Leucine: An anabolic alternative?

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ABSTRACT

The effects of excess dietary leucine on rats undergoing a strenous swimming exercise programme were investigated over a six weeks period on 36 male Wistar rats who were randomly divided into three groups – experimental, exercise control, and sedentary control. Variables measured were weight gain, swim time, and percentage of body weight of the gastrocnemius muscle. Analysis of variance for unequal groups with planned comparisons was used to compare the data. Results showed a significant difference ($P < 0.05$) in weight gain between all groups and a non-statistically significant average swim time improvement of 2.55 minutes for the experimental group versus controls. No differences were observed in the weight of the gastrocnemius. The findings suggest that leucine plays a role in maintaining lean body mass and is an oxidizable substrate in muscle during intensive aerobic exercise.

The use of anabolic steroids as an ergogenic aid by international athletes has received intensive public scrutiny since the revelation of steroid abuse at the recent Seoul Olympics.[1]

However, this diversion of restricted medical treatment is not new. In a survey of track and field athletes at the 1972 Olympic games, 68% of athletes competing in field events, sprints, and middle distance running admitted they had used anabolic steriods.[2] The steroids taken by athletes include the major male sex hormone testosterone and synthetic derivatives that have anabolic (tissue-building) and androgenic (masculinizing) capabilities.[3] Athletes use these drugs in the belief that they will increase lean body mass, muscular size, strength, endurance, and performance. Paradoxically, steroids may produce adverse effects, including hepatoxicity,[4] peliosis hepatitis,[5,6] kidney tumors, [7] accelerated atherosclerosis,[8] cancer of the prostate,[9] and psychotic disturbances.[10]

These potentially life-threatening consequences were paramount factors in the banning of steroids by the International Olympic Committee. Unfortunately, many athletes do not observe the ban choose to use steroids illegally. For this reason conventional alternatives to anabolic steroids require immediate exploration.

Leucine ($C_6H_{1N}O_2$), one of the branch-chained essential amino acids, has exhibited anabolic proclivity through increasing protein synthesis and inhibited protein degradation in young rats.[11,12,13,14] Studies on muscle tissue in vitro have implied that leucine has a significant anabolic effect when present in ten times the normal plasma levels.[15] Research on leucine and its anabolic effects in vivo is sparse. Therefore, this study was undertaken to examine the effect of the addition of excess leucine to the normal diet of rats undergoing a strenuous swimming exercise programme.

METHODS

Subjects

Thirty-six young male rats of the Wistar strain, ranging in initial weight from 75 to 115 grams, were used in this study. The rats were housed individually in wire cages in five tier metal racks. Each cage was equipped with a gravity-fed water bottle and had a feeding basket attached to it. The animals were maintained in a ventilated room on 12 hour dark/light cycles at 23.0°C.

EXPERIMENTAL PROTOCOL

On arrival at the laboratory the rats were randomly assigned to one of three groups: ex-

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perimental; exercise control; or sedentary control. The weight of each rat was recorded as they were handled. The exercise control and experimental groups were swum for 25 minutes on the day following arrival and for 1 hour on the following two days. After this pre-training period, weights (2% of body weight) were attached to the rat's upper body by means of an alligator clip. Cylindrical plastic tanks 69.0 cm in height, 42.0 cm in diameter at the bottom and 54.0 cm in diameter at the top were used for the swims. The depth of the water was 53.0 cm and was sufficient to prevent the rats from pushing off the tank bottom or supporting themselves with their tails. The water temperature was maintained at 34.0°C.

EXERCISE PROGRAMME

The exercise control and experimental group rats were swum on Monday, Tuesday, Thursday, and Friday mornings. The total swimming time for each rat ranged between 45 and 90 minutes with exhaustion being the criteria for removal from the water. The rats were pronounced exhausted upon failure to maintain their nostrils above the water surface.

Prior to each swimming session a subjective evaluation of the previous performance was made. On the basis of the evaluation additional weight was added to the load carried by each rat. The weight was attached by fixing a lead weight to a piece of waxed string, which was then connected to an alligator clip. After each training swim the rats were towel dried and returned to their respective cages.

At the end of the six weeks training period all the rats were swum to exhaustion while carrying a 10.2 grams weight attached to their chests. The rats swam in groups of three and swim time was recorded to the nearest second.

TREATMENT

For the experimental group leucine was added to the standard diet of Purina laboratory chow. The quantity of leucine required to increase intake ten times the normal dietary leucine was calculated using the analysis of nutritional content provided by the company. The required amount of laboratory chow was ground into a powder using a standard household food grinder. The ground food was weighed and the leucine added and mixed thoroughly. The resulting mixture was formed into a paste using a minimum amount of water. The paste was moulded into pellets, approximately the size of the original Purina chow, and dried overnight at low temperature in the laboratory oven. These pellets were fed to the rats in the experimental group ad libatum using the food hopper attached to the cage door.

SACRIFICIAL PROCEDURES

The rats were sacrificed the day after the final swim by injection of a lethal dose of sodium pentobarbital. Upon cessation of heartbeat the right gastrocnemius was excised, swabbed and weighed.

STATISTICS

To determine the specific relationships between the groups the method of statistical analysis used was an analysis of variance for unequal group sizes* with planned comparisons. The standard level of 0.05 was used to ascertain any significant changes in weight gain, swim time, and percentage of body weight of the gastrocnemius.

RESULTS

Figure 1 shows that weight gained by the experimental group was 38.83 grams less than that gained by the exercise control group, and 62.77 grams less than the sedentary group gain. The comparisons between experimental and exercise control, experimental and sedentary control, and exercise control and sedentry control were similarly significant ($P<0.05$).

Figure 2 reveals an increase in swim time by the experimental group of 2.78 minutes greater than the exercise control group, and 2.33 greater than the

*5 rats expired due to laboratory accidents.

Figure 1

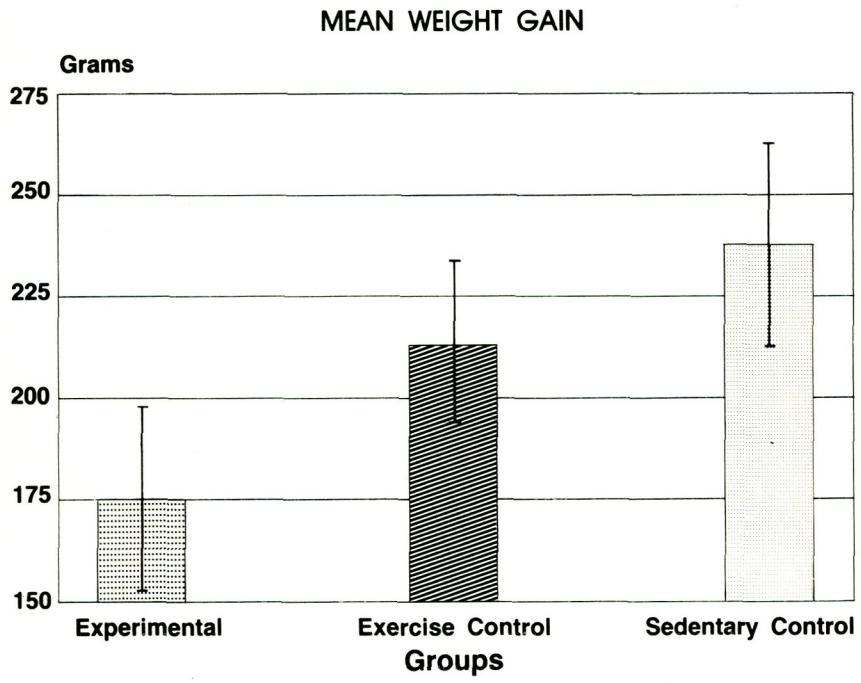
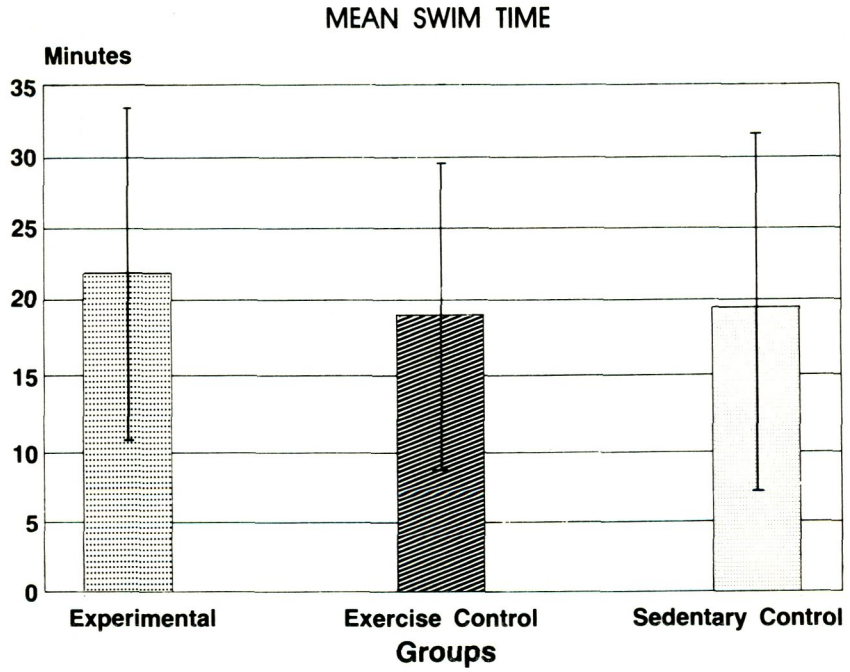


Figure 2



sedentary control group. However, these changes are not statistically significant.

There were no differences in the percentage of body weight of the gastrocnemmi. Mean percentage of body weight of the gastrocnemmi was 0.61 ± 0.01 for the experimental group, 0.63 ± 0.01 for the exercise control group, and 0.63 ± 0.01 for the sedentary control group.

DISCUSSION

The purpose of this study was to determine the effects of a ten fold increase of dietary leucine on swim time to exhaustion in young male Wistar rats. Supporting data were measurements of body weight gain and percentage body weight of the gastrocnemius muscle.

Due to technical reasons an accurate account of ingestion rate was not possible. However, the subjective observance of the principal investigator was that each animal consumed almost equal number of chow pellets.

The lower experimental group body weight gain is theorized to be due to increased utilization of leucine as exercise fuel during the endurance swimming [16] which may promote decreased body fat levels while maintaining or increasing lean body mass.

The excess leucine may have influenced maximal performance as evidenced by increased swim time but the improvement was not statistically significant. Fuge et al[17] propose that lower body weight enhances endurance, as smaller muscle fibers with large surface-area-to-mass ratios increase lactic acid diffusion out of the muslce fiber. During the approximate 20 minutes swim to exhaustion, and despite carrying a heavier attached percentage of body weight, the experimental group outswam the other groups by an average of 2.55 minutes. This result appears to support previous investigations [18, 19] that reported leucine to be an important oxidizable substrate in muscle and that amino acids may supply up to 10% of the energy used during exercise.

Intensive endurance training may alter the speed of protein synthesis or degredation, and lead to an elevated dietary requirement for amino acids.[20] Also, muscle fiber damage occurs with endurance exercise,[21] and additional amino acids may aid in regeneration of impaired fibers.

Absence of hyperthrophy in the gastrocnemmi after leucine ingestion and strenuous swimming exercise is consistent with reports of sacroplasmic protein augmentation rather than contractile protein development following endurance training.[22]

CONCLUSION

A ten-fold increase in dietary leucine in rats appears to simulate reported anabolic effects of lean body mass and performance enhancement. However, further clinical research on the effects of varying leucine dosages on total and structural proteins during intensive exercise is required, before definitive conclusion can be drawn and extrapolated to human subjects.

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