Relationship of body weight and volume of liver

A morphometrical and stereological study

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ABSTRACT

Objective: To examine the relationship between high-fat diet induced obesity and hepatomegaly, and to investigate whether obesity, or hepatomegaly, or both are related to gender.

Methods: This study was performed in 2003 at Ataturk University, Erzurum Turkey. Ten adult Sprague Dawley rats (5 male, 5 female) were fed with a diet that constituted highly of fat (30%) for duration of 3 months. Ten control animals (5 male, 5 female) were maintained with standard chow. At the end of the experiment, rats were sacrificed with Sevorane®. The body mass index (BMI) of all animals was calculated. Finally, the removed livers were histologically processed and the liver volume was estimated with unbiased Cavalieri method.

Results: The BMI was 4.8 ± 0.3 kg/m^2 in males; 4.2 ± 0.25 kg/m^2 in females (control group), and 5.92 ± 0.5 kg/m^2 in males and 5.425 ± 0.40 kg/m^2 in females (treatment group). The BMI of the 2 groups was significantly different (between both males and females, p<0.01; male to male, p=0.035; female to female, p=0.001). Moreover, weight values correlated with liver volume, especially in the treated females (r=1.000; p<0.01).

Conclusion: Thus, the results of the present study suggest that gender is a contributing factor for overweight and we found that hepatomegaly in women is more than men.

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Overall rates of obesity have increased dramatically in the United States and other modern societies.[1] Many African, and American women remain disproportionately represented among the overweight and obese.[1] High-fat diets leading to excessive energy intake are strongly linked to the increasing obesity worldwide.[2] The association between total dietary fat and cancer is still in doubt.[3] There is some evidence demonstrating associations between fatty diet and cancers of the breast, prostate, and colon, and so forth.[2,4] Dietary fat intake and obesity is also an independent risk factor for the development of diabetes and liver diseases.[5] Until today, few studies have documented about the effectiveness of gender on overweight or its association with hepatomegaly risk in obese individuals.[1,4] Animal research has contributed in numerous significant ways to advances in knowledge about health and disease. Within the animal models, there are number of factors that can be controlled; one of these factors is weight and height. Therefore, it may be performed an obesity model by any way, such as fatty diet and effects of this fatty diet induced obesity could be investigated. Height or weight of experimental animals can be measured, easily, and correctly. Also, volumes of any organ can be detected. Organ volumes can be estimated, without bias, using the Cavalieri principle of volume measurement by means of consecutive serial sections.[6,7] A simple approach to obtain such information was demonstrated over 300 years ago by the Italian mathematician Bonaventura Cavalieri. It is demonstrated that this simple, inexpensive stereological approach is well suited to rapid and accurate volumetric calculations on the basis of standard histological slides or magnetic images of liver tumors, cirrhosis, hepatitis, hepatomegaly, liver transplantation, hepatectomy, and other surgical applications of the liver.[8] It is also useful to examine structures that require assessment of changes in volume over time as an indicator of therapeutic effectiveness.[9-11] In this study, we aimed to examine the relationship between high-fat diet induced obesity and hepatomegaly, and to investigate whether obesity, or hepatomegaly, or both are related to gender.

Methods. Twenty adult Sprague Dawley rats, weighing between 150-200 gr were used. All animals were obtained from the Ataturk University Study Applying and Research Center, Ataturk University, Erzurum, Turkey. The study was carried out in 2003. The rats were maintained in the laboratory under controlled environmental conditions (12 hours light/dark cycle and room temperature $22-24^{0}C$ and mated overnight). Rats were housed in plastic cages (one animal in per cage) and given food and water ad libitum. A standard diet was used under supervision of the Animal Care Committee of the University. Control rats (n=10 animals, 5 male, 5 female) were fed by standard chow (7-10% fat, 68-70% carbohydrate, 18-20% protein, 1-2% vitamins and minerals; 210 kcal/100 gr/day). High fat diet, isoenergic with standard diet, was prepared and necessary vitamins and minerals were added (30% fat, 50-52% carbohydrate, 18-20% protein, 1-2% vitamins and minerals; 210 kcal/100 gr/day). For fatty diet; the chow, in powder form, was mixed with an additional 30% animal abdominal fat/kCal until it became homogenous in a dough-like consistency. This dough was shaped with a paste injector. Obtained chow blocks were dried and used for feeding. All animals were randomly assigned to 2 groups as control and study groups. While control rats (n=10 animals, 5 male, 5 female) were maintained on standard chow, and the study groups (n=10 animals, 5 male, 5 female) were fed with the specially prepared chow. The weight of all animals was measured every 10 days in order to determine any weight rise. At the end of this study, all animals were sacrificed by Sevorane® (Abbott, Ultane; Canada). All sacrificed animals' body weight and naso-anal length were recorded for indirect computation of body composition via body mass index (BMI). Thus, the obesity status of the animals was confirmed. Finally, livers were removed from all sacrificed rats and the liver volume was estimated, stereologically. On the basis of a pilot study, it was decided to select every 5th liver slice from a set of consecutive sections from each liver. The first chosen section was carried out randomly within the first 5 sections for each animal. Fifteen to 20 sections were sampled from each liver in a systematic random manner. Sampled sections were photographed with a modified light microscope, which has a camera attachment and dial indicators.[12] The Cavalieri principle was used for estimation of the volumes of brains and livers.[13,14] A point counting test grid was used for the estimation of sectioned liver areas (**Figure 1**). The point density of the point counting grid was designed again to obtain an appropriate coefficient of error (CE) for images of the serial liver sections.[15,16] For light microscopical procedures, CE and coefficient of variation (CV) were estimated according to Gundersen and Jensen' formula.[17] The test grid with systematic array of points is randomly placed on a computer screen. The volumes of the livers in each section were estimated with the following formula. Volume = t x a/p x SP. Where, "t" is the section thickness; "a/p" is the representing area of each point on the point counting grid, and "SP" is the total number of the points hitting the sections surface areas. All results were expressed as means ± SD. Student's t test, and Spearman's correlation analyses were performed using SPSS 13.0 for Windows (SPSS, Chicago, IL, USA). All p-values of less than 0.05 were considered to be significant.

Results. No animals died during the experimental period. The BMI of both groups were 4.8 ± 0.3 kg/m^2 in males and 4.2 ± 0.25 kg/m^2 in females of the control group, and 5.92 ± 0.5 kg/m^2 in males and 5.425 ± 0.40 kg/m^2 in females of the treatment group. The statistical difference between the BMI of the control and the treatment group was significant in both males ($p<0.01$) and females ($p<0.01$). Also, the BMI of males and BMI of females were different from each other in both the control (p<0.01) and the treatment group ($p<0.01$).

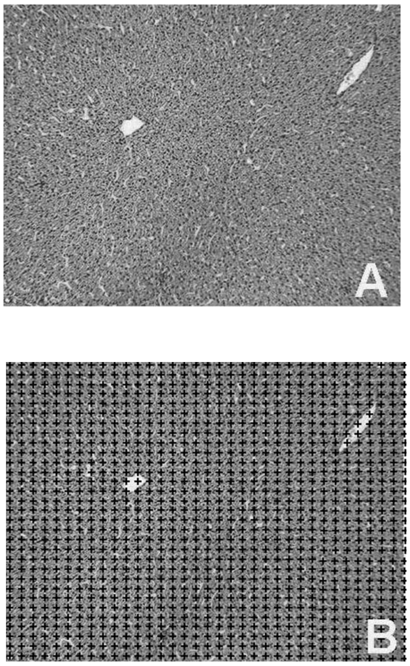

Figure 1 - Photomicrograph of **a)** selected liver section and **b)** selected liver section with superimposed point-counting grid.

At the beginning of the experiment, the weight of male rats was higher than female rats of the same age (p=0.024). When comparing the mean weight of the same gender of the 2 groups with each other, important differences were found (male to male, p=0.035; female to female, p=0.001). Also, there was a significant difference between mean weight of male rats and female rats of the same groups (p=0.000 in the control group, p<0.05 in the treatment group). In the control group, there was no difference between consecutive weight values in males or females (p>0.05). In the treatment group, there was a significant difference between consecutive weight values of females but not in that of males (males, p>0.05; females, p=0.01). We found a statistical difference between the last weight value of males and females in the control group (p<0.01), but not the treatment group. Moreover, the last weight of males (p<0.05) and the last weight of females (p<0.01) are different from each other in the 2 groups. The liver weight and volume measurement of the 2 groups are summarized in **Figures 2a & 2b,** and **3a & 3b**). In the control group, there was no correlation between mean weight values of all measurements and mean volume of livers in either males or females (r=0.50; p>0.05 for males, r=-0.319; p>0.05 for females) (**Figures 2a & 2b**). Also, there was no correlation between last weight values and mean volume of livers in either males or females of the control group (r=0.600; p>0.05 for males, r=-0.657; p>0.05 for females) (**Figures 2c & 2d**). In the treatment group, there were important correlations between mean weight values of all measurements and volume of livers in both males and females (r=0.90; p=0.019 for males and females) (**Figures 3a & 3b**). In addition, we detected significant correlations between last weight values and volume of livers in both males and females (r=0.90; p<0.05 for males, r=1.00; p<0.01 for females) (**Figure 3c & 3d**). Finally, the mean volume of livers in controls was less than the treatment group in males and females (p<0.05).

Discussion. In the present study, we focused on the body composition and liver volume of adult rats. We used periodical weight values, estimations of liver volumes, and we tried to understand the relationship between weight and liver size, and also to show that overweight and hepatomegaly could be affected by

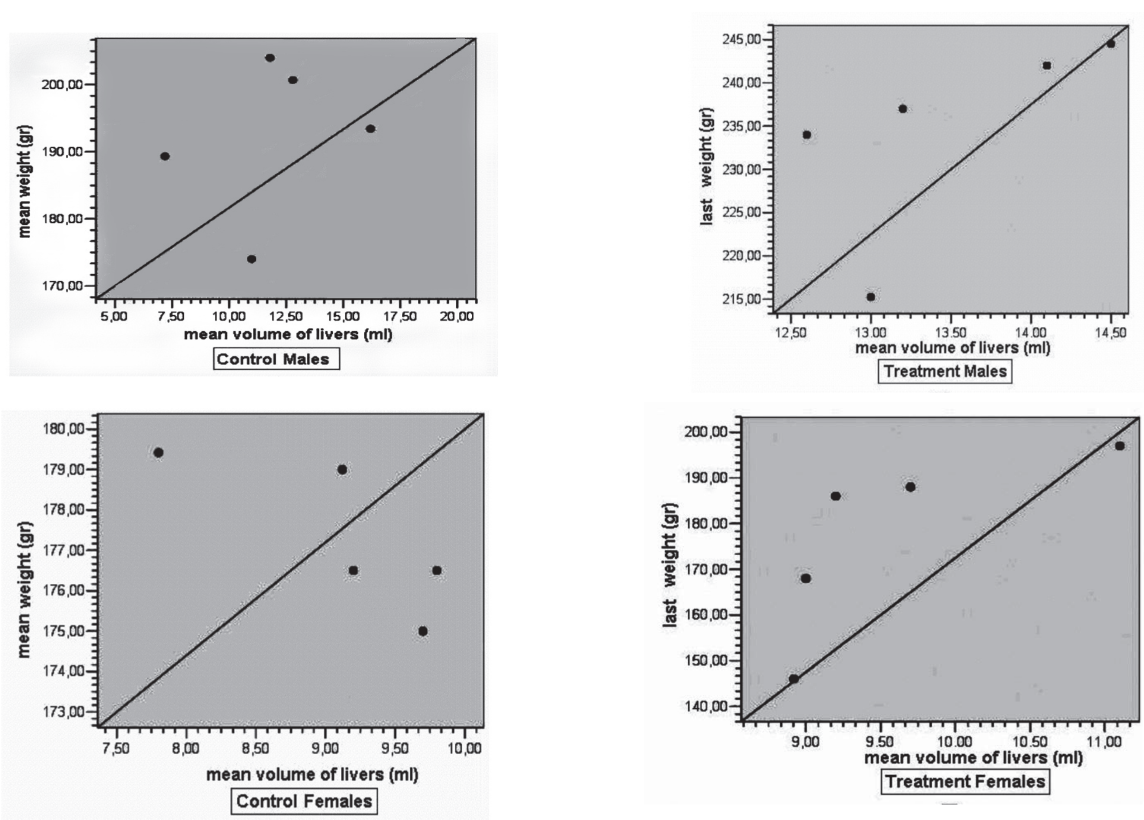

Figure 2 - Correlation between weight measurements and mean volume of livers. A, B; Correlation between mean weight values and mean volume of livers. C, D; Correlation between last weight values and mean volume of livers for control males and females.

gender. Generally, BMI is an easily obtainable, widely accepted measurement in human studies, showing correlations with percentage body fat.[18-20] We periodically measured the body weight and calculated BMI considering the mean body weight and mean height. When the results of the BMI measurements were evaluated between the 2 groups, the BMI of males increased from 4.8 ± 0.3 kg/m^2 to 5.92 ± 0.5 kg/m^2 (18.9%), and the BMI of females increased from 4.2 ± 0.25 kg/m^2 to 5.425 ± 0.40 kg/m^2 (22.6%). These results suggest that males of the control group gained more weight than the control females, however, in the treated group, females gained more than the treated males. At the beginning of the experiment, the weight of male rats, in same age group was higher than that of the female rats (p=0.024). The mean weight of the same gender of the 2 groups was significantly different from each other (male to male, p=0.035; female to female, p=0.001). This result indicates 2 important points firstly, our model is successful and animals put on weight; secondly, our finding that females gained more weight than males was again confirmed. Also, there was a significant difference between the mean weight of male rats and female rats of the same group (p=0.000 in the control group, p<0.05 in the treatment group), this result supports our finding that males of the control group gained more weight than the control females, and the treated group females gained more than the treated males. In the control group, there were no differences between consecutive weight values in measurements in either males or females (p>0.05). This finding resulted from the weight of the controls increasing gradually, and changing between fixed values (males, 170-200; females, 165-180). In the treatment group, there was a significant difference between consecutive weight values in measurements of females but not in that of males (males: p>0.05; females: p=0.01). It may be understood from these results that males are more resistant to gaining weight. Moreover, the last weight measurement of males (p<0.05), and the last weight measurement of females (p<0.01) were different from each other in the 2 groups. As seen from this result, obesity is an important risk factor for females. Routine physical examination of the liver cannot produce accurate information on the actual volume of the organ. Its major role in clinical diagnosis simply remains to define the characteristics of the lower edge of the organ.[21] The liver volume measurement must, therefore, be quantitative and reproducible and this can be achieved using routine histological liver slides.[22-24] In this study, we estimated volume of livers with the Cavalieri method; it is an unbiased stereological rapprochement. According to our results, in the control group, there was no correlation between mean weight values of all measurements and mean volume of livers in either males or females (r= 0.500; p>0.05 for males, r=-0.319; p>0.05 for females), as in this group, weight values were generally fixed and the volume of livers was different for each animals. Also, we did not find any correlation between last weight values and mean volume of livers in either males or females of the control group (r=0.600; p>0.05 for males, r=-0.657; p>0.05 for females) for the same reason. Contrary to controls, in the treatment group, we detected significant correlations between mean weight values of all measurements and the volume of livers in both males and females (r=0.90; p=0.019 for males and females). Furthermore, we found significant correlations between last weight values and volume of livers in both males and females (r=0.90; p<0.05 for males, r=1.00; p<0.01 for females). According to this finding, we report that the more body weight increases the more the volume of the liver enlarges. When comparing the liver volume in both groups, it was clear that the mean volume of livers in the controls was less than treatment group (in males and females, p<0.05), indicating hepatomegaly.

In conclusion, body weight relates to the volume of the liver in overweight persons, overweight is a very important risk factor for hepatomegaly, and overweight is a more important risk factor for women than men.

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Related topics

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