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OBESITY ATTENUATES FORMALIN-INDUCED TONIC PAIN IN BRITISH ANGORA RABBITS

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Abstract : Obesity is known to alter various physiological parameters including the pain sensitivity. There are conflicting reports on the pain sensitivity in obesity. In this context, the present study was aimed to investigate the tonic pain response in obese rabbit model. To achieve this aim, two groups of adult male British Angora rabbits were used. One of the groups was fed with standard rabbit chow and served as control. The other group was fed high fat diet (HFD) for 10 weeks to produce obesity. The standard formalin test was performed at the start and after 10 weeks of dietary regimen in both the groups. Timed behavioral responses (limping, elevation of paw, licking, biting, grooming etc.) were categorized and quantified with the help of standard pain rating scale. The total average pain rating score decreased significantly from 2.01 ± 0.02 to 1.47 ± 0.08 ($P<0.05$) in HFD group after 10 weeks of dietary regimen, whereas there was no change in the control group. A significant negative correlation was observed between body weight and pain rating score in HFD group of rabbits ($P<0.05$, $r=-0.62$). Results suggest that obesity attenuates the tonic pain responses induced by formalin in British Angora rabbits.

Key words : obesity, British Angora rabbit, tonic pain, formalin tests

INTRODUCTION

Obesity results from excessive deposition of fat in adipose tissue and is known to alter various physiological parameters leading to cardiovascular and metabolic dysfunctions (1, 2). An abnormal neuroendocrine function in obesity has been suggested to play key role in these issues (1–3). In addition, obesity has also been implicated in the alteration of pain

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response by modulating the opioid system in both human (4, 5) and animals (6–8). An elevated level of serum β-endorphin has been documented in the obese women exhibiting positive correlation with body weight (9, 10). In obese human, decreased sensitivity to pain was observed when pain was induced by needle pressure method (11) or using electrical stimulation method (12, 13). On the contrary, a lower threshold of pain was reported in nociceptive flexion reflex method (14). Similar conflicting observations (8, 15) were obtained in rat model of obesity for the tail flick latency test – a method that primarily assesses transient phasic pain. However, the effect of obesity on tonic pain response is not clear. Tonic pain is produced by inflammation, thus it is not possible to produce tonic pain in human. In order to overcome this difficulty formalin test in animal models have been utilized. Formalin test is known to evaluate tonic pain characterized by sustained irritating pain with close resemblance to clinical pain (16, 17). Therefore, present study was aimed to investigate the effect of obesity on formalin induced tonic pain in British Angora rabbits.

MATERIAL AND METHODS

Animals : The study was conducted on of fifteen adult male rabbits of British Angora breed weighing around 2 kg, available locally. The animals were housed separately in standard cages with controlled ambient temperature (26±2°C) and natural light/dark conditions. Food and water was given *ad libitum*. After two weeks of acclimatization in laboratory conditions, the animals were randomly divided into two groups. One of the groups was fed with standard rabbit chow to serve as control (n=6). The other group was fed with high fat diet (HFD) for ten weeks to produce obesity (n=9). The HFD was prepared following standard procedure (18) in which 10% fat (2/3rd corn oil and 1/3rd animal lard) was added to the standard normal rabbit chow. Food and water was provided *ad libitum* and their intake was monitored daily at 10:00 AM. The body weight was also measured once in every week. The obesity was confirmed in the HFD group of animals by measuring the body weight and skin fold thickness.

Formalin test (tonic pain test): After the animal got adapted with the test environment, 0.1 ml of 5% formalin was injected subcutaneously in to the centre of one of the paw of the hind limb (19) using fine needle. The recording of pain responses in the freely moving animal was started immediately after the injection. Quantification of the pain produced by the formalin injection was done on the basis of observable alteration in the behavior of the animal (16). The pain intensity was rated according to the standard numerical pain rating scale from 0-3 in decreasing order. Pain was quantified by measuring the time spent by the rabbit in each of the behavioral categories (limping, elevation of paw, licking, biting, grooming etc.) during each time block of 300 sec. (5 min) for a period of 60 min. The mean numerical ratings were calculated following the procedures described earlier (16, 19).

Statistical analysis : The data of individual rabbits were pooled and expressed in terms of mean ± SEM. The statistical significance was assessed by using paired/ unpaired *t*-test or two way ANOVA followed

by Tukey's multiple comparison test as required. The correlation between body weight and pain rating was investigated using linear regression analysis. P value <0.05 was considered significant.

The experimental protocol of the study was approved by the Postgraduate Research and Ethical Committee, B. P. Koirala Institute of Health Sciences, Nepal.

RESULTS

HFD feeding produced obesity in rabbits

At the end of 10 weeks of dietary regimen, development of obesity was confirmed in HFD fed rabbits by observing a significant increase in body weight (+24%) and skin fold thickness (+37%) as compared the initial values (P<0.05, paired *t*-test). On the other hand, in the control group, there was no significant change of body weight or skin fold thickness during the period.

Attenuation of pain scores in obese rabbits

The total average pain rating (TAPR) was not altered in the control group after 10 weeks. In contrast, the TAPR of HFD rabbits decreased significantly from the initial at the end of 10 weeks (P<0.05). The decrease was also significantly different from the time-matched control group. Further analysis of pain rating in 5 min time blocks of total 60 min, the responses were significantly lower than the initial (0 week) as well as from the corresponding control (10 week of control) group (P<0.05, two way ANOVA followed by Tukey's multiple comparison test; Fig. 1).

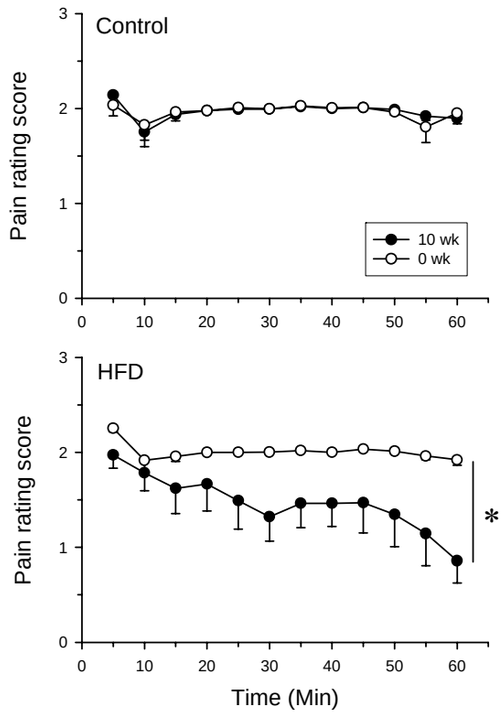

Fig. 1: Mean±SEM values of formalin induced pain rating scores at 5 min time block for 60 min. The upper graph shows the comparison of responses at 0 wk and 10 wk in control group of rabbits (n=6). There was no significant change in the responses at any of the time blocks. The lower graph depicts the comparison of responses at 0 wk (initial) and 10 wk in HFD group of rabbits (n=9).There was significantly diminished pain response after 10 weeks as compared to 0 week. Asterisk (*) indicates P<0.05 (two way ANOVA).

Pain rating varies inversely with body weight – in HFD group

To assess the relationship between body weight and pain response, linear regression analysis was applied, taking body weight as independent variable and pain parameter as dependent variable. Pain response was not having any relationship with body weight in the control rabbits (r=0.36, P>0.1). In case

of HFD group, there was a negative correlation of pain response with body weight ($r = -0.62$, $P<0.05$) as shown in Fig. 2.

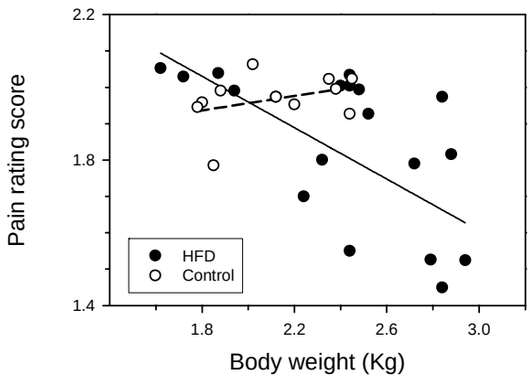

Fig. 2 : Linear regression analysis showing relationship between body weight and pain rating score in control and HFD fed rabbits. Open and filled circles indicate the individual data obtained from the control and HFD rabbits, respectively. The interrupted line (----) indicates the slope for control rabbits ($r = 0.36$, $P>0.1$). Solid line represents slope ($r = -0.62$, $P<0.05$) for HFD fed rabbits.

DISCUSSION

The results from our study using formalin test showed a significantly lower total average pain rating (TAPR) in HFD fed as compared to the initial as well as from the time-matched control group. This is indicative of the reduction in tonic pain sensitivity (increased pain threshold) in obese rabbits. The observation was further substantiated by the presence of a negative correlation of body weight with pain response in HFD group (Fig. 2). The similarity of basal pain rating scores of control and HFD animals at the beginning of the study indicated the absence of any factors/variables which could have affected the pain rating before any experimental intervention.

Formalin injection into the hind paw is known to induce a biphasic pain response by activating a transient receptor potential family cation channel (TRPA1) (20). The formalin test has several advantages over other models. In this test, spontaneous pain-related responses can be observed in a freely moving unrestrained animal. The long lasting pain responses closely resemble to those produced by tissue injury and inflammation of clinical situations (16, 17). The common behavioral parameters (altered motor activity) in nociception observed in this model have close approximation to human behavior. Although, formalin tests have been used in variety of animals including rat (16), mice (21) and rabbits (19), recording of pain score from several types of behavior is easier in rabbit model as compared to hyperkinetic rat/mice.

The lower pain response/ratings observed in this study could be argued in terms of sluggishness in motor activity as a result of obesity. This factor can easily be excluded because the pain rating score in control rabbits could not be correlated with the body weight. Conversely, there was significant negative correlation of pain scoring rate with the body weight in the HFD group of rabbits (Fig. 2). Thus, obesity evidently produced a diminished pain response.

The present result is in agreement with previous human data, which also showed that obese subjects were less sensitive to painful stimuli (11–13). The reason for decrease in pain sensitivity observed in obese rabbits cannot be ascertained from the present results. Increase in endogenous opioid levels has been reported in obese human (4, 5, 9, 10). Also, increased number of opioid

receptors has been found in obese animal models of obesity (6, 7). Thus the higher threshold of pain response observed in the present study may be associated with such changes. Further investigation is required to understand the underlying mechanisms responsible for modulation of pain sensitivity in obesity.

In conclusion, the results of present study revealed that obesity can attenuate the formalin induced tonic pain sensitivity in rabbits.

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