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Reduced Mitogenicity of Sera Following Weight Loss in Premenopausal Women

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Abstract

We investigated whether serum from normal weight women is less mitogenic and more apoptotic than sera from the same women in the overweight state. Sera from premenopausal women, age (mean $\pm$ SEE) 34.6 $\pm$ 0.53 years, who were randomized to caloric restriction (CR) ($n=13$), CR + aerobic exercise (AE) ($n=14$) or CR + resistance training (RT) ($n=20$) were used to culture endometrial cancer cells. Phases of the cell cycle were determined, proliferating cell nuclear antigen (PCNA) positivity was used to assess proliferation and apoptosis was assessed by determining cleaved caspase-3 and poly-ADP-ribose polymerase (PARP). Analyses showed that overall, cells grown in sera from the weight-reduced state had significantly more cells in G0/G1 and significantly fewer cells in the S and G2/M phases of the cell cycle than cells grown in sera from the overweight state. PCNA staining confirmed that cells grown in sera from the weight-reduced state had fewer proliferating cells. Cleaved caspase-3 and PARP were not different in cells grown in sera from the weight-reduced state compared to the overweight state. We conclude that weight loss with or without exercise could lower the risk for cancer through changes in serum that result in reduced cellular mitogenicity.

Introduction

The current high rates of overweight and obesity reflect positive energy balance due to low levels of physical activity and high caloric intake or a combination of both of these lifestyle factors. Excess body weight is a key public health issue because obesity is a major risk factor for several cancers (1) and obese individual are more likely to die from cancer relative to leaner individuals (2). This suggests that the accumulation of adipose tissue directly and/or indirectly influences cancer cell growth and proliferation.

Recent data has shown that weight loss can decrease the risk for several cancers in women (3). Caloric restriction (CR), a state of negative energy balance, is an effective weight loss method. Various animal models have consistently shown that CR not only results in a leaner phenotype but also retards the onset and progression of cancer (4,5,6) and can induce apoptosis in some models (7). In humans, data regarding the effects of CR on cancer incidence or risk have been limited to retrospective studies that have used cancer registries. Two epidemiological studies have reported decreased risk for breast cancer following CR (8,9) whereas two other studies reported higher risk for breast cancer following CR (10,11). The reasons for the conflicting data in these studies may be due in part to differences in the

length of time of exposure to CR, the degree of CR, age differences at the time of exposure and absence of physical activity data.

In addition to CR, animal models have shown that exercise conveys protection from cancer by decreasing the number and size of tumors and inducing apoptosis (12). Other animal studies have shown that the duration of exercise decreases tumorigenesis (13) and reduces metastatic disease (14). In humans, there is evidence that physical activity reduces the risks for a number of hormone-sensitive cancers in women (15,16). A recent randomized-controlled clinical trial showed that moderate-to-vigorous aerobic exercise decreased markers of proliferation in the colon (17) and up-regulated biomarkers associated with apoptosis (18) in patients at risk for colorectal cancer.

The mechanism underlying the increased risk for cancer with obesity and the lowered risk for cancer seen with CR and physical activity are unclear but may involve several factors that circulate in the blood and mediate cell proliferation such as adipocyte-derived cytokines, the insulin and insulin like growth factor axis and sex steroid hormones. In the present study we sought to determine whether weight loss through CR alone or combined with two different exercise regimens, aerobic or resistance training, lead to changes in serum that resulted in decreased cellular proliferation and increased apoptosis in human cells. To test this, we employed in vitro techniques described previously by Barnard et al (19) and used sera from the same group of overweight women [body mass index (BMI) 27-29] weight-reduced to a normal weight state (BMI $\leq$ 24.9) (20) to culture an endometrial adenocarcinoma cell line. One specific aim was to determine whether cells grown in media supplemented with sera from the weight-reduced state were less proliferative compared to cells grown in media supplemented with sera from the same women in the overweight state. A secondary aim was to determine whether caspase-3 and poly-ADP-ribose polymerase (PARP), indicators of apoptosis, were higher in cells supplemented with sera from the weight-reduced state compared to the overweight state. In addition, we sought to determine whether the method of weight loss, either CR alone or combined with aerobic or resistance training, modified cellular proliferation and apoptosis similarly.

Methods

Study Design

Biological samples and data were from a previous study described elsewhere (21). Briefly, premenopausal women with a BMI between 27-30, considered overweight by US National Institutes of Health criteria (20), were enrolled in a weight loss study at the University of Alabama at Birmingham (UAB) to reduce BMI to normal weight state (BMI $\leq$ 24.9). Women were eligible for the study if they were sedentary (defined as engaging in recreational or sport activity <1 time per week for the past year); having normal menstrual cycles; not taking oral contraceptives; negative for a history of eating disorders; normoglycemic (determined by 2-hour blood glucose concentration of <140 mg/dl following oral glucose load); non-smoker; not taking medications known to alter metabolism; and identified themselves as either African-American (AA) or European-American (EA). Women were randomized to one of three weight loss groups; [CR alone, CR + aerobic exercise (CR+AE) or CR + resistance training (CR+RT)], based on race, age and BMI. All women consumed an 800 kcal/day diet until BMI $\leq$ 24 was reached. The dietary composition was 20-22% of energy as fat, 18-22 % as protein, and 58-62% from carbohydrate and all food was provided to the participants throughout the study. In addition to the 800 kcal/day diet, the women in the CR+AE group performed aerobic exercise consisting of fast walking on a treadmill or light jogging or stationary cycling for 40 minutes at 80% maximum heart-rate three days per week. Women in CR+RT group performed resistance training consisting of upper and lower body weight training at 80% maximum lifting 3 days per week. All exercise was supervised

by an exercise physiologist at a UAB clinic. Written informed consent was obtained from all participants prior to testing. All procedures and protocols pertaining to the study were approved by the UAB Institutional Review Board.

Human sera collection

Sera were collected in Bectin Dickinson serum vacutainer® blood collection tubes at baseline and following weight loss after a 12-hour fast. All women were in the follicular phase of the menstrual cycle and were in a state of energy balance at the time blood collection. Energy balance was maintained for 4-weeks prior to and after weight loss. During this time participants consumed a diet based on their individual energy needs as determined by a Registered Dietitian. Participants were weighed at the General Clinical Research Center (GCRC) three days per week for the first two weeks and five days per week for the last two weeks to ensure weight maintenance. Energy balance was an essential component of our study design because negative energy balance has been shown to significantly affect serum growth factors (22).

Body composition measurements

Body mass index (BMI) was calculated using weight in kilograms divided by height in meters squared. Dual-energy X-ray absorptiometry (GE Medical Systems-Lunar Prodigy running ADULT software, version 1.35, Madison, Wisconsin,) was used to assess total and regional body fat composition. Computed tomography scanning with HiLight/Advantage Scanner was used to determine intra-abdominal adipose tissue (IAAT). A 5 mm scan at the L4-L5 region was taken while subjects were in the supine position with arms above their head. Cross sectional area of adipose tissue was analyzed using density contour as we have previously described (23). **Serum markers** –Adiponectin, insulin and leptin were measured in duplicate using double-antibody radio immunoassays (RIA) from Linco Research Products, Inc., St Charles, MO). The intra- and interassay coefficient of variation (CV) for adiponectin, insulin and leptin were 5.36% and 6.26%, 3.49% and 5.57%, and 5.0% and 5.6%, respectively. Total insulin-like growth factor 1 (IGF-1) and IGF binding protein (IGFBP)-1 were assayed in duplicate using immunoradiometric assays from Diagnostic Systems Laboratories (Webster, TX). The mean intra- and inter-assay CVs for IGF-1 were 4.14% and 7.69% and for BP-1 7.04% and 4.97%. Estradiol and testosterone were measured using an AIA-600 II immunoassay analyzer (TOSOH Bioscience, Toyoma, Japan). The mean intra- and interassay CVs were 4.20%, 12.9% for estradiol and 8.30%, 10.2%, for testosterone, respectively.

Cell culture conditions

ECC-1 cells, an endometrial adenocarcinoma cell line, were purchased from American Type Culture Company (CRL-2923, Manassas, Virginia). Cells were maintained according to the recommendations from ATCC with cells being cultured in RPMI 1640 medium with 5% fetal bovine serum (FBS) in a humidified incubator at 37° with 5% CO₂. Media was routinely changed every 2-3 days.

To test differences between sera from the same women in the overweight and weight-reduced states we used a mixture of phenol-red free RPMI containing 1.25% human serum and 3.75% charcoal-stripped FBS to achieve the total 5% serum recommended for this cell line. This mixture of sera was used because of limitations in the volume of human serum available from participants.

Cell cycle analysis

ECC-1 cells were seeded in 6-well cell culture treated plates (BD Falcon) at a density of 2.5×10^5 cells per well. Cells were initially cultured in 3 ml of phenol-red free RPMI supplemented with 0.5% charcoal-stripped FBS for 48 hours to slow growth and better synchronize the cells. The media was removed and replaced with 3 ml media containing human serum (3.75 μ l human serum, 11.25 μ l charcoal-stripped and 285 μ l phenol-red free RPMI). Following 48 hours of cell growth, cell cycle analysis was determined using the method previously described (24). Briefly, cells were harvested and washed three times in phosphate buffered saline (PBS) to remove trypsin and culture media. Cells were fixed in ice cold 100% ethanol. Following fixation cells were centrifuged at 400g for 5 minutes, washed in PBS three times to remove ethanol then stained with solution containing 0.2ml of 1mg/ml propidium iodide, 2mg DNase-free RNase, in 10ml of 0.1% triton-X100 PBS. Cells were incubated for 30 minutes before cell cycle analysis. Human serum-stimulated cells from both the pre- and post weight loss periods were assayed at the same time on the same culture plate. The assay was performed in triplicate. Approximately 10,000 events were captured and analyzed with Becton-Dickinson FACScan and MODFIT LT for Win32, version 3.2.1 software (Verity Software, Topsham, Maine).

Proliferating cell nuclear antigen (PCNA) immunocytochemistry

ECC-1 cells were seeded on coverslips inside 24-well culture dishes at a density of 2×10^4 in phenol red-free RPMI supplemented with 5% charcoal-stripped FBS. Media containing human sera from the overweight or weight-reduced state were added to the wells after 48 hours. Phenol-red free RPMI without serum and phenol-red RPMI with 5% FBS (C-media) served as negative and positive controls, respectively. The experiment was performed in duplicate. Following 48 hours of cell growth in media supplemented with human sera; coverslips were fixed with 3% paraformaldehyde PBS for 30 minutes at room temperature, washed in PBS three times, and incubated in 100% methanol for 10 minutes at -20°C . Subsequently, coverslips were washed in PBS three times and incubated overnight at 4°C with a mouse monoclonal antibody to PCNA (Invitrogen, Carlsbad, CA) at a concentration of 1:1000 in a solution of 10% goat serum 0.1% tween/PBS. The following day, coverslips were washed three times in 0.1% tween/PBS and incubated for 30 minutes at room temperature with Alexa Fluor[®] 594 goat anti-mouse secondary antibody (Invitrogen, Carlsbad, CA). Subsequently, coverslips were again washed three times in 0.1% tween/PBS and mounted on microscope slides using ProLong[®] Gold antifade reagent with 4',6-diamidino-2-phenylindole (DAPI). Cells were scored using a fluorescent microscope. Random fields of cells were visualized using a ultra-violet filter in order to view DAPI stained nuclei of cells. The number of cells in the field was counted and noted. The filter was then switched to 594nm in order to view cells positively stained for PCNA and the number of cells stained positive for PCNA was noted. The percentage of cells positive for PCNA was determined by dividing the number of positive nuclei by the number of nuclei visualized with DAPI staining and multiplied by 100.

Apoptosis assays

Cells were plated in 6-well tissue culture treated plates (BD Falcon) at a density of 2.5×10^5 in RPMI media supplemented with 5% FBS. Cells were allowed to reach 90% confluency then media with human sera was added to the wells. Eighteen hours later, cells were lysed using RIPA cell lysis buffer (1xTBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 0.004% sodium azide, 1% PMSF and 1% sodium orthovanadate). The protein content of cell lysates was quantified using bicinchoninic acid assay (BCA) (Thermo Scientific, Rockford, IL). Both cleaved caspase-3 (25) and cleaved poly-ADP-ribose polymerase (PARP) (26) are indicators of apoptotic activity therefore these markers were used to assess cellular apoptosis. Meso Scale Discovery (MSD) multi-spot apoptosis panel assay was used

to measure cleaved caspase-3, and cleaved PARP in cell lysates following manufacturer's instructions. In these assays 96-well plates supplied by MSD were read using a SECTOR™ Imager plate reader. The assay was performed in triplicate.

Statistical Analyses

The distribution of variables was assessed using normal quantile plots. The mean and standard error are shown for normally distributed continuous variables and the geometric mean and 95% confidence interval (CI) are shown for non-normally distributed variables. Differences in age among the weight loss groups were analyzed by analysis of variance (ANOVA). Repeated measures multivariate analysis of variance (MANOVA) was used to assess the change from baseline to follow-up for serum markers, body composition measurements and phases of the cell cycle. This allowed us to determine the changes in these factors over time, by weight loss group and whether there was an interaction between time x weight loss group. Post hoc analyses [analysis of covariance (ANCOVA)] was used to determine whether the differences in the groups were significantly different after adjusting for baseline values. Simple regression correlations were used to show associations between variables. ANOVA was used to test for differences in cleaved caspase-3 and cleaved PARP. All p-values were 2-sided and an alpha of 0.05 was considered statistically significant. JMP version 8.0 (SAS Institute, Inc., Cary NC) was used for statistical analyses.

Results

Sera from the overweight and weight-reduced time periods were available for 13, 14 and 20 participants randomized to the CR, CR+AE and CR+RT groups, respectively. The average age of the women was 34.6 ± 0.53 years. There were no differences in age among the three weight loss groups ($p=0.521$). Body composition measurements are shown in Table 1. Overall, there were significant decreases in body weight, BMI, lean body mass, total fat mass, percent fat and IAAT for all participants following the intervention ($p<0.0001$) and there were no significant differences among the three weight loss groups for these measurements. There was a significant time x group interaction for lean mass ($p=0.042$) indicating that the groups did not decrease lean mass similarly over the course of the weight loss. Post hoc analysis showed that the CR+RT group preserved more lean mass relative to the other weight loss groups.

Overweight and weight-reduced serum concentrations of adiponectin, estradiol, IGF-1, IGFBP-1, insulin, leptin and testosterone are shown in Table 1. Adiponectin and BP-1 significantly increased ($p<0.0001$, $p=0.0009$, respectively) and insulin and leptin significantly decreased following weight loss ($p=0.0006$, $p<0.0001$, respectively). There were no differences in adiponectin, BP-1, insulin or leptin among the three weight loss groups and the interaction term time x group was not statistically significant. There were no changes in serum IGF-1, estradiol or testosterone following weight loss.

The effects of growing cells in sera from the overweight and weight-reduced states on phases of the cell cycle are shown in Table 2. Overall, cells grown with the sera from the weight reduced state had a higher percentage of cells in G0/G1 phase of the cell cycle compared to cells grown with sera from the overweight state ($p=0.0007$). There were no differences among the three weight loss groups or a time x group interaction for the percentage of cells in G0/G1.

Cells grown with sera from the weight-reduced state had a lower percentage of cells in the S-phase compared to cells grown in media supplemented with sera from the overweight state ($p=0.002$). There were no differences in the percentage of cells in the S-phase among the three weight loss groups although there was a trend for a time x group interaction ($p=0.065$).

Post hoc analysis showed that the CR+RT group tended to not decrease the percentage of cells in the S-phase as the CR and CR+AE groups, although this did not reach statistical significance.

Cells grown with sera from the weight-reduced state had lower percentage of cells in G2/M phase compared to cells grown in media from the overweight state ($p=0.020$). There were no differences among the three weight loss groups for percentage of cells in the G2/M phase or a time x group interaction for the percentage of cells in G2/M phase.

The bivariate fit of the increase in the percentage of cells in the G0/G1 phase of the cell cycle by the decrease in the percentage of cells in the S-phase and G2/M phase are shown in Figure 1. There was a significant negative correlation between the increase in percent of cells in G0/G1 and the decrease in the percentage of cells in the S-phase of the cell cycle ($r=-0.81$, $p<.0001$). Similarly, there was a significant negative correlation between the increase in the percentage of cells in G0/G1 and the decrease in the percentage of cells in G2/M phase ($r=-0.69$, $p<.0001$).

To further assess the effects of growing cells in sera from the weight-reduced versus the overweight state on cellular proliferation, a random subset of 10 pairs of sera were used to grow ECC-1 cells. PCNA positivity was determined using immunocytochemistry. There was a decrease in PCNA staining in the cells grown in sera from weight-reduced women compared to the cells grown with sera from the same overweight women, although this did not reach statistical significance ($76\pm4.58\%$ vs $63\pm7.56\%$, $p=0.155$). There was a strong correlation between cells with the highest percentage change in PCNA positivity and cells with the highest change in the percentage of cells in the S-phase ($r=0.54$, $p=0.104$).

We investigated whether cells grown with sera from the weight-reduced state would be more apoptotic compared to sera from the overweight state in a subset of 20 pairs of sera. Compared to media supplemented with 5% FBS (C-media), human sera from both time periods had increased cleaved caspase-3 ($p\leq0.05$), however; there were no differences in either cleaved caspase-3 or cleaved PARP between cells grown with human sera from the overweight or weight-reduced periods, indicating that there was no difference in the apoptotic potential of the two sera.

Discussion

In this investigation we assessed the phases of the cell cycle of ECC-1 cells cultured in human sera to determine whether sera from normal weight women is less mitogenic compared to sera from the same women in the overweight state. Cells in the G0/G1 fraction of the cell cycle are considered to be arrested or preparing to enter the next phase of the cycle, the S-phase. Stimulation from mitogenic factors triggers the entry from G0/G1 to S-phase where DNA replication transpires. Cells in the G2/M phase are post DNA replication and preparing for cell division. One of the main findings from this study is that endometrial cancers cells cultured with sera from weight-reduced women had higher percentage of cells in the G0/G1 phase of the cell cycle and lower percentage of cells in the S and G2/M phases of the cell cycle compared to cells grown with sera from the same women in the overweight state. These differences in the cell cycle phases indicate that sera from the weight-reduced state are less mitogenic as a result of alterations in serum proteins due to decreased adiposity. Specifically, adiponectin significantly increased following weight loss as did IGFBP-1. Adiponectin has been shown to be associated with lower cellular proliferation in vitro (27) and higher IGFBP-1 results in decreased bioavailable IGF-1, a potent growth factor associated with cellular proliferation (28). Insulin and leptin both of which have been shown to be mitogenic in vitro (29) significantly decreased with weight loss. Taken as a

whole the in vivo changes in sera that occurred with weight loss, appear to be less favorable for cancer cellular proliferation in vitro.

Sera from the weight-reduced state increased G0/G1 phase similarly among the three weight loss groups. However, analyses of the S-phase indicated that the CR+RT group tended to have a lower change in the percentage of cells undergoing DNA synthesis relative to CR and CR+AE groups. The only statistically significant difference in serum proteins and body composition measurements among the three weight loss groups that we identified was the time x group interaction for lean mass that indicated that the CR+RT group preserved more lean mass following weight loss relative to the other groups. At this time it is unclear whether the preservation of lean mass or metabolic factors associated with the preservation of lean mass may have influenced serum factors that resulted in differences in cellular DNA synthesis of cells in vitro. Although it has been reported that CR and exercise likely reduce cancer risk through divergent mechanisms (30) it is unclear whether performing different types of physical activity results in similar cancer-protective benefits and this needs to be investigated further.

Our study confirmed one of our original hypotheses, that serum from overweight women is more mitogenic in ECC-1 cells grown in culture. ECC-1 cells, an endometrial adenocarcinoma cancer cell line, were used in this study because of the significant association between obesity and endometrial cancer (1). This cell line has also been shown to be stable over several passages and a preferable model for studying endocrine-related regulation of the endometrium (31). Similar in vitro studies have been previously reported in men that have investigated the effects of diet and exercise on prostate cancer cells grown in vitro (32, 33). To our knowledge only one other study has focused exclusively on a female population (19). In their study, Barnard et al. showed that sera from overweight/obese postmenopausal women is more proliferative in three different breast cancer cell lines relative to sera from the same women following two weeks of 1000kcal/day and daily exercise. These findings are similar to ours, although several important differences between their study and ours should be noted. The authors describe their study population as being in an “obvious state of negative energy balance” whereas special care was taken in our study to ensure that participants were in a state of energy balance. We chose this approach because it may not be practical for women of childbearing age to maintain negative energy balance for extended periods of time without decreasing fat stores and disrupting the normal hormonal milieu necessary for reproductive function. Further, Barnard et al reported significant decreases in estradiol and total IGF-1 whereas we did not find such changes in our population following weight reduction and restoration of energy balance. Growth factors such as IGF-1 have been shown to be significantly lower during times of negative energy balance in humans but return to baseline levels once energy balance resumes (22). This may explain why in their study they were able to detect greater reductions in the proliferation of cells grown in overweight/obese sera compared to sera from the same women following 2 weeks of negative energy balance whereas in our study the decrease in cellular growth was more modest. Another key difference is that our study population included only premenopausal women that were in the early follicular phase of the menstrual cycle and sex-steroid hormones were not altered following weight loss. Finally, our study used 1.25% of human serum due to limitations in the amount of sera available from study participants whereas Barnard et al used 10% human serum. However, even with this low concentration of sera we were able to see modest, yet statistically significant differences in phases of the cell cycle.

Our study did not confirm our second hypothesis that human sera from the weight-reduced state would be more apoptotic compared to sera from the overweight state. We did not find differences in the protein levels of cleaved caspase-3 or PARP in the cell lysates of cells

grown in sera from the overweight or weight reduced periods relative to one another. However, compared to phenol-red RPMI supplemented with 5% FBS (C-media) the human sera was generally more apoptotic and this likely reflects differences in inflammatory cytokines in human sera compared to fetal calf sera. Our results are in contrast to Bernard et al. (19) who showed that sera from women undergoing energy restriction and daily exercise for 14 days was more apoptotic relative to sera taken from the same women prior to starting the intervention. This may be due to dissimilar changes in both estrogen (34,35) and IGF-1 (36) which are anti-apoptotic in hormone-responsive cells. As stated previously, estrogen and total IGF-1 did not significantly change likely because our study population was premenopausal and in energy balance at the time of blood collection.

Our study has some limitations that should be noted. First, due limitations in the volume of sera available from the participants, we used a low percentage of human sera and therefore were able to detect minor, albeit, statistically significant changes in cell cycle phases. Whether these minor changes in vitro growth translate to clinical significance is unclear and requires further investigating. Second, inflammation is associated with overweight and obesity (37) and several inflammatory factors such as interleukin-6 and tumor necrosis factor-alpha are able to mediate cell proliferation (38, 39). We did not investigate these factors and therefore cannot rule out that these serum components may have changed with weight loss thus mediating an effect on cell proliferation. Finally, the underlying mechanism(s) or intracellular signaling pathway(s) that mediated the observed effects on cell proliferation in vitro remain undefined and require further investigating to better understand how sera from weight reduced individuals is less mitogenic relative to sera from the same individual in the overweight state.

Overall, this investigation showed that weight loss in healthy premenopausal women through CR alone or combined with exercise, results in changes in sera, that when applied to in vitro conditions, slowed endometrial cancer cell growth through increases in G0/G1 and decreases in S-phase and G2/M phases of the cell cycle. Because we did not see changes in serum sex-steroid hormones and endometrial cells are hormone-sensitive this suggests that changes in serum concentrations of adipocyte-derived cytokines and/or the insulin/IGF axis may be, in part, underlying mechanism. The results of our study provide further evidence that weight loss is likely an effective method to reduce cancer risk and that the addition of exercise with CR is not more beneficial than CR alone. Further efforts should be made through public health messages to promote weight loss as way to reduce obesity-related cancers in women.

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Figure 1.

The change in percentage of ECC-1 cells in the G0/G1 phase by the change in percentage of cells in the S-phase (A) and G2/M phase (B) of the cell cycle.

Figure 2.

No difference in the protein levels of cleaved PARP or cleaved caspase-3 in the paired cell lysates (n=20) from ECC-1 cells grown in sera from the over weight state relative to the weight-reduced state. Cell lysates from both the overweight and weight-reduced states contained higher levels of cleaved caspase-3 relative to cell lysate from ECC-1 cells grown in control media. Data are mean electrochemiluminescence (ECL) units $\pm$ standard error bars, * $p \leq 0.05$ from ANOVA

Table 1

Mean (95% CI) for baseline and weight-reduced body composition and serum concentration measurements.

	Baseline n=47	Weight- reduced n=47	P time	P for group	P for time x group
Wt, kg	78.4 (76.0, 80.7)	65.8 (63.7, 67.8)	<.0001	0.898	0.858
BMI	28.4 (28.0, 28.8)	23.8 (23.5, 24.2)	<.0001	0.59	0.861
Lean mass, kg	45.8 (44.5, 47.1)	44.7 (43.4, 46.0)	<.0001	0.917	0.042
Total fat mass, kg	32.6 (31.0, 34.1)	21.1 (19.7, 22.5)	<.0001	0.945	0.337
% fat	41.5 (40.4, 42.6)	31.9 (30.5, 33.2)	<.0001	0.994	0.060
IAAT ^I , cm ²	82.6 (72.4, 92.8)	47.2 (39.9, 54.4)	<.0001	0.305	0.742
Adiponectin, µg/ml	9.46 (8.48, 10.4)	12.3 (10.9, 13.7)	<.0001	0.706	0.654
Insulin, µIU/ml	10.7 (9.56, 11.8)	8.81 (7.94, 9.67)	0.0006	0.866	0.509
Leptin, ng/ml	23.3 (20.5, 26.1)	11.4 (9.54, 13.3)	<.0001	0.360	0.959
IGF-1, ng/ml	265.7 (241.6, 289.8)	263.8 (236.8, 290.7)	0.919	0.239	0.184
BP-1, ng/ml	14.4 (10.7, 19.7)	23.1 (18.4, 29.1)	0.0009	0.114	0.164
Estrogen, pg/ml	56.3 (47.0, 67.4)	73.0 (58.6, 90.9)	0.127	0.869	0.163
Testosterone, ng/ml	27.4 (23.6, 31.8)	27.7 (24.0, 31.8)	0.810	0.118	0.329

^IIAAT= Intra-abdominal adipose tissue.

Table 2

Percentage of ECC-1 cells in the G0/G1, S-phase and G2/M phase.

	CR ¹ n=13	CR+AE ² n=14	CR+RT ³ n=20	P for Time Group	P for Time x Group
G0/G1					
Over weight sera	53.8±1.45	55.0±1.42	56.1±1.05	0.0007	0.586
Weight-reduced sera	55.3±1.34	56.2±1.23	56.6±1.09		0.257
S-phase					
Over weight sera	28.4±1.32	28.2±1.30	26.8±1.05	0.002	0.690
Weight-reduced sera	27.5±1.18	27.6±1.32	26.7±1.00		0.065
G2/M					
Over weight sera	17.4±0.93	16.6±0.72	17.7±0.85	0.020	0.634
Weight-reduced sera	17.2±0.89	16.2±0.70	16.4±0.55		0.476

The assay was performed in triplicate and the data are mean ±SEE.

¹ CR=Caloric restriction

² CR+AE= Caloric restriction + aerobic exercise

³ CR+RT=Caloric restriction + resistance training.