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Penulis Artikel	:	Audrianto Suranto, **Sukmaningtyas Hermina**, Ngestiningsih Dwi, Batubara Lusiana
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ORIGINAL ARTICLE

Sleep Insufficiency Influence on Nitric Oxide Concentration and Systolic Blood Pressure in Medical Students

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ABSTRACT

Introduction: Medical students often experience sleep deprivation due to a large academic load. Sleep insufficiency is one hypertension modifiable risk factors, yet its pathophysiological mechanism is still under-researched. This study aims to find out the sleep quality profiles of medical students in Malang-Indonesia and explore the effects of sleep insufficiency on systolic blood pressure and Nitric Oxide (NO). **Methods:** A total of 153 medical students completed the Pittsburgh Sleep Quality Index questionnaires. Forty students (40) participants were randomly selected into two groups to explore the comparison of NO concentration and the systolic blood pressure. The systolic blood pressure was measured with a sphygmomanometer. NO concentration was assessed with ELISA using the saliva sample. **Results:** Most of the medical students (89.54 %) had poor sleep quality with the average sleep time for 4 hours. The independent t-test showed significant differences in systolic blood pressure and NO concentration between two groups ($p<0.05$). Nitric oxide negatively influenced systolic blood pressure ($p<0.05$, $R=-0.337$). **Conclusion:** Medical students experienced poor sleep quality and sleep deprivation. Sleep insufficiency increases the systolic blood pressure. The increase of NO concentration may indicate the normal vascular endothelial response due to sleep loss in young adults.

Keywords: Sleep quality, Sleep insufficiency, Medical students, Systolic blood pressure, NO

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INTRODUCTION

A remarkable number of studies in different countries worldwide reported the prevalent excessive loss of nocturnal sleep and poor sleep quality in medical students (1–10). The consequences of such sleep deprivation and poor sleep quality have been associated with the increased risk of hypertension. It has been reported that shorter periods of sleep were associated with higher risks for high blood pressure, and this was stronger in women than in men (11), though the other study showed male adolescents more susceptible, and other observations reported no preferential sex (12–14).

Interestingly, studies exploring the mechanisms of hypertension which is caused by night sleep insufficiency are still limited. Observations on night shift workers who experienced sleep insufficiency demonstrated a conversion of blood pressure status from dipper to non-dipper (15–17). The non-dipper state is associate with the endothelial dysfunction due to chronic activation of the sympathetic system and Renin Angiotensin Aldosterone system. Furthermore, sleep loss promote endothelial dysfunction that contribute to the decrease in NO circulation and thus induce hypertension in middle age animal model experiment (12,18–21).

Despite all of the findings, the correlation and the mechanism of sleep insufficiency and the hypertension is still controversial especially in young normotensive adults, although the response of orthostatic systolic blood pressure attenuate (22). The present study tried to observe the association between night sleep deprivation, systolic blood pressure and NO concentrations in medical students.

MATERIALS AND METHODS

Study Design and Subjects

A cross-sectional study was conducted to 153 eligible participants from four faculties of medicine in Malang, Indonesia. Their average night sleep duration, overall sleep quality score and systolic blood pressure were assessed. Forty students were selected and divided into two groups: (1) sleep deprivation groups (n=20) and (2) enough sleep groups (n=20) to further analyze NO concentration. Inclusion criteria of the participants were: (1) studying at the faculty of medicine; (2) not using anti-anxiety or antidepressant drugs; (3) not in psychology therapy program (4) not suffering from an infectious disease or having a history of previous infectious diseases. Each participant had signed informed consent for the study. The study was reviewed and approved by the institutional review board (052b/EC/KEPK-FKIK/2019).

Data collection and instrument

Student sleep quality was assessed by Pittsburgh Sleep Quality Instrument (PSQI) questionnaire, containing 7 domains which include (1) subjective sleep quality, (2) sleep latency, (3) sleep duration, (4) habitual sleep efficiency, (5) sleep disturbances, (6) use of sleep medication and (7) daytime dysfunction. The individual scores of each domain were accumulated to obtain a global score with cut-off score of 5. Global score ≤ 5 indicated good sleep and global score > 5 indicate poor sleep quality(23).

Systolic Blood Pressure Measurement

The subjects underwent blood pressure measurement using a sphygmomanometer (Riester-Novaecoline Germany) and stethoscope (Littman classic 3rd series) and were checked by Omron digital blood pressure monitor. Prior to assessment, a proper cuff was matched with the size of subject's arm. The circular cuff was placed on the arm where the examination was as high as the heart, with the bottom of the cuff 2-3 cm just above the cubital fossa.

The ear tip of stethoscope was placed right into the examiner's ear, while the diaphragm was lightly pressed over the brachial artery just below the cuff's edge. Rubber bulb was pumped until the brachial artery pulse was heard. The first sound that was listened to was systolic blood pressure. Rubber bulb was pumped again up to 20-30 mm Hg. The control valve was loosened slowly, so that mercury drops at a speed of 2 - 3 millimeters of Hg per second. The last pulse was called diastolic blood pressure.

Nitric oxide assay

Nitric oxide concentration was assessed with ELISA using Quantichrome ™ Nitric Oxide Kit (D2NO-100) Bioassay System. The subjects were divided into two groups based on sleep duration; < 5 hours and > 5 hours. The saliva sample was taken just before they went to sleep at night. The saliva collection was conducted on the same day for both groups. Participants were suggested not to eat within two hours before saliva collection and avoid any high NO_3^- foods. The participant seated with their head slightly tilted (approximately 45°). Immediately before the collection procedure, individuals gargled with water and the saliva was collected in Falcon sterile tubes for 5 minutes. The obtained saliva for each participant was approximately 5 ml. The saliva samples were stored frozen in the freezer at -80°C for later processing and analyzing. Prior to assay, the tubes were centrifuged at 2600 x g for 15 minutes at 4°C. The saliva supernatant was measured. Saliva and the standard were mixed with the working reagent, thus incubate for 10 minutes at 60°C. After the incubation process, centrifugation was performed once again to collect the pellet. The pellet was transferred to 96 well plates and read for Optical Density at 540nm (24–26).

Statistical Analysis

Participants' socio-demographic characteristics were presented as frequencies and proportions for categorical variables. The comparison between systolic blood pressure and NO concentration between the two groups was performed using independent t-test. All statistical analyses were performed with SPSS v. 22.0.

RESULTS

Demographic characteristic

Participant baseline characteristics were shown in Table I.

Table I : Demographic characteristics of the study population

No	Variable		Frequency (%)
1	Age (years)		
	1.	18	62 (42.48)
	2.	19	76 (49.67)
	3.	20	7 (0.04)
	4.	21	8 (0.05)
2	Gender		
	1.	Male	104 (68)
	2.	Female	49 (32)
3	BMI		
	1.	underweight	9 (5.9)
	2.	normal	93 (60.8)
	3.	overweight and obesity	51 (33.3)
4	Academic level		
	1.	First year	107 (69.93)
	2.	Second year	46 (30.06)
	3.	Third year	-
	4.	Fourth year	-

Body mass Index (BMI) is the weight in kilograms divided by the square of the height in meters. BMI was categorized based on Asia-Pacific classification. Obese was defined as BMI index of 25 or higher; overweight: 23-24.9 ; normal weight: 18.5–22.9 ; underweight <18.5
Data were available for 153 participants.

The Comparison of Systolic Blood Pressure in Two Groups

137 students (89.5%) had poor sleep quality (Fig.1) although 64.7% of them rated their sleep good and very good. Only 5.8 % of students reported the need to fall asleep > 15 min. Most of students (94.77 %) went to bed after 10 pm. Mean and SD of night sleep duration were 4.84 and ±1.231 h respectively. The detailed results of PSQI component was summarized in Table II.

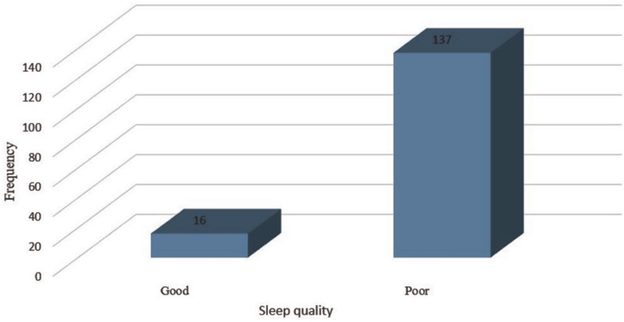

Fig. 1 : Profile of Sleep Quality of the Sample

Table II : Seven domains of PSQI in participants

No	Variable	Frequency (%)
1	Subjective sleep quality	
	1. Very good	6 (3.9)
	2. Good	93 (60.8)
	3. Fairly bad	53 (34.6)
	4. Very bad	1 (0.7)
2	Sleep latency	
	1. Very good	45 (17.4)
	2. Good	55 (21.2)
	3. Fairly bad	38 (14.7)
	4. Very bad	15 (5.8)
3	sleep duration	
	1. Very good	7 (4.6)
	2. Good	20 (13.1)
	3. Fairly bad	49 (32)
	4. Very bad	77 (50.3)
4	habitual sleep efficiency	
	1. Very good	129 (84.9)
	2. Good	2 (1.3)
	3. Bad enough	1 (0.7)
	4. Very bad	20 (13.2)
5	sleep disturbances	
	1. Very good	4 (2.6)
	2. Good	112 (73.2)
	3. Fairly bad	34 (22.2)
	4. Very bad	3 (2)
6	use of sleep medication	
	1. Very good	131 (90.6)
	2. Good	17 (6.6)
	3. Fairly bad	3 (1.2)
	4. Very bad	2 (0.8)
7	daytime dysfunction	
	1. Very good	6 (3.9)
	2. Good	39 (25.5)
	3. Fairly bad	74 (48.4)
	4. Very bad	34 (13.1)

Sleep quality indicator above were based on PSQI questionnaire. Data were available for 153 participants.

Systolic blood pressure was assessed twice in each group just before they went to sleep. The first group (sleep time > 5 h) was examined at 7-8 am, whereas the other group was taken at 11-12 am (sleep time < 5h). The results showed that the night systolic blood pressure between two groups differ significantly (p = 0.029). It was also found that there was signifcant difference between night and morning systolic blood pressure in sleep deprivation group (p-value = <0.000), but not in the other group (p = 0.148) (Fig.2).

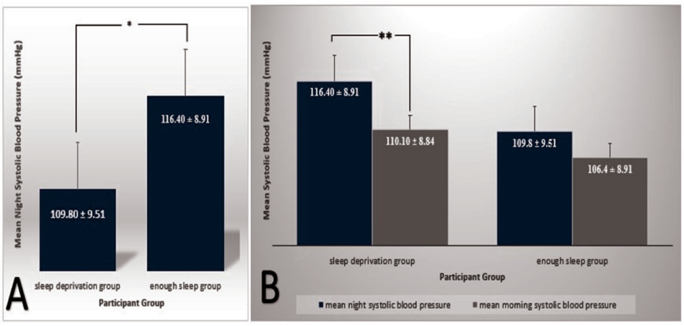

Fig. 2 : Comparison of Night Systolic Pressure between two groups and Night-Morning Systolic Pressure in each group

The Comparison of NO level in two groups

We found a significant difference of NO level between the two groups (p-value<0.05). The NO level was higher in sleep deprivation group compared with the enough sleep group (Fig.3).

The Relationship between NO and systolic blood Pressure

There was a significant correlation between NO and systolic blood pressure (p = 0.017, r = -0.377), which means the increase of NO will decrease the systolic blood pressure (Fig.3).

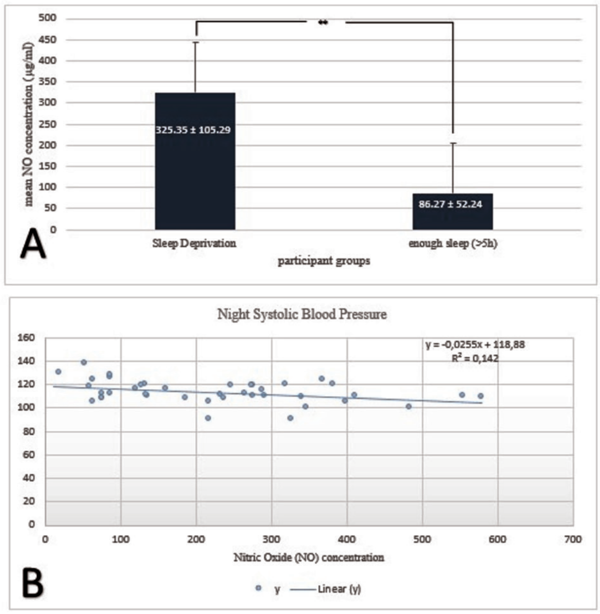

Fig. 3 : Correlation between NO and Systolic Pressure

DISCUSSION

Sleep is a vital process to maintain human homeostasis. Approximately one-third of human life is spent on sleep. From this current study, we can conclude that medical students in Indonesia especially in Malang experienced sleep deprivation and poor sleep quality. Previous reports have shown similar results worldwide, although our findings seem higher. A study in an Arabian region reported 66.7% of King Khalid Medical students experienced sleep duration while in Brazil around 40% (27). The mean of medical students' sleep duration in 13 countries was 6.3 h per night, whereas our finding showed it was 4 h (28).

Other activities besides study, tight schedule, ability to make proper time management, and full college task can motivate someone to delay their sleep (29). Heavy loads makes some students experience depression and anxiety or exhibit some headache that worsens their sleep (13,33–35). This study found 89.54% of students reported to have good sleep quality good though their average sleep duration was only 4 hours and the prevalence of daytime dysfunction was also high. We assume that sleep hygiene awareness among medical students was poor. This findings are similar with those of previous report that concluded that young adults had low sleep hygiene awareness (33,34), and better sleep hygiene awareness does not necessarily guarantee better sleep quality (33).

Several publications have revealed an association between sleep deprivation and the increased risk of blood pressure and hypertension (35–40). Our findings showed the night systolic blood pressure in sleep deprivation group was higher than that in enough sleep group. Furthermore, significant difference was found between morning and late-night systolic blood in sleep deprivation group but not in enough sleep group. Exposure to light in the midnight shifts the human internal clock (BMAL and CLOCK) and affects their targeted genes, which thus activating the symphatetic nervous system (41–44). The chronic sympathetic activation will further cause endothelial dysfunction marked by suppression of NO level (45,46). This mechanism consistent with Jiang's study in 2017 who reported that lack of REM sleep induced endothelial dysfunction in elderly rats (47). Investigation with Wistar rat treated unslept resulted in a decrease in NO production (48). Unlike the previous studies, our result showed that NO concentration was found higher in sleep deprivation group. The disruption of circadian rhythm in participants who experienced sleep deprivation will augment the sympathetic function (49). Indeed, the release of epinephrine and norepinephrine will generate vascular constriction which results in higher systolic blood pressure. However in this present study, due to young age of all study population, it is possible that the body system is in the process of adapting this changes by activating the endothelial NOS to produce and release NO.

This research has some limitations. First, the data for assessing sleep quality were obtained by self-report, and there could be potential recall bias. Second, the study did not collect information about other parameters that acted as confounding factors. Further study needs to complete the assessment validity of sleep with polysomnography and HBPM/ABPM. Longitudinal and interventional studies conducted in the animal models are warranted to provide further evidence of the association between sleep duration, time of sleep and endothelial dysfunction as the hallmark of cardiovascular diseases.

CONCLUSION

Most of the medical students experience sleep deprivation. Acute sleep insufficiency increases night NO concentration and systolic blood pressure. The knowledge about the importance of sleep physiology and sleep hygiene among medical students should become awareness for the Medical Institution.

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ORIGINAL ARTICLE

The Association Between Plasma Natural Antibodies and Inflammatory Biomarkers Two Weeks After Calving in Cows with No Dry Period

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ABSTRACT

Introduction: Improved energy balance, metabolic status, and natural antibodies (NAb) has been shown in cows with no dry period, however these cows showed increased inflammation status in early lactation. The aim of this study was to determine the association between plasma natural antibodies and inflammatory biomarkers in cows with no dry period during the first two weeks postpartum. **Methods:** Holstein-Friesian dairy cows (n=55) were selected. Before enroll to the experiment, cows were clinically healthy. Plasma samples were collected at week 1 and 2 after calving and were analyzed for NAb binding megantura-keyhole limpet hemocyanin and inflammatory biomarkers. **Results:** Cows with no dry period in this study had an improved energy balance and maintain NAb titers but increased ceruloplasmin (inflammatory biomarkers) in early lactation. In this study we found a significant correlation between NAb IgG binding KLH and haptoglobin in plasma ($P<0.01$). However, there were no correlations between albumin, cholesterol and NAb (IgG and IgM) binding KLH. **Conclusion:** This study demonstrate that cows with no dry period have an improved energy balance and maintained the level of natural antibodies in plasma. Moreover, IgG titers in plasma might be correlated with haptoglobin due to inflammation during calving until 2 wk postpartum.

Keywords: Continuous milking, Inflammation, Antibodies

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INTRODUCTION

During transition period, immune status in dairy cows were suppressed and need to be increased. It is known that dairy cows are characterized with immune suppression during transition period, which is related with severe negative energy balance (EB), and high rate of infection diseases and metabolic disorders (8). Innate immunity is the first line defense against infection (1), and natural antibodies (NAb) are a part of humoral innate immunity before get any antigenic stimulation (2). CD5+ B-1 cells produce natural antibodies in healthy individuals and NAb mainly consist of immunoglobulin M (IgM), IgG and IgA (3,4). In previous research, NAb binding keyhole limpet hemocyanin (KLH) were higher in cows with an improved EB in early lactation (7). Transition period is the crucial time for dairy cows especially in the first two weeks after calving. In early lactation, cows experienced negative EB, which is related to immunosuppression (9). Negative EB was not only related to NAb but also was associated with enhanced level of inflammatory biomarkers (10) and metabolic disorders (11) in dairy cows during early lactation.

In early lactation, increased disease rates are commonly reported among high-yielding dairy cows and characterized by the occurrence of an inflammatory response indicated by acute phase protein (APR) (12). Inflammation evokes white blood cells (WBC) to release of tumor necrosis factor-alpha (TNF-α) and (interleukin-1 and -6) (IL 1 or 6). As a consequence, TNF-α and IL-1 or 6 triggered the release of acute phase response (13). During the response of acute phase protein, positive acute phase reactants (+AP) including haptoglobin and ceruloplasmin were increased in plasma and negative acute phase reactants (-AP) including cholesterol and albumin were reduced in plasma (13,14).

Cows with no dry period had better energy and metabolic status (15), however these cows had higher ceruloplasmin and oxidative stress compared with cows with a 60-d dry period (10). In an earlier study, cows with no dry period had a higher plasma NAb (IgG) binding liposaccharide (LPS), and higher NAb (IgG and IgM) binding KLH and LPS in milk compared with cows with a short or conventional dry period (16). The relationship between inflammatory biomarkers and NAb titer in plasma during the first two weeks after calving in cows with no dry period are less known. The objective of this study was to determine the association between plasma NAb and inflammatory biomarkers (haptoglobin, ceruloplasmin, albumin and cholesterol) in the first two weeks after calving in cows with no dry period.

MATERIALS AND METHODS

Animals and Experimental Design

All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of Wageningen University. The registration number of the experimental protocol was 2010026. The experimental design was described in our earlier study (11). In present study, we investigated data of inflammatory biomarkers and NAb titers from cows with no dry period from earlier study (10). Holstein-Friesian dairy (n=55) were selected from the Dairy Campus research herd. Cows were housed in a freestall with slatted floor and cubicles. Cows were milked twice daily (0500 and 1630 h).

Rations

Ration composition was described earlier (11). Prepartum, cows with no dry period received a lactating cow ration supporting 25 kg of milk yield per day. Forage composition during prepartum and postpartum-treatments consisted of grass silage, corn silage, wheat straw, and a protein source with different ratio. Rations were isocaloric. Concentrate and forage were supplied separately and provided adlibitum.

Blood Sampling

Blood samples were taken from all cows (n=55) in the morning from the tail vein at week 1 and 2 postpartum. Blood samples collected in evacuated tubes containing litium-heparin and immediately put on ice. All blood samples were centrifuged at 3,000 × g for 15 min at 4˚C., frozen, and stored as plasma at -20˚C until use.

Laboratory Analysis

Natural antibody titers binding Megathura crenulata-derived KLH (Sigma, H7017 Sigma Aldrich Co., St Louis, MO) in plasma of cows were measured by an indirect enzyme-linked immunosorbent assay (ELISA) technique as outlined in previous study (16). In brief, titers of natural antibodies in plasma of IgG and IgM isotype were detected using 1:20,000. IgG and IgM were detected using diluted sheep anti-bovine IgG-heavy chain conjugated to horseradish peroxidase (Cat. No. E10-118P, Bethyl Laboratories) and rabbit anti-bovine IgM-whole molecule conjugated to horseradish peroxidase (Cat. No. A10-100P, Bethyl Laboratories), respectively. During ELISA, both IgG and IgM used four-step serial dilutions and started with the ratio 1:40. After washing, a substrate containing tetra methyl benzidine (TMB from Sigma) and 0.05% distillated water was added. The plates were incubated for 10-15 minutes at room temperature and the reaction was stopped by adding 1.25 M sulfuric acid. To measure the extinctions of the titers of IgG and IgM, a Multiskan reader with a wavelength of 450 nm was used.

Inflammatory biomarkers were measured using a clinical auto-analyzer (ILAB 650, Instrumentation Laboratory, Lexington, MA, USA). In current study, the level of total cholesterol, albumin, haptoglobin and ceruloplasmin was determined with the method described and were standardized for each assay (21).

Statistical Analysis

Data were analyzed using the Statistical Analysis System (SAS, version 9.4). To assess associations of NAb (IgG and IgM) titers binding KLH with inflammatory biomarkers (haptoglobin, ceruloplasmin, albumin and cholesterol), the titers from week one and two were added as a linear covariate to developed statistical logistic regression model. The regression coefficient (β) from the statistical model and the p-value corresponding to the β are displayed.

RESULTS

Inflammatory biomarkers and natural antibodies

In the current study, cows with no dry period were investigated for the associations between inflammatory biomarkers and natural antibodies in plasma in the first two weeks postpartum. Earlier study found that cows with no dry period in present study compared with a short or a conventional dry period had higher cholesterol, higher ceruloplasmin and tended to have higher haptoglobin levels in plasma in early lactation (10). The increase of haptoglobin levels in plasma was earlier related with high production of liver macrophages (known as Kupffer cells) during inflammation (22-24). Previous studies showed a positive relationship between plasma ceruloplasmin levels and clinical health problems in cows in early lactation (25-27).

Cows in the current study not only had higher inflammatory biomarkers (haptoglobin and ceruloplasmin) but they also showed increased NAb binding KLH in early lactation (16). Our earlier study showed that the higher NAb binding KLH was associated with mammary health in early lactation. The levels of inflammatory biomarkers and plasma antibodies binding KLH for cows with no dry period in the first two weeks after calving are shown in Fig 1, 2 and 3.

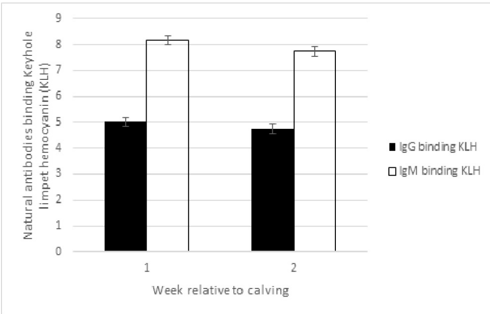

Fig. 1 : Natural antibodies for isotype IgG and IgM binding keyhole limpet hemocyanin in plasma of cows with no dry period in the first two weeks after calving . Values represent means ± SEM.

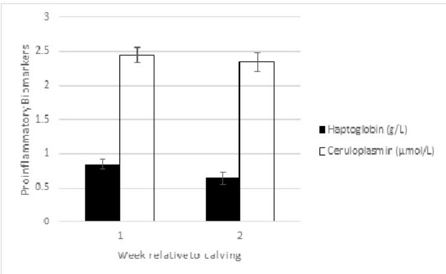

Fig. 2 : Proinflammatory biomarkers for haptoglobin and ceruloplasmin in plasma of cows with no dry period in the first two weeks after calving. Values represent means ± SEM.

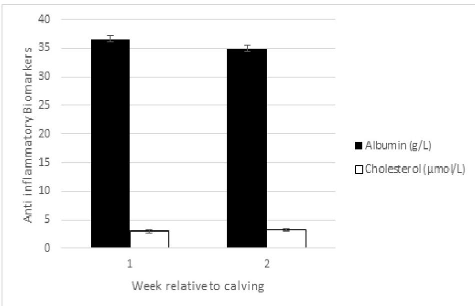

Fig. 3 : Antiinflammatory biomarkers for albumin and cholesterol in plasma of cows with no dry period in the first two weeks after calving. Values represent means ± SEM.

The association between inflammatory biomarkers and natural antibodies

The association between inflammatory biomarkers and NAb binding KLH in cows with no dry period in the first two weeks postpartum are shown in Table I. In the current study, we found a positive relationship between NAb IgG and haptoglobin levels in plasma (β = 0.97, *P* = 0.03). The increased plasma NAb IgG binding KLH levels were accompanied by an increased plasma haptoglobin levels. In addition, our study indicated that the increased plasma NAb IgG binding KLH levels tended to be related with increased ceruloplasmin levels (β = 0.45, *P* = 0.11), at least in the first two weeks after calving. The positive association between IgG and (+) acute protein response could be related with a severe inflammatory condition (14) of cows with no dry period in early lactation. In the current study, we did not find any association between IgM binding KLH with inflammatory biomarkers.

Table I : Regression coefficient (β) and *P*-value of plasma natural antibodies (NAb) binding keyhole limpet hemocyanin (KLH) related to haptoglobin, ceruloplasmin, albumin and cholesterol in dairy cows with 0-d dry period in the first two weeks after calving

Variable	IgG binding KLH		IgM binding KLH	
	β	*P*-value	β	*P*-value
Haptoglobin (g/L)	0.97	0.03	0.4	0.17
Ceruloplasmin (µmol/L)	0.45	0.11	-0.06	0.74
Albumin (g/L)	-0.01	0.82	-0.03	0.46
Cholesterol (µmol/L)	0.18	0.35	0.03	0.79

DISCUSSION

In the current experiment, cows with no dry period had improved EB and lower daily milk yield with similar dry matter intake (15). In addition, cows with no dry period had higher NAb titers (16) and inflammation status. It was suggested that the higher NAb titers for cows in plasma with no dry period are related with the improved EB (16). In our earlier study, specific plasma NAb were associated with high somatic cell count (SCC) and clinical mastitis. Our earlier study showed that "increasing plasma NAb titer for IgM binding KLH in the week before the occurrence of high SCC were associated with a decreased odd of high SCC occurrence. Moreover, increasing titers of IgM binding KLH or LPS in plasma in the three weeks before the incidence of the disease was associated with decreased odds of CM occurrence" (10 p. 8). It was suggested that NAb levels in plasma or in milk, may be an additional health biomarker to select for mastitis resistance in dairy cows (28,29).

In the current study, high SCC in cows with no dry period was not associated with inflammatory biomarkers (10). There were several causes for inflammation during transition period such as differentiation of mammary gland cell and high oxidative stress (30). A previous study showed that cows with subclinical mastitis (31) and clinical mastitis (32) had increased haptoglobin levels in plasma. Haptoglobin is an acute phase protein synthesized in the liver in response to inflammation (33) and it can be measured in serum. Haptoglobin binds to haemoglobin and so inhibits bacterial proliferation by reducing the availability of iron. Haptoglobin measurement has been of particular interest for detecting inflammation in cattle and dairy cows due to its virtual absence in the serum of healthy animals (34). Moreover, haptoglobin is more commonly available as a routine analysis compared with many other acute phase protein. Ceruloplasmin is plasma α-2 glycoprotein and one of the important positive acute phase protein. Ceruloplasmin plays an important role for immune system which help to transport copper in the blood by the enzymes lysyl oxidase and Cu-Zn superoxide dismutase. Moreover, ceruloplasmin involved in iron metabolism (ferroxidase) (35). As we know, copper improves immune function by acting on the levels of various enzymes mediating the antioxidant system and protects cells against oxidative damage. Low level of ceruloplasmin in plasma decreased phagocytosis and antimicrobial therefore increase inflammatory conditions (36).

In the previous study, some clinical health problems like fever, metritis, mastitis, retained placenta) were related with high levels of ceruloplasmin and a tendency for high haptoglobin levels in plasma (10). A previous study reported that clinical health problems has been associated with consequences of prolonged inflammations before calving (37). It seems that the increases of inflammatory biomarkers and NAb in plasma were correlated not only due to specific diseases or health problem but may be due to several causes of inflammation conditions in early lactation.

CONCLUSION

In conclusion, a positive correlation between haptoglobin and natural antibodies IgG binding keyhole limpet hemocyanin were found in the first weeks after calving in cows with no dry period. The association between components within immune responses showed complex cause-effect of defensive effect in the body. An inflammatory status and antibody responses attributed to negative EB should be disentangled in various subclinical and clinical health problems related with inflammation may partly explain the changes in inflammatory biomarkers in early lactation.

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