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Longitudinal evaluation of cancer-associated biomarkers before and after weight loss in RENEW study participants: Implications for cancer risk reduction☆,☆☆

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Abstract

Introduction—Obesity is a major risk factor for the development of endometrial cancer (EC). An improved understanding of biologic mechanisms associated with weight loss, including alteration in inflammation, hormonal balance, and cancer antigens expression may lead to the development of effective cancer prevention strategies. The goal of this study was to explore

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longitudinal biomarker changes in obese women who underwent weight loss intervention, testing the hypothesis biomarker levels can be altered through intentional weight loss.

Methods—Serum samples from 89 participants with Class II and Class III obesity and 43 non morbidly obese comparisons were obtained in Re-Energize with Nutrition, Exercise and Weight Loss (RENEW) study as previously reported. Twenty-one bead-based xMAP immunoassays were utilized, including cancer-associated antigens, cytokines, chemokines, and hormones. One-way repeated measures ANOVA was used to examine the association between changes in biomarker expression levels over time (baseline, 6 months and 12 months). Linear mixed effects models were used to examine longitudinal relationships between biomarker expression levels.

Results—Mean levels of VEGF, soluble E-selectin, GH, adiponectin, IL-6, IL-7, CA-125, and IGFBP-1 significantly differed between time periods. In adjusted mixed linear models, decreasing BMI was significantly associated with lower levels of soluble E-selectin and IL-6 and increases in GH, adiponectin, and IGFBP-1.

Conclusions—This is one of the first efforts to explore changes in cancer-associated biomarkers in a cohort of weight loss research participants at high risk for EC development. Our findings demonstrate that changes in the expression of markers can be achieved with weight loss intervention.

Keywords

Obesity; Weight loss; Cancer biomarkers; Interleukins; Endometrial cancer

Introduction

Obesity is a major risk factor for the development of many cancers, including endometrial, ovarian, pancreatic, post-menopausal breast, prostate, kidney, gallbladder, liver, and esophageal cancers [1-10]. The magnitude of risk varies substantially by the type of cancer, with endometrial cancer (EC) showing the largest increase in risk associated with obesity. Compared to normal-weight women, obese women (body mass index (BMI) >30 kg/m^2) have a five-fold greater relative risk of developing EC [11,12]. In addition to BMI, other measures of adiposity, such as waist circumference, have been associated with increased risk of EC [13]. The pathophysiological and biological mechanisms underpinning these associations are the focus of current investigations. Recent large prospective multiethnic study confirmed that heavier weight and obesity (BMI≥30 kg/m^2) increase endometrial cancer risk, as well as the presence of a dose–response relation with BMI [9].

An improved understanding of the biologic consequences of weight loss may hold the key to better preventive strategies for cancers associated with excess adiposity. Although associations have been repeatedly found between excess weight and cancer risk, the evidence for protective effects of intentional weight loss in healthy individuals is now also beginning to emerge [14]. In a recent review Wolin et al. suggested that obesity causes a substantial proportion of all cancers, and emerging evidence suggests that adult weight loss reduces cancer risk [5]. Insulin resistance has been widely hypothesized to be involved in the increased cancer risk associated with obesity, but several other candidate systems are receiving increasing attention, including insulin-like growth factors, adipokines, obesity-

related inflammatory markers, and several others [15,16]. Several energy balance-related host factors, including leptin, adiponectin, steroid hormones, insulin, insulin-like growth factor (IGF)-1, and others are known to influence tumor progression; these have been implicated as key contributors to the complex effects of obesity on cancer [17]. Similar pathways including adipokines, chemokines, adhesion molecules, and interleukins have been implicated in EC risk [18-24].

In our previous investigations, we evaluated multimarker panels that included several factors involved in cancer and chronic inflammation, including IL-6, IL-8, prolactin, soluble E-selectin, IGF binding protein 1(IGFBP1), and others, utilizing multiplexing approaches of Luminex Technology. Our group previously reported that increased serum levels of several inflammatory cytokines are associated with ovarian and pancreatic cancers in case–control studies [25], with similar data for other malignancies including EC [19]. We observed differences in biomarker expression between obese and lean EC patients (unpublished data). Additionally, we developed panels of markers that showed significant potential to detect early cancers [26-28]. Furthermore, Luminex technology was shown to be a reliable tool for measuring large number of analytes in healthy individuals [29-31]. Thus, we wanted to expand the knowledge we accumulated in the area of cancer biomarkers to evaluate biomarker changes in healthy individuals who are at high risk for cancer development. The long term goal of this effort is to expand our knowledge and our research methods for developing cancer prevention studies by utilizing data from obese research participants who intentionally lose weight.

To realize this goal, potential mechanisms linking selected cytokines, adipokines, cancer antigens, and adhesion molecules to cancer risk can be studied prospectively in women undergoing weight loss through participation in research interventions. It is well known that very few individuals are able to intentionally lose weight and sustain weight loss. Bariatric surgery is known to be an effective long term weight loss option for morbidly-obese individuals [32], for whom other methods of weight loss have not been effective. Studies are also now beginning to confirm the expected lowering of cancer risk following bariatric surgery [33]. Particularly relevant to the current study, recent research has also begun to demonstrate that long-term weight loss after bariatric surgery is accompanied by a decreased pro-inflammatory state, accompanied by reduction in the expression of markers like sE-selectin [34]. Since bariatric surgery is invasive and not suitable for all obese individuals, we investigated changes in twenty-one cytokines, chemokines, and other markers in individuals undergoing intentional weight loss through a diet and exercise program. The goal of this study was to evaluate the association between changes in weight and changes in biomarkers associated with cancer development in severely obese women who participated in a longitudinal study examining the effect of dietary and exercise interventions on weight loss conducted at the University of Pittsburgh as part of the Re-Energize with Nutrition, Exercise and Weight Loss (RENEW) study [35]. We hypothesize that intentional weight loss may reduce the risk of obesity-associated cancers through modification of biomarkers of inflammation, insulin resistance, and cell adhesion. This line of research is especially important in the field of gynecologic malignancies, as exploratory reports investigating the link between weight loss through bariatric surgery and obesity associated cancer risk reduction demonstrate favorable effects in women [36].

Methods

Participant population

For the present analysis, we selected female participants with class II obesity (35 $kg/m^2 \leq BMI < 40\ kg/m^2$) and class III obesity ($BMI \geq 40\ kg/m^2$) who were originally enrolled in the parent RENEW Study. Eighty-nine participants, assessed up to 3 times each (baseline, 6 months, and 12 months) were included in the present study. Details about patient recruitment and study design have been highlighted in our previous publication [35]. Briefly, the RENEW study was a randomized intervention trial designed to determine the efficacy of a weight loss and physical activity intervention on the adverse health effects of severe obesity. One group of participants was randomized to diet and physical activity for 12 months (D-PA), while the second group of participants (D-DPA) had the identical dietary intervention, but with physical activity delayed for 6 months. The SenseWear Pro Armband (BodyMedia, Pittsburgh, PA) was used to provide an objective measure of physical activity. These two interventions were selected to gain insight on how diet and exercise impact the mechanisms that may contribute to obesity and whether staging influences behavioral incorporation of the exercise component.

Recruitment in the RENEW trial included mass mailings using voter registration and motor vehicle lists, news releases, the University of Pittsburgh's Health Science News Bureau, local newspapers, television stations, and placement of posters in various community locations. The non-morbidly obese comparison group, consisting of forty-three non-morbidly obese females, was primarily recruited by way of fliers and phone within the University of Pittsburgh Medical Center (UPMC). Of these, 20 were normal-weight ($18\ kg/m^2 \leq BMI < 25\ kg/m^2$), 11 were overweight ($25\ kg/m^2 \leq BMI < 30\ kg/m^2$), and the remaining 12 were Class I Obese ($30\ kg/m^2 \leq BMI < 35\ kg/m^2$). For the purposes of this study, the type of exercise intervention was not the focus. We were interested in the association between the magnitude of weight loss and change in cancer-associated biomarkers. Finally, while the parent RENEW study included both males and females, we limited this analysis to females only, as our main research question is the relationship between weight loss and development of female cancers.

Sample storage and laboratory assays

Fasting blood samples were obtained at the Endocrinology and Metabolism Research Center of the University of Pittsburgh following standard blood collection and processing protocol. Samples were obtained from participants who agreed to participate in RENEW study and signed the informed consent. Sera were separated by centrifugation, immediately aliquoted, frozen and stored at –80 °C. Never thawed 1 mL serum samples were sent on dry ice to the Luminex Core Facility at the University of Pittsburgh Cancer Institute, where they were stored at –80 °C until they were assayed. The xMAPTM bead-based technology (Luminex Corp., Austin, TX) permits multiplexed analysis of several analytes in one sample. Twenty-one bead-based xMAPTM immunoassays were utilized in this study: cancer-associated antigens 125 and 15-3, carcinoembryonic antigen (CEA), interleukins (IL) 2, 6, 7, 8, 10,IL-1 receptor alpha (RA), tumor necrosis factor alpha (TNF-a), IGFBP1,IGFBP2, vascular endothelial growth factor (VEGF), eotaxin, soluble E-selectin, thyroid stimulating hormone

(TSH), prolactin, growth hormone (GH), resistin, adiponectin, and IGF-1. These markers were chosen because they have been associated with pro-inflammatory milieu, as well as with EC development and progression in previous studies [17,19,22,37]. Inter-assay and intra-assay variability of each assay was 3.5–5% and 7–15%, respectively.

Each bead-based assay has been validated in comparison with appropriate standard ELISA based on the same antibody pair and demonstrated 89–98% correlation. Recovery from serum was 70–120% (data presented on Luminex Core Facility website for in-house assays; performance of purchased assays was in agreement with that described by the manufacturer). Assays were performed according to manufacturers' protocols as previously described [26]. Samples were analyzed using the Bio-Plex suspension array system (Bio-Rad Laboratories, Hercules, CA). For each analyte, 100 beads were analyzed and means were calculated. Analysis of experimental data was performed using four-parametric-curve fitting to the standard analyte curves, excluding all values falling outside the range of standard curve.

Statistical analysis

Descriptive statistics—All statistical analyses were done with SAS version 9.2 (SAS Institute Inc., Cary, NC). Basic statistics were used to summarize the characteristics of the 89 intervention participants and 43 non-morbidly obese comparisons. Age, BMI, weight, waist circumference, and steps (measured by SenseWear Pro Armband) were included as continuous variables; race was dichotomized into ‘Caucasians or ‘African-American’; time period was categorized as baseline, 6 months, or 12 months; smoking status was categorized as ‘smoker’ or ‘non-smoker’.

Cross-sectional association between BMI and cancer-associated biomarkers —Cross-sectional analyses comparing obese participants and non-morbidly obese comparisons at baseline were performed. Biomarker expression levels were log transformed and linear regression was used to examine the relationship between baseline biomarker expression and BMI controlling for age, race, and smoking status. Intervention and comparison group participants were included in this cross-sectional analysis. The goal of this step was to identify cross-sectional associations between BMI and biomarkers to support hypotheses for the longitudinal analysis. CEA was excluded from analyses as the values for this marker fell outside the range of the standard curve. Il-1RA was excluded because of similar concerns regarding values outside of standard curve and bimodal distribution of remaining values. For all other biomarkers that we analyzed, ≥92.5% of readings were inside the standard curve range; for analytic purposes only values within the curve range were included.

Longitudinal evaluation of biomarker changes—Repeated measures analysis of variance (ANOVA) was used to determine whether biomarker expression differed significantly across time periods for the 89 intervention participants, ignoring all other factors. The biomarkers that showed significant differences in expression between the three time points were entered into a mixed linear model that examined the relationship between

biomarker expression, BMI, steps, and time period, adjusted for age, race, and smoking status.

An interaction between BMI and time period was examined to assess the relationship between biomarker expression and BMI changes over time. All statistical tests were two-sided and the α-level for significance was set at $p<0.05$. Given the exploratory nature of this study, no adjustment for multiple tests was used. Potentially influential data points were determined by Cook's distance and models were run with and without outliers. No outliers had a significant effect on results and all results are presented using the complete dataset.

Results

Demographic and anthropometric characteristics

Demographic, weight, height, and BMI characteristics of the 89 subjects from the RENEW study and the 43 non-morbidly obese comparison subjects are shown in Table 1. Mean age of the participants was 47 years and 25% of the study sample was African-American. At baseline, mean BMI and weight were 43.8 kg/m^2 and 116 kg, respectively, indicating an obese study sample. Comparison participants had a similar age distribution to cases. Through participation in the RENEW study, participants lost an average of 12.8 kg from baseline to the 12 month assessment, with the largest change occurring in the first 6 months of the intervention. Similarly, BMI decreased an average of 5 kg/m^2 over the 12-month period, with 4 kg/m^2 lost in the first 6 months of follow-up. Waist circumference significantly decreased by 10.77 cm over the 12-month period, with 7.65 cm lost in the first 6 months of follow-up.

Cross-sectional analysis at baseline

Among all participants (N=132), cross-sectional analysis showed that BMI was significantly associated with the following eight bio-markers at baseline: CA 15-3, soluble E-selectin, GH, resistin, adiponec-tin, IL-8, CA-125 and IGFBP-1 (Table 2). Parameter estimates in Table 2 reflect the direction and magnitude of the changes that occur in bio-marker expression level with each unit change of BMI. A direct relationship between decreasing BMI and decreasing levels of soluble E-selectin, resistin, and adiponectin was observed, while inverse associations between decreasing BMI and increasing levels of GH, IL-8, CA-125, CA 15-3, and IGFBP-1 were observed. No significant interactions between race and BMI (or waist circumference) were found for any of the biomarkers.

Longitudinal evaluation

In the first 6 months of follow-up, BMI change of the participants ranged between 11.7 kg/m^2 lost to a gain of 1.0 kg/m^2 (data not shown). Between baseline and 12 months, BMI change ranged from 21.9 kg/m^2 lost to 1.0 kg/m^2 gained (data not shown). Ignoring all other factors, eight markers significantly differed in expression from baseline to at least one follow-up time point: VEGF, soluble E-selectin, GH, adiponectin, IL-6, IL-7, CA-125, and IGFBP-1 (Table 3). Soluble E-selectin, VEGF, IL-6, IL-7, and CA-125 decreased over time while GH, adiponectin, and IGFBP-1 significantly increased. No statistically significant

differences were observed in biomarker changes between D-PA and D-DPA experimental groups.

Biomarker-specific linear mixed effect models were created that contained age, race, smoking status, time period, steps, BMI, and an interaction between BMI and time period (Table 4). Parameter estimates (beta values) in Table 4 provide information on the direction and magnitude of the relationship between each factor and the biomarker of interest, while simultaneously adjusting for all other covariates in the model. Using the biomarker GH for example, a beta value of –0.068 for BMI indicates that GH expression is estimated to decrease by 0.068 for every 1 unit increase in BMI. Age, smoking status, and steps were not significantly associated with any biomarker. Adiponectin, CA-125, and IGFBP-1 levels were all significantly higher in Caucasians compared to African-Americans, adjusting for age, time period, and smoking status. BMI and GH expression were significantly and inversely associated with each other (parameter estimate –0.068, p=0.01), such that lower BMI levels were associated with higher levels of GH. Conversely, BMI and expression of IL-6 were directly related (parameter estimate 0.044, p=0.002), such that decreasing BMI was associated with lower levels of IL-6. Also, IGFBP-1 and BMI are inversely associated with one another (controlling for time period, age, race, smoking status, and steps). The interaction (BMI*time period) was only significant for IGFBP-1 (Table 4), indicating that the association between BMI and IGFBP level is time dependent. For example, the difference in the estimated IGFBP expression between a participant with a BMI of 30 and another participant with a BMI of 38 will be greater at 6 months than at baseline and even greater at 12 months than at 6 months.

Discussion

We explored changes in biomarkers associated with cancer development in a cohort of severely obese women who are at high risk of cancer development undergoing a weight loss intervention and we found that levels of some of these biologic markers can potentially be normalized with weight loss. Our findings demonstrate that changes in the expression of multiple markers can be achieved with weight loss intervention in as little as six months. Expression of VEGF, soluble E-selectin, GH, adiponectin, IL-6, IL-7, CA-125, and IGFBP-1 significantly differed between at least two time points. Of these eight markers, a significant association between change in bio-marker expression and change in BMI was noted for GH and IL-6. None of the markers was significantly associated with age and smoking status. IGFBP1 was significant in the interaction between BMI and time.

The novelty of our study is evaluating multiple biologic markers associated with endometrial cancer development in a group of high risk patients who successfully lost weight. Our observations regarding weight loss and biomarker change in the current study are consistent with previously published studies examining some of these bio-markers. For example, increases in GH and IGFBP-1 levels among individuals who lose weight have been reported previously [1]. IGFBP-1 is an important regulator of circulating insulin-like growth factor (IGF-1), which has powerful effects on cell growth and proliferation. The increase in IGFBP-1 in people who lose weight implies a decreased bioavailability of IGF-1. GH is a major stimulus of IGF-1 production. In obese individuals, GH secretion is reduced

compared to that of normal weight individuals, possibly due to negative feedback inhibition [38]. Calorie restriction studies show that GH secretion can be restored in normal-weight individuals but rarely in obese individuals.

Similarly, our finding that serum adiponectin levels increased with weight loss is consistent with a previous prospective study of patients who lost weight after bariatric surgery [39] and with several cross sectional correlation studies. Previous studies showed that circulating levels of adiponectin are inversely associated with body mass [40]. Since it has been hypothesized that adiponectin may have anti-inflammatory effects, especially in endothelial cells and macrophages, increases in adiponectin with weight loss may offer a protective effect for cancer development [41]. Specifically, previous findings suggest that adiponectin exerts energy-homeostatic and anti-inflammatory effects in the endometrium, and these effects might be relevant to pathological and physiological endometrium-related events [42].

Soluble E-selectin is an adhesion protein that is involved in endothelial function. In a sample of morbidly obese patients, Vazquez et al. [43] reported significant reductions of circulating soluble E-selectin following bariatric surgery. The mechanism underlying this change can be related to the role of soluble E-selectin in inflammatory processes. Soluble E-selectin expression is higher than normal in a pro-inflammatory milieu, resulting in greater microvascular permeability. Since obesity is associated with chronic inflammation, weight loss may alter the expression of some of the inflammatory factors and thus potentially lead to decreased risk of cancer.

Increasing IGFBP-1 levels were associated with increased EC risk in a population based case–control study [22]. A panel consisting of prolactin, GH, eotaxin, E-selectin, and TSH was effective in separating known cancer cases from controls in our previous case–control research [19]. Carcinogenic mechanisms associated with leptin and adiponectin have been described by Renehan [15]. Thus, modifying these markers with weight loss could be a key to preventing cancer.

The present study had several limitations and strengths that should be emphasized. Although our ultimate goal is to build a program for obesity associated cancer prevention, cancer was not being assessed as a study outcome. This is mainly attributable to a limited follow up period of this study, and it is unlikely that we would observe a meaningful difference in the rate of EC or any obesity associated cancer development within such a short follow up period. Similar problem has been reported by Wolkin and Colditz, who indicated that it is unlikely that in the near future a cancer prevention trial will focus on weight loss as a primary prevention strategy, mainly due to the fact that cancer is an inappropriate outcome to assess in such trials [44]. Instead of addressing EC as an endpoint, this study was intended to provide a foundation for advancing understanding and future prevention and management of obesity associated cancer.

Another limitation of this study was that the list of biomarkers chosen for investigation was not comprehensive. Findings of this study suggest the importance of additional research to explore other markers, especially in the inflammatory pathways.

The major strength of this study was the assessment of a diverse set of biomarkers and the investigation of their relationship with weight change over time in a prospective study, which is rare in the literature. Furthermore, we simultaneously examined the possible contributions of physical activity (objectively measured in steps) and weight loss (measured as change in BMI) to biomarker changes, and concluded that changes in BMI showed a stronger relationship with changes in biomarkers than to changes in physical activity. To our knowledge, these findings are novel. Finally, within the RENEW study, a large proportion of African-American women were recruited and participated in the intervention, which is a strength of our study as it suggests the generalizability of the findings. It should be noted however, that the study was not adequately powered to explore possible differential effects by race.

Substantial intentional weight loss is increasingly being documented to reduce the risk of obesity related cancers [45,46], however, existing studies are small and underpowered. It is important to point out that the effect of weight loss on cancer development remains an under investigated area, which is the rationale behind the current research. These results demonstrate that the magnitude of weight loss that can be accomplished by an effective behavioral weight loss intervention (RENEW) is associated with significant changes in biologic markers that are thought to be associated with cancer development.

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Table 1

Participant characteristics.

	Intervention participants (n=89)	Comparisons (n=43)	All participants (n=132)
Age, mean (SD)	47.28 (6.18)	42.60 (6.30)	45.76 (6.58)
Race, n (%)			
Caucasian	67 (75.28)	32 (74.42)	99 (75.00)
African-American	22 (24.72)	11 (25.58)	33 (25.00)
Smoking Status, n (%)			
Smoker	10 (11.24)	10 (23.26)	20 (15.15)
Non-smoker	79 (88.76)	33 (76.74)	112 (84.85)
Intervention type, n (%)			
Immediate exercise (D-PA)	47 (52.80)	NA	NA
Delayed exercise (D-DPA)	41 (46.07)	NA	NA
Weight (kg), mean (SD)			
Baseline	116.59 (15.43)	70.79 (12.68)	101.67 (25.99)
6 months	106.19 (14.47) [a]	NA	NA
12 months	103.81 (17.09) [b]	NA	NA
Height (cm), mean (SD)			
Baseline	163.11 (6.34)	164.53 (6.53)	163.57 (6.41)
BMI (kg/m^2), mean (SD)			
Baseline	43.84 (5.40)	26.11 (4.39)	38.06 (9.76)
6 months	39.93 (5.18) [c]	NA	NA
12 months	38.89 (6.16) [d]	NA	NA
Waist circumference (cm), mean (SD)			
Baseline	122.55 (11.26)	85.11 (11.64)	110.35 (20.95)
6 months	114.90 (9.89) [e]	NA	NA
12 months	111.78 (12.57) [f]	NA	NA

SD = standard deviation.

[a] Paired *t*-test for difference in weight from baseline to 6 months: $p<0.001$.

[b] Paired *t*-test for difference in weight from baseline to 12 months: $p<0.001$.

[c] Paired *t*-test for difference in BMI from baseline to 6 months: $p<0.001$.

[d] Paired *t*-test for difference in BMI from baseline to 12 months: $p<0.001$.

[e] Paired *t*-test for difference in waist circumference from baseline to 6 months: $p<0.001$.

[f] Paired *t-test for difference in waist circumference from baseline to 12 months:* $p< 0.001$.

Table 2

Association between biomarker[a] at baseline and BMI[b].

Biomarker	N	Parameter estimate for BMI[c]	*P*
VEGF	117	−16.39	0.16
Eotaxin	132	−0.69	0.28
TNF-alpha	129	0.02	0.45
CA 15-3	132	−0.004	**0.004**
Soluble E-selectin	116	0.44	<**0.001**
TSH	132	0.04	0.10
Prolactin	132	0.47	0.25
GH	132	−0.06	<**0.001**
Resistin	131	0.53	<**0.001**
Adiponectin	131	0.15	**0.02**
IGF-1	132	−0.0006	0.95
IL-2	84	−0.03	0.65
IL-6	81	0.01	0.87
IL-7	128	−0.08	0.18
IL-8	127	−0.04	**0.01**
IL-10	131	0.13	0.57
CA-125	98	−0.31	**0.01**
IGFBP-1	132	−0.20	<**0.001**
IGFBP-2	132	0.097	0.47

[a] Paired t-test for difference in weight from baseline to 6 months: $p<0.001$

[b] Paired t-test for difference in weight from baseline to 12 months: $p<0.001$.

[c] Paired t-test for difference in BMI from baseline to 12 months: $p<0.001$.

Table 3

Mean levels of biomarkers at baseline, 6 months, and 12 months, mean (sd).

Biomarker (unit)	Baseline (N=89)	6 months (N=88)	12 months (N=59)	*p*-value[a]
VEGF (pg/ml)	619.75 (586.12)	615.58 (587.99)	522.78 (501.25)	**0.003**
Eotaxin (pg/ml)	103.14 (70.94)	99.48 (67.03)	100.22 (64.38)	0.67
TNF-alpha (pg/ml)	6.98 (3.00)	6.80 (2.86)	7.12 (2.88)	0.57
soluble E-selectin (ng/ml)	33.54 (12.68)	29.93 (10.69)	30.10 (11.58)	<**0.001**
TSH (mU/l)	3.58 (2.32)	3.84 (4.1)	4.15 (3.64)	0.63
Prolactin (μg/l)	22.45 (51.98)	19.52 (18.71)	20.62 (15.62)	0.06
GH (ng/ml)	0.43 (0.77)	0.81 (1.03)	1.07 (1.25)	<**0.001**
Resistin (ng/ml)	17.62 (6.88)	17.69 (6.45)	16.97 (5.59)	0.41
Adiponectin (μg/ml)	11.52 (7.64)	12.62 (6.16)	13.75 (6.91)	<**0.001**
IGF1 (ng/ml)	1.39 (1.22)	1.72 (2.36)	1.70 (1.88)	0.43
IL-2 (pg/ml)	2.28 (3.29)	2.75 (4.05)	2.37 (3.89)	0.56
IL-6 (pg/ml)	6.58 (4.30)	6.51 (6.46)	5.14 (4.24)	<**0.001**
IL-7 (pg/ml)	7.26(4.91)	7.11(4.39)	6.05(3.36)	**0.003**
IL-8 (pg/ml)	3.87 (1.62)	4.11 (1.61)	4.39 (2.43)	0.24
IL-10 (pg/ml)	10.56 (24.79)	7.76 (9.50)	7.50 (11.54)	0.85
CA 15-3 (pg/ml)	0.09 (0.12)	0.11 (0.12)	0.12 (0.16)	0.31
CA-125 (pg/ml)	9.88 (6.40)	9.16 (5.80)	8.96 (9.16)	**0.002**
IGFBP-1 (ng/ml)	3.26 (3.41)	3.70 (3.17)	4.11 (3.53)	<**0.001**
IGFBP-2 (ng/ml)	15.00 (15.91)	15.17 (14.67)	16.41 (16.10)	0.61

Note: Biomarkers with significant *p*-values (<0.05) are in bold.

[a] *p*-Value from repeated measures ANOVA, biomarkers were log transformed for ANOVA.

Table 4

Longitudinal analysis examining association between biomarkers, BMI, and steps adjusted for covariates.

EFFECT	BIOMARKER															
	Adiponecitin		CA-125		GH		IGFBP-1		IL-6		IL-7		VEGF		Soluble E- selectin	
	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
Intercept	16.297	<0.01	2.985	<0.01	5.451	<0.01	7.057	<0.01	0.147	0.88	1.651	0.03	5.708	<0.01	9.864	<0.01
Age	0.007	0.36	−0.007	0.41	0.033	0.12	0.02	0.08	−0.005	0.71	−0.007	0.49	−0.013	0.41	0.001	0.89
African-Americana[a]	**−0.362**	**0.001**	**−0.326**	**0.01**	0.340	0.27	**−0.411**	**0.01**	−0.089	0.61	−0.128	0.39	−0.292	0.19	0.076	0.45
Smoke	0.037	0.68	−0.155	0.26	0.093	0.79	0.116	0.49	−0.094	0.55	−0.038	0.79	0.070	0.72	−0.096	0.29
Steps	0.000	0.38	0.000	0.71	0.000	0.99	0.000	0.42	0.000	0.36	0.000	0.52	0.000	0.91	0.000	0.66
BMI	−0.010	0.15	−0.009	0.35	**−0.068**	**0.01**	−0.006	0.65	**0.044**	**0.002**	0.013	0.23	0.022	0.08	0.010	0.13
Time period[b]																
6 months	0.088	0.71	0.012	0.98	1.325	0.24	**1.058**	**0.03**	−0.369	0.48	0.291	0.45	−0.121	0.74	−0.330	0.22
12 months	0.339	0.17	−0.038	0.93	1.600	0.18	**1.839**	**<0.001**	−0.088	0.87	−0.134	0.74	−0.365	0.35	−0.447	0.07
BMI*time (6 months)	−0.0005	0.93	−0.004	0.69	−0.022	0.41	**−0.023**	**0.04**	0.009	0.45	−0.005	0.57	0.004	0.65	0.006	0.40
BMI*time (12 months)	−0.005	0.36	−0.005	0.67	−0.023	0.42	**−0.041**	**0.001**	0.000	0.99	0.001	0.92	0.007	0.43	0.008	0.16

[a] Baseline race is ‘Caucasian’.

[b] Baseline time period is ‘baseline’.