Improvement in glucose tolerance and skeletal muscle glucose transport in broiler chickens treated with PPARγ agonist Troglitazone

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Carbohydrate metabolism in chickens is characterized by hyperglycemia and insulin resistance compared to that observed in mammals. We previously reported that although gene of GLUT4 (an insulin-responsive glucose transporter) is deficient in chickens (Seki et al., 2003), GLUT1 and GLUT8 are expressed, to a very small extent, in skeletal muscles (Kono et al., 2005). Furthermore, it has been evidenced that glucose transport across the plasma membrane of skeletal muscles is stimulated by insulin injection in growing chicks (Tokushima et al., 2005). In the insulin-mediated glucose transport in mammals, peroxisome proliferator-activated receptor-gamma (PPARγ) plays a crucial role. The present study was undertaken to assess the involvement of PPARγ in the glucose tolerance and skeletal muscle glucose transport in chickens by using troglitazone, a PPARγ agonist. Broiler chickens aged 1 to 2 week were orally administered with troglitazone (50 mg/kg body weight/day) 2 times a day for 17-22 days. Plasma glucose and NEFA concentrations were decreased, to a small extent, by troglitazone administration for 22 days. In an oral glucose tolerance test, troglitazone suppressed an increase in plasma glucose concentrations following glucose loading (2 g glucose/kg BW). A decrease in the plasma glucose concentration in insulin-injected (40 μg/kg BW) chickens was partly intensified by troglitazone administration for 22 days. Troglitazone administration for 17 days augmented insulin-mediated glucose transport, being determined by 2DG uptake, in skeletal muscles (extensor digitorum longus (EDL), pectoral superficialis and pectoral profundus) of chickens. These results suggest that PPARγ is involved in the regulation of carbohydrate metabolism species-specific to chickens and troglitazone improves insulin resistance through modulation of skeletal muscle glucose transport.

Keywords: broiler chickens, PPARγ, troglitazone, skeletal muscle, glucose transport

Introduction

Glucose is an essential fuel source and metabolic substrate in animal cells. The glucose uptake across the plasma membrane in animal cells, catalyzed by a family of facilitative transport proteins

known as glucose transporter (GLUT), plays a crucial role in the whole body glucose homeostasis. It is commonly accepted that GLUT4 plays a major role in the insulin-stimulated glucose transport through the blood stream in mammalian tissues (Watson and Pessin, 2001).

On the other hand, carbohydrate metabolism in chickens is characterized by hyperglycemia and insulin resistance compared to that observed in mammals (Belo et al., 1976; Akiba et al., 1999). We previously reported that although gene of GLUT4 (an insulin-responsive glucose transporter) is deficient in chickens (Seki et al., 2003), GLUT1 and GLUT8 are expressed, to a very small extent, in skeletal muscles (Kono et al., 2005). Furthermore, it has been evidenced that glucose transport across the plasma membrane of skeletal muscles is stimulated by insulin injection in growing chicks (Tokushima et al., 2005). Thus, the glucose metabolism, including glucose transport across the plasma membrane of skeletal muscles, might be unorthodox and not well understood to date.

Peroxisome proliferation-activated receptor gamma (PPARγ) is a member of the nuclear receptor superfamily. PPARγ is expressed in many cell types, including muscle cells, adipocytes, epithelial cells, and endothelial cells (Spiegelman,1997; Law et al., 2000). In addition, thiazolidinedione, a specific agonist of PPARγ, has insulin-sensitizing properties in skeletal muscles and improves insulin resistance in human and rodents (Rangwara et al., 2004). In chickens, we have identified broiler chicken PPARγ cDNA (AB045597) with 2,089 base pairs and predicted to encode a 475 amino acid (Sato et al., 2004). However, to date metabolic involvement of PPARγ in the glucose transport mechanism in skeletal muscles of chickens characterized by hyperglycemia and insulin resistance has not been determined.

The present study was undertaken to assess the involvement of PPARγ in the glucose tolerance and skeletal muscle glucose transport in chickens by using troglitazone, a PPARγ agonist.

Materials and methods

Male broiler (Cobb) chickens were housed individually in wire cages in a room with controlled temperature (25±3 ºC) and fed on a commercial diet prior to the commencement of experiments. The animal care and use committee of Graduate School of Agricultural science of Tohoku University approved all procedures.

One or two-week-old chickens were orally administered twice a day into crop with troglitazone (50 mg/kg body weight/day) for 17 or 22 days. At 31 or 36 day of age, skeletal muscles (EDL: extensor digitorum longus; PS: pectoralis superficialis; PF: pectoralis profundus) were taken for 2DG glucose uptake determinations, after collecting blood samples. Plasma glucose and non-esterified fatty acids (NEFA) concentrations were measured by the commercial assay kit (Wako Pure-Chemicals, Osaka, Japan). Transport of 2DG across the muscle cell membrane was estimated by measuring 2DG6P content in the muscle following 2DG injection, in reference to the procedure reported by Ueyama et al. (2000). In chickens received troglitazone for 22 days were subjected to the glucose tolerance test in that chickens were starved for 12 h and orally administered with glucose (2 g/kg body weight). Blood samples were taken at 0, 15, 30, 60 and 90 min after the glucose administration

The SAS® applications package was used for statistical calculations (SAS Version 6.03, SAS Institute Inc., Cary, NC). The level of significance used in all studies was $P < 0.05$.

Results and discussion

Body weight and abdominal fat weight were significantly increased by 22 days administration of troglitazone (Table 1). Plasma glucose concentration was significantly decreased by troglitazone, suggesting that troglitazone administration for more than 3 weeks exerts hypoglycemic effects in chickens, as being similarly shown in Zucker Diabetic Fatty rats treated with a thiazolidinedione (Upton et al., 1998). Troglitazone decreased plasma NEFA concentrations (Table 1). This is in accordance with findings obtained with rodents (Upton et al., 1998) and suggests that the mode of action of PPARγ agonist in chickens is associated, at least in part, with modulation of lipid metabolism, in particular changes in plasma NEFA concentration.

Table 1 Effects of troglitazone administration for 22 days on body weight, abdominal adipose tissue weight, liver weight, plasma glucose and NEFA concentrations in broiler chickens

	Control	Troglitazone (50 mg/kgBW/day)
Body weight (g)	1617 ± 71	1816 ± 30 *
Abdominal fat weight (% BW)	0.37 ± 0.06	0.78 ± 0.20*
Liver weight (% /BW)	3.75 ± 0.18	3.93 ± 0.21
Plasma glucose (mg/dl) after 12 h starvation	221.4 ± 5.5	204.5 ± 6.0*
Plasma NEFA (mEq/l) after 12 h starvation	0.82 ± 0.05	0.51 ± 0.09 *

Results are given with the SD of the mean values (n=5).

Asterisks (*) indicate significant difference ($P < 0.05$) compared to control group

In the glucose tolerance test performed in chickens received troglitazone for 22 days, plasma glucose concentrations at 15 and 30 min after the glucose administration were significantly lower than those in the control chickens (Figure 1). The lowered plasma insulin concentrations, to a small extent, in chickens injected with troglitazone were shown together with the decrease in plasma glucose in this experiment (data not shown). These results follow findings in diabetic subjects that plasma glucose concentration in oral glucose tolerance test was significantly reduced by administration of pioglitazone for 16 weeks (Miyazaki et al., 2001). It is therefore likely that PPARγ agonist heightens insulin sensitivity and lowers plasma glucose concentration after glucose loading, resulting in improvement of insulin resistance in chickens as similar as mammals. However, because the hypoglycemic effect of troglitazone was shown only in case of the high dose (50 mg/kg body weight) in chickens compared to the effective dose in human subjects (less than 10 mg), the role of PPARγ in glucose homeostasis of chickens might be less extent than that in mammals.

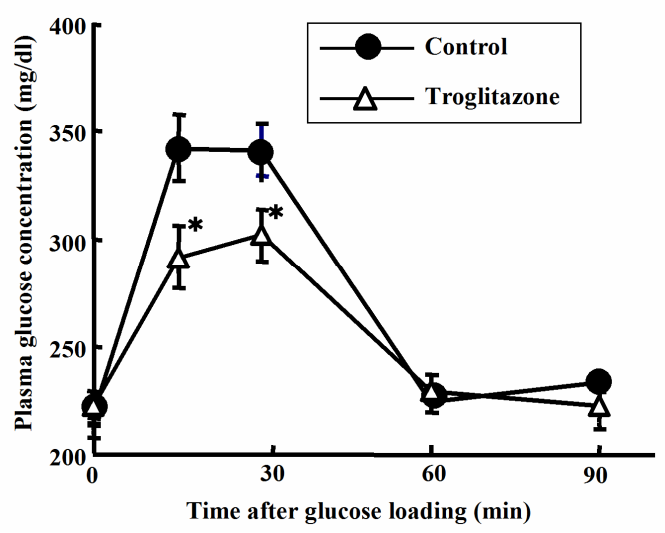

Figure 1.

Changes in plasma glucose concentration in oral glucose tolerance test of chickens administrated with troglitazone (50 mg/kgBW/day) for 22 days. Chickens were orally administered with glucose (2 g/kgBW) following 12 h starvation. Blood was taken at 0, 15, 30, 60 and 120 min after the glucose administration.

Results are given with the SD of the mean values (n=5).

Asterisks (*) indicate significant difference ($P < 0.05$) compared to control group.

Figure 2 shows uptake of 2DG in skeletal muscles of insulin-treated chickens administered with troglitazone, estimated by tissue 2DG6P concentration following 2DG injection. Troglitazone significantly increased 2DG uptake in EDL and pectoral (superficailis and profundus) muscles compared to that in the control chickens received no troglitazone, showing that insulin-stimulated glucose uptake in skeletal muscles was increased by PPARγ agonist.

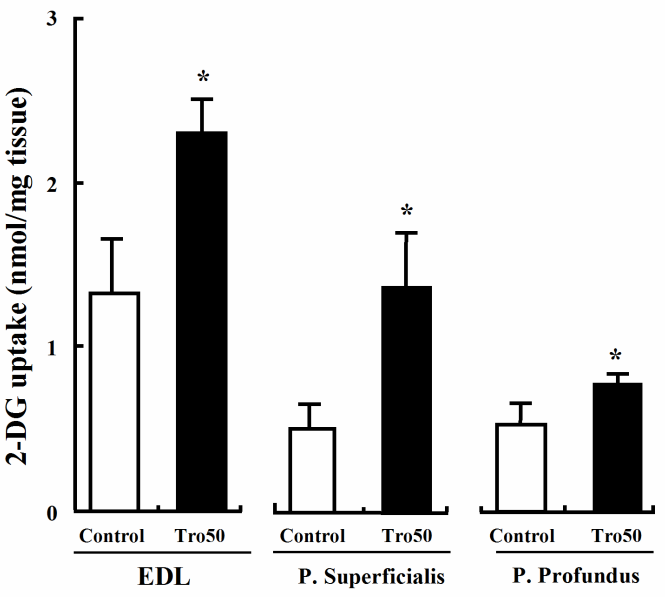

Figure 2.

Insulin-stimulated 2-deoxy-D -glucose (2-DG) uptake in extensor digitorum longus muscle (EDL), pectoralis superficialis muscle (P. Superficialis), and pectoralis profundus muscle (P. Profundus) of chickens administrated with troglitazone (50 mg/kgBW/day) for 17 days.

Tro50: Troglitazone 50mg/kgBW/day.

Results are given with the SD of the mean values (n=4).

Asterisks (*) indicate significant difference ($P < 0.05$).

Zierath et al. (2005) showed that in vivo troglitazone treatment increased 2DG uptake in isolated soleus muscle and corrected insulin resistance in ob/ob mice. Considering glucose homeostasis in human postprandial state, more than 70 % of glucose is cleared by skeletal muscles (DeFronzo et al., 1992). This state must occur in chickens as well. It is likely that torglitazone, a PPARγ agonist, improves insulin resistance in chickens, probably being mediated by the stimulation of glucose transport in skeletal muscles.

In conclusion, PPARγ has a certain properties in the regulation of carbohydrate metabolism species-specific to chickens.

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