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Short Communication

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Aging effect on plasma metabolites and hormones concentrations in riding horses

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Abstract
Age effects on plasma metabolites, hormone concentrations, and enzyme activities related to energy metabolism were investigated in 20 riding horses. Animals were divided into two groups: Young (3-8 years) and aged (11-18 years). They were clinically healthy, and not obese. Plasma adiponectin (ADN) concentrations in aged horses were significantly lower than those in young horses (mean±SE, 6.5±1.3 μg mL^{-1} vs, 10.9±1.7 μg mL^{-1}, Mann-Whitney U test, respectively; P=0.0233). Plasma non-esterified fatty acid levels and Insulin and malondialdehyde concentrations in aged group tended to increase compared to those in young group although there were not significant differences statistically. In aged group, malate dehydrogenase/lactate dehydrogenase (M/L) ratio, which is considered an energy metabolic indicator, did not change significantly compared to that in young group. Present data suggest that aging may negatively affect nutrition metabolism, but not induce remarkable changes in M/L ratio in riding horses.
Keywords: Adiponectin, Aging, Horses.

Introduction

Nutrition metabolism and immune function are generally subject to change with aging in animals. In particular, lipid metabolism is down regulated in overweight animals with aging (Kawasumi *et al*., 2014). Prevalence of overweight and obesity has increased in recent years in dogs (Tvarijonaviciute *et al*., 2012), cats (Martin *et al*., 2014), and riding horses (Robin *et al*., 2015). Obesity is defined as the accumulation of excess amounts of adipose tissue in the body, and is the risk factor for decreased longevity, hypertension, diabetes, lameness, and certain types of cancer (German *et al*., 2010). Obesity is also associated with inflammation and immune cell recruitment of adipose tissue, muscle, and the intima of atherosclerotic blood vessels (Pillon *et al*., 2015). In obese animals, aberration of adipocytokine secretion is frequently observed with hyperlipidemia. Obesity is often associated with hypoadiponectinemia (Nakatsuji *et al*., 2014).

In general, obesity and overweight are caused by excess calorie and physical inactivity. Domestic dogs and cats tend to decrease physical activity with aging and excess weight. Riding horses usually maintain adequate level of physical activity compared to domestic dogs and cats. In this study, plasma metabolite, hormone concentrations, and enzyme activities in riding horses at different ages were measured to investigate the aging effects on nutrition metabolism.

Materials and Methods

Animals

Twenty riding horses (Thoroughbred, female n=3, male n=17) examined in this study were maintained at Japan Horseback Riding Club (Saitama, Japan). They were divided into two groups: Young group (3-8 years old, average 7.1±0.5) and aged group (11-18 years old, average 14.1±0.7). All male horses were gelding (Table 1). The degree of obesity of horses was assessed by a six-scale equine body conditioning score (EBCS), established by Carroll and Huntington scoring system (Carroll and Huntington, 1988): Very thin [1], thin [2], fair [3], good [4], fat [5] and very fat [6] as modified by Robin (Robin *et al*., 2015).

Horses were fed 5.2-6.4kg of hay cube, 3.0-4.0 kg of Italian ryegrass, 0-1.3 kg of wheat bran, 0-1.8kg of barley at 6:00 and 16:00 daily. Each riding horse was exercised by walking at 100-110 m/min for 10-30 minutes, trotting at 200-220 m/min for 10-30 minutes and cantering at 300-350 m/min for 15 minutes 1-3 times daily for 6 days each week. Exercise amount of each riding horse depends on various riding lesson menu based on each rider's skill level: Beginner, intermediate, and senior levels. Ethical approval for this study was obtained from Japan Horseback Riding Club.

Blood sampling and analysis

Blood samples were taken from jugular veins of horses into heparinized tubes. Plasma was recovered by centrifugation at 1200 g, for 5 min at 4°C and stored at -80°C until use. Glucose (GLU), total cholesterol (TC), triglyceride (TG), total protein (TP), blood urea nitrogen (BUN) and creatinine (CRE) concentrations and alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities were

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measured using an auto-analyzer (JCA-BM2250, JEOL Ltd., Tokyo, Japan) with the manufacture's reagents at Monolis Inc. (Tokyo, Japan). Activities of plasma lactose dehydrogenase (LDH) (Kaloustian *et al*., 1969) and malate dehydrogenase (MDH) (Bergmeyer and Bernt, 1974) were measured by previously described methods, respectively. The plasma MDH/LDH ratio was calculated as MDH activity divided by LDH activity. Plasma NEFA and MDA concentrations were measured using a commercial kit, NEFA-C test (Wako Pure Chemical Industries, Inc., Tokyo, Japan) and NWLSS™ Malondialdehyde assay (Northwest Life Science Specialteis, LLC, Vancouver, Canada), respectively. Plasma Insulin (INS) and adiponectin (ADN) concentrations were measured with commercial ELISA kits, Lbis Rat T insulin kit (SHIBAYAGI Co., Gunma, Japan), and mouse/rat adiponectin ELISA kit (Osuka Pharmaceutical Co., Ltd, Tokyo, Japan), respectively. Plasma superoxide dismutase (SOD) activity was measured using a commercial kit, NWLSS™ Superoxide Dismutase Activity Assay (Northwest Life Science Specialties, LLC, Vancouver, Canada).

Statistical analysis

Results are presented as means±SE. Statistical significance was determined by Mann-Whitney U test. The significance level was set at $P<0.05$.

Results and Discussion

All profiles of horses examined in this study were shown in Table 1. Animals were judged as EBCS 4 by the modified Carroll and Huntington EBCS scoring system (Carroll and Huntington, 1988). They were healthy and not obese as shown in Fig. 1. Their body weight ranges were estimated from 450 kg to 500 kg.

As shown in Table 2, plasma ADN concentrations in aged horses significantly decreased compared to those in young horses (mean±SE, 6.5±1.3 μg mL^{-1} vs 10.9±1.7 μg mL^{-1} Mann-Whitney U test, respectively; $P=0.0233$) although there were no remarkable differences in plasma GLU, TG, TC, BUN, CRE, TP, ALT and LDH levels between young and aged group. ADN is a cytokine secreted from adipose tissue, which regulates glucose and lipid metabolism, increases insulin sensitivity, and has an anti-inflammatory effect (Balsan *et al*., 2015). In obese animals, plasma ADN concentrations decrease significantly (Park *et al*., 2014, 2015). On the other hand, plasma ADN concentrations decrease in clinically healthy dogs with aging have been reported (Mori *et al*., 2012).

Plasma NEFA level, INS and MDA concentration, and SOD activity in aged group tended to increase compared to those in young group (mean±SE, NEFA: 83.9±44.9 μmol mL^{-1} vs 11.8±3.7 μmol mL^{-1}, INS: 0.63±0.15 ng mL^{-1} vs 0.43±0.06 ng mL^{-1}, MDA: 1.58 ±0.10 μmol L^{-1} vs 1.37±0.05 μmo^{-1} L^{-1}, SOD: 14.5±5.9 U mL^{-1} vs 7.8±4.6 U mL^{-1}) although there

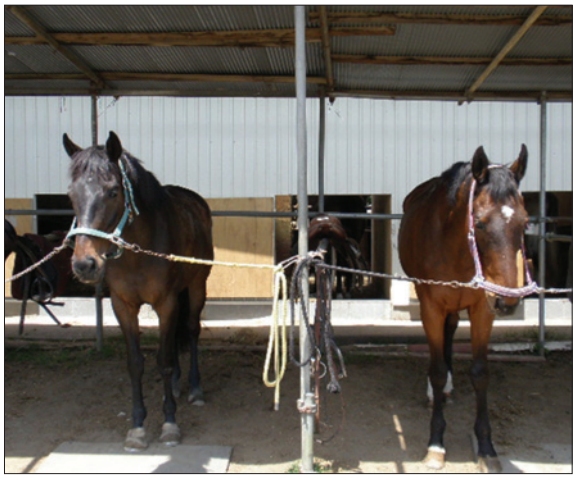

Fig. 1. Representative horses in this study. Left: A young horse with EBCS 4 (Thoroughbred, female 7 years old). Right: An aged horse with EBCS 4 (Thoroughbred, gelding 18 years old).

Table 1. Profile of the sex, gelding, age and breed in young and aged horses.

Group	*Sex*	*Gelding*	*Age (years)*	*Breed*
Young group				
1	Male	○	8	Thoroughbred
2	Female	-	8	Thoroughbred
3	Male	○	8	Thoroughbred
4	Male	○	8	Thoroughbred
5	Male	○	8	Thoroughbred
6	Male	○	7	Thoroughbred
7	Male	○	7	Thoroughbred
8	Female	-	7	Thoroughbred
9	Male	○	7	Thoroughbred
10	Female	-	3	Thoroughbred
			Av.: 7.1	
Aged group				
1	Male	○	18	Thoroughbred
2	Male	○	12	Thoroughbred
3	Male	○	11	Thoroughbred
4	Male	○	13	Thoroughbred
5	Male	○	15	Thoroughbred
6	Male	○	15	Thoroughbred
7	Male	○	16	Thoroughbred
8	Male	○	14	Thoroughbred
9	Male	○	12	Thoroughbred
10	Male	○	15	Thoroughbred
			Av.: 14.1	

Table 2. Comparison of biomarker levels between young and aged riding horses.

Biomarker	*Young group (10)*	*Aged group (10)*	*P value*
GLU (mg dL^{-1})	98.3±2.3	99.9±1.4	0.3431
TG (mg dL^{-1})	16.1±3.4	14.7±1.4	0.5425
TC (mg dL^{-1})	71.0±2.9	74.7±4.0	0.5697
NEFA (μmol L^{-1})	11.8±3.7	83.9±44.9	0.2266
BUN (mg dL^{-1})	17.6±0.7	18.8±0.6	0.2442
CRE (mg dL^{-1})	1.1±0.0	1.2±0.1	0.5118
TP (g dL^{-1})	6.5±0.1	6.7±0.1	0.2703
INS (ng mL^{-1})	0.43±0.06	0.63±0.15	0.3536
ADN (μg mL^{-1})	10.9±1.7	6.5±1.3*	0.0233
MDA (μmol L^{-1})	1.37±0.05	1.58±0.10	0.0953
SOD (U mL^{-1})	7.8±4.6	14.5±5.9	0.3703
AST (IU L^{-1})	261.3±33.8	232.7±10.8	0.7913
ALT (IU L^{-1})	6.8±1.1	6.2±0.4	0.6625
ALP (IU L^{-1})	258.8±16.3	248.8±13.5	0.5452
MDH (IU L^{-1})	384.1±43.1	374.3±18.7	0.9349
LDH (IU L^{-1})	206.9±24.7	200.6±10.7	0.9349
M/L ratio	1.92±0.13	1.89±0.09	0.4624

The numbers in parentheses indicate the number of animals examined. Data are expressed as mean±standard error (SE). *Significant (P<0.05) when compared against young group (Mann-Whitney U test).

were no significant differences statistically. In aged group, M/L ratio as an energy metabolic indicator, has significantly changed compared to that in young group (mean±SE, 1.89±0.09 vs 1.92±0.13). Increase in plasma NEFA concentration in aged horses appear to be related to decrease in ADN concentration. Increase in plasma NEFA concentration induces nonspecific binding to Toll-like receptors leading to pro-inflammatory phenomenon in obese animals (de Heredia *et al*., 2012). Very slight inflammation is suspected to be caused in aged healthy horses with decreasing plasma ADN concentrations as previously reported in obese animals (German *et al*., 2010). Increasing tendency in plasma MDA concentration in aged horses is considered to be related to low ADN concentration as ADN is associated with the reduction of oxidative stress (Wang *et al*., 2014). Increase in plasma SOD activities seems to reflect increase in MDA concentrations in aged healthy horses.

Malate dehydrogenase (MDH) is a crucial enzyme for the malate-aspartate shuttle, one of the NADH shuttles that produce ATPs from glucose metabolism. Lactate dehydrogenase (LDH) is an enzyme that catalyzes the conversion of lactate to pyruvate consuming cytosolic NADH. MDH/LDH activity ratio (M/L ratio) is considered to be a good indicator for evaluating energy metabolism in animals including riding horses (Hirakawa *et al*., 2012) as long term intensive exercise training was found to lead to a higher M/L ratio in race horses (Li *et al*., 2012). In this study, M/L ratio did not change significantly in aged horses. Lower grade of changes in nutrition metabolism may not induce remarkable changes in M/L ratio (Okada *et al*., 2015).

This study has several limitations. First, we had a limited number of healthy riding horses in our study. Second, because the animals involved in the study were randomly selected, classification of age group was not appropriate. Third, blood sampling was not performed after fasting. Fourth, an equal number of males and females to further study the effect of sex was not possible.

Since aging may predispose to glucose and lipid metabolic abnormality (Hoenig *et al*., 2011), a further study will be needed in more horses at various ages.

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Conflict of interest

The authors declare that there is no conflict of interest.

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