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A well-balanced diet combined or not with exercise induces fat mass loss without any decrease of bone mass despite bone micro-architecture alterations in obese rat

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ABSTRACT

The association of a well-balanced diet with exercise is a key strategy to treat obesity. However, weight loss is linked to an accelerated bone loss. Furthermore, exercise is known to induce beneficial effects on bone. We investigated the impact of a well-balanced isoenergetic reducing diet (WBR) and exercise on bone tissue in obese rats. Sixty male rats had previously been fed with a high fat/high sucrose diet (HF/HS) for 4 months to induce obesity. Then, 4 regimens were initiated for 2 months: HF/HS diet plus exercise (treadmill: 50 min/day, 5 days/week), WBR diet plus exercise, HF/HS diet plus inactivity and WBR diet plus inactivity. Body composition and total BMD were assessed using DXA and visceral fat mass was weighed. Tibia densitometry was assessed by Piximus. Bone histomorphometry was performed on the proximal metaphysis of tibia and on L2 vertebrae (L2). Trabecular micro-architectural parameters were measured on tibia and L2 by 3D microtomography. Plasma concentration of osteocalcin and CTX were measured. Both WBR diet and exercise had decreased global weight, global fat and visceral fat mass ($p<0.05$). The WBR diet alone failed to alter total and tibia bone mass and BMD. However, Tb.Th, bone volume density and degree of anisotropy of tibia were decreased by the WBR diet ($p<0.05$). Moreover, the WBR diet had involved a significant lower MS/BS and BFR/BS in L2 ($p<0.05$). Exercise had significantly improved BMD of the tibia possibly by inhibiting the bone resorption, as evidenced by no change in plasma osteocalcin levels, a decrease of CTX levels ($p<0.005$) and trabecular osteoclast number ($p<0.05$). In the present study a diet inducing weight and fat mass losses did not affected bone mass and BMD of obese rats despite alterations of their bone micro-architecture. The moderate intensity exercise performed had improved the tibia BMD of obese rats without any trabecular and cortical adaptation.

1. Introduction

Because obesity is associated with many serious clinical complications, it has become a serious public health problem. The risk of developing a condition of excess of body fat mass depends on lifestyle factors such as high energy food intake and inactivity. Therefore, the association of a well-balanced diet with exercise appears as a useful strategy to treat obesity. On the one hand, it is important to improve diet quality of obese subjects by reducing the consumption of saturated fatty acid and sucrose and by increasing intakes of fibers, vitamins and minerals. On the other hand, chronic physical activity and exercise, by increasing energy expenditure and body metabolism, help to lose fat mass. Thus, the main issue in obesity treatment is to lose the excessive fat mass by engaging an increased physical practice and appropriate food consumption. Several studies using caloric restrictive diets showed that this type of dietary intervention can reduce obesity in obese humans [1–5] and rats [6–9] but no studies to date have tried to modify only the macronutrient distribution. Thus, information on the effects of macronutrients composition in diet without caloric restriction on body weight and composition is lacking.

It has been reported that a weight loss induced by a caloric restrictive diet is linked to a concomitant accelerated bone loss. Studies conducted in obese women have found that such diets are associated with significant decreases in bone mass and total bone mineral density (BMD), as well an increased risk of fracture [10–12]. This bone loss can be proportional to the amount of weight loss [13,14]. It has been shown that a moderate weight loss induced by a caloric restriction can increase bone resorption [15]. Several mechanisms can explain this bone loss; including reduced mechanical loading, dietary factors such as reduced calcium [16] and hormone dysregulations [15].

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Regular exercise, known to induce beneficial effects on bone [17,18], could attenuate weight loss-induced bone loss. In human, there are conflicting reports on whether exercise incorporated into a weight loss program can protect against bone loss. Both diet-induced or exercise-induced weight loss have been accompanied with bone loss [19]. More specifically, weight loss with or without exercise can decrease total body and lumbar spine BMD and exercise can prevent weight loss-induced femoral neck bone loss [4]. In contrast, the conclusions of a recent study showed that a combination of a reducing diet and exercise did not induce changes in total and lumbar spine BMD but exercise attenuated the decrease of total hip BMD observed in weight loss induced by the diet alone [5]. These results suggested that bone response to weight loss combined with exercise could be site-specific. More recently, Shah K. el al [20] concluded that the addition of exercise to a weight-loss therapy among obese older adults prevented the increase in bone turnover and attenuated weight-loss-induced reduction in hip BMD. Despite these discrepancies, including exercise as a part of a weight-loss program seems to be particularly important in obese subjects to minimize bone loss.

The mechanisms of bone response during a weight loss therapy as well as the possible osteoprotective effect of exercise remain unclear. Although animal models allow studying mechanistic aspects of bone tissue, the literature reports few data on the effects of weight loss on the bone health. Energy restriction inducing weight loss in lean female rats results in bone loss at most sites [21] and this effect seems to be more detrimental to bone tissue in lean rats compared with obese rat [22]. Matthey et al. [23] showed that exercise induced fat mass loss, increased bone mineral density of genetically obese and diabetic rats (alteration of leptin receptor). To date, no study has investigated the effect of a well-balanced isoenergetic diet in combination with exercise, on the body composition and bone tissue of obese feeding rats.

Therefore, the purpose of this study was to investigate, in obese rats, the bone response to body fat loss induced by a well-balanced reducing diet and exercise.

2. Material and methods

2.1. Animal care, training protocol and experimental diet

All experimental designs and procedures were prepared in accordance with the current legislation on animal experience in France and were approved by the ethical committee for animal experimentation (CREEA Auvergne, CE1-09). Seventy four 11-month old Wistar male (CERJ Janvier®, Le Genest Saint-Isle, France) were included in the study. All rats had previously been fed with a high fat/high sucrose diet (HF/HS) for 4 months to induce obesity (95 kcal/days). Fourteen randomly selected rats were sacrificed to body composition analysis and bone investigation before treatment (BT, n = 14). The 60 other rats were divided into 4 groups of 15 rats. Two groups pursued the HF/HS diet and were assigned to exercise (HF/HS Exo) or sedentary scheduled (HF/HS Sed). The two other groups were given a well-balanced reducing diet (WBR) and were assigned to exercise (WBR Exo) or not (WBR Sed). Rats were individually housed in a temperature-controlled room (20–22 °C) and a reversed light–dark cycle (light on 20 h00–08 h00) was maintained. The rats had free access to water. Training protocol consisted on running on treadmill 5 days a week for 8 weeks. During the first 10 days, rats were gradually familiarized to the treadmill by a progressive increase of duration and speed starting from 6 m/min for 15 min to 10 m/min for 50 min. To avoid measuring the acute effects of exercise, we stopped exercise sessions 2 days before sacrifices. The composition of the experimental diets is reported in Table 1. In the WBR diet, we mainly removed sucrose and fat provided by lard resulting in a decrease of saturated fatty acids and cholesterol compared to the HF/HS diet. As consequence, the omega3/omega6 ratio was higher in WBR diet (0.59) than in HF/HS diet (0.36). Although the diet differed in dietary composition, each diet provided similar daily amounts of cellulose, vitamins and minerals to the animals based on the four groups being fed the same daily caloric intake. In order to ensure that all groups consumed equal amounts of calories each day, the diets were prepared in individual ramekins and removed daily. Finally, 95 kcal of food per day were given to all rats throughout the study. The body mass of the rats was recorded weekly.

2.2. Total body composition, central fat mass and bone mineral density by DXA

A Hologic QDR 4500 DXA device was used with an internal adapted collimator for small animal measurements (Hologic QDR Software for Windows XP version, Copyright© 1986–2002 Hologic Inc.). One week before sacrifice, total body composition, central fat mass and BMD of all animals were assessed. Rats were anaesthetized before measurements using an intra-peritoneal injection of a solution of Acepromazine Vetranquil® (0.5 ml/kg of body weight) and Ketamine Imalgène® (0.75 ml/kg of body weight). After anesthesia, rats were positioned ventrally on a reference film to reproduce the position. The coefficients of variation (CV) were determined for these parameters from six repeated measurements with repositioning on eight animals. DXA-derived lean tissue mass was used as a surrogate of muscle mass. CVs were 1.20%, 4.19% and 0.81% for global mass, global fat and BMD, respectively. The validity and protocols for the measure of central fat mass (CFM) have been published recently [24]. Briefly, CFM can be distinguished using DXA by identifying it as a specific region of interest within the analysis program. Fat mass from this region was strongly correlated with weighed visceral fat mass ($r = 0.94$; $p < 0.001$) and had been validated to be a useful predictor of visceral fat mass.

2.3. Dissection of rats

Rats were fasted for 12 h before sacrifice. They were euthanized by decapitation under isoflurane anesthesia. Visceral fat mass was assessed by weighing the total perirenal and peri-epididymal adipose tissues. The weights of these two tissues were combined to form the *ex-vivo* fat mass. Right tibia and L2 vertebrae (L2) were removed for bone microarchitecture and histomophometric analyses. Left tibia and right femur were removed for densitometric analyses and mechanical tests respectively.

2.4. Abdominal circumference measure

Abdominal circumference (AC) was assessed on all rats on the largest circumference of the rat abdomen using a plastic non extensible measuring tape (Rollfix, Hoechstmass®, Germany) with an accuracy of 0.1 cm. Rats were placed in ventral position. It was shown that the AC measure could be a useful biometric technique for assessing *in-vivo* abdominal fat mass storage in fat rats [24]. The CV for AC measures (2.69%) was determined following three analyses on 13 rats. The same operator repositioned the measuring tape three times.

2.5. Oral glucose tolerance test

All rats were subjected to an oral glucose tolerance test (OGTT) one week before sacrifices. After 13 h fasting, the rats were weighed and blood samples were collected from the tail vein using heparinized capillary tubes without anesthesia. The rats were then given a glucose load solution by gavage (1 g/kg of body weight) and vein tail blood was collected 15, 30, 60, 90 and 120 min later. The blood samples were centrifuged at 13,000 *g* for 3 min to obtain the plasma which was stored at −80 °C and subsequently assayed for glucose (bioMérieux® SA, Marcy-l'Etoile, France) and insulin (Millipore Corporation, Billerica, MA, USA). The glucose and insulin responses