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Insulin effect on lipogenesis and fat distribution in three genotypes of ducks during overfeeding

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ABSTRACT

In waterfowl, the response to overfeeding differs from one genotype to the other. Pekin ducks generally store lipids in the peripheral tissues while Muscovy and mule ducks promote hepatic lipid storage. A possible reason for these various susceptibilities to hepatic steatosis could be a difference in insulin sensitivity. We suggest a resistance to insulin in Pekin ducks. In the present work we investigate the action of insulin on glucose and lipid metabolisms for the three overfed genotypes. Regardless of the kind of genotype, all ducks appear to be sensitive to insulin: their glycemia is lower when the animals are treated with insulin. Insulin-treated Muscovy and Pekin ducks present a lower increase in total body weight (−16.5% for Muscovy; −8.3% for Pekin); and a significantly lower liver weight than the controls (−9.6% and −18.3%). The percentage of total lipids in the liver is higher in the controls than in the insulin-treated Pekin and mule ducks (respectively −40.4% and −34.7%), which means a decreased hepatic lipogenesis. Pekin ducks present a higher pectoral muscle weight when the individuals are insulin-treated (+9.7%). Lipoprotein lipase (LPL) activity appears to be significantly higher in insulin-treated Pekin and Muscovy ducks (1.39 and 3.38 times greater than controls). Insulin-treated mule ducks present a decrease of muscle and abdominal lipid storage compared to controls (−11.6% and −13.8%). In this experiment, exogenous insulin has induced an increase of lipid oxidation and has led to a less favorable use and storage of dietary glucose. The hypothesis of insulin-resistance of Pekin ducks is not verified.

1. Introduction

In some waterfowl species, the induction of hepatic steatosis by overfeeding allows the production of fatty liver. However, the metabolic response to overfeeding is extremely variable and depends, among others, on the genotype, suggesting a genetic determination. Previous studies have clearly demonstrated that Pekin ducks have a low hepatic steatosis, the result of the export of lipids to the peripheral tissues, while Muscovy and mule ducks provide heavier fatty livers (Guy et al., 1999; Davail et al., 2003a; Baeza et al., 2005).

Furthermore, the intensity of the hepatic lipogenesis and its equilibrium with the secretion of lipids in the blood via lipoproteins may be a cause for the development of hepatic steatosis (Saadoun and Leclercq, 1987; Hermier et al., 1991). The differences in overfeeding performances between different genotypes can be explained by a greater or lesser imbalance between the synthesis and the secretion of triglycerides (TG) in the blood. In mule and Muscovy ducks, a major part of the synthesized TG would not be excreted and would contribute to the fattening of the liver (Hermier et al., 2003). On the other hand, in Pekin ducks TG are more exported via the VLDL (very light density lipoproteins). This improved export is associated to a higher activity of lipoprotein lipase at the end of the overfeeding period (LPL, an enzyme catalyzing the hydrolysis of TG into fatty acids to their incorporation in underlying tissues — i.e. adipocytes and myocytes) which contributes to a peripheral fattening. Studies have shown that the LPL activity is significantly inhibited by overfeeding in Muscovy and mule ducks whereas in Pekin ducks this inhibition does not appear (Andre et al., 2007).

Among the factors that could explain this difference, we suspect a hormonal influence. Indeed, the pancreatic hormones are actively involved in the regulation of metabolic pathways. Davail et al. (2003b) suggested a resistance to insulin in Pekin ducks. Overfeeding can induce an oxidative stress which can represent a pathogenic factor leading to insulin resistance (Ceriello and Motz, 2004). We therefore, intend to study the action of insulin on fat distribution, lipoprotein lipase activity and glucose and triglyceride plasma levels for the three genotypes during overfeeding. We will also test the hypothesis of insulin resistance.

Insulin is typically the hormone of the post-prandial state. Insulin stimulates the glycogenesis (Parkes and Grieninger, 1985), controls

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the use of glucose in insulin dependent tissues (Thomas-Delloye et al., 1999; Yang, 2010), stimulates hepatic lipogenesis (Bedu et al., 2002), and facilitates the synthesis and the activation of the LPL (Leclercq, 1984; Rosato et al., 1997; Knutson, 2000). The interest of studying the metabolic role of insulin is therefore obvious in the case of animals subject to overfeeding.

2. Materials and methods

2.1. Animals

The animals used were male ducks from the Pekin (*Anas plathyrhynchos*, genotype F 29), the Muscovy (*Cairina moschata*) and the mule genotypes. The latter is a sterile hybrid of the male Muscovy duck and the female Pekin duck. Birds of each genotype, hatched on the same day, were bred under natural light and temperature conditions at the Experimental Station for Waterfowl Breeding (INRA Artiguères, France). The total strength was 28 Pekin ducks, 28 Muscovy ducks and 28 mule ducks. From hatching to 6 weeks of age, they were fed ad libitum with a starting diet providing 17.5% crude proteins (11.9 MJ/kg) and then submitted to a restricted feeding from the age of 6 weeks to the age of 10 weeks (230 g/day with a growing diet, 11.9 MJ/kg and 15.5% crude proteins). With the same growing diet, the pre-overfeeding period started at 10 weeks of age with 5 days of restriction (200 g/day) and 6 days of gradual increase of the amount of food (from 220 to 320 g/day). At the age of 12 weeks, the animals were bred in individual cages and were overfed twice a day for 14 days with a mixture of 35% corn-flour, 25% corn-grain and 40% water (13.8 MJ/kg). In order to observe an overfeeding proportional to the total body weight for the three genotypes, the observation was performed on the basis of the ingestion capacity of Muscovy ducks (which is the weakest ingestion ability: 6% of the total body weight for the first meal until 11% at the end of the overfeeding period). Finally, the average amount of feed ingested by ducks during the overfeeding period was: 9748 g for mule, 11,569 g for Muscovy and 9670 g for Pekin.

2.2. Experiments

During the overfeeding period, each genotype was distributed into 2 groups: the controls (14) and the insulin treated (14).

2.2.1. Insulin injections

The doses of insulin injected were determined according to endogenous insulin plasma levels and from experimental doses used in literature (Schmidtke et al., 1998; Pal et al., 2002; Albuquerque et al., 2008). As it exhibits a relative resistance of birds to insulin of mammalian origin (Akiba et al., 1999; Tesseraud et al., 2007), the doses used to obtain some effects were relatively high compared to previous measurements of endogenous level of insulin in Pekin and Muscovy ducks (10 to 30 μUI/ml plasma respectively before and after overfeeding (Berradi et al., 2004; Davail et al., 2003a). Insulin (Insulin glargine: LANTUS, 10 UI/ml, Sanofi-AVENTIS, France, long acting, human insulin analog, ADN recombinant on *Escherichia coli*) was administrated through intramuscular injection: 2 UI/kg during the first four days and 1 UI/kg from the fifth day until the end of the experiment. The insulin doses were then decreased by half on the three genotypes because the Muscovy ducks presented a certain weakness and digestion troubles, suggesting insulin overdose. Two injections per day were performed 15 min before feeding. Meanwhile, the controls were injected with physiological serum (NaCl, 9 g/l).

2.2.2. Blood sampling

During the overfeeding period, blood samples were collected 70 min after the first meal on the 2nd, 5th, 8th, 10th and the 14th days on both the controls and the treated animals. Blood was withdrawn through puncture of the occipital venous sinus, collected in an EDTA/heparin vacuum tube. Samples were centrifuged 2000 *g* for 10 min at 2 °C. Plasma was then frozen at −20 °C for further analysis.

2.2.3. Heparin injection

The lipoprotein lipase (LPL) activity was assessed on the post-heparin plasma collected on the 2nd, 8th, and 14th days during the overfeeding period. Heparin injections (400 UI/kg) were performed on all animals, through the wing vein, 60 min after the meal. Blood was collected 10 min later.

2.2.4. Tissue analyses

At the end of the overfeeding period, animals were bled after electronarcosis and plucked. The carcasses were refrigerated 24 h at 4 °C. Then, they were eviscerated: liver, muscles (*pectoralis major*), subcutaneous and abdominal fat were removed, weighed and sampled. Samples were frozen at −20 °C for further analysis.

2.2.5. Total lipid extraction

The total contents of lipids in the liver and muscles were determined according to the Folch et al. (1957) method.

2.2.6. Plasma analyses

Triglycerides and glucose were quantified using enzymatic kits (triglycerides LDB Kit, Biodirect; Glucose RTU Kit, Biomérieux, France). The LPL activity was measured according to the Saez et al. (2010) method.

2.2.7. Statistical analyses

The genotypes, together with the insulin treatment and the interactions were tested. Values were expressed by means ± SEM. All statistical analyses were carried out using R, version 2.15.1 (The R Foundation for Statistical Computing). For all data, normality and homogeneity tests of the variances were performed. Insulin effects on animal performances were tested by ANOVA without considering the genotype at first, and then species by species. Differences were considered significant at $P \leq 0.05$.

3. Results

In general, regardless of the species, significant interactions between genotype and insulin treatment appeared; especially for feed efficiency, body weight (BW) gain, abdominal fat and percentage of lipids in the liver.

3.1. Body weight (Table 1)

Before overfeeding, Muscovy ducks displayed a higher body weight (BW) than Pekin and mule ducks. Pekin ducks were also heavier than mule ducks. At the end of the overfeeding period, Muscovy ducks had the highest BW and BW gain. We found no significant differences between Pekin and mule ducks for these two last parameters.

The insulin treatment decreased the BW gain in Muscovy and Pekin ducks (−16.5% and −8.3%, respectively). It had no effect on the body weight gain of mule ducks.

The analysis of feed efficiency is consistent with these initial observations with a higher overall efficiency in Muscovy ducks ($P<0.001$) compared to the two other genotypes. We did not find any differences between mule and Pekin ducks.

It displayed an interaction between feed efficiency and insulin treatment with a significant decrease in Muscovy (−16.1%; $P<0.001$) and in Pekin ducks (−8.4%; $P<0.05$). No effect was observed in mule ducks.