Effects of aging on the plasma levels of nesfatin-1 and adiponectin

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Abstract. Gastric and adipose tissue secrete a number of hormones that are involved in energy metabolism. The biological functions of these hormones, including their effects on aging, are currently under investigation. Adiponectin was shown to be directly involved in appetite and the control of body weight. However, the effects of aging of nesfatin-1, an appetite-suppressing peptide that was recently identified, have not yet been fully elucidated. The aim of this study was to determine the effects of aging on the plasma levels of nesfatin-1 and adiponectin. Our results demonstrated no significant differences in the nesfatin-1 plasma levels among three age groups (2, 6 and 24 months) of female BALB/c mice. The plasma nesfatin-1 levels/visceral fat (VF) ratio in the 24-month-old mice was significantly lower compared to that in the 2- and 6-month-old mice. In addition, there were no significant differences in the plasma adiponectin levels among the three age groups. The plasma adiponectin levels/VF ratio in the 24-month-old mice was significantly lower compared to that in the 2- and 6-month-old mice. In conclusion, there were no age-related changes in the plasma levels of nesfatin-1 and adiponectin, although the ratio of plasma levels of nesfatin-1 and adiponectin per VF was decreased with advancing age. Our results indicated that nesfatin-1 and adiponectin may be involved in controlling energy balance during aging.

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Introduction

The hypothalamus integrates signals originating in the brain, peripheral circulatory system and gastrointestinal tract, in order to regulate food intake and the expenditure of energy (1). The hypothalamic arcuate nucleus (ARC) plays a major role in the integration of signals regulating appetite. A neuronal circuit inhibits food intake via the expression of pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), whereas a different circuit stimulates food intake via the expression of neuropeptide Y (NPY) and Agouti-related peptide (AgRP), which are two potent orexigenic peptides (2,3). This network responds to several hormonal and metabolic signals, including leptin, insulin, ghrelin, corticosterone and glucose (4).

Adiponectin, which is secreted by adipose tissue, may be a direct signal involved in appetite and the control of body weight (BW) (5). Adiponectin plays a central role in the stimulation of food intake by activating AMP-activated protein kinase (AMPK) in the ARC. Previous studies on the effects of adiponectin on energy expenditure demonstrated that oxygen consumption was significantly decreased by adiponectin. By contrast, adiponectin-knockout mice exhibited reduced food intake and increased oxygen consumption. Furthermore, the phosphorylation of AMPK was significantly suppressed, the expression of NPY was significantly reduced and the expression of POMC was increased in the ARC of adiponectin-knockout mice following fasting (6).

Nesfatin-1 was recently identified as an anorexigenic factor ssociated with melanocortin signaling in the hypothalamus. Nesfatin-1 is an 82-amino acid residue derived from the nucleobindin-2 (NUCB2) peptide, possibly through proteolysis by prohormone convertases (7). Nesfatin-1/NUCB2 immunoreactive cells were found to be expressed in the pancreas, stomach, duodenum, adipose tissue, central amygdaloid nucleus, hypothalamus, nucleus accumbens, cerebellum and

microdissected lumbar spinal cord in rodents, suggesting that nesfatin-1/NUCB2 may be crucial in the physiological regulation of carbohydrate metabolism, gastrointestinal function and nutrient absorption (8-14). Furthermore, the nesfatin-1-induced inhibition of food intake may be mediated through the inhibition of orexigenic NPY neurons (15) and the activation of POMC and CART (16).

Over the last few years, numerous studies demonstrated that the NPY, CART and POMC mRNA expression levels and the plasma levels of the respective proteins are altered with advancing age (4,17-19). However, although it was demonstrated that nesfatin-1 suppresses appetite via the regulation of NPY, CART and POMC, and that adiponectin regulates energy metabolism in association with NPY and POMC (6,7,20), the mechanisms underlying the changes in nesfatin-1 and adiponectin levels with advancing age have not been fully elucidated. The present study aimed to investigate the effects of aging on the plasma levels of nesfatin-1 and adiponectin.

Materials and methods

Laboratory animals and breeding environment. A total of 23 female BALB/c mice, aged 2, 6 and 24 months, were obtained from CLEA Japan Inc., (Tokyo, Japan). The mice were housed in breeding rooms at a temperature of 22±2˚C and a humidity of 55±10%, under a 12-h light cycle, with the the light period initiated at 7 a.m. daily. A normal diet (CE-2 containing 11% kcal fat, 59% kcal carbohydrate and 30% kcal protein; CLEA Japan Inc.) and water were freely available. All the experiments were approved by the Animal Experimental Ethics Committee of the Kagoshima University Graduate School of Medical and Dental Sciences (Kagoshima, Japan).

Blood and tissue sampling. The mice were deprived of food for 6 h prior to tissue sampling. The rectal temperature was measured using a digital thermometer (Technol Seven Co. Ltd., Yokohama, Japan) in a room maintained at 22±0.5˚C. A lubricated thermocouple was inserted 1.5 cm into the rectum of conscious mice. Blood samples were obtained from the orbital sinus under diethyl ether anesthesia. Immediately after collection, the blood samples were transferred to chilled tubes containing EDTA-2Na (1 mg/ml) and aprotinin (500 U/ml), centrifuged and stored at -80˚C until analysis. For peptide measurements, the samples were not further aliquoted, nor were they repeatedly frozen and thawed. The mice were sacrificed by cervical dislocation. The liver and visceral fat (VF) were removed and weighed.

Measurement of nesfatin-1 and adiponectin. The plasma nesfatin-1 levels were measured using a commercial ELISA kit (Phoenix Pharmaceuticals, Belmont, CA, USA) following the manufacturer's instructions. The plasma adiponectin levels were determined with an adiponectin ELISA kit (Otsuka Pharmaceutical Co., Tokyo, Japan).

Statistical analysis. Statistical analysis was performed using SPSS/PASW Statistics for Windows, version 18.0 (SPSS, Inc., Chicago, IL, USA). The nesfatin-1 and adiponectin data were analyzed using analysis of variance followed by the post hoc least significant difference test. The correlation between plasma nesfatin-1 and adiponectin levels, VF and other related variables were assessed by two-way invariant correlations (Pearson's correlation coefficient). P<0.05 was considered to indicate a statistically significant difference.

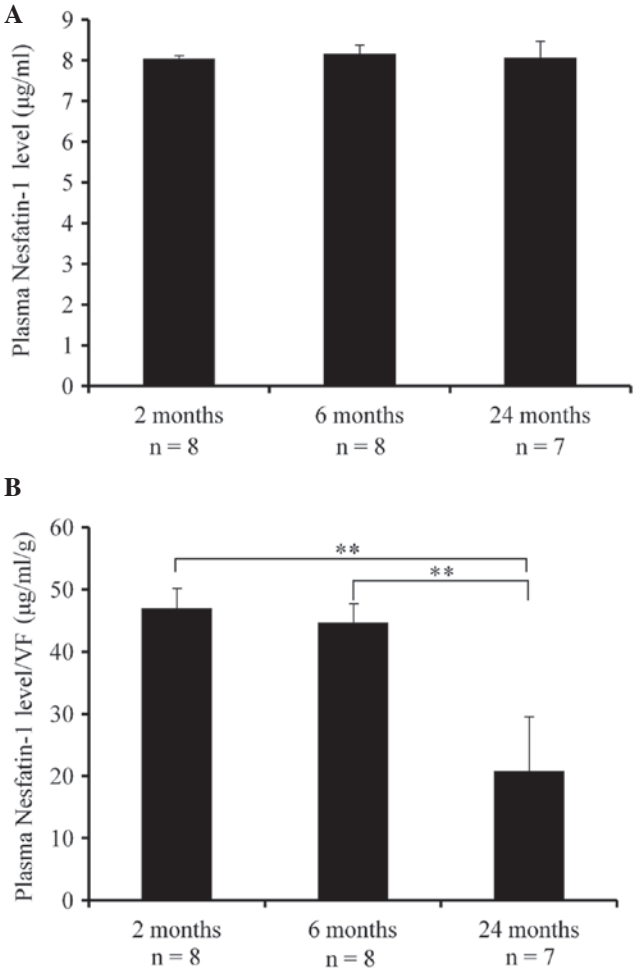

Figure 1. (A) Plasma nesfatin-1 levels and (B) plasma nesfatin-1 level/VF ratio in female BALB/c mice aged 2, 6 and 24 months. Data are expressed as means ± standard error of the mean (SEM). **P<0.01.

Results

Changes in plasma nesfatin-1 levels (µg/ml) and nesfatin-1 level/VF ratio with age. The plasma nesfatin-1 levels and the plasma nesfatin-1 level/VF ratio of the mice are presented in Table I. There were no significant differences between the groups regarding the plasma nesfatin-1 levels [F (2,20)=0.068] (Fig. 1A). However, there were significant differences in the plasma nesfatin-1 level/VF ratio among the three age groups [F= (2,20)=7.19, P<0.01], with the ratio in the 24-month-old group being significantly lower compared to that in the 2- and 6-month-old groups (P<0.01) (Fig. 1B).

Changes in plasma adiponectin levels (µg/ml) and in plasma adiponectin/VF ratio with age. The plasma adiponectin levels and plasma adiponectin level/VF ratio of the mice are presented in Table I. There were no significant differences in the plasma adiponectin levels between the different

Table I. Comparison of plasma nesfatin-1 and adipotectin levels among the three age groups (2, 6 and 24 months) of BALB/c mice.

Variables	2 months	6 months	24 months	Difference by group
Plasma nesfatin-1 level (μg/ml)	8.04±0.07	8.17±0.23	8.07±0.41	N.S.
Plasma nesfatin-1 level/VF	46.98±3.29	44.7±3.01	20.83±8.66	24 vs. 2, 6 months, P<0.01
Plasma adiponectin level (μg/ml)	32.07±1.49	38.61±1.70	33.01±3.47	N.S.
Plasma adiponectin level/VF	185.79±13.62	211.14±15.32	68.65±15.29	24 vs. 2, 6 months, P<0.001

Data are expressed as means ± standard error of the mean (SEM). The nesfatin-1 and adiponectin data were analyzed using analysis of variance followed by a post hoc least significant difference test. VF, visceral fat; N.S., non-significant.

Table II. Comparison of body weight, visceral fat, liver weight and body temperature among the three age groups (2, 6 and 24 months) of BALB/c mice.

Variables	2 months	6 months	24 months	Difference by group
Body weight (g)	18.95±0.17	25.56±0.49	26.69±0.30	2 vs. 6, 24 months, P <0.01
Visceral fat (g)	0.18±0.01	0.19±0.02	0.61±0.11	24 vs. 2, 6 months, P <0.01
Liver weight (g)	1.02±0.03	1.37±0.05	1.32±0.04	2 vs. 6, 24 months, P <0.01
Temperature (˚C)	38.19±0.12	37.40±0.17	35.72±0.34	2 vs. 6 months, P <0.05 2 vs. 24 and 6 vs.24 months, P <0.01

Data are expressed as means ± standard error of the mean (SEM). Body weight, visceral fat, liver weight and temperature were analyzed using analysis of variance followed by a post hoc least significant difference test.

age groups [F (2,20)=2.488] (Fig. 2A). However, there were significant differences in the plasma adiponectin level/VF ratio [F (2,20)=25.39, P<0.001], with the plasma adiponectin level/VF ratio being significantly lower in the 24-month-old group compared to that in the 2- and 6-month-old groups (P<0.001) (Fig. 2B).

Changes in body and liver weight with age. There were significant differences in BW and liver weight among the three age groups [F (2,20)=143.96, P<0.01 and F (2,20)=25.44, P<0.01, respectively]. The BW and liver weight were significantly lower in the 2-month-old group compared to the 6- and 24-month-old groups (P<0.001) (Table II).

Changes in the VF level with age. There were significant differences in the VF levels among the three age groups [F (2,20)=16.64]. The VF level was higher in 24-month-old group compared to that in the 2- and 6-month-old groups (P<0.01) (Table II).

Changes in body temperature with age. There were significant differences in the body temperature among the three age groups [F (2,20)=32.31]. The body temperature was higher in the 2-month-old group compared to that of the 6- and 24-month-old groups (P<0.05 and P<0.01, respectively), and that of the 6 months group was significantly higher compared to that of 24 months group (P<0.01) (Table II).

Correlation analysis. There was no significant correlation of the plasma nesfatin-1 and adiponectin levels with BW, liver weight, VF and body temperature.

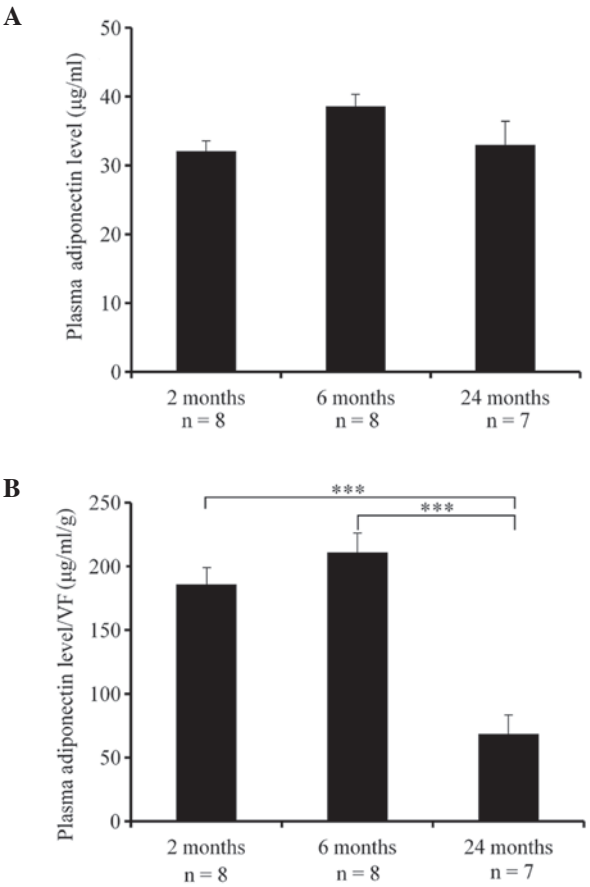

Figure 2. (A) Plasma adiponectin levels and (B) plasma adiponectin level/VF ratio of female BALB/c mice aged 2, 6 and 24 months. Data are expressed as means ± standard error of the mean (SEM). ***P<0.001.

Discussion

Nesfatin-1 was identified as a novel appetite-suppressing peptide in 2006 and studies on its physiological effects, distribution and effects on energy metabolism are currently underway (7,9-12,16). It was previously demonstrated that fasting for 24 h decreased NUCB2 mRNA expression in a pool of enriched small gastric endocrine cells (21) and significantly reduced nesfatin-1 plasma levels in rats (11). Li *et al* (22) reported that older healthy human subjects (average age, 47.3 years) exhibited a higher mean fasting plasma nesfatin-1 concentration compared to that of young healthy subjects (average age, 19.4 years); no differences in plasma nesfatin-1 levels were observed between healthy male and female subjects.

Previous studies demonstrated that the NPY content in the hypothalamus was significantly increased in rats with streptozotocin-induced diabetes mellitus and in spontaneously diabetic Brattleboro rats, and that the increased NPY content of the hypothalamus may result in diabetic hyperphagia (23,24). Moreover, the vasoconstrictive properties of NPY may contribute to the mechanism underlying hypertension in obesity (25,26). In aging studies, it was demonstrated that plasma NPY levels and NPY gene expression in the hypothalamus increased with age (17-19). However, the POMC mRNA levels in the ARC were not found to be affected by age in the basal state, whereas an age-associated increase of CART mRNA in the ARC and an age-associated decrease in CART mRNA in the PVN were previously reported (4). The prevalence of diabetes and hypertension were shown to increase with age (27,28). Those findings suggested that changes in NPY, CART and POMC with age may be associated with the risk of onset of diabetes or hypertension. The nesfatin-1-induced inhibition of food intake may be mediated through the inhibition of orexigenic NPY neurons (15) and the activation of POMC and CART (16). If nesfatin-1 levels increase with age, the risk of onset of diabetes or hypertension may decrease via the activation of NPY, CART and POMC. In our study, there was a marginal change in the plasma nesfatin-1 levels in mice aged 2-24 months, although the plasma nesfatin-1 level/VF ratio was markedly decreased with advancing age. These results indicated that nesfatin-1 may be relatively insufficient when taking into consideration the changes in the amount of VF with age. To the best of our knowledge, this study was the first to report the effects of age on plasma nesfatin-1 concentrations in mice.

Previous studies demonstrated that the plasma adiponectin levels increased with age (18,29,30). By contrast, Takenouchi *et al* (31) reported that there was no change in the plasma adiponectin levels associated with age. In our study, the plasma adiponectin levels in mice exhibited no differences with advancing age, which is in accordance with the findings of Takenouchi *et al* (31). However, the plasma adiponectin level/VF ratio was decreased from 6 to 24 months of age. This result indicated that, as with nesfatin-1, adiponectin may be relatively insufficient when taking into consideration the changes in the VF amount with age. Adiponectin, an adipose tissue-derived protein, possesses antidiabetic, anti-atherogenic and insulin-sensitizing properties. Adiponectin induces a decrease in circulating free fatty acids (FFA) through increasing fatty acid oxidation by skeletal muscles, decreases liver FFA influx and stimulates glucose uptake by adipocytes and myocytes through the activation of AMPK (32-35). A decrease in the triglyceride content of the muscles and the liver results in increased insulin sensitivity. In addition to its metabolic effects, adiponectin possesses anti-inflammatory and atheroprotective properties that affect endothelial vascular function (36). Thus, we hypothesized that the relative insufficiency of nesfatin-1 and adiponectin per VF with age may be associated with an increased risk of the progression of aging or cardiovascular and cerebrovascular diseases.

In conclusion, our results demonstrated that the concentrations of plasma nesfatin-1 and adiponectin, which are crucial in regulating food intake and energy metabolism, per VF, are significantly decreased with age and these changes may affect the onset and progression of various diseases.

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