A Study on the Effect of Short Term Complete Food Deprivation on Bone Impairment in Adult Female Mice

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Abstract — Severe malnourishment has been associated with alterations in bone metabolism and turnover. However, to our knowledge, no previous studies have analyzed the bone microstructure of adult mice subjected to complete fasting. This study aimed to identify the adverse effects of complete food deprivation on lower limb bones and alterations in the physical characteristics of the femur via histomorphometric analysis in mice. Twelve-week-old adult C57BL/6J mice were randomly divided into ad libitum (AL) and three-day water-only fasting (FA) groups. Bone histo-morphometric analysis was used to assess the differences in the microstructure of bones after the three-day fasting period. The histo-morphometric data indicated that the bone resorption parameters, including the number of multinucleated osteoclasts (Mu.N.Oc/B.Pm), osteoclast surface (Oc.S/BS), and eroded surface (ES/BS), were similar between the groups. However, the bone formation parameters, including the osteoid surface (OS/BS), osteoid thickness (O.Th), osteoblast surface (Ob.S/BS), and number of osteoblasts (N.Ob/B.Pm) based on the bone perimeter, were significantly decreased in the FA group compared with the AL group. Thus, bone resorption was unaffected, whereas bone formation was severely impaired following complete food deprivation. Furthermore, the OS/BS was significantly reduced, which indicates that the bone mass should be reduced. However, there was no significant reduction in the osteoid thickness (O.Th); thus, the bone mass remained relatively stable and was not significantly different between the groups. These novel findings provide intriguing insights that may be relevant to the increased incidence of osteoporosis in individuals with complete food deprivation and other diseases associated with chronic undernutrition.

Keywords - fasting, bone mass, histomorphometry assay, osteoblast, osteoclast

I. INTRODUCTION

Bone hist-omorphometry is used to analyze a two-dimensional bone tissue section; a microscopic image is used as the object in this process. Parameters, such as the BV/TV, OS/BS, O.Th, Ob.S/BS, N.Ob/B.Pm, Mu.N.Oc/B.Pm, Oc.S/BS, and eroded surface (ES/BS) of the bone microstructure, can be identified through target image analysis. Compared with the measurement technology used to determine bone mineral density, bone histomorphometry is a superior technique because it can qualitatively identify the morphology of bone tissue. Moreover, it can provide an objective measure of bone biology performance, including the thickness and void ratio of the cortex, the area and thickness of the trabecular bone, and the amount of trabecular bone connection. Furthermore, it can also determine the rate of formation of new bones and the microstructure of bones [13-15]. This histomorphometric data can provide critical information regarding the bone microstructure, which can be used in research studies that investigate dietary-induced osteoporosis.

Nutrition plays an important role in the prevention of many diseases. Furthermore, a balanced diet ensures that the body receives essential nutrients, which influence body weight, organ mass, and bone mass [1]. Severe chronic undernutrition has been associated with alterations in bone metabolism. For example, the incidence of osteoporosis is increased in patients with anorexia nervosa, an eating disorder characterized by self-starvation [2,3]. Elderly women with dietary restriction over time have been reported to exhibit decreased bone turnover [4,5]. Moreover, a reduction in bone formation has been associated with the degree of energy restriction in young women who exercise[6]. Similar findings have originated from animal studies. Marked effects on bone metabolism have been identified in male mice subjected to food restriction [7-10]. Several studies have also demonstrated the effects of short-term complete food deprivation on bone turnover. For example, Grinspoon et al. have reported approximately 50% reductions in bone formation markers in women who underwent complete fasting for 4 days with the exception of ad libidum water[11]. Bone turnover was also reduced by 24 hr of fasting in male undergraduate students [12]. However, to the best of our knowledge, no previous study has conducted a detailed microstructural analysis of bone parameters in animals or humans following complete fasting. In this study, we conducted a comprehensive microstructural analysis of the following parameters: the trabecularbone volume (bone volume/tissue volume, BV/TV), osteoid surface (OS/BS), osteoid thickness (O.Th), osteoblast surface (Ob.S/BS), number of osteoblasts...
To the best of our knowledge, no previous studies have investigated the bone microstructure of adult mice following complete fasting. In the current study, adult female mice were subjected to a 3-day period of complete fasting. The aim of the study was to identify the effects of complete short-term fasting on lower limb bones, as well as alterations in the physical characteristics of the femur via histomorphometric analysis of food-restricted mice.

II. MATERIALS AND METHODS

A. Animal model

Twelve week-old (18.2 ± 0.7 g in weight) C57BL/6J female mice were housed in the Hangzhou Normal University Animal Center. The mice were provided with food and water ad libitum and maintained on a 12-hour light/dark cycle, under controlled temperature and humidity conditions. Prior to the study, all experiments were reviewed and approved by the Animal Care and Use Committee of Hangzhou Normal University, Zhejiang, China. The mice were randomly divided into two groups (n = 8/group): the ad libitum (AL) control group and an experimental group that underwent complete fasting with ad libitum water for three days (FA). Three days were chosen based on a pilot experiment that was conducted in our group, which indicated mice could endure extreme fasting conditions for 3 days. At the start of the study, the mice in the FA group were transferred to a new clean cage without access to food at Zeitgeber time 20 (ZT20). These mice were subsequently sacrificed at ZT8 on the third day of food deprivation [8]. The body weights of the mice were monitored during the experimental period. The left femur of each mouse was harvested, preserved in 70% ethanol, and subsequently processed for histomorphometric analysis.

B. Histomorphometric analysis of the femur

Each femur was cut into two parts: the proximal femur and the middle/distal femur. The proximal metaphysis was stained with Villanueva Osteochrome Bone Stain (Merck, Darmstadt, Germany) for seven days. The specimens were subsequently dehydrated, defatted sequentially in increasing concentrations of ethanol (70%, 95%, 99.5%, and 100%) and acetone, respectively, and embedded in methyl methacrylate (Wako, Osaka, Japan). Six-µm-thick longitudinal sections of the proximal metaphysis were cut on a Leica RM2255 microtome (Leica, Inc., Nussloch, Germany). The samples were then transferred onto a chromium/gelatin-coated slide and dried overnight under pressure at 42 °C and cover slipped with acrylic resins (Maruto, Tokyo, Japan). These sections were analyzed using histomorphometric software (System Supply Co., Nagano, Japan). The nomenclature, symbols, and units have been recommended by the Nomenclature Committee of the American Society for Bone and Mineral Research [16].

C Statistical Analysis

The data are expressed as the mean ± standard deviation (S.D.). Significant differences between the AL and FA groups were identified using Student’s t-tests. The effects were considered to be statistically significant when p < 0.05.
IV. CONCLUSION

The aim of this study was to evaluate the effects of short-term complete fasting on bone mass via the measurement of morphological variations and subsequently discuss the potential factors that influenced osteoblasts rather than osteoclasts. Weight loss, especially via energy restriction, has been associated with bone status in previous studies [1,17,18]. However, previous studies have not reported a quantification of the morphological changes in osteoblasts following complete fasting. The current histomorphometric analysis clearly demonstrated the transformation of the femur, including the invariability in bone resorption and the variability in bone formation. The bone volume relative to the tissue volume and the surface and number of osteoclasts were not significantly different between the AL and FA groups. In contrast, the surface and N.Ob/B.Pm significantly decreased in the FA group following three days of complete food fasting. These findings suggest that bone resorption was unaffected; however, bone formation was severely impaired as a result of the fasting period. In addition, the OS/BS was significantly reduced in the FA group, which indicates that the bone mass should be reduced in this group. Nevertheless, there was no significant reduction in the O.Th, and the bone mass remained stable. These findings indicate that a significant reduction in body weight caused by three days of complete fasting influenced bone metabolism and negatively affected bone in female mice.

The osteoid is the un-mineralized portion of the bone matrix that forms prior to bone tissue maturation. It is composed of fibers and ground substances, such as Type I collagen, chondroitin sulfate, and osteocalcin. Osteoblasts initiate the process of bone tissue formation by secreting the osteoid. When the osteoid becomes mineralized, it transforms into a new bone tissue along with the adjacent bone cells. The current findings indicate that the O.Th did not significantly change during the fasting period; however, the surface was significantly reduced, which suggests that there was a remarkable decrease in the number or area of cells that secrete osteoid. Furthermore, the number and surface of osteoblasts were significantly decreased.

The current findings regarding the detailed analysis of the bone microstructure indicate that during the fasting period, bone loss occurred due to changes in osteoblasts rather than osteoclasts. This finding provides novel insights that may be relevant to individuals with osteoporosis following food deprivation or other chronic diseases associated with malnourishment, such as the eating disorder anorexia nervosa. Nevertheless, several limitations must be considered in the interpretation of these findings. For example, the current study was performed on a small number of animals, who were subjected to complete food deprivation for only three days. Thus, the microstructural analysis of bone must be conducted in a larger sample size that includes chronically malnourished individuals. Furthermore, the changes in bone marrow cells should also be examined via in vitro experiments.

Figure 2. Bone histomorphometric analyses following three days of complete food fasting in adult female mice. The trabecular bone volume (bone volume/tissue volume, BV/TV), osteoid surface (OS/BS), osteoid thickness (O.Th), osteoblast surface (Ob.S/BS), number of osteoblasts (N.Ob/B.Pm), number of multinucleated osteoclasts (Mu.N.Oc/B.Pm), osteoclast surface (Oc.S/BS), and eroded surface (ES/BS) are shown. Data represent the mean ± standard deviation (SD) of five mice. **p < 0.01 vs. control.

Previous studies have demonstrated that metabolic acidosis may play an important role in impairing bone metabolism in individuals who are severely undernourished [19]. Furthermore, in vitro studies have demonstrated that metabolic acidosis simultaneously inhibits the functions of osteoblasts [20]. In summary, these findings suggest that metabolic acidosis may affect bone turnover in vivo and contribute to bone demineralization during severe...
undernutrition[6]. Nevertheless, the underlying mechanism of the changes that were identified in the current study remain unclear. Future studies should investigate the potential mechanism that decreases bone formation during states of severe food restriction.

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REFERENCES