ORIGINAL ARTICLE

EFFECTS OF FREE & RESTRICTED REMOBILIZATION ON THE BODYWEIGHT AND MID THIGH CIRCUMFERENCE OF RABBIT

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ABSTRACT

Objective: To find out the effects of free and restricted remobilization on the generalized body weight and mid thigh circumference of rabbit. For this purpose, knee joints of rabbits were immobilized first.

Study Design: Randomized Controlled Trials (RCT)

Place and Duration of the study: Study was carried out in Army Medical College, Rawalpindi and National Institute of Health Sciences, Islamabad from April 2006 to April 2007.

Materials and Methods: Twenty four adult New Zealand White rabbits were randomly divided into three groups using random numbers table. Group I served as a control group whereas the left knee joint along with ankle joint of the experimental groups II & III were immobilized in extension in a plaster of Paris cast for 4 weeks. At the end of 4 weeks plaster cast was removed and group II i.e. free mobility group and group III i.e. restricted mobility group (caged animals) were remobilized for another 4 weeks. The generalized body weight and mid thigh circumference of all the animals were taken at the start of experiment, at the end of 4 weeks of immobilization and at the end of 4 weeks of remobilization in all groups.

Results: Immobilization caused significant reduction in the body weight and mid thigh circumference of both the experimental groups. On remobilization the body weight increased but significant difference remained between the experimental and control groups. Although weight gain is more in the caged animals during remobilization phase but statistically insignificant difference was found when free & restricted mobility groups were compared. The difference in the mean mid thigh circumference between control and free mobility groups was statistically insignificant whereas the difference between control and restricted mobility groups was significant at the end of 4 weeks of remobilization. Recovery in caged animals was slower as compared to free mobility group in terms of mid thigh circumference but when these two groups were compared statistically insignificant difference existed at the end of 8 weeks.

Conclusion: Immobilization induced reduction in body weight and mid thigh circumference which is to a great extent reversible in both free and restricted mobility groups. Both the groups responded almost equally to immobilization stress and confinement did not prevent restoration of bodyweight and mid thigh circumference, however complete recovery of the body weight and mid thigh circumference to initial control values did not occur in 4 weeks.

Keywords: Immobilization, Remobilization, Body weight, Mid thigh circumference.

INTRODUCTION

Immobilization of animals by a plaster cast is one of the popular methods used for the treatment of musculoskeletal injuries and is also one of the most widely used methods for immobilization in animal models[1,2]. The impact of immobilization on the body weight and skeletal muscles has been investigated in animal models. Zarzhevsky et al[3]. 2001 reported that several weeks of immobilization contributed to decrease both the body weight and weight of skeletal muscles in rats. Coutinho et al[4]., 2002 also described significant loss of body weight during 21 consecutive days of immobilization. Faraday et al[5]. 2005 also described that immobilization stress decreased both feeding and body weight in female rats[5]. However, the data on the effect of free verses restricted remobilization on the body weight was not documented.

It was reported that mid thigh circumference served as an indicator for assessing the nutritional status of individual[6,7].

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Heerkens et al., 1987 reported a decrease in circumferential dimensions due to immobilization of human knee. They further reported that the difference between immobilized and unaffected leg remained even after 81 days of remobilization and this decrease in circumferential dimensions indicated atrophy of thigh and calf muscles[8]. Studies conducted in the past also reported the recovery from atrophic changes of skeletal muscle during remobilization[9-11]. However, the effect of free and restricted remobilization on mid thigh circumference of rabbit hind limb was not reported. In most of the studies animals were euthanized and skeletal muscles were then studied directly to see the atrophic changes. Therefore measurement of mid thigh circumference in animal models is an indirect way of assessing the nutritional status of the animal and atrophic changes in muscles. In view of above the objective of the present study was to immobilize the stifle joint of rabbit first and then to see the subsequent effects of free and restricted remobilization on the body weight and mid thigh circumference of rabbit.

MATERIALS AND METHODS

These randomized controlled trials (RCT) were carried out at Army Medical College, Rawalpindi and National Institute of Health Sciences (NIH), Islamabad. In this study twenty four adult New Zealand white rabbits [approx. ages 4-6 months; weights 1.3-1.7 kg] were taken. Animals were randomly divided into three groups using random numbers table. Each group comprised of 08 animals. Group I served as control. The left knee joints along with ankle joints of groups II & III were immobilized in extension in a long leg padded plaster of Paris cast. The animals remained ambulant during the period of immobilization (Fig.1).

All the animals were given water and standard animal house diet ad libitum prepared at National Institute of Health (NIH), Islamabad. At the end of 4 weeks of immobilization plaster cast was removed and animals were remobilized for another 4 weeks by either allowing free mobility as in group II or restricted mobility in a cage (Fig. 2) as in group III.

The body weight and mid thigh circumference (MTC) of all the animals were taken at different intervals. Mid thigh circumference was measured by a measuring tape. First a diagonal length was taken from the epicondylus medialis (the most prominent point on medial side of lower end of femur of rabbit) to the most prominent ventral point on the crista iliaca of os coxa (Hipbone). Then the mid point of this diagonal is taken which approximately lies 5.5 cm above the knee joint. At this point mid thigh circumference of thigh was measured. Data was entered in a database using SPSS for windows version 11. The statistical significance of difference between two means was evaluated by Student's't' test. The comparison was done between the groups by Independent Sample't' test. The difference was regarded as statistically significant, if 'p' value was less than 0.05.

RESULTS

General Observations

All the animals survived and remained active during the duration of experimental period. During immobilization intake of food among the experimental groups was reduced as evident by decreased intake of standard diet in 24 hrs assessed by left over food, however after remobilization food intake improved as the animals consumed almost whole of the diet in 24 hrs in both the groups II and III. The general condition of the animals remained healthy and they tolerated the plaster cast very well and remained ambulant with the plaster cast. Any subsequent cracks in the plaster cast were duly repaired. None of the animals developed any complications. After the removal of the cast in groups II & III left leg was examined and mid thigh circumference was taken.

The body weights of all the animals were taken at different intervals. Initial body weight (W1) was taken at the start of experiment. Then the weights were taken at 4 weeks of immobilization (W4) and at the end of 8 weeks (W8) i.e. 4 weeks of immobilization and 4 weeks of remobilization. The Mean body weights (g) of all animals in three groups were recorded as W1, W4 and W8 in (Table 1).

Comparison of mean body weights between groups:

W1: The mean body weight of group I was 1592.50 ± 47.72g, group II was 1622.50 ± 30.63g and group III was 1568.75 ± 27.93g (Table 1) and the difference in the mean body weights between groups I & II, I & III and II & III was statistically insignificant (p > 0.05, Table 2).

W4: The mean body weight of group I was 1611.25 ± 46.03 g and group II was 1346.25 ± 43.79 g and group III was 1362.50 ± 40.03g (Table 1, Fig. 3) and the difference in the mean body weights between groups I & II, I & III was statistically significant (p < 0.05, Table 2). However, the difference in the mean body weights between groups II and III was statistically insignificant (p > 0.05, Table 2).

W8: The mean body weight of group I was 1645.00 ± 45.00g and group II was 1378.75 ± 38.05g and group III was 1395.75± 37.42g (Table 1, Fig. 4) and the difference in the mean body weights between groups I and II and I & III was statistically significant (p < 0.05, Table 2). However, the difference between the mean body weights of group II and III was statistically insignificant (sp > 0.05, Table 2).

Mid thigh circumference (MTC) of animals in centimetres (cm) was taken at different intervals. Initial MTC (C1) was taken at the start of experiment. Then the Mtc was taken at 4 weeks of immobilization (C4) and at the end of 8 weeks (C8) i.e. 4 weeks of immobilization and 4 weeks of remobilization. The mean MTC of all animals in three groups was recorded as C1, C4 and C8 in (Table-3).

Comparison of mean MTC between groups:

C1: The mean MTC of group I was 12.36 ± 0.17cm, group II was12.46 s± 0.18cm and group III was 12.20 ± 0.15cm (Table 3) and the difference in the mean Mtc between groups I and II, I & III and II & III was statistically insignificant (p > 0.05, table 4).

C4: The mean MTC of group I was 12.42± 0.15cm, group II was 11.93 ± 0.16cm and group III was 11.88 ± 0.08cm (Table-3, Fig.5) and the difference in the mean MTC between groups I and II and I & III was statistically significant (p < 0.05, Table 4) and between groups II & III was statistically insignificant (p > 0.05, Table 4).

C8: The mean MTC of group I was 12.55 ± 0.15cm, group II was 12.18 ± .13cm and group III was 12.12 ± 0.11cm (Table 3, Fig. 6) and the difference in the mean MTC between groups I & II and II & III was statistically insignificant (p > 0.05, Table 4). However, the difference between groups I & III was statistically significant (p < 0.05, Table 4).

Figure 1: Animals were allowed free mobility following immobilization

Figure 2: Showing animal of restricted mobility group in a cage

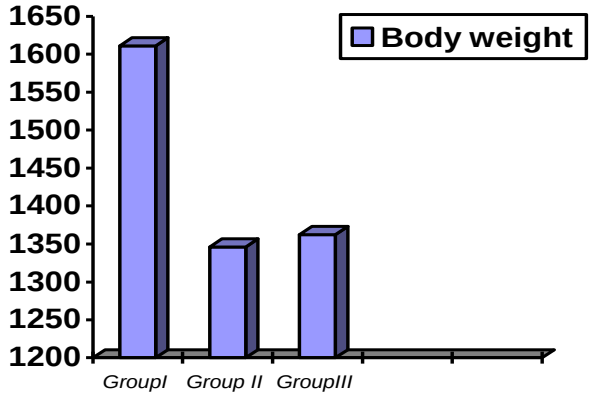

Figure 3: Comparison of mean bodyweights W4 between groups

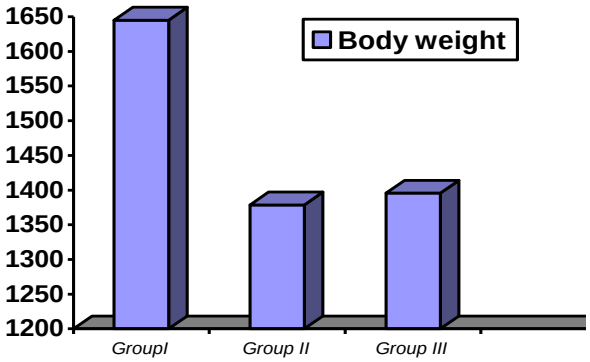

Figure 4: Comparison of mean bodyweights W8 between groups

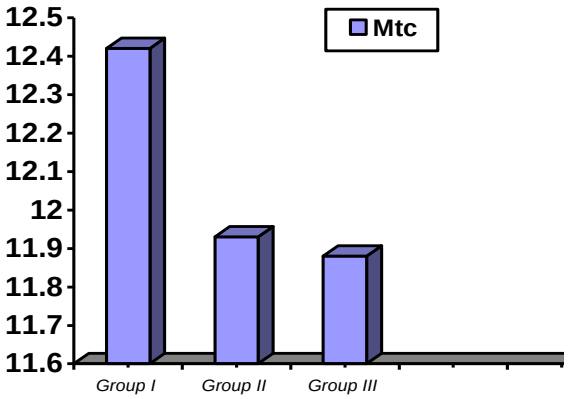

Figure 5: Comparison of mean MTC C4 between groups

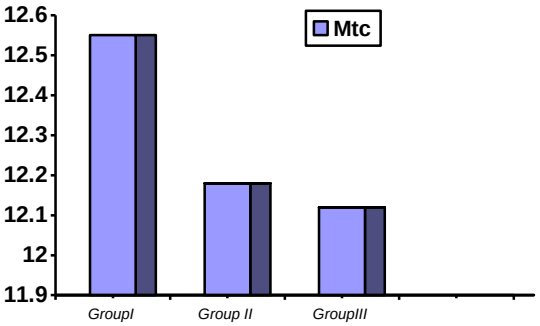

Figure 6: Comparison of mean MTC C8 between groups

DISCUSSION

During immobilization intake of food among the experimental groups was reduced, this could be because the animals were under stress but on remobilization their appetite gradually improved as the stress of immobilization was over. These findings were similar to Martha et al. (2005) who suggested that stress of immobilization decreased both feeding and body weight[12]. Plaster cast was well tolerated by all the animals and they remained ambulant with it on three feet. This was in accordance with Wadood (2002) and Jortikka et al. (1997) who mentioned that the animals remained active and mobile during immobilization[1,13]. Maximum leg stiffness was observed on the day of removal of plaster cast

Table 1: Mean body weight (g) W1, W4 and W8 of each group

Group No	Mean ± SE (W1)	Mean ± SE (W4)	Mean ± SE (W8)
Group I	1592.50 ± 47.72	1611.25 ± 46.03	1645.00 ± 45.00
Group II	1622.50 ± 30.63	1346.25 ± 43.79	1378.75 ± 38.05
Group III	1568.75 ± 27.93	1362.50 ± 40.03	1395.75 ± 37.42

W1: Initial body weight
W4: Body weight at four weeks
W8: Body weight at eight weeks

Table 2: Comparison of mean body weights W1, W4 & W8 between groups

Body Weight	Group No.	Statistical Significance of difference
W1	I & II	P>0.05
	I & III	P>0.05
	II & III	P>0.05
W4	I & II	*P<0.05
	I & III	*P<0.05
	II & III	P>0.05
W8	I & II	*P<0.05
	I & III	*P<0.05
	II & III	P>0.05

** Statistical difference between groups is significant.*

Table-3: Mean MTC (cm) C1, C4 & C8 of each group

Group No	Mean ± SE (C1)	Mean ± SE (C4)	Mean ± SE (C8)
Group-I	12.36 ± 0.17	12.42 ± 0.15	12.55 ± 0.15
Group-II	12.46 ± 0.18	11.93 ± 0.16	12.18 ± 0.13
Group-III	12.20 ± 0.15	11.88 ± 0.08	12.12 ± 0.11

C1: Initial Mtc
C2: Mtc at four weeks
C3: Mtc at eight weeks

Table-4: Comparison of mean MTC C1, C4 & C8 between groups

MTC	Groups No.	Statistical Significance of difference (p value)
CI	I & II	p > 0.05
	I & III	p > 0.05
	II & III	p > 0.05
C4	I & II	*p < 0.05
	I & III	*p < 0.05
	II & III	p >0.05
C8	I & II	p >0.05
	I & III	*p < 0.05
	II & III	p > 0.05

*** Statistical difference between groups is significant.**

but thereafter the stiffness reduced on passive flexion and the resistance became almost equal to the contralateral limb after 7-8 days in both free and restricted mobility groups. Namba et al[16]. (1991) also mentioned that joint stiffness increased 2.6 times the pre injury level in limbs that were immobilized for 3 weeks[14]. Also it was suggested that nontraumatic immobilization of joints in rats, dense connective tissue remodels in such away that mobility is unaffected after 2 weeks, but became quite limited by 6 weeks[15].

Body Weight

At the start of the experiment, there was no significant difference between the mean body weights W1 among the groups. This was because of little variation in the initial body weights of all groups, which range between 1300 to 1700 g. The animals gained weight gradually in 8 weeks which could be attributed to proper feeding, housing, mobility and environment of animals. When comparison of mean body weights W4 was done between groups I & II and I & III, significant decrease in body weights was found following 4 weeks of immobilization. This was in accordance with Laurilla et al[16]. (1991) who also found a total 11% reduction in body weights after six weeks of immobilization[16]. Coutinho et al[4]. (2002) had observed similar findings and reported that immobilization contributed to decrease both the body weight and skeletal muscle. The reason for reduction in body weight of animals could be immobilization stress that resulted in decreased feeding and probably atrophic changes in the musculature of immobilized limb.

However, the findings in present study were not in agreement with Jortikka et al[13]., 1997 who reported no change in generalized body weight even after 11 weeks of immobilization. This could be because of the difference in immobilization technique and environment of animals which induced less amount of stress. Although the mean body weights W8 increased at the end of 4 weeks of remobilization but they never reached near the control values, therefore when mean body weights of groups I & II and I & III were compared still significant difference was found. Perhaps this was because immobilization induced moderate degenerative changes in the knee joint[17] resulting in a continuous stress during remobilization phase and hence the animals had not gained the weight they had lost. When mean body weights of groups II and III were compared after 4 weeks of immobilization (W4) and also at the end of 4 weeks of remobilization (W8), statistically insignificant difference was found. Perhaps the reason was that both the groups had received similar type of stress and therefore responded equally in the remobilization phase.

Mid Thigh Circumference (MTC)

The mean MTC between all groups at the start of experiment was insignificant (p > 0.05). This could be correlated with the initial body weights W1 of these groups, which were also statistically insignificant. When mean Mtc C4 of experimental groups II and III was compared between the groups I & II and I & III at the end of 4 weeks of immobilization, significant decrease was found. This decrease in mean Mtc could be related to under nutrition[6,7] of animals during immobilization phase due to constant stress. This could also be attributed to disuse atrophy of skeletal muscles sduring immobilization[11]. Heerkens et al[8]. (1987) also concluded that immobilization resulted in decrease in circumferential dimensions of thigh and calf muscles of human knee. They added

further that this difference in circumferential dimensions was present even after 81 days of remobilization. Comparison of mean Mtc C8 at the end of 4 weeks of remobilization between groups I & II was statistically insignificant and I & III was significant. Perhaps this could be due to lack of free mobility and physical activity in caged animals. This was in accordance with Kannus et al., 1998 who reported that immobilization induced atrophy is a reversible phenomena if remobilization is intensified by physical training[18]. However, when mean Mtc C4 was compared between groups II and III, statistically insignificant difference was found showing that immobilization stress affected both the groups equally. Mean Mtc C8 improved in both the groups during remobilization indicating that there was some gain in muscle bulk with joint loading and motion. Also it was noted that the recovery in caged animals was slow as compared to freely mobile animals, yet statistically insignificant difference was found between groups II and III at the end of 8 weeks. Also this duration was not enough to completely restore the mid thigh circumference to control values.

CONCLUSION

Immobilization stress induced significant reduction in the body weight and mid thigh circumference of animals which is to a great extent reversible in both free and restricted mobility groups. Although recovery of mid thigh circumference in caged animals was slower as compared to freely mobile group, yet both the groups responded almost equally to immobilization stress and confinement did not prevent restoration of bodyweight and mid thigh circumference. However, complete recovery of the body weight and mid thigh circumference to initial control values did not occur in 4 weeks.

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