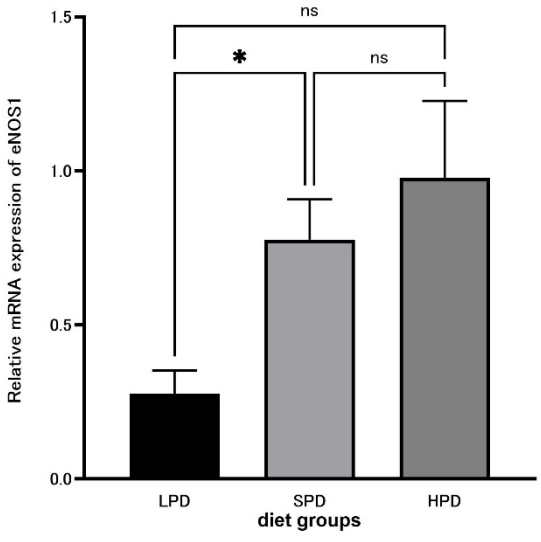

(**c**)

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Figure 5. (**a**) Comparison of mRNA CuZn-SOD expression in the three rat groups. qRT-PCR of CuZn-SOD expressed as a ratio of GAPDH. Data was analyzed using Brown–Forsythe test, Welch's ANOVA, and Dunnett's T3 multiple comparison test. Values without a common letter differ, $p < 0.05$. LPD vs. SPD * $p = 0.02062$, LPD vs. HPD n.s. ($p = 0.2113$), SPD vs. HPD n.s. ($p = 0.9991$). Bars represent the standard error (SE) of the mean. (**b**) Comparison of mRNA EC-SOD expression in the three rat groups. qRT-PCR results of EC-SOD expressed as a ratio of GAPDH. Data was analyzed using Brown–Forsythe test, Welch's ANOVA, and Dunnett's T3 multiple comparison test. Values without a common letter differ, $p < 0.05$. LPD vs. SPD * $p = 0.01906$, LPD vs. HPD n.s. ($p = 0.1432$), SPD vs. HPD n.s. ($p = 0.9480$). Bars represent the standard error (SE) of the mean. (**c**) Comparison of mRNA eNOS expression in the three rat groups. qRT-PCR results of EC-SOD expressed as a ratio of GAPDH. Data was analyzed using Brown–Forsythe test, Welch's ANOVA, and Dunnett's T3 multiple comparison test. Values without a common letter differ, $p < 0.05$. LPD vs. SPD * $p = 0.01359$, LPD vs. HPD n.s. ($p = 0.06006$), SPD vs. HPD n.s. ($p = 0.8579$). Bars represent the standard error (SE) of the mean.

3.5. P53 and NRF-1

No significant difference was observed for p53 and NRF-1 at any concentration.

4. Discussion

As far as we know, this study is one of the few to show the relationship between protein intake and Sirtuin 1 expression. The result of the present study in rats revealed a significant difference ($p = 0.03853$) between the LPD and the SPD groups in terms of Sirtuin 1, and a significant difference ($p = 0.004914$) was also observed between the LPD and HPD groups. The results showed that there was a significant increase in the Sirtuin 1 expression level according to the protein intake (Figure 4). Based on these results, it is considered that sustained high protein intake decreases oxidative stress and increases Sirtuin 1 activity. Petzke et al. also reported that oxidative stress was reduced by continuous high protein intake [6]. The results of this study also indicate the possibility that an appropriate level of protein contributes to the prevention of aging. A large amount of Sirtuin 1 research has involved calorie restriction [7,8]. Under calorie restriction, the aging rate was found to be reduced by decreasing the metabolism rate, and as a result, the Sirtuin 1 activity increased. Additionally, Sirtuin 1 is known to have a hepatoprotective effect in high-fat diets [9].

A significant difference ($p = 0.02062$) was observed in the SPD group compared with the LPD group in terms of CuZn-SOD. CuZn-SOD significantly lowered the expression level in the LPD group (Figure 5a). This means that the expression level of CuZn-SOD is reduced under low protein concentrations. Moreover, a significant difference ($p = 0.01906$) was observed between the LPD and the SPD groups for EC-SOD as well as for CuZn-SOD (Figure 5b). This means that, similarly, the expression level of EC-SOD is reduced under low protein intake conditions. A significant difference ($p = 0.01359$) between the LPD and the SPD groups was also observed for eNOS (Figure 5c). This indicates that the SOD activity reduces when the protein intake is insufficient, thus supporting the findings reported by Huang et al. [10,11].

In the LPD group, the expression of CuZn-SOD, EC-SOD, and eNOS appeared to be reduced, and in the low protein intake group, the expression of these SOD groups was suppressed. The antioxidant reaction in the SOD group did not function sufficiently, causing an increase in ROS; an increase in oxidative stress leads to failures within the organism. Accordingly, it is suggested that the intake of an appropriate amount of protein in human beings promotes the production of SOD groups, reduces oxidative stress, and leads to the prevention of diseases caused by various oxidative stresses [2].

Diet intake and body weight gain were also characterized in this study. At weekly intervals, the rate of body weight gains slowed and showed a nearly equal weight gain trend in the SPD and the HPD groups, but tended to be especially low in the LPD group compared with the other two groups (Figure 1, Table 3). In addition, the diet intake reduced along with weight gain in all groups (Figure 2). As a result, hunger decreased, and diet intake decreased with weight gain. The lower body weight

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gain in the LPD group may be due to preference; one report noted that high protein intake in rats leads to a reduction in food intake for getting tired of the intake high protein. [12].

In this study, no significant differences in serum total protein levels were observed between each concentration group (Table 4). However, a significant difference in serum albumin levels was observed, being higher in the SPD group (Figure 3a,b). This result is also consistent with previous study suggesting that serum total protein is not affected by protein intake [13]. According to an epidemiological study in Japan, there was no significant difference in serum albumin between males and females, a significant inverse correlation with age was observed, and it was reported that the intake of animal protein showed a weak positive correlation with serum albumin [14].

A variety of views exist on how the difference in protein intake under equal energy intake influences the life span and the occurrence of disease. A previous study examined the relationship between protein intake and survival [15]. In this study, we conducted molecular biological research using rats to verify the relationship between protein intake and aging, the onset of diseases such as lifestyle-related diseases, and carcinogenesis.

The previous study in rats conducted by McCay in 1935 revealed a relationship between calorie restriction and survival [1]. Dietary calorie restriction increases survival in various organisms and has been shown to protect humans and other mammals against age-related conditions, including atherosclerosis, cancer, and renal disease [16,17]. Further, aging and lifestyle-related disease are strongly related to arteriosclerosis. An appropriate protein intake level seems to contribute to the prevention of aging, lifestyle-related diseases, and carcinogenesis.

A total of seven sirtuins (Sirtuin 1-7) are known in mammals [18]. Sirtuin 1, which gave significant results in this study, is known as an aging-related enzyme. A recent study showed that aging and aging-related diseases are associated with decreased levels of nicotinamide adenine dinucleotide (NAD^+) and the decreased activity of Sirtuin 1 [19,20]. Although NAD^+ is a coenzyme present in all species, Sirtuin 1 is known to be affected as the NAD^+-dependent deacetylase Sirtuin, and NAD^+ is used as a substrate for Sirtuin 1 enzyme reactions [19,20].

SOD is an antioxidant enzyme expressed in whole-body tissues and is thought to protect the body from oxidative stress through the efficient disproportionation of superoxide to H_2O_2 and O_2, SOD is very important for reduction of oxidant stress, and primary products of many ROS production reactions [21]. As other enzymes that erase ROS, the existence of catalase and glutathione peroxidase (GPx) is also known, and both functions involve the reduction of free hydrogen peroxide to water, also important. It was not mentioned about GPx in this article, but GPx is known plays a key role in the maintenance of redox homeostasis within the SOD group.

Typically, there are three forms of SOD in mammals [22]. As SOD isozymes, CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) are reported to exist. CuZn-SOD is known to be primarily localized in cytoplasmic mitochondria, whereas EC-SOD is known to be the only extracellularly secreted protein among the SOD isozymes [23,24].

EC-SOD is known to inhibit the acceleration of the arteriosclerosis reaction. EC-SOD's reactions to the progress of arteriosclerosis include the inhibition of the differentiation of monocytes into macrophages, the suppression of the disappearance of macrophages by the adsorption of modified LDL such as oxidized low-density lipoprotein (oxLDL), and the suppression of the abnormal proliferation of vascular smooth-muscle cells [25]. Although large amounts of ROS are produced during the course of these conditions, it has been reported that EC-SOD suppresses the production of ROS [26].

NRF-1 (nuclear respiratory factor-1) is known as a nuclear gene involved in ATP production in mitochondria. ATP is produced from acetyl-CoA produced by the phosphorylation of glucose in the cytoplasm during the TCA cycle and oxidative phosphorylation in mitochondria. Oxidative phosphorylation occurs at four protein complexes in the electron transport chain, and NFR-1 is known as an activator of one of these protein complexes, cytochrome C. In this study, there was no significant difference in the expression level of NRF-1 between the different protein concentrations

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administered. This may mean that protein intake does not affect the mitochondrial TCA cycle or other nuclear respiratory processes [27,28].

To our knowledge, this is one of the few studies to show a relationship between protein intake and gene expression, and we attempted to analyze the expression levels of six anti-aging, antioxidant, and cancer-suppressing enzyme genes. The qRT-PCR method was used, which is a technique for amplifying and detecting a specific cDNA by combining the functions of reverse transcription and quantitative PCR or real-time PCR. The PCR amplification reaction is monitored in real time, and the amount of cDNA in the sample is measured quantitatively. Compared with the real-time PCR method, which measures the amount of PCR products according to the presence or absence (shading) of bands at a predetermined number of cycles, this method is reported to have higher sensitivity, a higher quantitative and dynamic range, and high reproducibility [29]. So this is considered to be an appropriate PCR method for this study.

In addition, there are several methods for comparing gene expression levels using qPCR. These include the absolute quantification method, which creates a standard curve using a standard sample containing a known copy number, the relative quantification method, which creates a standard curve using a standard sample, and the comparative Ct method (ΔΔCt method). In this study, the analysis was performed using the comparative Ct method (ΔΔCt method). The ΔΔCt method is used in many gene expression studies because it allows for easy comparison of relative gene expression levels without creating a standard curve [30], [31].

Recently, maintaining ADL in the elderly has become important from the perspective of preventing frailty and sarcopenia [32], and diagnostic methods have been established [33]. Among these studies, adequate protein intake has been shown to maintain skeletal muscle mass and prevent frailty [34,35]. A study of elderly Japanese women indicated that a low protein intake was one of the causes of frailty [36]. Lower serum albumin levels have also been reported to increase the risk of death in older adults [37,38].

Although high protein intake increases SOD activity in the rat brain, it has been reported that reduced glutathione (GSH) levels result in increased oxidative stress [39,40]. As a result, protein metabolism in the brain cannot be discussed in the same manner as in other organs. Although a low-protein diet is recommended in patients with renal impairment, some reports state that malnutrition occurs and survival is shortened, while others state that normal protein intake does not affect renal function [41]. A cautious discussion is needed about the relationship between protein intake and renal function [42].

A recent study also reported a relationship between the anti-aging-related enzyme Sirtuin 1 and the tumor suppressor protein p53 [43]. In the results of the current study, no p53 activity was observed in either group. An increase in Sirtuin 1 activity might account for the suppression of p53 activity. There are also reports that Sirtuin 1 activity has an impact on cancer treatment [44]. Although the kidneys are organs that are likely to be affected by aging, a study using a mouse model of diabetic nephropathy reported that the Sirtuin 1 expression level in glomeruli was decreased, and that Sirtuin 1 suppressed the progression of CKD [45]. As such, it is known that Sirtuin 1 is involved in the prevention of aging- and lifestyle-related diseases and cancer; thus, future research in this field is greatly anticipated.

5. Conclusions

A significant difference in Sirtuin 1 expression was observed between the LPD and SPD groups, and between the LPD and HPD groups. Moreover, a significant difference in CuZn-SOD, EC-SOD, and eNOS expression was observed between the LPD and SPD groups. This means that an appropriate amount of protein intake in humans contributes to the prevention of aging, aging-related diseases, and lifestyle-related and arteriosclerotic diseases caused by oxidative stress, and may help to extend the life span.

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