Obesity Decreases Serum Selenium Levels in a Mammary Tumor Zucker Rat Model

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Abstract

Previously, we reported that obese Zucker rats had increased susceptibility to DMBA-induced mammary tumors compared to lean Zucker rats. In that experiment, 36% of the obese ovariectomized rats developed mammary tumors while lean ovariectomized rats developed no mammary tumors. The obese sham-operated rats developed mammary tumors (59%) compared to 30% of the lean sham-operated rats. On the other hand, several lines of evidence suggest that lower serum selenium (Se) may play an important role in increasing the risk of several types of cancers (e.g., colon, breast and prostate cancers). In the present study, we used this Zucker rat model to examine the effect of obesity on Se status, and the serum Se level was determined by graphite furnace atomic absorption spectrometry. We found that the serum Se levels did not differ by ovariectomy when comparing the combined sham-operated groups with the combined ovariectomized groups; similarly, there was little difference among the four sub-groups. However, obesity decreased the serum Se levels in the combined obese groups (480 ± 10.9 ng/ml) when compared with the combined lean groups (511 ± 10.3 ng/ml) (P<0.05). In summary, our data demonstrate for the first time that obesity decreases serum Se levels in an animal model and suggest that serum Se may play an important role mammary carcinogenesis.

Keywords: Obesity; Mammary tumours; Serum selenium; Zucker rat

Introduction

Overweight (BMI 25-29.9 kg/m^2) and obesity (BMI $\geq$ 30 kg/m^2) have reached epidemic proportions, affecting two-thirds of Americans and an estimated 2.3 billion people worldwide. These conditions increase the risk for type 2 diabetes, cardiovascular disease, and cancer [1,2]. Breast cancer is the second leading cause of death in women in US. The American Cancer Society has estimated that 229,315 women will be diagnosed with new cases of breast cancer (29% of total new cancer cases) and 38,552 women will die from breast cancer, 14% of estimated total cancer deaths in 2012 [3]. Also, there is increasing epidemiological evidence that increasing body weight is associated with increased risk for postmenopausal, but not premenopausal breast cancer [4]. For example, a recent large population-based cohort study of more than 145,000 Austrian women that examined the relationship between overweight, obesity, and cancer found that the incidence of breast cancer was positively associated with high BMI only after 65 years of age [5]. Similarly in animal models, higher body weight has been associated with increased incidences of both spontaneous and chemically induced mammary tumors [6-8].

Although obesity increases breast cancer risk, the potential for nutrients to mitigate obesity-driven carcinogenesis is unclear. Epidemiological evidence indicates that selenium (Se) status is inversely associated with cancer risk, and results from numerous studies with a variety of animal models and in several clinical trials show that high Se intake effectively reduces the risk of mammary, prostate, lung, colon, and liver cancer [9-14].However, the largest of these trials, the SELECT trial [15], found no protection by Se against prostate cancer, at least over a short (5 year) period of study in subjects of relatively high Se status (plasma Se: 136 ng/ml). This observation is consistent with those of the NPC trial [14,16,17], which found Se treatment to reduce cancer risk only for subjects in the lowest tertile of baseline plasma Se level (<106 ng/ml) [13]. Thus, Se supplementation may not benefit all individuals, and it has been suggested that Se supplementation may yield anti-cancer benefits in individuals who are below a certain threshold of Se status but not Se deficient. In regard to cancer risk, we recently found that that obese Zucker rats had increased

susceptibility to DMBA-induced mammary tumors compared to lean Zucker rats [18-20]. In view of the fact that obesity increases cancer risk and Se status is inversely associated with cancer risk, we hypothesized that obesity-enhanced cancer may reduce serum Se level and used the Zucker rat model to determine the extent to which obesity decreases serum Se in the present study.

Materials and Methods

Experimental design

Female Zucker rats were sham-operated (lean, n=30; obese fa/fa, n=27) or ovariectomized (lean, n=31; obese fa/fa, n=25) at the age 40 days by Harlan Industries (Indianapolis, IN). These animals were housed at animal facilities at the Arkansas Children's Hospital Research Institute two per cage in polycarbonate cages and allowed ad libitum access to water and a semi-purified AIN-93G diet (Teklad, Madison, WI), as was reported previously [18]. At the age of 50 days, all rats received via gavage 65 mg/kg DMBA (Sigma Chemical Co., St. Louis, MO) [18] and were sacrificed 135 days post-DMBA treatment [20]. All rats were weighed twice weekly.

This study was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Arkansas for Medical Sciences, and the rats were maintained in accordance with the guidelines for the care and use of laboratory animals [21].

Selenium plasma analysis

Plasma and serum samples (30 µL) were diluted with 20 µL

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of laboratory purified water and 150 μL of modifier, 0.09% $PdCl_2$ (Palladium chloride, Alfa Aesar, Ward Hill, MA), and 1.5% $NiNO_3$ (nickel nitrate hexahydrate, Alfa Aesar, Ward Hill, MA) for analysis. Next, 20 μL of sample mix, 5 μL of 3% hydroxylamine hydrochloride (Eastman Kodak Company, Rochester, NY), and 5 μL of laboratory purified water were transferred by auto-sampler to the graphite furnace tube of the Perkin Elmer Analyst 800 instrument (Perkin Elmer Corp., Wellesley, MA).

Statistical analysis

Results are given as means ± SEM. The serum Se levels were analyzed by 2-way ANOVA to test for effects of status (sham or OVX), weight (obese or lean), and their interaction using the Proc Mixed procedure in SAS version 9.2 (SAS Institute). Tukey contrasts were used to compare individual group means. Differences with a P value <0.05 were considered significant.

Result

The mammary tumor incidence and body weight was previously reported [20]. To determine the effect of obesity on plasma Se status in DMBA-induced mammary tumor rat model, serum Se level was determined by graphite furnace atomic absorption spectrometry. The serum Se levels of L/S, L/O, O/S, and O/O group were (504 ± 7.8 ng/ml), (518 ± 10.8 ng/ml), (490 ± 20.1 ng/ml), and (469 ± 19.2 ng/ml), respectively, and these Se levels did not differ among these 4 groups (Figure 1). Similarly, the Se levels of the combined lean and obese sham-operated groups and the combined lean and obese ovariectomized groups were (498 ± 10.3 ng/ml) and (493 ± 10.8 ng/ml), respectively, and the Se levels of these two groups did not differ (Figure 2). Interestingly, the Se level of combined lean groups (511 ± 10.3 ng/ml) was significantly greater than that of combined obese groups (480 ± 10.9 ng/ml) (Figure 3).

Discussion

Our previous studies showed that obesity increases the susceptibility of ovariectomized Zucker rats to DMBA-induced mammary tumors [20]. This observation is consistent with the fact that overweight/obesity is now established as a risk factor (second only to smoking) for cancer [22,23]. Although hundreds of studies with animal and cell models have shown various Se-compounds to reduce/delay tumorigenesis [12,13], Se supplementation may only yield anti-cancer benefits in individuals who are below a certain threshold of Se status but not Se
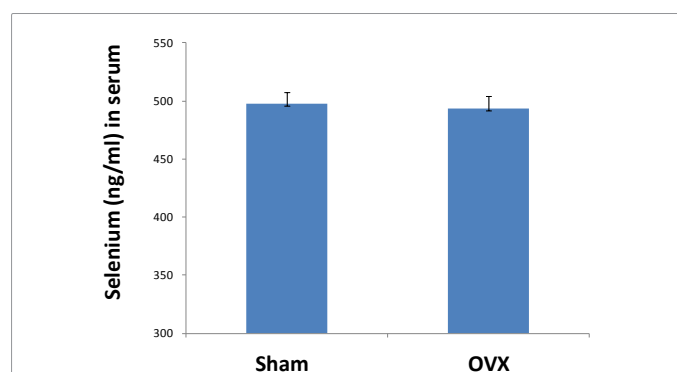

Figure 1: The serum Se levels of lean and obese sham operated and ovariectomized (OVX) group sat the end of the experiment (135 days). Values are means ± SEM, lean (sham, n=30; OVX, n=31) and obese fa/fa (sham, n=27; OVX, n=25).

Figure 2: The serum Se levels of the combined sham-operated groups and the combined ovariectomized (OVX) groups at the end of the experiment (135 days). Values are means ± SEM, sham (n= 57) and OVX (n= 56).
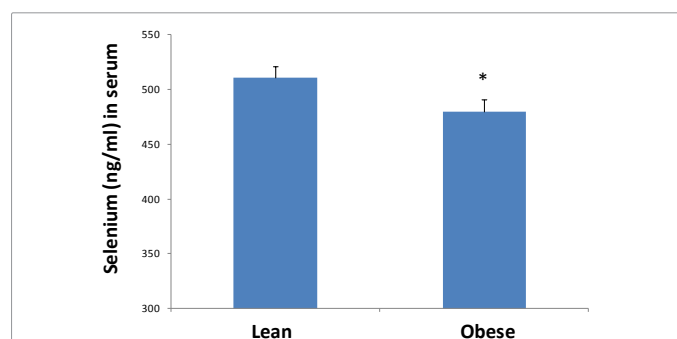

Figure 3: The serum Se levels of the combined lean groups and the combined obese groups at the end of the experiment (135 days). Values are means ± SEM lean (n=61) and obese fa/fa (n=52). *Difference between lean groups and obese groups, *P<0.05.

deficient (plasma Se: 70-106 ng/ml) in human clinical trials [24,25]. The number of subjects in these clinical trials that were overweight/ obese raises the question of whether adiposity affects Se status. In the present study, we found that the serum Se levels in combined sham-operated group and combined ovariectomized rat group did not differ (Figure 2). However, the serum Se level of combined lean rat group was greater than that of the combined obese rat group (Figure 3). This observation demonstrated for the first time that obesity decreased serum Se level in an obesity-enhanced cancer animal model.

In this experiment, we used the standard rat model for breast cancer development, the DMBA-induced mammary tumor model, which has been used for past 60 years to investigate the effects of diets on breast cancer prevention or promotion. The model requires younger animals to establish breast cancer development. Our results indicate that obesity is major risk factor for breast cancer development and for reduction of serum selenium in this model in which serum selenium reduction appears to be another risk factor for breast cancer development.

Interestingly, it should be also noted that there was a non-statistical trend of obesity-effect on decreasing serum Se level between the lean and obese sham operated groups as well as between the lean and obese ovariectomized groups (Figure 1). The present study is one of only a few animal studies with a large subject number (n > 50/group) with all of the animals fed the same AIN-93G diet. These data indicate that the effect of obesity on serum Se level needs to be determined with a larger number of experimental subjects. Our finding will have an important health implication in human population and suggests that obese population may have lower serum Se level than that of lean population
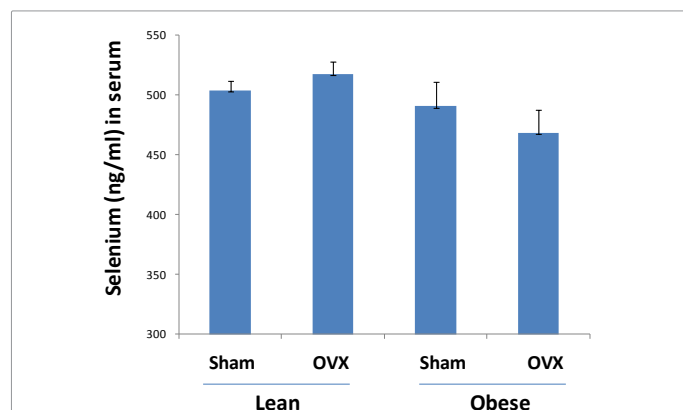
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even when they have the same dietary patterns. Thus, a future human feeding-control study on obesity-effect on decreasing serum Se level is warranted. Because low serum Se level contributes to the high cancer risk [9-14], our data also partly explain the connection of obesity and high cancer risk.

Acknowledgements

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