Adiponectin and its receptor genes' expression in response to Marek's disease virus infection of White Leghorns

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ABSTRACT Marek's disease virus (**MDV**) causes T-cell lymphoma in susceptible chicken and is also related to an imbalance of the lipid metabolism. Adiponectin is a circulatory cytokine secreted from adipose tissue and exerts critical metabolic functions. Although the associations between adiponectin and diseases, including lipid disorder and noncardiac vascular diseases, have been reported, little is known about the relationship between MDV infection and adiponectin. Here, we challenged white Leghorns from Marek's disease (**MD**)-susceptible and MD-resistant lines with MDV at 7 D of age and then explored the body weight and plasma lipoprotein levels at 21 D after MDV infection. Meanwhile, adiponectin and the expression of its receptors were detected using quantitative real-time PCR and Western blot. The results showed that MDV infection induced body weight loss in all the experimental birds. Meanwhile, the concentrations of total cholesterol and high-density lipoprotein were lower after the infection, although there was no significant difference ($P > 0.05$). However, the infection did not affect adiponectin circulating levels in plasma. MD-susceptible birds had much lower plasma adiponectin than MD-resistant birds ($P < 0.01$). In abdominal fat, there was no significant difference in *adiponectin* mRNA level. Still, we observed a significant decrease in adiponectin protein concentration, as well as *adipoR1* and *adipoR2*, at both mRNA and protein levels in the infected compared with the noninfected MD-susceptible chickens. In the spleen, MDV infection significantly reduced the *adiponectin* mRNA expression but increased the protein in MD-susceptible chickens, which decreased both *adipoR1* mRNA expression and protein levels. Also interestingly, the *adipoR1* mRNA expression level was significantly increased in MD-susceptible chickens in the liver after MDV infection. All findings in the present study provided interesting insights into adiponectin metabolism in chickens after MDV infection, which helps to advance the understanding of lipid metabolism in response to herpesvirus infection.

Key words: Marek's disease virus, herpesvirus infection, lipid metabolism, poultry, health

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INTRODUCTION

Marek's disease virus (**MDV**) is a highly oncogenic herpesvirus that infects chickens and causes deadly lymphoma in susceptible chickens. Herpesvirus has been shown to disturb the lipid metabolism, as it causes atherosclerotic plaque formation (Hajjar et al., 1986; Dai et al., 2017). Lipid analysis of the arterial smooth muscles in MDV-infected birds revealed a significant increase in nonesterified fatty acids, cholesterol, cholesterol esters, squalene, phospholipids, and triacylglycerol. Furthermore, excess lipid biosynthesis triggers the cellular deposition of lipid droplets in herpesvirus-infected cells (Fabricant et al., 1981; Hajjar et al., 1986; Dai et al., 2017; Boodhoo et al., 2019). In MDV-infected primary chicken embryo fibroblasts, different lipid metabolites have different expression patterns, which may increase in fatty acid synthesis or breakdown of lipids or both (Boodhoo et al., 2019).

Adiponectin is a circulatory cytokine secreted from adipose tissues and exerts critical metabolic functions. Adiponectin is an adipokine hormone, which circulates as a heavy-, medium-, and light-molecular-weight isoforms

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in mammals (Tsao et al., 2002, 2003; Waki et al., 2003). Adiponectin exerts its biological effects by binding to 2 structurally and functionally distinct G protein–coupled, seven-transmembrane receptors, adiponectin receptors 1 and 2 (Yamauchi et al.,2014). Adiponectin is the most abundant protein in human adipose tissue, and the most abundant hormone in human plasma (Arita et al., 1999), which has multiple beneficial effects on glucose utilization and insulin sensitivity, thereby aiding in the prevention of type 2 diabetes and cardiovascular diseases (Whitehead et al., 2006; Katsiki et al., 2017). As previously shown, serum adiponectin level is positively correlated with plasma high-density lipoprotein (**HDL**) cholesterol concentrations, and there are significant inverse relationships between very-low-density lipoprotein (**VLDL**) and low-density lipoprotein (**LDL**) levels as well as adiponectin (Mantzoros et al., 2005; Altinova et al., 2007; Chan et al., 2011; Tsuzaki et al., 2012; Izadi et al., 2013). Decreased plasma adiponectin levels are associated with the development of insulin resistance, type 2 diabetes, and cardiovascular diseases in humans and rodents. Adiponectin reduces lipid accumulation in macrophages (Tian et al., 2009) and prevents the conversion of macrophage to foam cells, an important step in atherogenesis (Ouchi et al., 1999; Engin, 2017). Adiponectin improves NF-kB–mediated inflammation and abates atherosclerosis progression in apolipoprotein E-deficient mice (Wang et al., 2016).

Adiponectin and its receptors were also found in avian species, which are widely expressed in peripheral tissues, such as adipose tissue, skeletal muscles, liver, diencephalon, testicles, and ovarian tissues (Ramachandran et al., 2007, 2013; Ocon-Grove et al., 2008; Zhang et al., 2017). In chicken, adiponectin plays important roles in energy homeostasis, body weight, lipid metabolism, and insulin sensitivity (Yan et al., 2013, 2014; Gamberi et al.,2016; Ruan and Dong, 2016). The characterized adiponectin protein in chicken is a predominant multimeric heavy-molecular-weight isoform that is larger than 669-kDa mass. Under reducing conditions and heating up to 70°C to 100°C, a majority of the multimeric adiponectin in chicken plasma and adipose tissue was reduced to oligomeric (64-kDa) or monomeric (30-kDa) forms or both (Hendricks et al., 2009). The plasma concentrations of macromolecule adiponectin and adipose expression levels of adiponectin and adiponectin receptors 1 and 2 were increased when the chickens had high-fat diet (Chen et al., 2018). In a study using 2 distinctly and highly inbred lines of chickens, one highly susceptible and the other relatively resistant to Marek's disease (**MD**) (Venugopal, 2000), both the adiponectin receptors and cholesterol expression patterns were characterized. Higher plasma adiponectin levels were observed in the MD-resistant line of birds than in the MD-susceptible chickens during growth (Yuan et al., 2012).

However, the relationship between the lipid metabolism and MDV infection remained unknown, especially the effects of MDV infection on adiponectin, its receptors, and cholesterol in serum. In this study, therefore, the expression of adiponectin and its receptors in response to MDV infection in MD-susceptible (**line 7_2**) and MD-resistant (**line 6_3**) chickens were investigated. We anticipated that the findings from this study would advance our understanding about the effect of MDV infection on lipid metabolism and inflammation.

MATERIALS AND METHODS

Animals and Challenge Trial

Seven-day-old specific pathogen-free chicks were sampled from the MD-resistant line 6_3 and the susceptible line 7_2, which were raised at USDA, Agriculture Research Service, Avian Disease and Oncology Laboratory at East Lansing, Michigan. The chickens from each line were divided into 2 groups: One group was inoculated intraabdominally with a partially attenuated very virulent plus strain of MDV, 648A passage 40, with a viral dosage of 500 plaque-forming units and the other group was not inoculated as control. The chickens were housed in negatively pressured biosafety level 2 isolators. Feed and water were supplied *ad libitum*. All procedures in handling, housing, feeding, sampling, and euthanization followed the Institutional Animal Care and Use Committee guidelines established and approved by the Avian Disease and Oncology Laboratory and the University of Maryland's standard animal use guidelines (R-08-62).

Phenotype Data

All the experimental chickens were weighed and then euthanized to take blood and tissue samples at 21 D after infection (**dpi**). Abdominal fat, liver, spleen, breast muscle with bone, and leg muscle with bone were collected from noninfected (n = 6) and infected (n = 5) chickens in line $6_3$and from noninfected (n = 9) and infected (n = 5) chickens in line 7_2. Then, the samples were snap-frozen in liquid nitrogen and stored at −80°C. The abdominal fat, breast muscle with bone, and leg muscle with bone over body weight ratios (BW ratios) were calculated and presented as percentages.

Plasma HDL and LDL/VLDL Cholesterol Levels

Lipoprotein concentrations were measured for noninfected (n = 5) and infected (n = 5) chickens in each line. Plasma was separated from whole blood by centrifugation at 2,000g for 20 min and kept at 4°C until analysis. Plasma total cholesterol, HDL cholesterol, and LDL cholesterol were measured using an HDL and LDL/VLDL cholesterol quantification kit (BioVision, Exton, PA).

Ingenuity Pathway Analysis

The gene expressions from microarray data were from our previous publication (Yu et al., 2011). Data

normalization and differential gene expression analysis were performed using the limma package in R. Dye bias was removed by normalizing within the array using loess normalization, and normalization between arrays was carried out using quantile normalization. We compared the *adiponectin*, *adipoR1*, and *adipoR2* expression level of the infected chickens to those of noninfected chickens at 21 dpi in lines 6_3 and 7_2. To check biological functions of adiponectin and its receptors, the ingenuity pathway analysis was conducted out for differentially expressed genes.

Quantitative Real-Time PCR Analysis

To study genes' expression, total RNA samples were extracted from spleen, abdominal fat, and liver of noninfected (n = 4) and infected (n = 4) chickens in lines 6_3 and 7_2 by using an RNeasy mini kit (Qiagen Inc., Valencia, CA). RNA quality was evaluated using a Nanodrop-2000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE). Only samples with A260/A280 ratios of 1.8-2.0 and A260/A230 ratios greater than 2.0 were included in subsequent analyses. RNAs were reverse-transcribed to cDNA using an ImProm-II Reverse Transcriptase kit (Promega, Madison, WI) following the manufacturer's instructions. The quantitative real-time PCR (qPCR) was performed using an iQ SYBR Green supermix (Bio-Rad, Hercules, CA) following the manufacturer's recommendations in a CFX96 Real-Time system (Bio-Rad, Hercules, CA). The primers used to amplify *adiponectin*, *adipoR1*, *adipoR2*, and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) are given in Supplementary Table 1. *GAPDH* was used as an endogenous control. The qPCR reaction program was 95°C for 10 min, 40 cycles of 95°C for 15 s, 57°C for the 30 s, and 70°C for 30 s, with a melting curve analysis (65°C–95°C) in the last cycle to evaluate amplification specificity. All samples were analyzed in triplicate and normalized to *GAPDH* mRNA levels. The expression of genes was calculated via the $2^{-\Delta\Delta\ Ct}$ method.

Western Blot Analysis

Protein was extracted from spleen, abdominal fat, and liver of noninfected (n = 2) and infected (n = 2) chickens in lines 6_3 and 7_2. The samples were incubated with ice-cold lysis buffer (10 mM Tris-HCl, 150 mM NaCl, 1% NonidetP-40, 0.5% sodium deoxycholate, 0.1% SDS) containing phenylmethylsulfonyl fluoride and centrifuged at 4°C for 30 min at 12,000 × *g*. Protein concentration was quantified using a BCA Protein Assay Kit (Novagen, Madison, WI). The protein samples were stored at −80°C for subsequent analyses. Protein samples were prepared for electrophoresis by heating with SDS-PAGE Laemmli buffer (50 mmol Tris-HCl, 10% glycerol, 2% SDS, 0.05% bromophenol blue, pH 6.8) containing 10% β-mercaptoethanol (Promega, Madison, WI) for 10 min at 100°C. Chicken plasma samples (0.5 μL) from noninfected (n = 3) and infected (n = 3) chickens in each line were used directly for Western blot analysis to determine plasma adiponectin levels. Each tissue protein sample (20 μg) was subjected to SDS-PAGE analysis with 10% separating gel and 4% stacking gel. Proteins from the SDS gel were transferred onto polyvinylidene difluoride (**PVDF**) membranes, and the membranes were blocked overnight at 4°C. The PVDF membrane was incubated with specific primary antibody (rabbit antichicken adiponectin, adipoR1, adipoR2, and β-actin) for 1 h at 37°C on a shaker platform (Hendricks et al., 2009; Tiwari et al., 2015). After 3 washes for 5 min each in a 0.05% TBS-Tween-20 solution, the PVDF membrane was incubated with an appropriate secondary antibody donkey antirabbit IgG for 1 h at 37°C. The membranes were then incubated with enhanced chemiluminescence detection reagent (GE Healthcare, Piscataway, NJ). Anti–β-actin antibody was used as control. The adiponectin, adipoR1, adipoR2, and β-actin primary antibodies were diluted to 1:40,000, 1:2,000, 1:2,000, and 1:1,000 before use, respectively. The secondary antibodies were diluted to 1:5,000. The relative gray-scale of protein bands was measured by using Image J (version 1.52q; https://imagej.nih.gov/ij/index.html).

Statistical Analysis

The statistic estimates are presented as mean ± standard deviation. The data were analyzed with 2-way ANOVA followed by Tukey's test for comparison among different groups using SAS (version 9.4; SAS Institute Inc., Cary, NC).

RESULTS

MDV-Infection Impact on Phenotypic Characteristics

To check the influence on BW and tissue-to-BW ratios, phenotypic data analyses were assessed on the differences between the noninfected and infected groups in each of the 2 lines of chickens. Our data showed that MDV infection might have hindered body weight gain. Still, the difference between the infected (286.38 ± 21.07 g) and noninfected group (358.40 ± 19.24 g) was not statistically significant in line 6_3 ($P > 0.05$). In line 7_2, the body weight of the infected group (194.26 ± 21.07 g) was significantly lower than that of the noninfected group (353.30 ± 15.71 g), as depicted in Figure 1 ($P < 0.01$). No significant difference was detected statistically in abdominal fat-to-BW ratio of noninfected or infected bird groups between line 7_2 and line 6_3 ($P > 0.05$). The abdominal fat-to-BW ratio, leg muscle with bone-to-BW ratio, and breast muscle with bone-to-BW ratio were not significantly different between the infected and noninfected chickens of each line ($P > 0.05$).

Plasma Lipoprotein Levels

The total cholesterol levels were not significantly different between noninfected and infected chickens in line 6_3 or line 7_2 ($P > 0.05$). However, it is noted here

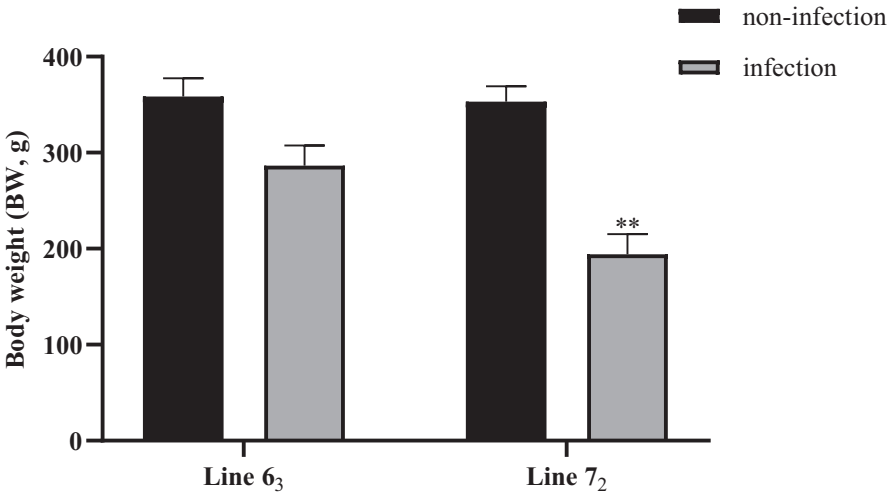

Figure 1. Body weight for chickens after MDV infection. Body weight were measured for noninfected (n = 6) and infected (n = 5) chickens in line 6_3, and for noninfected (n = 9) and infected (n = 5) chickens in line 7_2.

that the line 7_2 had higher total and HDL cholesterol concentrations than those of line 6_3 in both noninfected and MDV infection group ($P < 0.01$; Figure 2A). In addition, although no significant difference in the concentration of LDL + VLDL cholesterol was detected between noninfected and infected chickens in both lines ($P > 0.05$), it can be seen that MDV infection caused the different direction of change in LDL + VLDL cholesterol level in line 6_3 and 7_2, which means a decrease of LDL + VLDL concentration in line 6_3 (0.085 ± 0.015 μg/μL vs. 0.074 ± 0.004 μg/μL) but an increase in line 7_2 (0.087 ± 0.014 μg/μL vs. 0.100 ± 0.011 μg/μL) (Figure 2A). Moreover, an analysis of lipoprotein ratios also revealed that MDV infection induced a slight decrease in the LDL + VLDL ratio and a slight increase in the HDL ratio in line 6_3, whereas the opposite change in the cholesterol ratios was observed in line 7_2 (Figure 2B).

Expression of Adiponectin and Its Receptors From Microarray Analysis

We examined the expression levels of *adiponectin*, *adipoR1*, and *adipoR2* at 21 dpi using the published microarray analysis data (Yu et al., 2011). The data showed that the 3 genes had no discernable alteration in response to MDV infection in the spleen of line 6_3 birds ($P > 0.05$). However, a significant decrease in *adiponectin* expression ($P < 0.05$) and an increase in *adipoR2* expression ($P < 0.05$) were detected in line 7_2 birds (Table 1).

Biofunction Categories Associated With Adiponectin and Its Receptors

Compared with genetic categories in the ingenuity pathway analysis database, the differentially expressed genes were categorized according to biological functions. As adiponectin possesses antiatherogenic, antidiabetic, and anti-inflammatory properties (Tilg and Moschen, 2006), we investigated the alterations of 6 biofunction categories associated with adiponectin functional properties during MDV infection, such as cardiovascular system development and function, cardiovascular disease, inflammatory response, inflammatory disease, lipid metabolism, and metabolic disease (Table 2). All 6 categories were enriched ($P < 0.01$) after viral infection in MD-susceptible and MD-resistant lines of chickens. Moreover, the biofunction category "lipid metabolism" was represented in a similar level in both lines of chickens. However, the other categories, especially "cardiovascular system development and function" and "cardiovascular disease", were overrepresented in line 7_2, as compared to line 6_3.

qPCR Analysis for Adiponectin and Its Receptor Genes

To validate the data mentioned previously, *adiponectin*, *adipoR1*, and *adipoR2* expression in spleen were quantified by qPCR. As shown in Figure 3A, there were no significant differences in expressions of the 3 genes between the noninfected and infected group in line 6_3, which was consistent with the results of the microarray data. As for line 7_2, a similar trend of microarray and qPCR results was found for *adiponectin* and *adipoR2*, indicating downregulation of *adiponectin* and upregulation of *adipoR2* after MDV infection. However, it should be noted that after MDV infection, adiponectin in line 7_2 showed much lower expression level than that in microarray analysis (Table 1, Figure 3A). In addition, the expression level of *adipoR1* showed no obvious difference in microarray data, whereas it showed significant decrease in RT-qPCR data in line 7_2 after MDV infection ($P < 0.01$). As adiponectin is primarily synthesized by adipocytes, we next measured their expression levels in abdominal fat and liver by qPCR. The results revealed no significantly different expressions between noninfected and infected groups in line 6_3 for *adiponectin* in abdominal fat and *adipoR1* and *adipoR2* in abdominal fat and liver. However, in line 7_2, there were significantly

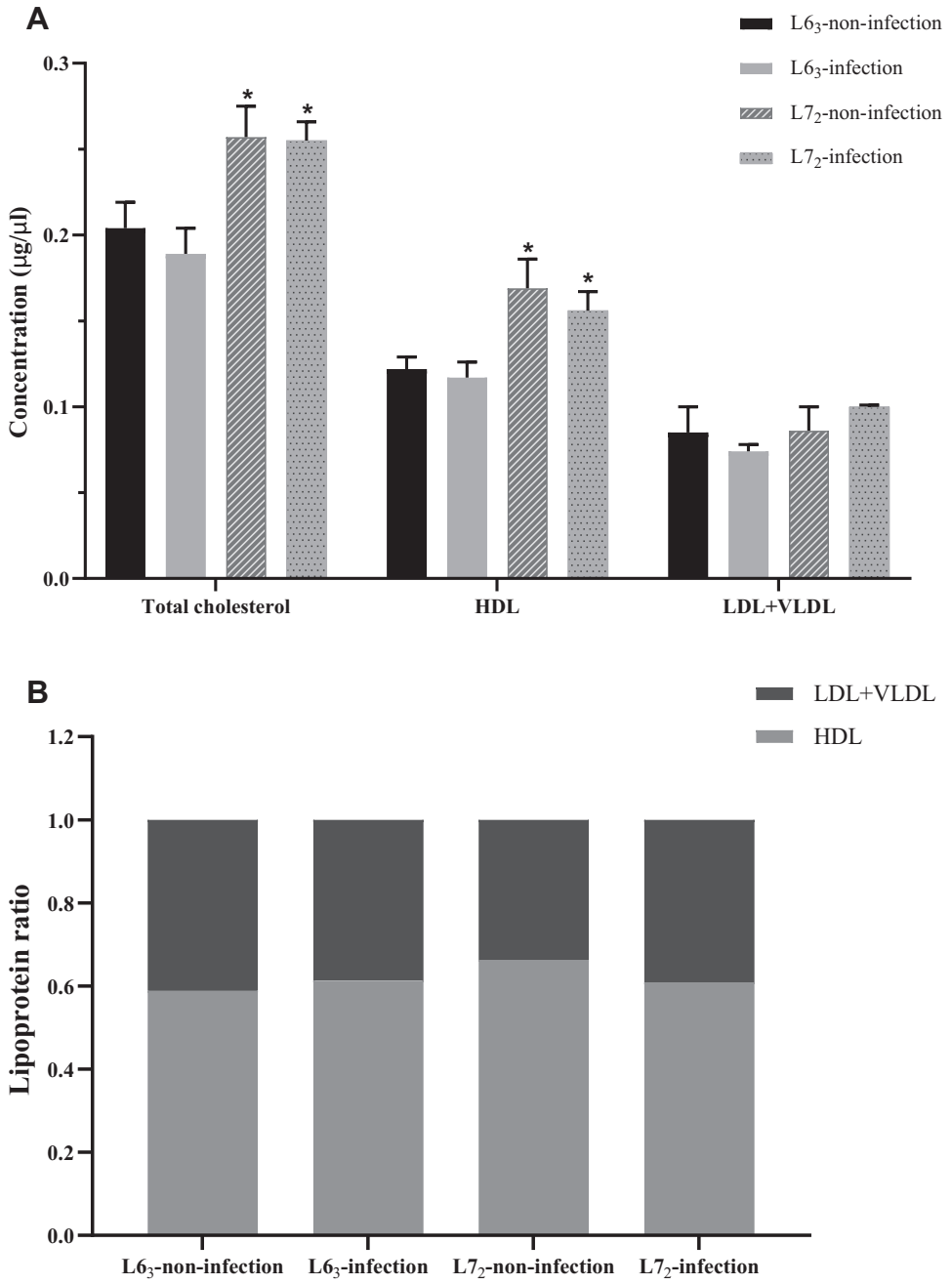

Figure 2. Plasma lipoprotein status for chickens after MDV infection. (A) Plasma lipoprotein concentration (μg/μL). (B) Plasma lipoprotein ratio. Lipoprotein concentrations were measured for noninfected (n = 5) and infected (n = 5) chickens in each line. Total cholesterol (μg/μL) = HDL cholesterol + (LDL + VLDL) cholesterol. *$P < 0.05$.

decreased expressions of *adipoR1* and *adipoR2* in abdominal fat ($P < 0.01$) and increased expressions of *adipoR1* in the liver ($P < 0.05$) after MDV infection. In addtion, no alterations were detected for *adiponectin* in abdominal fat and *adipoR2* in the liver in line 7_2 after viral infection (Figure 3B).

The Protein Concentration of Adiponectin and Its Receptors

We first detected the adiponectin protein level in plasma. The results indicated that only a 30-kDa monomer of adiponectin was yielded, and viral infection had no detectable effect on adiponectin level in both lines, although line 7_2 had obviously lower adiponectin level in plasma than line 6_3 regardless of MDV infection (Figure 4A). In abdominal fat also, only 30-kDa monomeric isoform was detected, and it was significantly decreased in the MDV infected group compared with that in the noninfected group of line 7_2 birds ($P < 0.05$) (Figure 4B). In the spleen, both 64-kDa adiponectin oligomeric and 30-kDa monomeric isoforms were observed. There was no significant difference in each of the 2 isoforms between noninfected and infected groups in line 6_3. However, there was a notably increased expression of 30-kDa monomeric isoform in line 7_2 infected group (Figure 4C).

Table 1. *Adiponectin* and its receptors' expression in microarray analysis.

Genes	Gene expression log_2fold change at 21 d after MDV infection in each line (infection/noninfection)	
	Line 6_3	Line 7_2
Adiponectin	−0.372	−0.946*
AdipoR1	−0.122	0.109
AdipoR2	0.229	0.997*

The number represent the log_2fold change of gene expression after MDV infection. The numbers >0 means the expression level is increased after MDV infection, while the numbers <0 means the expression level is decreased after MDV infection. * Represents statistically significant ($P < 0.05$).

Meanwhile, the protein concentration of adipoR1 and adipoR2 in response to MDV infection had no notable difference in line 6_3 or line 7_2 ($P > 0.05$) (Figure 4B). In the spleen, the results showed that MDV infection led to a significant decrease in adipoR1 concentration in line 7_2 ($P < 0.05$), but not in line 6_3. No significant difference in adipoR2 expression was found among all 4 groups (Figure 4C). In the liver, there was no significant difference detected in both lines after MDV infection (Figure 4D).

DISCUSSION

MDV is an *Alpha herpesvirus* that causes a deadly lymphoproliferative disease in chickens (Benditt et al., 1983; Jarosinski et al., 2006). Interestingly, besides the transformation of CD4+ T cells, MDV causes atherosclerosis by disturbing the lipid metabolism in infected birds (Benditt et al., 1983). The associations between adiponectin and lipid disorder and noncardiac vascular diseases have been reported (Katsiki et al., 2017). However, little is known about the effect of MDV infection on adiponectin, its receptors, and cholesterol in serum. The present study compared phenotypic characteristics, plasma lipoprotein levels, adiponectin, and its receptor expression patterns in MD-susceptible and MD-resistant chickens in response to MDV infection. One of the hallmarks upon MDV infection is bodyweight loss (Morimura et al., 1996). The results of this study again demonstrated that BW was decreased in the infected groups regardless of MD-susceptible or MD-resistant chickens. However, it is noted here that the MD-susceptible chickens suffered a more serious impact on BW loss than the resistant birds in response to MDV infection.

Table 2. Biofunction categories associated with *adiponectin* and its receptors.

Terms	*P* value	
	Line 6_3	Line 7_2
Cardiovascular system development and function	3.85E-03	7.40E-10
Cardiovascular disease	5.41E-05	2.46E-07
Inflammatory response	8.04E-05	7.07E-06
Inflammatory disease	5.54E-04	2.57E-08
Lipid metabolism	1.21E-04	1.45E-04
Metabolic disease	3.29E-05	2.80E-08

The biofunction categories were from ingenuity pathway analysis database.

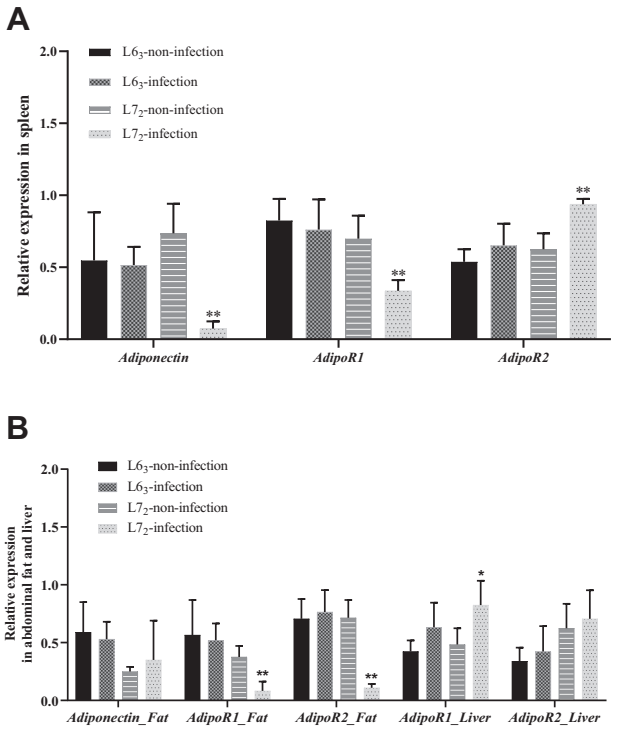

Figure 3. *Adiponectin*, *adipoR1*, and *adipoR2* mRNA levels after MDV infection. (A) mRNA Levels in spleen. (B) mRNA Levels in abdominal fat and liver. The mRNA levels were measured by qPCR for noninfected and infected chickens in lines 6_3 and 7_2, respectively (n = 4). *$P < 0.05$, **$P < 0.01$.

In physiological conditions, HDL plays a critical role in the development of atherosclerotic lesions by several mechanisms. HDL removes the excess cholesterol from peripheral tissues, including the arterial wall, and delivers it to the liver for excretion into the bile (Barter et al., 2007; Drew et al., 2012; Rohrl and Stangl, 2013; Marques et al., 2018). The line 7_2 birds had significantly higher total cholesterol and HDL concentration than line 6_3 regardless of MDV infection ($P < 0.01$). Meanwhile, MDV infection caused a decrease of LDL + VLDL concentration in line 6_3 but an increase in line 7_2. The analysis of lipoprotein ratios also revealed that MDV infection induced a slight decrease in the LDL + VLDL ratio and a slight increase in the HDL ratio in line 6_3, whereas the opposite change in the cholesterol ratios was observed in line 7_2, which suggested that the MDV-susceptible and MDV-resistant lines might have different lipid metabolism patterns because of their different genetic background (Luo et al., 2012; Mitra et al., 2012; Perumbakkam et al., 2013; Cheng et al., 2015; Xu et al., 2018).

As the lipid metabolism disorder was also proposed as an inflammatory disease (Ross, 1999; Geovanini and Libby, 2018) and spleen plays an important role in the inflammatory response and the pathogenesis of MD (Schat, 1981), the genes expressed in the spleen at 21 dpi were analyzed using our published microarray data (Yu et al., 2011); no discernable alteration in *adiponectin* and its receptors was found in the spleen of line 6_3 in

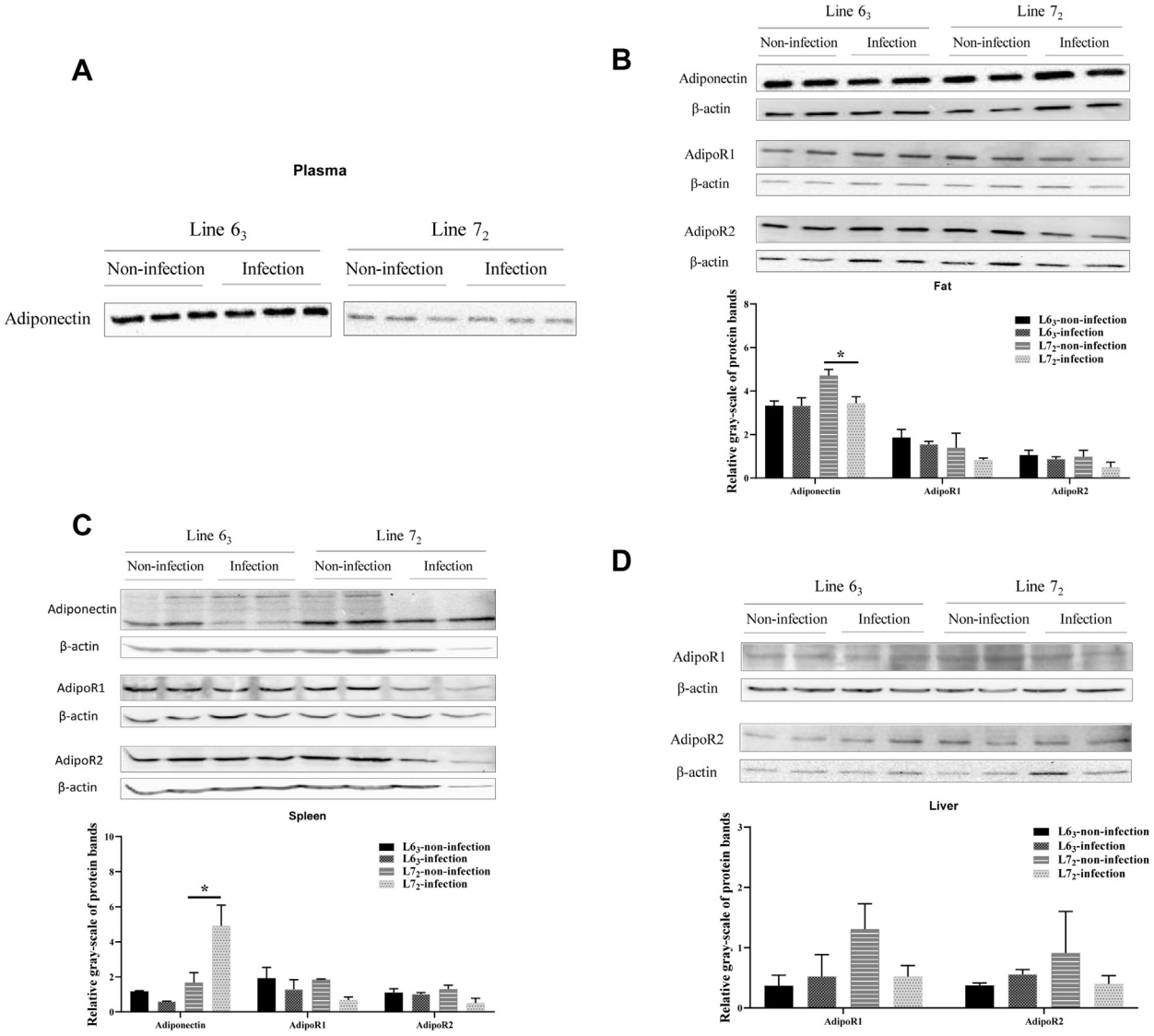

Figure 4. Adiponectin, adipoR1, and adipoR2 protein levels after MDV infection. (A) Adiponectin in plasma (n = 3). (B) Adiponectin, adipoR1, and adipoR2 in abdominal fat (n = 2). (C) Adiponectin adipoR1, and adipoR2 in the spleen (n = 2). (D) AdipoR1 and adipoR2 in the liver (n = 2). $^*P < 0.05$.

response to MDV infection ($P > 0.05$). However, line 7_2 showed a significant decrease in *adiponectin* expression and an increase in *adipoR2* expression ($P < 0.01$) at 21 dpi, which indicated that *adiponectin* and its receptors might be involved in the process of herpesvirus. Previous reports showed that adiponectin signaling, which is involved in glucose and lipid metabolism, regulates energy homeostasis and interacts with insulin signaling in mammals (Yamauchi et al., 2002; Ryu et al., 2014). Adiponectin deficiency leads to increased oxidative stress and inflammation (Lee et al., 2012; Wang et al., 2016). The function and pathway analysis of the microarray data showed that MDV infection led to a high enrichment of adiponectin associated with cardiovascular system development and function, cardiovascular disease, inflammatory disease, and metabolic disease. MDV infection might affect several biofunctions associated with atherosclerosis, and *adiponectin* and its receptors may play an important role in MD-susceptible line during MDV infection. However, the relationship between MDV infection and the roles of adiponectin need to be further studied and verified. Circulating adiponectin concentrations were proposed to have a crucial role in the pathogenesis of atherosclerosis and cardiovascular diseases associated with obesity and metabolic syndrome (Arita et al., 1999; Funahashi et al., 1999; Ghadge et al., 2017). We measured the plasma adiponectin in response to MDV infection in 2 chicken lines. In our model, MDV infection did not affect adiponectin circulating levels in plasma. However, line 7_2 had a greatly reduced plasma adiponectin level compared with that of line 6_3 regardless of MDV infection, and both lines showed an opposite pattern in the concentration of total cholesterol and HDL. Our results in chicken were not in total agreement with reports that plasma adiponectin was shown a strong positive correlation with HDL level in mammals (Matsubara et al., 2002; Bansal et al., 2006; Chan et al., 2009; Fujiwara et al., 2009; Geloneze et al., 2009; Kawamoto et al., 2011). We hypothesize that adiponectin signaling in chickens may bear some unique

differences in compositions and functions of adiponectin signaling in contrast to mammals. Furthermore, we detected the expression pattern of *adiponectin*, *adipoR1*, and *adipoR2* in different tissues. In abdominal fat, our data demonstrated that there was no significant difference in *adiponectin* mRNA level but a significant decrease of protein concentration in the infected group compared with the noninfected group of the line 7_2 chickens, which suggested that MDV infection affected the expression of adiponectin on protein level other than mRNA level. The decrease of adiponectin concentration in fat after MDV infection might lead to a reduction in insulin-stimulated glucose uptake via inhibition of AMP-activated protein kinase in adipocytes (Wu et al., 2003; Wang et al., 2007; Yamauchi et al., 2007). Meanwhile, the MDV infection resulted in a decrease of the adipoR1 and adipoR2 at mRNA and protein levels in line 7_2. The decline in *adipoR1* and *adipoR2* expression should lead to a decrement in adiponectin binding to the cell membrane, and this turns into attenuation in the adiponectin effects (Engin, 2017). In the spleen, both 64-kDa adiponectin oligomeric and 30-kDa monomeric isoforms were found, and MDV infection significantly reduced the mRNA expression level but increased the protein level in line 7_2. The increase of adiponectin in spleen might trick an increase in gene expression of the anti-inflammatory factors (Yokota et al., 2000; Ouchi et al., 2001; Wang et al., 2016), which need to be further investigated in chicken. Perhaps, different multimerized forms of adiponectin in various molecular weights in response to MDV infection might be different, even opposite, in the spleen of chickens. In contrast, the viral infection resulted in a discernable decrease in *adipoR1* mRNA and protein expression levels. In the light of that adipoR1 is of high affinity for globular adiponectin but low affinity for the full-length protein of adiponectin (Yamauchi et al., 2003, 2007) and may be associated with activation of AMP-activated protein kinase pathway and NF-kB pathway (Ouchi and Walsh, 2007; Yamauchi et al.,2007; Wang et al., 2016). In addition, *adipoR1* is widely expressed in various tissues, whereas *adipoR2* is abundantly expressed in the liver (Yamauchi et al., 2007). MDV infection did not affect the expression levels of *adipoR2*, which significantly increased the expression of *adipoR1* at the mRNA level, yet there was no difference in adipoR1 protein. Based on these results, we speculate that the possible effects of viral infection on the adiponectin pathway in various tissues could be different.

In conclusion, our findings of the present study implied that MDV infection affects the expression of *adiponectin* and its receptors, which may influence lipid metabolism and inflammation and may distinctly differ between different genetic lines of chicken. However, the full biochemical mechanism warrants further investigations.

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Conflict of Interest Satatement: The authors did not provide a conflict of interest statement.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.psj.2020.06.004.

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