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Effect of Insulin on the Secretion and Content of Triglyceride in the Chicken Liver Slices

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Abstract: Triglyceride (TG) secretion from liver and its storage in abdominal cavity of the broiler affects the net meat yield. So agents that affect the hepatic TG secretion may be important for poultry breeders. At present study, we have shown short term effects of insulin on the chicken liver TG secretion at the first, third, fifth and seventh weeks of breeding. For this purpose, 10^{-10}, 10^{-8} and 10^{-6} M insulin were added to the liver slices of roosters and TG secretion and content followed after 4h and 8h incubation. Results showed, while 4h incubation had no effect on TG secretion from the liver slices at the first, third and fifth weeks, in the seventh week 10^{-10} and 10^{-8} M insulin drastically decreased TG secretion 32% and 35% below the basal levels and 10^{-6} M insulin increased it 31% above the basal levels. In 8h incubation, while 10^{-6} M insulin at the first, third and fifth weeks decreased TG secretion below the basal levels (58%, 54%, and 39%), it showed 29%, 121%, and 128% increase in the seventh week when compared to the basal level, 10^{-10} and 10^{-8}M insulin, respectively. At the first and seventh weeks TG content did not show response to insulin but in the third and fifth weeks showed a dose dependent increase. Insulin mainly decreases TG secretion from the chicken liver yet increases TG content as an age dependent manner.

Key words: Insulin, chicken, liver, triglyceride

Introduction

Lipid storage, particularly in the abdominal cavity, is a major concern in poultry breeding because it affects the meat yield (Hermier, 1997). This fat pad is mainly due to hepatic lipogenesis (Leveille *et al*., 1975; Pullen *et al*., 1990; Griffin *et al*., 1992). For manipulating of this fat pad some effectors on TG synthesis and secretion are studying. Eventhough, Mooney and Lane (1981) showed that there is no correlation between synthesis and secretion of lipid in the liver, there is a controversy about the effect of insulin on the synthesis and secretion of TG from the chicken hepatocytes. Tarlow *et al*. (1977), believe insulin increases fatty acid synthesis and VLDL formation in the primary culture of chicken hepatocytes. Moreover it has been shown that high doses of insulin increase lipogenesis and inhibit apoB synthesis so that cause a decrease in the VLDL formation and secretion. Such condition is accompanying with TG storage as cytoplasmic vesicles. In this respect, Laurin and Cartwright (1993) and Duerden and Gibbons (1990) showed insulin decreases TG secretion and increases cytosolic storage in chicken hepatoctes. More ever, Legrand *et al*. (1996) showed that 10^{-9} M and 10^{-6} M insulin increase TG synthesis in a dose-dependent manner during 24 h and 48 h culture of chicken hepatocytes. They have shown while10-9 M insulin increases TG secretion, 10^{-6} M insulin had no effect.

There are a few studies to address the effect of insulin on the synthesis and secretion of VLDL-TG in chicken hepatocytes. To our knowledge, there are a few reports on the short-term effects of insulin on TG secretion in chicken hepatocytes. The objective of the present study is to explore the short-term effects of insulin on TG Secretion from chicken liver with different ages.

Materials and Methods

Chemical materials including: insulin (Sigma Co; I5500), MEM (Sigma Co; 4642), hexane (Merck Co; 822280), isopropanol (Merck Co; k995), chloroform (Merck Co; 822265), methanol (Merck Co; 6008), acetyl acetone (Merck Co; 800023), triolein (sigma Co; T7140), BSA (Merck Co; 126604), meta-periodate (Merck Co; 6597), commassie blue G250 (Merck Co; 15444), glacial acetic acid (Merck Co; 90056), sulfuric acid (Merck Co; 713), ammonium acetate (Merck Co; 115), sodium sulfate (Merck Co; 6645), natrium hydroxide (Merck Co; 6462) and kalium hydroxide (Merck Co ;5012) were purchased from suppliers.

Sixty four apparently healthy roosters were studied in this experiment. For this purpose, in the end of each week (the first, third, fifth and seventh weeks) 16 roosters were decapitated and their livers cultured in the presence of 0, 10^{-10}, 10^{-8}, and 10^{-6} M insulin on the basis of Pullen *et al*. method (1990). Briefly, after removing of liver, 1g weighed and cut into 1×1×2 cm blocks. All blocks were immersed into the ice-cold MEM, removed their capsules and non-parenchymal tissues. Three blocks were prepared from each liver. All blocks were cut into 0.5mm thickness slices by a cutter.

Slices were bloated on a filter paper and incubated in a

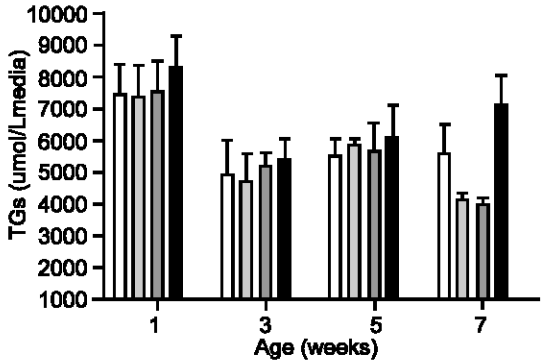

Fig.1: Effect of 0, 10^{-10}, 10^{-8}, 10^{-6}) M insulin on TG secretion (Mean ± SD) from the chicken liver slices after 4 h incubation. Only at the seventh week an increase has been slightly seen in response to 10^{-10} ($p = 0.043$) and 10^{-8} M insulin ($p = 0.024$) when compared to the control level (0 M) of insulin.

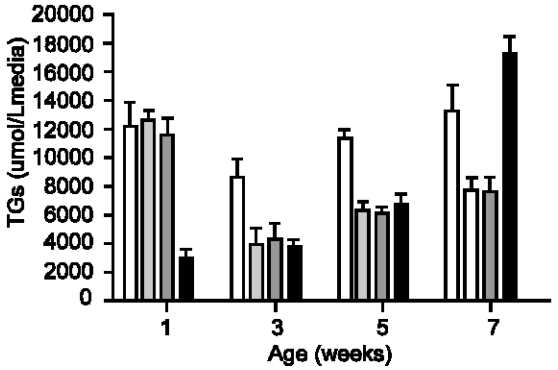

Fig. 2: Effect of 0, 10^{-10}, 10^{-8}, 10^{-6} M insulin on TG secretion (Mean ± SD) from the chicken liver slices after 8 h incubation. In response to 10^{-10} and 10^{-8} M insulin a decrease has been shown at the third week ($p<0.001$), fifth week ($p<0.001$) and seventh week. ($p=0.001$) when compared to the control level. While 10-6 M insulin make decreases in TG secretion at the first, third and fifth weeks ($p<0.001$) it slightly make increases at the seventh week ($p=0.013$).

flask with 3 ml MEM at 37°C for 0, 4, and 8h. All blocks gassed (95% O_2 /5% CO_2) for 20s in the initiation of experiment and after then were incubated (60 oscillation/min at 37°C).Flasks were repeatedly gassed each 1.5 h. In the end of experiment, media were separated with 0.8 µm filter paper from the liver slices Lipids were extracted from the media and liver slices. For liver slices briefly, 9 ml of extraction solution [hexane: isopropanol (3:2 v/v)] was added to 0.5 g of liver slice and homogenized by glass beads for 8h at room temperature. After homogenization, organic phase was separated by centrifugation at 2000×g for 10 min and used for TG assay (Hara and Radin, 1978). Lipids were extracted from the media by adding 3 ml of chloroform: methanol (80:20, v/v) to each media, decanted for 10 min and the organic phase separated for TG assay. Total cell protein was measured by Bradford method (1976), after 4h dissolving in 0.1 N sodium hydroxide.TG was measured by the method of Neri and Frings (1973), both in tissue extract and media.

Results

As was shown in Fig. 1, TG secretion in 4h incubation shows no difference among different doses of insulin (10^{-10}, 10^{-8}, 10^{-6}, and 0 M) at the first third and fifth weeks. While in the seventh week, 10^{-10} and 10^{-8} M insulin decrease TG secretion below the basal level (22% and 28%), 10^{-6} M insulin increases it above the basal level (27%) and also when compared to10^{-8} and 10^{-10} M insulin (80% and 64%). In 8h incubation of the first week liver slices (Fig. 2), Merely 10^{-6} M insulin makes 58%, 56% and 59% decreases in TG secretion when compared to 0, 10^{-8} and 10^{-10} M insulin, respectively. At this week other doses do not show difference between each other and when compared to control (0 M insulin). At the third and fifth weeks all doses (10^{-6} , 10^{-8} and 10^{-10} M insulin) make decrease in TG secretion below the basal level. The amount of decrease for the former are 54%, 49%, 53% and for the latter are 39%, 46%, 43%, respectively. At the seventh week, 10^{-6} M insulin make 29, 128 and 121% increase in TG secretion when compared to 0, 10^{-8} and 10^{-10} M insulin, respectively.

As was shown in figure 3, there is no difference among the effects of different doses on TG content after 4 h incubation at the first week ($p>0.05$). At the third week, 10^{-6} M insulin increases TG content (18%, 21%, 5%), when compared to 0, 10^{-10} and 10^{-8} M insulin. Moreover, such increases have shown at the fifth week when compared to 0 and 10^{-10} M insulin (70% and 43%). Furthermore, at this week 10^{-8} M insulin shows 48% increase in TG content above the basal level. At the seventh week, there is no difference among different doses ($p = 0.186$).

Fig. 4 shows 8h incubation of the liver slices at the first, third, fifth and seventh weeks. At the first week, any difference was seen among different doses. 10-6 M insulin make increases in TG content both at the third week (80%, 63%, 38%) and fifth week (75%, 86% 33%) when compared to 0, $10^{-10}$and 10^{-8} M insulin respectively. At the fifth week, 10^{-8} M insulin shows 31% and 40% increase when compared to the control and 10^{-10} M insulin, respectively. In contrast to such effects there were any differences between doses when compared to each other and the basal level in the seventh week.

Discussion

For the short term effect of insulin we have shown, in spite of an increase in TG secretion in response to 10^{-6}

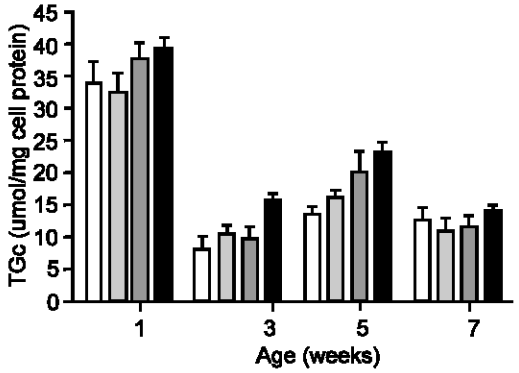

Fig. 3: Effect of 0, 10^{-10}, 10^{-8}, 10^{-6} M insulin on TG secretion (Mean ± SD) from the chicken liver slices after 4 h incubation. An increase in TG content has been seen in response to 10^{-6} M insulin at the third week ($p<0.001$) and fifth week ($p<0.001$) and in response to 10^{-8} M insulin at the fifth week ($p<0.001$) when compared to the control level. No effect has been seen in response to 10^{-10} M insulin.

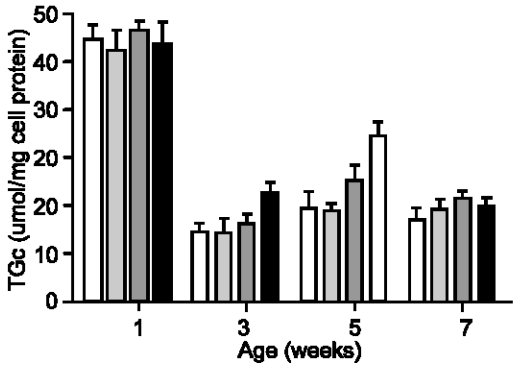

Fig. 4: Effect of 0, 10^{-10}, 10^{-8}, 10^{-6} M insulin on TG secretion (Mean ± SD) from the chicken liver slices after 8 h incubation. In response to 10^{-6} M insulin an increase has been seen at the third week ($p=0.001$) and fifth week ($p<0.001$) in the chicken liver TG content. At the fifth week, an increase in TG content has been seen in response to 10^{-8} M insulin ($p=0.039$).No effect has been seen in response to10^{-10} M insulin.

M insulin, other doses (0, 10-10, 10^{-8}, 10^{-6} M) did not show any change in TG secretion of the chicken liver at the first, third, and fifth weeks. Such effect can be due to time insufficiency, i.e.Tarlow *et al.* (1977) believed a minimum time (about 4 h) is necessary for taking insulin response in the chicken primary hepatocyte culture. Furthermore, different effects of 10^{-6} M insulin seem to be age dependent manner. In agreement with our findings, Duerden and Gibbons (1990); Patsch *et al.* (1983) and Spark *et al.* (1986) showed insulin decreased TG secretion from the rat liver. Similar effects of insulin have been shown in the HepG2 cells by Pullinger *et al.* (1989) and Dashti and Wolfbauer (1987). These effects of insulin on the formation and secretion of VLDL can be done through two ways: 1) through the blockage of newly synthesized TG to the VLDL (Brown and Gibbons, 2001) and 2) by means of apoB degradation in the hepatocytes (Spark *et al.*, 1986).

Decrease of TG secretion in response to 10^{-6} M insulin in both 4h and 8h incubation shows its negative correlation with age. Nonetheless, Laurin and Cartwright (1993) showed stimulatory effects of insulin on TG synthesis as dose dependent manner which is negatively correlated with age. Moreover, Tarlow *et al.* (1977) showed an increase in VLDL-apoB secretion in response to 0.5 µg/ml of insulin. Such an effect has been considered as unspecific effect of insulin .Furthermore, it has been shown 48 h incubation of chicken hepatocytes with 10-9 M insulin increased TG secretion (Legrand *et al.*, 1966).

At present study, while 4h and 8h incubation of liver slices at the first and seventh weeks had no effect on TG content, its content showed an increase at the third and fifth weeks as dose dependent manner. Increasing effect of insulin on TG content of chicken hepatocyte has been attributed to induced lipogenesis and inhibition of apoB synthesis. In this case TG accumulates as cytoplasmic storage. (Brown and Gibbons, 2001). In study on the rat liver, Drrington (1983), Spark *et al.* (1986) and Patsch (1983) showed insulin dependent degradation of apoB which was followed by increased cytoplasmic TG storage. Moreover, it has been shown induced lipogenic enzymes of TG synthesis by insulin (Laurin and Cartwright, 1993).

In conclusion, while in 4h incubation insulin has no effect on TG secretion in none weeks, in 8h incubation a decrease has been shown at the first, third and fifth weeks. Moreover, increased TG secretion was shown with 10^{-6} M insulin in the seventh week. On the other hand, while TG content showed no response to insulin at the first and seventh weeks, increase of TG content has been seen at the third and fifth weeks as a dose-dependent manner.

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Abbreviations: Triglyceride (TG), Very Low Density Lipoprotein (VLDL), Apolipoprotein B (apoB)