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Clenbuterol induced changes in cholesterol and triglyceride levels of *gastrocnemius, pectoralis* and heart of rat under work induced stress

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Work induced stress led to decreased cholesterol and fluctuating triglyceride levels in *gastrocnemius* and *pectoralis* muscles in rats. But the drug (clenbuterol, 2 mg kg^{-1} day^{-1}) treatment increased cholesterol and triglyceride levels in both the muscles. However, heart showed decreased cholesterol and increased triglyceride level in the animals under work stress, but at the same time drug treatment led to a significant increase in levels of the two lipid fractions, inferring towards deleterious effect of the drug on heart.

Keywords: Beta agonist, Clenbuterol, Heart, Lipids, Skeletal muscles, Work induced stress

Lipids like triacylglycerols (triglycerides), phospholipids, and sterols are of metabolic importance in mammals as they are the most concentrated source of energy to the organisms. Lipolysis in the fat cells is regulated by the cAMP levels, which in turn are regulated by several hormones like ACTH, chemical agents like catecholamines and certain drugs[1]. β-adrenergic agonists like clenbuterol are known to have muscle anabolic and lipolytic action[2]. They are involved in the body weight gain, particularly of the muscles[3-5]. Clenbuterol treatment in adult rats increased skeletal muscle mass and changed body composition similar to those seen in young rats[6]. The rate of total protein degradation increases by exercise and involves increased flux through the lysosomal pathway, while the breakdown of myofibrillar protein remains unchanged[7]. β-adrenergic agonists because of their sympathomimetic behaviour have tremendous potential in muscle wasting disorders accompanied with potentially deleterious effects like slow to fast fibre transformation in muscles which can be checked by endurance exercise[8]. Clenbuterol being a powerful muscle anabolic metabolite reduces the exercise capabilities of rats when chronically administered and at the same time produces deleterious effect on cardiac muscle[9]. The drug is also known to increase the heart weight [10]. The aim of present study is to see, firstly the effect of work induced stress on *gastrocnemius, pectoralis* muscles and heart. Secondly, to see the effect of β-adrenergic agonist clenbuterol on muscles under work stress and heart.

Adult Wistar male rats (120-150 g) obtained from Central Research Institute (CRI) Kasauli were maintained in the animal house of the department under suitable hygienic conditions (16 hr day light; 24° ±2°C), fed upon Hindustan Lever Feed and water *ad libitum*. They were divided into three groups. Group-I animals were normal and served as control. Group-II animals were denervated (sciatic nerve of left hind limb was cut ~2 cm) as per the method of Dhingra *et al* [11]. As a result of denervation the contralateral limb i.e. the right hind limb had to carry maximum body weight hence subjected to continual work induced stress or exercise. Because of imbalance created in its movements due to paralyzed hind limb, the *pectoralis* muscle was also subjected to work induced stress. Group-III included animals which were denervated and were given β-agonist clenbuterol (Sigma Chemical Co. USA) intraperitoneally in the thigh region of the denervated limb.

Clenbuterol was administered at a dose of 2 mg kg^{-1} day^{-1} till 15 days of post-denervation. This dose was derived from previous studies which suggests that clenbuterol dose in the range of 1-2 mg kg^{-1} day^{-1} is effective in producing hypertrophy and protein accumulation[6,12]. Duration of the drug treatment was kept 15 days because earlier studies suggest that maximum increase in muscle mass and organ weight are obtained within a fortnight and prolonged treatment shows no significant difference[5].

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Rats of all the three groups were maintained under similar experimental conditions for 30 days and were sacrificed on day 7 and 30 of post-denervation by cervical dislocation. Only healthy animals were employed for the purpose and the rest were discarded. At least 4-6 animals from each group were sacrificed at each stage. *Gastrocnemius* of the contralateral limb, *pectoralis* and heart were excised immediately and processed for the quantitative estimation of cholesterol and triglycerides. Lipids were extracted as per the method of Folch[13].The lipid extract hence prepared was utilized for the quantitative estimation of cholesterol and triglycerides. Cholesterol was estimated by the colorimetric method of Stadman[14] using acetic anhydride and concentrated sulphuric acid. Optical density was read at 680 nm in Bausch and Lomb Spectronic-20. Standard calibration curve was drawn using various concentrations of cholesterol. Triglycerides were estimated by the method of Vanhandel and Zilversmith[15] using arsenic trioxide and chromotropic acid. Optical density was read at 570 nm in Bausch and Lomb Spectronic-20. Standard calibration curve was drawn using various concentrations of tripalmitin.

Statistical significance was determined by Student's *t*-test[16] to find out significance of main difference among groups. Differences were assumed significant at $P<0.01$ and $P<0.001$.

Gastrocnemius and *pectoralis* muscles and heart of rat behave similarly under work induced stress and drug treatment, when compared for their cholesterol levels at 7 and 30 day post stress stage (Table 1). Contrary to the age related study on cholesterol content in the human muscles and various tissues[17,18] present study shows a decrease in same at day 30 when compared to day 7.Work induced stress decreased the cholesterol levels significantly from a very high (6.113±0.230 mg/g to 2.47±0.008 mg/g) in *gastrocnemius* at the 7th day stage, which is in confirmation with similar study done on chicks[19]. Because of the work induced stress the lipids present in the muscles got rapidly utilized for the generation of energy and therefore a reason for their dilution.

β-adrenergic agonists have anabolic effects on the skeletal muscles[2-5] but the chronic administration of the same is known to produce deleterious effects upon the cardiac muscles[9,20]. Cholesterol levels in the drug treated animals, in all the three muscles were significantly high when compared with those under stress which suggests that, clenbuterol brings about lipolysis in the adipose tissue and their mobilization into the muscles, when needed. In contrast to the lower cholesterol level in *gastrocnemius* of treated animals than the normal, *pectoralis* and heart show higher cholesterol levels than the normal which suggests that these two muscles were comparatively under less stress than *gastrocnemius*. At day 30, cholesterol levels in the drug treated animals came down in all the muscles when compared with same group at day 7, confirming withdrawal effect of the drug. Therefore, the present data suggest that work stress leads to the rapid utilization of the lipids and the drug treatment brings about fat mobilization from the adipose tissue to the muscles. As far as the increase of cholesterol content in the heart with β-agonist treatment is concerned, it occurs because of the stiffening of heart due to collagen infiltration in its ventricles[9], which in turn reduces the cardiac output thereby increasing the lipid level and hence proves to be deleterious to the heart.

Few studies have been done on the triglycerides in relation to work stress or β-agonist treatment[10,21] During the present investigation, fluctuating triglyceride levels were observed in the muscles. In mammals,

Table1 — Effect of clenbuterol [2mg kg^{-1} day^{-1}] treatment on 7 and 30 day post stress stage on cholesterol and triglyceride levels[mg/g fresh tissue wt] in *gastrocnemius and pectoralis* muscles and heart of rat under work induced stress

[Values are mean ± SE from 6 observations in each group]

	Pectoralis				*Gastrocnemius*				Heart			
	Cholesterol		Triglyceride		Cholesterol		Triglyceride		Cholesterol		Triglyceride	
Groups	7	30	7	30	7	30	7	30	7	30	7	30
C	4.56 ± 0.189	1.973 ± 0.083	211.99 ± 4.758	66.44 ± 17.945	6.133 ± 0.230	2.578 ± 0.153	177.91 ± 3.118	356.17 ± 29.773	0.717 ± 0.064	0.706 ± 0.110	75.21 ± 3.222	75.44 ± 6.014
D[WIS]	2.76 ± 0.051**	1.538 ± 0.032**	164.521 ± 29.414	74.94 ± 9.616	2.47 ± 0.008**	1.514 ± 0.038**	259.19 ± 47.896	64.95 ± 0.085**	0.656 ± 0.033	0.561 ± 0.094	299.41± 16.171**	103.66 ± 9.991
WIS +Cb	4.79 ± 0.035**	2.221 ± 0.003**	294.98 ± 11.380*	192 ± 2.984**	3.46 ± 0.044**	1.982 ± 0.025**	233.54 ± 43.042	150.59 ± 11.710**	1.256 ± 0.069**	1.198 ± 0.369	377.38 ± 43.986	124.88 ± 31.270

P value: *<0.01; ** <0.001

C = control; D = denervated; WIS = work induced stress; Cb = clenbuterol

triglycerides found in the cytoplasm of the adipose cells are used only under stress, when sufficient glucose is not present to meet the body requirements. When the animals under work stress were compared for their triglyceride levels at 7 and 30 day, it was found that the triglyceride level was comparatively higher on day 7 than on day 30 (Table 1). Present data suggest that initially work stress acted as a stimulant for the triglyceride synthesis, due to the activation of lipogenetic and inhibition of lipolytic processes. However continuous work stress induced metabolic aberrations resulting in the abnormal functioning of the muscle mitochondria at different times, which led to fluctuating triglyceride levels in some muscles[19].

The triglyceride levels in the *gastrocnemius* and *pectoralis* muscles and heart of drug treated animals were higher than the normal muscles and the muscles under stress, but when the triglyceride levels were compared on 7 and 30 day stage of work stress and drug treatment, its level declined on day 30 as compared to day 7 in all the muscles which can be attributed to the withdrawal effect of drug. β-agonists are known to produce thermogenic effects[22,23] because of which the fats are known to burn. But this thermogenic response is maintained for a small period, that too at higher dose, which after some time leads to desensitization of β-adrenergic receptors[24], virtually bringing lipolysis to halt in the adipose tissue hence leading to resynthesis of triglycerides and reducing their accumulation in the muscles. The increased triglyceride content in the heart may be because of the impairment of β-oxidation of free fatty acids in the heart[25] and hence synthesis of triglycerides. Therefore, present study shows that the animals under stress use up lipids for the generation of energy and clenbuterol mobilizes the fats into muscle tissue but at the same time proves deleterious to the heart because of excessive lipid accumulation in it.

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