Effect of organic selenium food supplementation and fasting on adipose tissue lipid concentrations and lipoprotein lipase activity in broiler chickens

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The aim of this study was to investigate the effect of the organic selenium supplementation and fasting on lipid concentrations and lipoprotein lipase activity in adipose tissue of Ross 308 broiler chickens. Chickens were divided into two groups: first group were fed with standard diet (control group) and second were fed the same diet supplemented with organic selenium (Sel-Plex group). Adipose tissue samples were collected at the age of 42 days and after 48-hours food deprivation and triacylglycerol, total and free cholesterol concentrations and lipoprotein lipase activity were assayed. Adipose tissue total cholesterol concentration in the chickens fed with organic selenium supplementation was significantly lower (P<0.05) compared with control after fattening period. Fasting significantly decreased total and free cholesterol (P<0.05) concentrations in control chickens and increased free cholesterol concentration (P<0.05) in organic selenium supplemented chickens. Lipoprotein lipase activity in chicken adipose tissue of both control and Sel-Plex groups were significantly increased (P<0.05) after fasting. In both groups, no changes in triacylglycerol concentrations were observed after fattening period and 48-hour food deprivation (P>0.05). Present research suggests that organic selenium supplementation influences cholesterol metabolism in adipose tissue by decreasing total cholesterol concentration during the fattening period and increasing free cholesterol concentration after 48-hour food deprivation.

Keywords: adipose tissue; organic selenium; fasting; lipids; lipoprotein lipase

Introduction

Selenium is an essential trace element that needs to be present in the human and animal diet to maintain normal physiological functions (Goldhaber, 2003). Selenium exerts its influence on physiology as an integral component of proteins. Selenoproteins regulate antioxidant defence mechanism (Mahmoud and Edens, 2003), thyroid hormone synthesis and metabolism (Köhrle, 1999) and immune response (McKenzie et al., 1998).

Selenium food supplementation is associated with many metabolic processes preventing appearance of degenerative processes and different diseases. As supplement in food, selenium can be organic (selenomethionine and selenocysteine) or inorganic (selenite or selenate) forms. Organic selenium had an advantage in reducing oxidative stress in mammals (Vinson et al., 1998) as well as in birds (Mahmoud and Edens, 2003).

Iizuka et al. (2001) were studied the effects of inorganic selenium on lipid metabolism in rats fed a high-cholesterol diet. Authors demonstrated that selenium suppressed the triacylglycerol, total cholesterol and free fatty acid concentrations in the serum and inhibited the amount of liver triacylglycerols and cholesterols. Asha and Indira (2004) concluded that the suplementation of inorganic selenium and ascorbic acid reduced alcohol induced hyperlipidemia by decreased synthesis and increased catabolism. Organic selenium is an effective antioxidant and hypolipidemic agent in normal hamsters (Vinson et al., 1998).

Adipose tissue contains one of the largest pools of triacylglycerols and cholesterols in man as well as in animals (Angel and Farkas, 1974). Triacylglycerol is the most concentrated form of energy available to biological tissues (Gibbons et al., 2000). Cholesterol accumulates within the adipocytes in two cell compartments: plasma membrane and lipid droplet (Kraus and Hartman, 1984). Furthermore, cholesterol in adipocytes might serve as intracellular signal for the size of fat cell, linked to triacylglycerol stores (Le Lay et al., 2004).

The metabolism of lipids in birds differs from that in man in two important respects (Chapman, 1980). First, portomicrons are secreted directly into the portal circulation because intestinal lymphatic system is poorly developed in avian species and second, the liver is the main site of lipogenesis in birds (Hermier, 1997).

Animals deprived of food deplete glycogen reserves after a short period and then they begin to metabolize fat reserves. If starvation persists, the animals may begin to use protein for glucose synthesis (Guyton and Hall, 2003.)

Lipoprotein lipase (LPL) is key enzyme in lipoprotein metabolism. It is synthesized in the parenchymal cells of extrahepatic tissues including adipose tissue, skeletal and cardiac muscle, and is translocated to its physiological site of action in the capillaries. LPL catalyzes the hydrolysis of circulating triacylglycerols, allowing their utilization by peripheral tissues (Karpe et al., 1998). The activity of LPL is influenced by nutritional and hormonal status (Llado et al., 1999), strains and gender (Galan et al., 1994). Bergö et al. (1996) showed that in rat adipose tissue are both active and inactive form of LPL. Fasting decreased amount of active form of LPL in adipose tissue redirecting triacylglycerols in other tissues.

Early studies showed that fasting have different effects on lipid metabolism in man (Sävendahl and Underwood, 1999) and in animals (Yasuhara et al., 1991). The time required for depletion of all carbohydrate and lipid reserves varies with size of the bird and amount of initial reserves of glycogen and triacylglycerols (Blem, 2000; Lamošová et al., 2004).

In poultry production, duration of fattening period is shorter and body development is very intensive. Birds have more intensive metabolism and higher body temperature compared with mammals. The aim of this study was to investigate the effect of the organic selenium supplementation on lipid concentrations and lipoprotein lipase activity in adipose tissue of broiler chickens after fattening period and 48-hours food deprivation.

Materials and methods

The experiment was performed on Ross 308 chickens of both sexes. One day old chickens were housed in experimental room. Ambient temperature of the experimental room was set at 32ºC at the time of placement, and over the 4-weeks growing period ambient temperature was reduced to 24ºC. By the end of experiment ambient temperature was 20ºC and lights were on continuously.

Chickens were randomly divided into two groups at the age of seven days. Control group fed with standard diet and Sel-Plex group were fed the same diet supplemented with 0.3 ppm organic selenium (Sel Plex™, Alltech). Until day 42, food and water were given *ad libitum*.

On the 42nd day, ten chickens of control and ten chickens of Sel-Plex group were sacrificed and adipose tissue samples were removed and stored at - 80 ^{o}C until analyses.

The rest of the animals were subjected to 48-h fasting with free access to tap water. After the period of fasting, ten chickens of control and ten chickens of experimental group were also sacrificed and adipose tissue samples were removed and stored at - 80 ^{o}C until analyses.

Adipose tissue lipids were extracted according to the procedure of Folch et al. (1957). Total cholesterol and triacylglycerols were measured using commercial kit (Randox, UK) and free cholesterol was assayed as described Sobel and Mayer (1945).

To assay LPL activity, adipose tissue samples were homogenized with acetone and fibrous mat was incubated in NH_3-HCl buffer (Korn and Quigley, 1957) containing 2 IU heparin/mL (Levy et at., 1990.) Clear supernatants obtained after centrifugation were incubated with triolein emulsion (Herbos dijagnostika, Croatia). Free fatty acid (FFA) released during the incubation was determined colorimetrically (Randox, UK). One unit of enzyme activity was defined as µmol FFA released per minute (Sato et al., 1997).

Results and disccusion

Avian adipose tissue has very limited capacity for fatty acids synthesis and most of the accumulated fat is derived from the diet or synthesized in the liver (Griffin et al., 1991). Adipose tissue is the most important site of cholesterol storage in man as well as in animals (Angel and Farkas 1974). Triacylglycerols stored in adipose tissue is the most concentrated form of energy available to all tissues. Lipids and especially triacylglycerols may be stored in adipocytes, hepatocytes and growing oocytes in avian species (Hermier, 1997).

Tablica 1. Lipoprotein lipase activity, triacylglycerol, total and free cholesterol concentrations in adipose tissue of chickens fed standard diet (control) and chickens fed same diet supplemented with organic selenium (Sel-Plex) before and after fasting

	LIPOPROTEIN LIPASE U/g tissue	**TRIACYLGLYCEROLS** mol/g tissue	**TOTAL CHOLESTEROL** mmol/g tissue	**FREE CHOLESTEROL** mmol/g tissue
before 48-h fasting				
control	1.32	0.91	244.32	2.12
Sel-Plex	2.06	1.01	118.59$^{\#}$	1.84
after 48-h fasting				
control	5.10*	1.00	98.73*	1.26*
Sel-Plex	5.05**	0.86	112.17	2.63$^{\#}$ **

Values are expressed as means ± standard deviation; Significant difference between control and Sel-Plex groups $^{\#}$(P<0.05); Significant difference before and after 48-h fasting between the control groups *(P<0.05) and Sel-Plex groups **(P<0.05)

Direct measurement of adipocytes total cholesterol and triacylglycerols before and after fasting suggests mobilization triacylglycerols at rates different from cholesterol mobilization (Kraus and Hartman 1984). In our investigation, no changes in triacylglycerol concentrations in adipose tissue were observed et the end of fattening period and after 48-hour food deprivation but at the same time, serum triacylglycerol concentrations in broilers decreased after fattening period and 48-hour food deprivation (Beer Ljubić et al., 2005.) suggest transport triacylglycerols from blood to periferal tissues. Our data on triacylglycerol concentration in adipose tissue were opposite to previous findings (Angel and Farkas, 1974). Angel and Farkas (1974) demostrated that fasting for 6 days decreased triacylglycerol content in adipose tissue continuously.

In present study, fasting significantly decreased total (P<0.05) and free cholesterol (P<0.05) concentrations in control chickens and increased free cholesterol concentration (P<0.05) in organic selenium supplemented chickens.

In agreement with previously published results, fasting increased serum cholesterol concentrations. 24 hours of fasting increased serum cholesterol concentrations in *Suncus murinus* (Yasuhara et al., 1991) and laying hen (Peebles et al., 2004) while 48 hours of fasting increased serum cholesterol levels in broiler chickens (Beer Ljubić et al., 2005.) and japanese quail (Lamošová et al. 2004). Furthermore, serum cholesterol levels remained increased after 9 days of starvation in rabitts (Klauda and Zilversmit, 1975). Cholesterol stored in the adipose tissue and liver is released into blood and that is main source of the hypercholesterolemia observed during fasting (Swaner and Connor, 1975; Lamošová et al., 2004.)

LPL plays an important role in regulating rates of triacylglycerols deposition in adipocytes according to the energy needs of the organism. In our research, 48-hours food deprivation incresead

(P<0.05) LPL activity in adipose tissue unexpectedly, but no effect on triacylglycerols. On the other hand, Ladu et al., (1991) demonstrated that LPL activity in rat adipose tissue decreased in starvation from first to sixth day of fasting. Sato et al. (1997) showed species related differences between chickens and rats adipose tissue LPL. 24 hours of fasting decreased LPL activity in rat adipose tissue but did not affect LPL mRNA levels (Lee et al., 1998). However, after 3 days of starvation LPL continued to decrease in common with LPL mRNA levels in rat adipose tissue (Lee et al., 1998). Sato and Akiba (2002) indicated specificitiy of physiological response on broiler chickens LPL because LPL mRNA levels in broilers adipose tissue were not modified by 48-hours food deprivation or by refeeding for 48 hours.

Present research suggests that organic selenium supplementation influences cholesterol metabolism in adipose tissue by decreasing total cholesterol concentrations during the fattening period and increasing free cholesterol concentrations after 48-hours food deprivation. The data from this investigation show a clear trend toward increasing adipose tissue lipoprotein lipase activity in chickens of Sel-Plex group, whereas LPL activity were significantly increased after fasting in both groups. Selenium must function through specific selenoproteins. They may be modulating the various enzymes and some of them are probably included in lipid metabolism. Therefore, only further studies are required to investigate the mechanism by which selenium affects metabolism in adipose tissue.

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