Plasma Lipid Profiles and Redox Status are Modulated in a Ketogenic Diet-Induced Chicken Model of Ketosis

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Ketone bodies such as β-hydroxybutyrate and acetoacetate have physiological functions in addition to being used as an energy source. In order to assess the effect of elevated ketogenesis on blood lipid profiles and redox status, a ketogenic diet (KD), a high-fat, low-carbohydrate, and low-protein diet, was fed to chicken for 4 weeks. Plasma β-hydroxybutyrate, but not acetoacetate, concentrations were significantly increased by KD feeding for 2 and 4 weeks. The KD also induced elevation of plasma non-esterified fatty acid (NEFA) and total cholesterol concentrations, whereas plasma triglyceride concentration was decreased. Plasma total antioxidant activity in chicken with ketosis induced by KD was lower than that of the control. However, the level of plasma TBARs, an oxidative stress marker, was also reduced by KD feeding. A reduction of energy intake was observed in chickens fed the KD; therefore, the effect of a restricted diet (RD) was also investigated. Plasma β-hydroxybutyrate concentration, lipid (total cholesterol and NEFA) concentration, and redox status were not affected by RD feeding. These data suggest that a high-fat, low-carbohydrate, and low-protein diet induces ketosis by elevating blood β-hydroxybutyrate concentration in chicken. Under conditions of ketosis induced by the KD, total antioxidant capacity was reduced along with a modulation of the blood lipid profile in chicken.

Key words: antioxidant activity, ketone body, ketogenic diet, lipid concentration


Introduction

Ketone bodies such as acetoacetate and β-hydroxybutyrate are produced primarily in the liver as a result of the incomplete oxidation of long chain fatty acids (McGarry and Foster, 1980). They readily serve as fuel for extrahepatic tissues, including the brain, skeletal muscles, intestines and kidneys. Enhancement of ketogenesis, defined as ketone body formation, leading to high ketone body concentrations in the blood, occurs under a variety of physiological and pathological conditions in mammals, including food deprivation, high-fat diets, and diabetes (Robinson and Williamson, 1980); therefore, ketone bodies are thought to be used as an energy source under these conditions. High ketone body concentrations in the blood are also observed in chicken, where it has been shown that blood ketone body concentration is high at around the time of hatching, compared to 14-day-old chicks (Linares et al., 1993; Ohtsu et al., 2003). Thereby, it appears that ketone bodies can also be utilized as fuel in extrahepatic tissues in chicken under these conditions.

In addition to their function as an energy source, ketone bodies have specific effects on physiological status and metabolism. For example, ketone bodies directly inhibit hepatic glucose production, adipocyte lipolysis (Henry et al., 1990), glucose utilization by extrahepatic tissues (Robinson and Williamson, 1980), and glutamine metabolism in the small intestine (Hanson and Parsons, 1978) and kidneys (Lemieux et al., 1980) in mammals. Moreover, in chicken, it has been reported that ketone bodies modulate protein turnover (Wu and Thompson, 1990) and amino acid metabolism (Wu and Thompson, 1988) in the skeletal muscles.

The ketogenic diet (KD), a high-fat, low-carbohydrate, and low-protein diet, induces ketosis by enhancing of ketogenesis in mammals (Kennedy et al., 2007). KD has been used in the treatment of refractory childhood epilepsy and neurological disorders (Gasior et al., 2006). The mechanism underlying KD-induced protection from these diseases has been suggested that to be the inhibition of oxidative stress and/or an increase in antioxidant capacity (Coppola et al., 2002; Gasior et al., 2006; Peterson et al., 2005; Reger et al., 2004; Sirven et al., 1999; Ziegler et al., 2003). However, there
have been several reports that KD or ketosis can induce oxidative stress under pathological conditions such as diabetes (Jain et al., 1999). Therefore, although it is thought that ketone bodies modulate redox status, the precise effects on this process remain unclear.

KD also induces weight loss or slower weight gain in mammals, despite having high lipid content (Dashti et al., 2007; Kennedy et al., 2007). In addition, since ketone bodies are mediators of fatty acid β-oxidation in the liver, the effect of KD or ketosis on plasma lipid levels and lipid metabolism has been investigated in mammals. Some (Chesney et al., 1999; Dekaban, 1966), but not all (Katyal et al., 2000; Schwartz et al., 1989), previous studies have indicated that plasma total cholesterol and triglyceride concentrations were increased under KD-induced conditions of ketosis. However, it was also shown that serum triglycerides were decreased by KD in a type 2 diabetic patient (Yancy et al., 2005). Thus, the effects of ketosis induced by KD on plasma lipid profiles are not completely understood.

The metabolism and physiological mechanisms of chicken differ from those of mammals. For instance, blood glucose levels are twice as high in chickens as those of the majority of healthy mammals, and insulin sensitivity is relatively low. Moreover, the liver is the main organ for fatty acid synthesis in chickens, whereas this process occurs in the adipose tissue in mammals (Gondret et al., 2001). Furthermore, the laying hen has more peculiarities in its metabolism, especially in lipogenesis, than the broiler chickens. Lipogenesis in the liver is highly accelerated because of estrogen, the primary female sex hormone, and blood lipid concentrations in the laying hen are extremely high compared to broilers. Therefore, it is of great interest and importance to investigate changes in physiological status, including redox status, under conditions of ketosis in the laying hen, in order to understand the basic poultry physiology. As mentioned above, in poultry, blood ketone body concentrations are high and can be used as fuel under some conditions, and it is thought that the redox status is modulated differently in response to ketosis than in mammals. Based on this evidence, we investigated the modulation of redox status and blood lipid profiles during ketosis induced by feeding chickens with a high-fat, low-carbohydrate, and low-protein “ketogenic diet”, as a model of ketosis induction in the laying hen.

Materials and Methods

All experiments in this study followed the recommendations within the Guide for the Care and Use of Agricultural Animals in Agricultural Research of the National Institute of Livestock and Grassland Science (Tsukuba, Japan). Animal use protocols were approved by the animal experiments committee at the institute. Prior to experiments, birds were housed in individual cages, fed with a commercial diet ad libitum, and allowed free access to water.

Experiment I

Twenty female White Leghorns (29-week-old) were randomly divided into 2 groups (10 replicates of 2 dietary treatments); 1) control diet (CD) corn-soybean meal (2.92 Mcal/kg ME, 17% CP), 2) high-fat, low carbohydrate, and low protein ketogenic diet (KD) (Table 1). Birds were fed ad libitum and allowed free access to water. Two and 4 weeks after starting the experimental diets, blood samples were collected from 5 birds randomly selected from each group.

Experiment II

In experiment I, the energy intake of the KD group was about 55% of that of animals fed the CD (data not shown). Therefore, a restricted feeding (RD) group was added in experiment II, in order to control for the effect of reduced energy intake in the KD group. Thirty birds were divided into 3 groups randomly; 1) CD, 2) KD, and 3) restricted feeding of the CD (RD) group. Birds in the RD group were given 194kcal of the CD each day. Blood was collected from 5 birds randomly selected from each group. Two and 4 weeks after starting the experimental diets, blood samples were collected from 5 birds from each group at 2 and 4 weeks of treatment.

Measurement of Ketone Bodies

Plasma acetoacetate and β-hydroxybutyrate concentrations were measured using a commercial kit (Sanwa Kagaku kenkyusyo Co., Ltd., Nagoya), based on the method described by Williamson et al. (1974a, 1974b). Plasma glucose, Non-esterified fatty acid (NEFA), triglyceride and total cholesterol concentrations were measured using commercial assay kits (Wako Chem., Osaka).

Total Antioxidants Capacity

Plasma total antioxidant capacity was determined using a commercial assay kit, called “PAO” (NIKKEN SEIL Co., Tokyo).

Lipid Peroxidation (TBARS)

Plasma lipid peroxidation was measured by thiobarbituric acid reactive substance (TBARS) (Yagi, 1976). Briefly, 4.0 ml H2SO4 (12 N) and 0.5 ml phosphor tungstic acid (10%
w/v) were added to 20 μl plasma sample and incubated for 5 min at room temperature. Samples were centrifuged and the supernatant was removed. Pellets were then dissolved in 2.0 m/ H₂SO₄ (12 N), and 0.3 m/ phosphotungstic acid (10% w/v) was added. After centrifugation of samples, pellets were dissolved in 4.0 m/ H₂O and 1.0 m/ TBA reagent (0.34 % w/v 4, 6-dihydroxy-2-mercaptopyrimidine in 25% v/v acetic acid) and incubated at 95°C for 60 min. Samples were then cooled, 5.0 m/ n-butanol added and mixed well. After centrifugation, the n-butanol phase was collected, and fluorescence was measured at a wavelength of 553 nm after excitation at 515 nm.

**Statistical Analysis**

The SAS application package (SAS institute, 1988) was used for statistical calculations. Data were analyzed using the Student’s t-test in experiment I, and by the GLM procedure, followed by Tukey’s multiple range test in experiment II.

**Results**

**Experiment I**

Plasma β-hydroxybutyrate concentration in the CD group was 347 ± 45 μM (average ± SE.) at 2 weeks and 463 ± 80 μM at 4 weeks after feeding the experimental diet. KD feeding increased the plasma β-hydroxybutyrate concentration significantly at 2 and 4 weeks (Fig. 1a). By contrast, acetoacetate concentration was not affected by KD feeding (Fig. 1b). Thus, the results demonstrate that a high-fat diet, low-carbohydrate, and low-protein “ketogenic diet” induced ketosis by increasing β-hydroxybutyrate concentrations in chicken.

Plasma glucose levels were not significantly different between the CD and KD groups (data not shown). NEFA concentration was significantly increased by KD (Fig. 2a), whereas plasma triglyceride concentration was markedly decreased by KD compared CD groups (Fig. 2b). KD administration for 4 weeks, but not 2 weeks, increased plasma total cholesterol concentration significantly (Fig. 2c).

Total plasma antioxidant activity (PAO) was significantly reduced in KD-fed chickens compared to CD-fed chickens after 4 weeks (Fig. 3a) and plasma TBARs was significantly reduced after both 2 and 4 weeks by KD feeding (Fig. 3b).

**Experiment II**

In experiment I, energy intake in the KD group was about 55% of that of the CD group. Therefore, in experiment II, a RD group was added to control for the effect of the reduced energy intake in the KD group. Energy intake in the RD and KD groups was approximately 59% and 85%, respectively, of that of the CD group in experiment II.

Plasma β-hydroxybutyrate concentration in the KD group was significantly higher than in both the RD and CD groups (Fig. 4). Thus, KD feeding induced ketosis irrespective of energy intake reduction in chickens.

The effects of KD feeding on plasma NEFA and triglyceride concentrations observed in experiment I were also confirmed. RD administration did not affect plasma NEFA concentration (Fig. 5a), neither did we note was a significant difference in plasma triglyceride concentrations between RD and KD groups (Fig. 5b). Plasma total cholesterol concentrations in the KD group were higher than those in the CD and RD groups after 4 weeks of treatment, but this was not significant (Fig. 5a).

RD administration for 2 and 4 weeks did not affect plasma total antioxidant activity, whereas this was reduced in the KD group compared to the CD group, as shown in experiment I (Fig. 6a). Plasma TBARs was also unaffected by RD administration, whereas it was decreased after KD administration for 2 and 4 weeks, consistent with the results of experiment I (Fig. 6b).

![Fig. 1. Effect of feeding a high-fat, low-carbohydrate, low-protein, ketogenic diet (KD), for 2 and 4 weeks on plasma β-hydroxybutyrate (a) and acetoacetate (b) concentrations in chicken. Vertical bars indicate standard error of the mean. * P<0.05 compared with control diet (CD).](image-url)
Fig. 2. Effect of feeding a KD on plasma NEFA (a), triglyceride (b) and total cholesterol (c) concentrations in chicken. Vertical bars indicate standard error of the mean. *$P<0.05$ and **$P<0.01$ compared with control diet (CD).

Fig. 3. Effect of feeding a KD on plasma total antioxidant activity (a) and TBARS (b). Vertical bars indicate standard error of the mean. *$P<0.05$ and **$P<0.01$ compared with control diet (CD).

Fig. 4. Change in plasma $\beta$-hydroxybutyrate concentration in chickens fed with a control (CD), restricted (RD), or ketogenic (KD) diet. Vertical bars indicate standard error of the mean. Means ($n=5$) with different letters (a and b) differ significantly ($P<0.05$).
Discussion

A high-fat, low-carbohydrate, and low-protein diet is known to induce ketosis in mammals. Therefore, we first investigated whether a similar diet also acts as a “ketogenic diet” in chickens by increasing plasma ketone body concentrations. Plasma β-hydroxybutyrate concentration was increased by KD administration irrespective of energy intake reduction. By contrast, acetoacetate concentration was not affected by KD administration in experiment I. Although there is limited information about the effect of KD administration on blood acetoacetate concentrations, there are reports that acetoacetate levels in blood were elevated by KD administration in rats (Al-Mudallal et al., 1996) and humans (Musa-Veloso et al., 2002). However, we and other research groups have shown that acetoacetate concentrations in the blood were much lower than those of β-hydroxybutyrate levels, and remained unchanged even when the blood β-hydroxybutyrate concentration was elevated in newly hatched chicks (Linares et al., 1993; Ohtsu et al., 2003). Therefore, the evidence suggests that blood acetoacetate concentration may be constant, and that ketone bodies circulate mainly as β-hydroxybutyrate, in chicken. Here, we show that a high-fat, low-carbohydrate, and low-protein diet induces ketosis by increasing β-hydroxybutyrate in chicken.

Since the KD is high in fat, and ketone bodies are in-

Fig. 5. Change in plasma NEFA (a), triglyceride (b), and total cholesterol (c) concentrations in chickens fed with control (CD), restricted (RD), or ketogenic (KD) diet. Vertical bars indicate standard error of the mean. Means (n=5) with different letters (a and b) differ significantly (P<0.05).

Fig. 6. Change in plasma total antioxidant activity (a) and TBARs (b) in chickens fed with control (CD), restricted (RD), or ketogenic (KD) diet. Vertical bars indicate standard error of the mean. Means (n=5) with different letters (a and b) differ significantly (P<0.05).
termediate metabolites of fatty acid β-oxidation, it is believed that plasma lipid levels and metabolism are modulated under conditions of ketosis induced by KD. Kennedy et al. (2007) demonstrated that plasma NEFA concentration was elevated under ketosis induced by KD feeding in mice. However, it has also been reported that blood ketone body concentrations were increased by KD administration without elevation of blood NEFA levels. Here, we showed that plasma NEFA concentration is increased by KD irrespective of the effect of reduced energy intake in chicken. Under normal physiological conditions of active ketogenesis, adipose tissue-derived fatty acids are the principal source of circulating NEFA. Circulating NEFA is transported to the liver, where ketogenesis is induced through β-oxidation. By contrast, it has been thought that, under KD feeding conditions, NEFA derived from dietary fat may be the main source of ketogenesis, since the KD includes a very high proportion of fat. Therefore, our results suggest that KD led to an increase in the liver uptake of NEFA, resulting in increased ketogenesis and blood β-hydroxybutyrate concentration in chicken.

Long-term periods of KD maintenance led to decreased plasma triglyceride levels in diabetic patients (Yancy et al., 2005; Dashni et al., 2007). Therefore, we investigated the effect of KD on plasma triglyceride concentrations in chicken. Our results indicate that both KD and RD administration reduced plasma triglyceride concentrations compared to CD administration. It is thought that blood triglyceride concentrations are affected by egg production in laying hens. In this study, the egg production was markedly reduced by KD (data not shown). Therefore, it is supposed that the plasma triglyceride reduction by KD may be due to the reduction of egg production and energy intake in part. However, it has previously been suggested that triglyceride synthesis in the liver is reduced by KD in mice, since fatty acid synthesis-related gene expression was reduced (Kennedy et al., 2007). Furthermore, we have also observed that KD tends to decrease plasma triglyceride concentrations in broiler chickens (unpublished data). Accordingly, it is possible that plasma triglyceride concentrations could also be reduced in chickens through reduction in fatty acid synthesis in the liver under conditions of ketosis induced by KD in a similar way to mice.

KD administration modulates oxidative stress status in mammals (Sirven et al., 1999; Coppola et al., 2002; Ziegler et al., 2003; Reger et al., 2004; Peterson et al., 2005; Gasior et al., 2006); therefore, we asked whether redox status is affected under conditions of ketosis induced by KD administration in chicken. KD reduced plasma total antioxidants activity, irrespective of energy intake reduction, since RD feeding did not affect plasma total antioxidant activity. As total antioxidant activity expresses the ability to protect from reactive oxygen species, these data suggested that KD reduced oxidative stress resistance in chicken. However, the KD also reduced plasma TBARs irrespective of energy intake reduction. Although this phenomenon is difficult to understand, one possible explanation may be that, since KD markedly reduced the plasma triglyceride concentrations in chicken, the substrate of peroxidation by free radicals was also markedly reduced, leading to consequent reduction of TBARs. Therefore, as antioxidants actively scavenge free radicals, the plasma total antioxidant activity was reduced as a consequence of KD. Another possible mechanism may be due to acidosis. Ketosis also induces acidosis, as ketone bodies are acidic in nature. It has been reported that activities of antioxidant enzymes such as glutathione peroxidase, glutathione S-transferase, and glutathione reductase, are decreased under conditions of acidosis (Ying et al., 1999). Therefore, it is possible that the acidosis induced by ketosis could be the cause of the induction of oxidative stress in chickens. Further investigation is required to assess the effect of KD feeding and ketosis on oxidative status; however, we demonstrated here that redox status is modulated under conditions of ketosis in chicken.

Blood β-hydroxybutyrate concentration is elevated in chicken by starvation (Linares et al., 1992) and at around the time of hatching (Linares et al., 1993; Ohtsu et al., 2003). As we show here, antioxidant capacity is reduced under condition of ketosis and high blood β-hydroxybutyrate concentrations in chicken. Therefore, it is likely that oxidative stress is induced under these conditions. In fact, it has been reported that fasting reduced blood total antioxidant status and glutathione peroxidase activity in poultry (Milinkovic-Tur et al., 2007) and, although the cause of the reduction of antioxidant status is not clear, ketosis could be a possible contributing factor. Therefore, it may be important to prevent the effects of oxidative stress under situations assumed to induce ketosis.

In summary, we showed here that a high-fat, low-carbohydrate, and low-protein diet induced ketosis by elevating blood β-hydroxybutyrate concentrations in chicken. Under condition of ketosis induced by KD in chicken, total antioxidant capacity was reduced, although the lipid peroxidation was also reduced, and the lipid profile modulated.

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References


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