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Effect of diazepam treatment on metabolic indices in trained and untrained rats

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Exercise training, like diazepam, is commonly employed as a means of reducing anxiety. Both diazepam and exercise training have been shown to modify carbohydrate and lipid metabolism as well as influence calcium metabolism in skeletal muscle. As receptor binding and thereby efficacy of diazepam has been demonstrated to be modulated by the lipid environment of the receptor, and changes in calcium levels can affect a number of intracellular signalling pathways, we sought to determine if the interaction of both chronic diazepam and exercise training would modify selected metabolic indices in an animal model. For this purpose, muscle and liver glycogen, blood glucose and plasma free fatty acids (FFA) were measured in sedentary, exercise trained and exercise trained, acutely exhausted animals. Alterations in lipid and carbohydrate metabolism were observed in all experimental groups. Diazepam treatment alone exerts metabolic consequences, such as elevated muscle glycogen and plasma FFA and depressed blood glucose levels, which are similar to those observed with exercise training. When animals are acutely exercised to exhaustion, however, differences appear, including a reduced rise in plasma FFA, which suggests that long-term diazepam treatment does influence exercise metabolism, possibly as a result of effects on the sympatho-adrenal system.

Keywords: exercise, FFA, glucose, glycogen

Diazepam (Vallium, Roche) is used widely in the treatment of anxiety (20). It is a centrally acting drug belonging to the benzodiazepine group of tranquillizers. In addition to having some anxiolytic properties, diazepam has sedative, hypnotic, muscle relaxant and anti-convulsant properties (2, 3). Exercise-training, like diazepam, is also

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commonly employed as a means of reducing anxiety. Both diazepam and exercise training have been shown to modify carbohydrate (9, 17) and lipid (9, 15) metabolism as well as increase calcium uptake by skeletal muscle (18). Recently, Farmer et al. (8) have noted some practical implications of the combined effects of diazepam and exercise. The combination of the two perturbations is involved in cardiac vagal withdrawal at the beginning of exercise in humans. Contractions initiated after diazepam treatment resulted in a significant reduction in heart rate change and thus the drug therapy may be responsible for training bradycardia. As receptor binding and thereby efficacy of diazepam has been demonstrated to be modulated by the lipid environment of the receptor (25) and changes in calcium levels can affect a number of intracellular signalling pathways (24), we sought the determine if the interaction of both chronic diazepam and exercise training would modify selected metabolic indices in an animal model.

Materials and Methods

Thirty-six male Sprague-Dawley rats (Ferme et Laboratoires Canadiens, St. Constant, Quebec) with initial body weights of 180–210 gr were used in the experiment. The animals were kept in individual cages with stable temperature, humidity and artificial illumination. Three experimental groups were studied. Group one (n=12) was sedentary; the second group (n=12) was exercise trained. The third group (n=12) was exercise trained and acutely exhausted by running on a treadmill. Half of the animals in each group were given a daily (5× per week) intramuscular injection of diazepam (Valium, Roche) at a dose of 5 mg/kg body weight. Animals were fed and watered *ad libitum* and were treated according to the guidelines outlined in Care of Experimental Animals: A Guide for Canada. The study was approved by the ethics committee of the Université de Montréal.

Rats in both trained groups were exercised on a rodent treadmill at a moderate exercise intensity. The speed of the treadmill and the duration of the training sessions were progressively increased over 4 weeks, until the rats were capable of running at a speed of 1.6 km/h, for one hour, 5 days a week. The animals then trained an additional 11 weeks at this pace, prior to sacrifice. All animals were weighed on the morning of sacrifice.

The sedentary and trained groups were sacrificed at rest. The trained-exhausted group was sacrificed immediately after the last training session which was a run to exhaustion. Exhaustion was defined as the point at which the rats were incapable of running further and could not attain an upright position when placed on their backs on the treadmill.

At the time of sacrifice, the animals were lightly anaesthetized with ether (14), their abdomens opened, and 10–12 ml of blood withdrawn from the abdominal aorta into heparinized syringes. One ml of whole blood was used for the estimation of blood glucose concentration by a modified Nelson-Somogyi technique (5). Duplicate samples

of plasma were used for determination of free fatty acids (FFA) as described by Dole and Meinertz (7). Parts of the liver and gastrocnemius muscle were quickly removed at the time of sacrifice and immediately frozen in liquid nitrogen. Liver and skeletal muscle glycogen levels were determined according to the method of Lo et al. (13).

All data are displayed as means ± standard error. An ANOVA was performed initially to determine the effects of exercise training and exhaustion within the treated and untreated groups and significance was determined using a Scheffe F test. Thereafter the effect of diazepam was determined using unpaired student's *t*-test for each state of the animal (sedentary, trained and exhausted). A *p* value less than 0.05 was considered significant.

Results

Body weights of the animals prior to the start of the program (T1) and on the day of sacrifice (T2) are found in Table I. Groups did not differ in body weight at T1. All exercised animals weighed less than their sedentary counterparts at T2.

Table I

Body weights of sedentary, exercise trained and exercise trained, exhausted animals with and without chronic diazepam treatment (5 mg/kg body weight) at the start and finish of the exercise training regimen (15 weeks)

	Sedentary		Trained		Trained Exhausted	
	T1	T2	T1	T2	T1	T2
Untreated	193.8 ± 8.3	572.4[a] ± 60.1	191.2 ± 11.3	506.0[ab] ± 43.6	193.6 ± 10.2	482.0[ab] ± 31.6
Diazepam Treated	194.7 ± 12.1	550.8[a] ± 49.6	187.8 ± 8.7	488.0[ab] ± 37.4	191.2 ± 16.4	512.0[ab] ± 49.0

T1 = weight at the start of the training regimen or diazepam treatment
T2 = weight at the termination of the experiment after 15 weeks
a = significant increase in weight throughout the study by similar groups (P<0.01)
b = significantly different from similarly treated sedentary group (P<0.05)

The effects of the drug, training and acute exhaustive exercise following training, on blood glucose, plasma FFA, and liver and skeletal muscle glycogen are presented in Figure 1.

Effects of exercise

Gastrocnemius muscle glycogen levels were higher in exercise trained, non-diazepam treated animals by 113% ($p<0.01$) when compared to the sedentary condition. No discernable effects on liver glycogen, blood glucose and plasma FFA levels were observed as a result of training alone.

After exhaustive exercise liver glycogen concentrations were significantly lower ($p<0.01$) in both treated and untreated rats when compared to the trained levels.

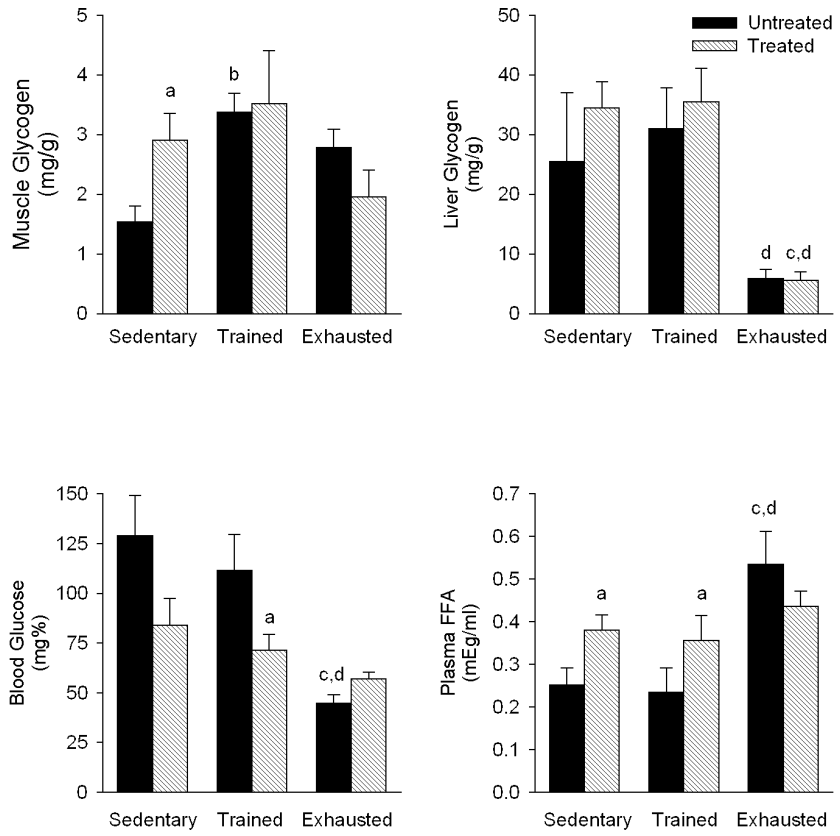

Fig. 1. Muscle glycogen, liver glycogen, blood glucose and plasma free fatty acid (FFA) levels in untreated and diazepam treated sedentary, exercise trained, and exercise trained, acutely exhausted animals. Values are means ± SE. a = $p<0.05$ untreated vs treated; b = $p<0.05$ sedentary vs trained; c = $p<0.05$ sedentary vs exhausted; d = $p<0.05$ trained vs exhausted

Blood glucose concentrations were also lower with exhaustion in both groups. Free fatty acid concentrations were higher at the point of exhaustion in the untreated rats than in their sedentary or trained only counterparts ($p<0.01$).

Sedentary rats treated with diazepam had higher levels of glycogen (75%, $p<0.015$), when compared to the untreated sedentary rats, in the gastrocnemius muscle. In the trained and trained-exhausted rats, diazepam treatment caused no significant differences in muscle glycogen content. Diazepam treated animals also exhibited elevated plasma FFA levels in both the sedentary and trained conditions.

Effects of exercise plus diazepam

Blood glucose levels were lower in trained diazepam treated than trained untreated animals. Diazepam treated sedentary and diazepam treated trained animals both had higher plasma FFA concentrations than the untreated groups, although the levels did not differ between these two treated groups.

Discussion

Sedentary state

In the present study diazepam administration, in sedentary rats, brought about higher values in the glycogen content of the gastrocnemius muscle. This difference in skeletal muscle glycogen storage could be explained by a stimulating effect of the drug on lipolysis (12), with a subsequent increase in plasma FFA utilization by the muscle, and thus the preservation of the glycogen stores. The higher plasma FFA concentrations further suggest a lipolytic effect of the drug. Interestingly, the tendency towards a decrease in plasma glucose observed in the sedentary diazepam treated animals, would indicate that the glucose/fatty acid cycle is unaffected. Plasma triglycerides have been reported to increase in patients receiving benzodiazepine therapy (12). It has also been demonstrated that diazepam administration elevates the plasma level of FFA in rabbits (4) and rats (11). The mechanism of action of benzodiazepines on lipolysis has not been established, although diazepam has been found to enhance plasma lipoprotein lipase activity (4). It is also possible that benzodiazepines enhance insulin secretion (11).

Trained state

As expected, trained animals had higher exercise training increased muscle glycogen levels (1, 10, 23). Our results suggest that this effect is not further enhanced when combined with diazepam treatment, since skeletal muscle glycogen content was equal in both treated and untreated trained groups. Whereas the glycogen content was the same, the effect of training on the absolute change in skeletal muscle glycogen was different in the two groups. Training resulted in higher skeletal muscle glycogen content in the untreated animal (?113%), whereas the change was only 25% in the treated rats. The interplay between fat and carbohydrate metabolism, known as the glucose-fatty acid cycle (19, 21), may offer an explanation for this unexpected finding. It is quite possible that a feedback mechanism is in effect, by which elevated FFA impair further glucose uptake. Rodgers and Vranic (21) suggest that the effect of FFA levels on carbohydrate metabolism is not a function of FFA concentration but a result of the effect of FFA levels on insulin and/or the sensitivity of insulin receptors. As well, Marette et al. (16) have recently demonstrated that glucose can reduce plasma membrane GLUT 4 protein content in rat skeletal muscle. They hypothesize that this could be a protective

mechanism against excessive glucose uptake under certain conditions. Liver glycogen, blood glucose and plasma FFA levels were not altered significantly by training. These observations might suggest an upper limit for the muscle glycogen content (10).

Exhausted state

Exercise to exhaustion resulted in lower blood glucose and higher plasma FFA levels in untreated rats. Non-significant changes observed in the treated animals were a consequence of already depressed blood glucose and elevated FFA levels. Lower FFA concentrations and smaller changes in FFA in the exhausted treated rats could also be related to the decreased activation of the sympatho-adrenal system caused by the administration of diazepam (2, 25), although without a direct measure of catecholamine levels or receptor sensitivity, this possibility remains speculative. Activation of the adrenergic system is thought to be important for the increased lipolysis observed during exercise (22).

The release of catecholamines from the adrenal medulla is not as essential for controlling the mobilization of carbohydrate, as it is for lipids, during exercise (9). Possibly as a consequence, the reduction in liver glycogen content after exhaustive exercise was similar in the untreated and treated groups. However, skeletal muscle glycogen content was lower in the exhausted rats treated with diazepam than the untreated exhausted rats. This lower glycogen content could simply reflect an accommodation by the muscle for a decrease in FFA supply or be due to a direct effect of diazepam (6).

In summary, this investigation has demonstrated minor alterations in lipid and carbohydrate metabolism in exercised rats treated with diazepam. These results suggest that diazepam treatment alone exerts metabolic consequences, such as elevated muscle glycogen and plasma FFA which are similar to those observed with exercise training. When animals are acutely exercised to exhaustion, however, differences, including a reduced rise in plasma FFA, appear, which suggests that long-term diazepam treatment does influence exercise metabolism, possibly as a result of effects on the sympatho-adrenal system.

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