Seasonal Variations of Lipoprotein Metabolism in Hibernating Indian Common Toad Duttaphrynus melanostictus (Schneider, 1799)

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Abstract: During hibernation, fat is known to be the preferred source of energy. A detailed analysis of different lipoproteins, as well as free cholesterol, was conducted to investigate lipid abnormalities during hibernation. The levels of total lipoproteins and total cholesterol in the serum of Indian toad were found to increase significantly in hibernation as compared with the active state. Indian common toad showed significantly higher (p<0.05) concentrations of total serum cholesterol, high density lipoprotein cholesterol (HDL) and low density lipoprotein (LDL) cholesterol during the hibernating state in comparison with the active state. In conclusion, serum cholesterol and lipoprotein fractions are increased during hibernation in Indian toad.

Key words: Hibernation ∙ Toad ∙ Cholesterol ∙ HDL ∙ LDL

INTRODUCTION

Hibernation occurs during exposure to low temperatures and, under normal conditions, occurs principally during winter seasons when there are lengthy periods of low environmental temperatures. Hibernating animals conserve energy, especially during winter when food is short, tapping energy reserves, body fat, at a slow rate. It is the animal’s slowed metabolic rate which leads to a reduction in body temperature and not the other way around. A related form of dormancy is known as estivation. Many animals estivate when they are exposed to prolonged periods of drought or during hot, dry summers. For all practical purposes, hibernation and estivation in animals are indistinguishable, except for the nature of the stimulus, which is either cold or an arid environment.

The cholesterol transporting through the blood, lipoproteins have cell-targeting signals which direct the lipids they carry to certain tissues. For this reason, there are many types of lipoproteins within blood, generally called, in order of increasing density: chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). The cholesterol within all the various lipoproteins is identical, although some cholesterol is carried as the “free” alcohol and some is carried as fatty acyl esters referred to as cholesterol esters. However, the different lipoproteins contain apolipoproteins which serve as ligands for specific receptors on cell membranes. In this way, the lipoprotein particles are molecular addresses that determine the start and endpoints for cholesterol transport.

Transient elevation of serum lipid levels suggests a more rapid improvement in catabolism than the anabolism of lipid during early stage. The decline in thyroid hormone concentration has its behavioral and physiological response that leads to increased cholesterol concentration in serum, which is found to be the main metabolite during the hibernating phase. Food restriction and physiological preparation for hibernation is coincident with depressed plasma concentration of thyroid hormone [1]. Ackerman [2] measured cyclic fluctuations in hepatic lipid in Rana esculenta and concluded that maximum fatty infiltration occurs in July and again in winter months corresponding with period of maximum ovarian growth. She also showed that triglycerides and cholesterol ester account for the major hepatic fluid fluctuations. Hibernating ground squirrels had also significantly higher very low density lipoprotein plus low density lipoprotein cholesterol (VLDL + LDL) concentrations than did pre-hibernating ground squirrels. Hibernating squirrels additionally exhibited significantly higher (P < 0.005) total plasma cholesterol concentration per high density lipoprotein cholesterol concentration ratios than did pre-hibernating squirrels [3].
hibernating hedgehog there was a threefold rise in the brown fat FFA content which suggests an intense breakdown of fats (hydrolysis of triglycerides) in this tissue during hibernation [4]. In bear during hibernation, the body fat is doubling of its summer cholesterol level, yet it does not suffer the usual human afflictions such as hardening of arteries and gall stone formation [5].

Duttaphrynus melanostictus is a species of toad that is common in South Asia. It inhabits southwestern and southern China (including Taiwan and Hainan) and throughout southern Asia from northern Pakistan and Nepal through India to Sri Lanka andaman Islands, Sumatra, Java, Borneo and Bali. The species grows to almost 20 cm long. Commonly disturbed in open areas, villages, towns and only seen occasionally in primary forest. The species breeds during the monsoons. In this study a detailed analysis of different lipoproteins as well as free cholesterol were conducted to investigate lipid abnormalities during hibernation.

MATERIALS AND METHODS

Animals: Ten adult common Indian toads (Duttaphrynus melanostictus), each weighing 80-100 g were collected from a selected site in Midnapur (22°15′N 87°39′E) in late November, 2007 (air temperature 21.4°C-23.5°C) before the onset of hibernation and other group in mid Feb (air temperature 7.4 - 10.2°C) when hibernation was in late phase, from the mud hole of the same site. Another group was analyzed from the same site in late March (air temperature 22-25°C) when they just aroused from hibernation. Another group was collected from same site at monsoons July (air temperature 32-35°C).

Method: From hibernating and non-hibernating individuals blood samples were drawn via cardiac puncture using 21 gauge needle and 5 ml syringe immediately after euthanasia. We used pithing euthanasia. Blood sample were collected immediately after collection. (Animals handling was approved by ethical guideline laid down by the committee for the purpose of control and supervision of experimental animals (CPCSEA) constituted by the Animal Welfare Division of Government of India on the use of animals in scientific research.

Cholesterol reacts with Ferric chloride in the presence of acetic acid. The red color thus produced is measured calorimetrically. Optical density measured at 560nm or using a yellow green filter against distilled water set at zero. Lipoprotein lipase hydrolyses triglycerides to glycerol and free fatty acids. The glycerol formed with ATP in the presence of glycerol kinase forms glycerol 3 phosphate which is oxidized by the enzyme glycerol phosphate oxidized to form hydrogen peroxide. The hydrogen peroxide further reacts with phenolic compound and 4 aminoantipyrine by the catalytic action of peroxide to form a red coloured quinonemine dye complex. Intensity of the colour formed is directly proportional to the amount of triglycerides present in the sample. Optical density measured at 505nm (green filter). HDL and LDL/VLDL quantification kit is based on PEG precipitation method in which HDL and LDL/VLDL are separated and cholesterol concentrations are determined using cholesterol esterase/cholesterol dehydrogenase reagent. In this reaction, NAD is reduced to NADH. The optical density of formed NADH at 340 nm is directly proportionate to cholesterol concentration in the sample.

Statistical Analysis: Statistical analysis was done by using Microcal 6.0, statistical analysis. Each biochemical experiments were performed at least three times with 5 toads in each experimental group. Students t-test was performed to compare the means with a level of significance of P<0.05. Results have been represented as Mean ±SEM unless mentioned otherwise.

RESULTS

Result showed marked increase in serum cholesterol during hibernating phase (154±2.56) in comparison with the non hibernating (93±2.26) individuals as shown in Fig. 2. During the breeding season, cholesterol (202±2.94) ,HDL (49±2) LDL (116±2.05), VLDL (37±2.05) and triglyceride (189±2.33) reached their highest value due to reproduction success (Figs. 1 and 3). During pre hibernation state in the month of November they reserved fat materials and maintain cholesterol (170±2.43), LDL (124±2.07), HDL (38±1.25). During hibernation they depleted the reserve fat and during late hibernation in mid February, serum averages were 154±2.56, 105±1.45 and 36±1.15 for cholesterol, HDL and LDL, respectively. A significant difference (p<0.05) was shown for these three parameters during hibernating and non hibernating period. But triglyceride and VLDL concentration in serum decreased significantly during hibernation in comparison to non hibernating active individuals.
Fig. 1: Scatter diagram showing changes in serum HDL and LDL levels (mg/dl) throughout the year including during different phases of hibernation: entering hibernation (Nov), late hibernation (Feb) and after the hibernation (late-March) and rainy season (Jun-Jul). Both the levels of HDL (*P<0.05) and LDL (*P<0.05) showed significantly higher concentration during hibernation phase compared to non hibernating individuals.

Fig. 2: Bar diagram showing changes in cholesterol level (mg/dl) during different phases of hibernation viz. entering hibernation (Nov), late hibernation (Feb) and during arousal phase from hibernation (late March) and rainy season (Jun-Jul). Significant difference (*P<0.05) of cholesterol concentration during hibernation and non hibernation.

Fig. 3: Bar diagram showing changes in Triglyceride and VLDL levels (mg/dl) during different phases of hibernation viz. entering hibernation (Nov), late hibernation (Feb) and during arousal phase from hibernation (Late March) and rainy season (Jun-Jul). Both the levels of VLDL (*P<.05) and Triglyceride (**P<.05) showed significantly higher concentration during hibernation phase compared to arousal phase.
DISCUSSION

During hibernation, fat is known to be the preferred source of energy. Result showed significantly higher concentrations (p<0.05) of total serum cholesterol, high density lipoprotein cholesterol (HDL) and very low density lipoprotein (VLDL) cholesterol. Increased metabolic dependency towards lipids in hibernating condition is not only response to lower temperature but part of circannual homeostatic adjustment that at least partly regulated by thyroid hormone. The inverse relationship between serum thyroid hormone level and cholesterol level has been known for over years. Cholesterol 7 alpha hydroxylase (CYP7A) is the key enzyme involved in converting cholesterol to bile acid in liver. CYP7A expression was low in hypothyroidism and reactivated when hypothyroidism is restored when thyroid hormone is added back. Serum cholesterol levels are also elevated during hypothyroidism and are also reversed when thyroid is added back. CYP7A levels are reduced in hypothyroidism and are directly activated by thyroid hormone [6]. Much of the fat reserve goes into gonad maintenance, especially in females during monsoon so the fat reserve was highest during breeding seasons. At the late phase of hibernation toads usually exploited their reserve fat as a main energy source. At that time of hibernation toad generally did not eat, drink as a result body weight was reduced due to exclusively loss of body fat. Serum lipid concentration was also low immediately after arousal from hibernation but increased when feeding commences. During the late phase of hibernation triglyceride newly synthesize from liver to supply energy at that crucial phase. The significant differences (p<0.05) in the lipoprotein cholesterol concentrations observed in this study suggest that lipoprotein metabolism in pre-hibernators was significantly different from that in hibernators and was a reflection of the marked biochemical and physiological adjustments these animals must undergo during their transition from pre-hibernation to hibernation.

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REFERENCES