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Effects of diet and/or exercise on the adipocytokine and inflammatory cytokine levels of postmenopausal women with type 2 diabetes☆

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Abstract

This study examined the independent and combined effects of diet and exercise on adipocytokine and inflammatory cytokines in postmenopausal women with type 2 diabetes. Using a randomized, controlled design, 33 women (age, 50-70 years) were assigned to diet alone (D), exercise alone (EX), or diet + exercise (D + E) for 14 weeks. Before and after the interventions, blood samples for adipocytokines and inflammatory markers were drawn, a meal test was performed, and abdominal fat distribution was measured by magnetic resonance imaging (MRI). Body weight decreased ~4.5 ± 0.6 kg ($P < .05$) after the D and D + E interventions, whereas only small changes in body weight were found with the exercise-alone intervention. Plasma C-reactive protein levels were decreased by ~15% with all 3 interventions, whereas leptin levels were reduced with the D and D + E intervention (D: pre = 48.7 ± 6.0, post = 38.9 ± 5.0 ng/mL; D + E: pre = 38.5 ± 6.0, post = 22.9 ± 5.0 ng/mL; $P < .05$) with no differences between groups. There was a trend for leptin levels to decrease in the EX group ($P = .06$). Plasma resistin levels were not altered by the 3 interventions from pre- to posttreatment (D: pre = 6.9 ± 0.6, post = 6.2 ± 0.4 ng/mL; D + E: pre = 5.6 ± 0.6, post = 5.7 ± 0.4 ng/mL; E: pre = 6.2 ± 0.6, post = 5.9 ± 0.6 ng/mL, $P > .05$), and no differences in adiponectin and tumor necrosis factor α (TNF-α) levels were found. Visceral adipose tissue and tumor necrosis factor α were the only predictors of calculated insulin resistance ($P < .05$), explaining 43% of the variability. A typically prescribed weight loss program with lifestyle changes resulted in few changes in adipocytokines and inflammatory cytokines in older women with type 2 diabetes, suggesting that dramatic weight loss or clinical interventions are needed.

1. Introduction

Recent research has demonstrated that adipose tissue is an active endocrine tissue, which secretes hormones, such as adiponectin, resistin, and leptin, referred to as adipocytokines [1,2]. Adipocytokines appear to contribute to inflammation, atherosclerosis, and may be involved in the etiology of type 2 diabetes, possibly constituting the missing link between obesity and insulin resistance (IR) [1,2].

Abnormal levels of adipocytokines may contribute to IR. In human beings, the physiological role of resistin on obesity and IR is unclear, with some studies reporting a significant relationship between resistin levels and obesity and IR markers [3,4], whereas others do not [5-10]. Likewise, adiponectin has been linked to IR such that low adiponectin levels are found with obesity and may contribute to IR and atherogenesis [11,12]. Administration of adiponectin to animals has resulted in a significant reduction in hyperglycemia, hyperinsulinemia, and IR [13] and prevented diet-induced weight gain through increased rates of lipolysis and fatty acid oxidation [14]. Leptin alters metabolism by mediating appetite and energy expenditure via feedback mechanism on the hypothalamic satiety-regulating centers [15] and has also been linked to obesity-related IR [16,17]. Leptin resistance is associated

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with the development of IR in individuals with type 2 diabetes [18], and in animals, administration of leptin reverses IR [19].

Interleukin (IL)-6 and tumor necrosis factor α (TNF-α), 2 major pro-inflammatory cytokines, are secreted in significant amounts from adipose tissue [20-22], and consequently obese women (healthy and diabetic) have higher cytokine levels than healthy lean women [23,24]. IL-6 and TNF-α are also known to regulate C-reactive protein (CRP) release from the liver and increase plasma CRP levels [25]. Furthermore, increased levels of IL-6 and TNF-α are associated with deterioration of glycemic control, increased IR, and dyslipidemia, contributing to the dysfunctional metabolic status of obese and type 2 diabetic individuals [26-28].

Weight loss through diet alone or diet and exercise interventions results in elevations [29-39] or no change [35,40] in adiponectin levels and reductions in leptin levels [29-39] in obese healthy individuals. On the other hand, only one study to date has examined resistin levels after weight loss [41]. Lifestyle modifications of diet and exercise elevate adiponectin levels, reduces leptin levels but do not affect resistin levels in obese individuals [41].

Most of the previous research has focused on obese individuals. The few studies conducted on individuals with type 2 diabetes [41-43] have provided conflicting results from those found on obese individuals, indicating that either this population does not respond in the same manner to lifestyle interventions or that more dramatic weight loss is necessary for changes to occur. Furthermore, limited research exists on the effects of exercise as the sole intervention on these adipocytokines in individuals with type 2 diabetes. Exercise without weight loss has been shown to decrease visceral fat in obese men [44], suggesting that exercise may also alter the adipocytokines even if no weight loss occurs.

The purpose of the present study was to investigate the independent and combined effects of diet and exercise on the adipocytokines (resistin, adiponectin, leptin) and the inflammatory cytokines (TNF-α, IL-6, and CRP) in postmenopausal women with type 2 diabetes. We hypothesized that the combination of diet + exercise would result in greater changes in adipocytokines and inflammatory cytokines than diet or exercise alone, and that these changes would be related to improvements in glycemic control.

2. Methods

2.1. Experimental design

The subjects were randomly assigned to 1 of 3 interventions: diet alone (D), exercise alone (EX), and diet + exercise (D + E) for 14 weeks. Before the start and at the end of the intervention period, all women had an exercise stress test, fasting blood samples drawn, a meal test performed, and body composition measured (total body fat and abdominal fat distribution).

2.2. Subjects

Forty women with type 2 diabetes (50-70 years old) were recruited to participate in this study. All women were postmenopausal for a minimum of 1 year (8.9 ± 1.3 years), obese (body mass index [BMI] > 30 kg/m^2), and diagnosed with type 2 diabetes for at least 1 year (3.0 ± 0.6 years). Twenty-two of the subjects were on a stable dose of oral hypoglycemic agents for a minimum of 1 year (sulfonylureas [n = 3], metformin [n = 14], combination of sulfonylureas and metformin [n = 5]), whereas 11 women were not taking any medication for diabetes. Women on thiazolidinediones, insulin, and β-blockers were excluded. Of the 33 women, 10 (D + E = 4, D = 2, EX = 4) were on hormone replacement therapy (7.6 ± 2.5 years) and their dose did not change over the course of the study. Eleven women were on angiotensin-converting enzyme inhibitors. None of the women participated in any type of regular physical activity or diet treatment for the prior year and all have stable weight. All women were in good health with no major complications related to diabetes, such as cardiovascular disease and neuropathies. Before participation in the study, the women signed an informed consent approved by both the Syracuse University and SUNY Upstate Medical University institutional review boards.

2.3. Exercise stress test

On the first visit, a continuous exercise stress test was performed on the treadmill to determine aerobic fitness and to detect any abnormalities using a protocol previously described [45]. Metabolic data were collected using open circuit spirometry, which was calibrated against known gases before each test (Cosmed Quark b^2 metabolic cart, Rome, Italy). The electrocardiogram was continually monitored throughout the exercise stress test and evaluated by a cardiologist for any cardiac abnormalities.

2.4. Blood sampling and meal test

On a subsequent visit, the subjects reported to the Human Performance Lab between 7:00 and 8:00 AM after a 12-hour overnight fast. The women were instructed to take their oral hypoglycemic drugs as prescribed and to abstain from any type of exercise for 48 hours before testing. A venous blood sample was drawn for measurement of fasting blood glucose, insulin, hemoglobin A_{1c} (HbA_{1c}), lipid concentrations (high-density lipoprotein [HDL], low-density lipoprotein [LDL], very low-density lipoprotein, triglycerides [TRIG], total cholesterol [TC]), resistin, adiponectin, leptin, CRP, TNF-α, and IL-6 concentrations. Following this sample, the meal test was initiated with the subject consuming 2 cans of Ensure (66 g carbohydrate, 29 g protein, 11 g fat; Abbott Laboratories, Abbott Park, Ill). The meal test was used to more closely reflect the response to a typical meal and prevents the blood glucose levels from increasing too high. A 5-mL blood sample was drawn every 30 minutes for 4 hours.

2.5. Body composition

Percentage of body fat was determined by 2 methods: the hydrostatic weighing test [45] and air plethysmography using the BodPod (Life Measurement Inc, Concord, Calif). The hydrostatic weighing test was used at the beginning of the study, but because of technical problems was replaced by the BodPod. All subjects were evaluated by the same piece of equipment before and after the intervention.

Abdominal fat distribution was determined by magnetic resonance imaging (MRI) with techniques previously reported [45]. Total abdominal fat volume was determined by summating ~40 consecutive, 1-cm slices. Subcutaneous abdominal adipose tissue (SAT) was calculated from total visceral abdominal adipose tissue (VAT) from the total abdominal fat tissue (total abdominal fat) (SAT = total AT − VAT) of each slice. Test-retest reliability for repeated image analysis was $r = 0.9999$ ($P < .0001$) [45].

2.6. Exercise intervention

The EX intervention consisted of a supervised walking program 3 to 4 times per week for 60 minutes at 65% to 70% $\dot{V}O_2$ peak and approximately 1050 to 1250 kJ were expended per exercise session. During this intervention, all subjects were asked to continue their typical eating patterns.

2.7. Diet intervention

The D intervention consisted of a high-monounsaturated fat (HMF) diet composed of 40% fat (30% monounsaturated, 5% polyunsaturated, and 5% saturated), 40% carbohydrates (15% simple and 25% complex carbohydrates), and 20% protein. Weight maintenance energy consumption was determined using resting metabolic rate [45] and daily activity levels, and 2510 kJ/d was subtracted from this weight maintenance value. Nutritional consulting was provided at the beginning of the study and at weekly intervals, and a 1-day dietary recall was completed every 2 weeks. This group refrained from any type of regular physical activity during the 14 weeks of the intervention.

2.8. Diet + exercise intervention

The D + E intervention combined the above interventions, such that on exercise days there was a ~1460-kJ deficit from the diet and ~1050-kJ deficit from the exercise, whereas on the non-exercise days a ~2510-kJ deficit was produced from the diet alone.

2.9. Blood analysis

Blood was collected, centrifuged, and stored at −80°C for later analysis. Blood glucose and lipid concentrations (HDL, LDL, very low-density lipoprotein, TRIG, TC) were measured using the Cholestech, LDX automated analyzer (Hayward, Calif). HbA_{1c} was commercially analyzed by the Diabetes Technologies, Inc (Thomasville, Ga). Plasma insulin concentrations were analyzed in duplicate using commercial radioimmunoassay kits (Diagnostic Products Corporation, Los Angeles, Calif). The sensitivity, and intra- and inter-assay coefficients of variation of the assay were 2.02 pmol/L, and 3.0% and 14.5%, respectively. Plasma levels of CRP were determined by a commercial enzyme-linked immunosorbent assay (ELISA) (DSL Systems, Webster, Tex). TNF-α and IL-6 concentrations were determined by a quantikine high-sensitivity ELISA assay (R&D Systems, Minneapolis, Minn), with assay sensitivity

Table 1
Baseline subject characteristics and changes in metabolic and abdominal fat distribution from pre- to post-intervention

Dependent variable	D + E		D		EX	
	Pre	Change	Pre	Change	Pre	Change
Age (y)	57.4 ± 1.7		58.5 ± 1.7		55.5 ± 1.7	
Duration of diabetes (y)	2.86 ± 1.05		4.15 ± 1.1		2.91 ± 1.05	
Body height (cm)	163 ± 22		163 ± 22		161 ± 22	
Body weight (kg)	89.5 ± 5.9	−5.4 ± 1.3	92.4 ± 5.9	−4.6 ± 1.4	92.9 ± 5.9	−1.7 ± 0.8
BMI (kg/m^2)	33.7 ± 1.9	−2.0 ± 0.4	34.3 ± 1.9	−1.4 ± 0.4	35.9 ± 1.9	−0.6 ± 0.2
Total body fat mass (kg)	31.2 ± 3.0	−5.4 ± 0.4*	30.9 ± 3.2	−4.7 ± 0.5*	34.3 ± 3.0	−1.3 ± 0.6
Fasting glucose (mmol/L)	9.1 ± 0.8	−2.4 ± 0.2*	9.5 ± 0.8	−1.3 ± 0.2*	8.19 ± 0.8	+0.6 ± 0.3
HbA_{1c}	6.8 ± 0.5	−0.5 ± 0.2	7.3 ± 0.5	+0.5 ± 0.3	6.4 ± 0.8	+0.2 ± 0.3
Insulin (pmol/L)	94.9 ± 27.4**	−48.3 ± 26.1*	31.9 ± 11.3	−4.1 ± 5.5	32.2 ± 21.1	−15.8 ± 14.2
Insulin resistance	31.4 ± 4.5***	−3.5 ± 5.4*	19.0 ± 3.2	−0.38 ± 3.0	41.7 ± 11.1***	−6.0 ± 3.0*
TC (mg/dL)	207 ± 14	−15.2 ± 3.3*	224 ± 14	−12.6 ± 8*	217 ± 15	0.3 ± 8
LDL (mg/dL)	118 ± 11.6	−10.6 ± 3.4	134 ± 12	−2.2 ± 9.7	139 ± 16.1	1.5 ± 8.2
HDL (mg/dL)	48 ± 3.5	−4.1 ± 2.7*	49.2 ± 3.5	−7.5 ± 3.1*	48.5 ± 3.4	0.4 ± 3.6
TRIG (mg/dL)	212.4 ± 48.5	−20.6 ± 26.4	240.3 ± 48.2	−62.3 ± 28.0	147.1 ± 17.0	18.9 ± 18.7
Total abdominal tissue (cm^3)	15892 ± 1455	1789 ± 700*	13952 ± 1500	−416 ± 130*	15380 ± 1455	763 ± 231*
Visceral adipose tissue (cm^3)	5912 ± 524	760 ± 320*	4785 ± 524	−360 ± 152	5204 ± 524	529 ± 187*
Subcutaneous adipose tissue (cm^3)	9987 ± 1059	−1037 ± 613*	9331 ± 1100	−221 ± 111*	10495 ± 1060	553 ± 195*

Mean ± SEM; n = 33. Insulin resistance as calculated from Tanaka et al [46].

* $P < .05$ vs pre values.

** $P < .01$ vs the other groups.

*** $P < .05$ vs the diet-only group.

of 1.6, 0.12, and 0.04 ng/mL for CRP, TNF-α, and IL-6, respectively. The intra- and inter-assay coefficients of variation for CRP were 3.9% and 3.7%, and for TNF-α were 3.5% and 14%, respectively. The intra-assay coefficient of variation for IL-6 was 4.2%. All pre- and post-intervention samples were measured in the same assay. Insulin resistance was calculated using the Homeostasis Model Assessment–Insulin Resistance (HOMA-IR) equation by Tanaka et al [46].

Plasma leptin levels were determined by a commercial ELISA (DSL Systems). Adiponectin and resistin concentrations were determined by quantikine high-sensitivity ELISA assay (R&D Systems). The sensitivity of these assays was 0.05 ng/mL, 0.08 pg/mL, and 0.06 ng/mL for leptin, adiponectin, and resistin, respectively. The intra- and inter-assay coefficient of variation for adiponectin were 8.5% and 6.5%, respectively, and the intra-assay coefficient of variation for leptin and resistin were 3.4% and 2.5%.

2.10. Statistical analysis

Comparison of the descriptive data for the 3 interventions was conducted using a 2-way analysis of variance; if significant differences in the main effect ($P < .05$) were seen, post hoc comparisons were conducted. To control for the effects of fat mass changes on these variables, an analysis of covariance was also run using total body fat mass and abdominal fat distribution as covariates. (Variables that were not normally distributed were log transformed

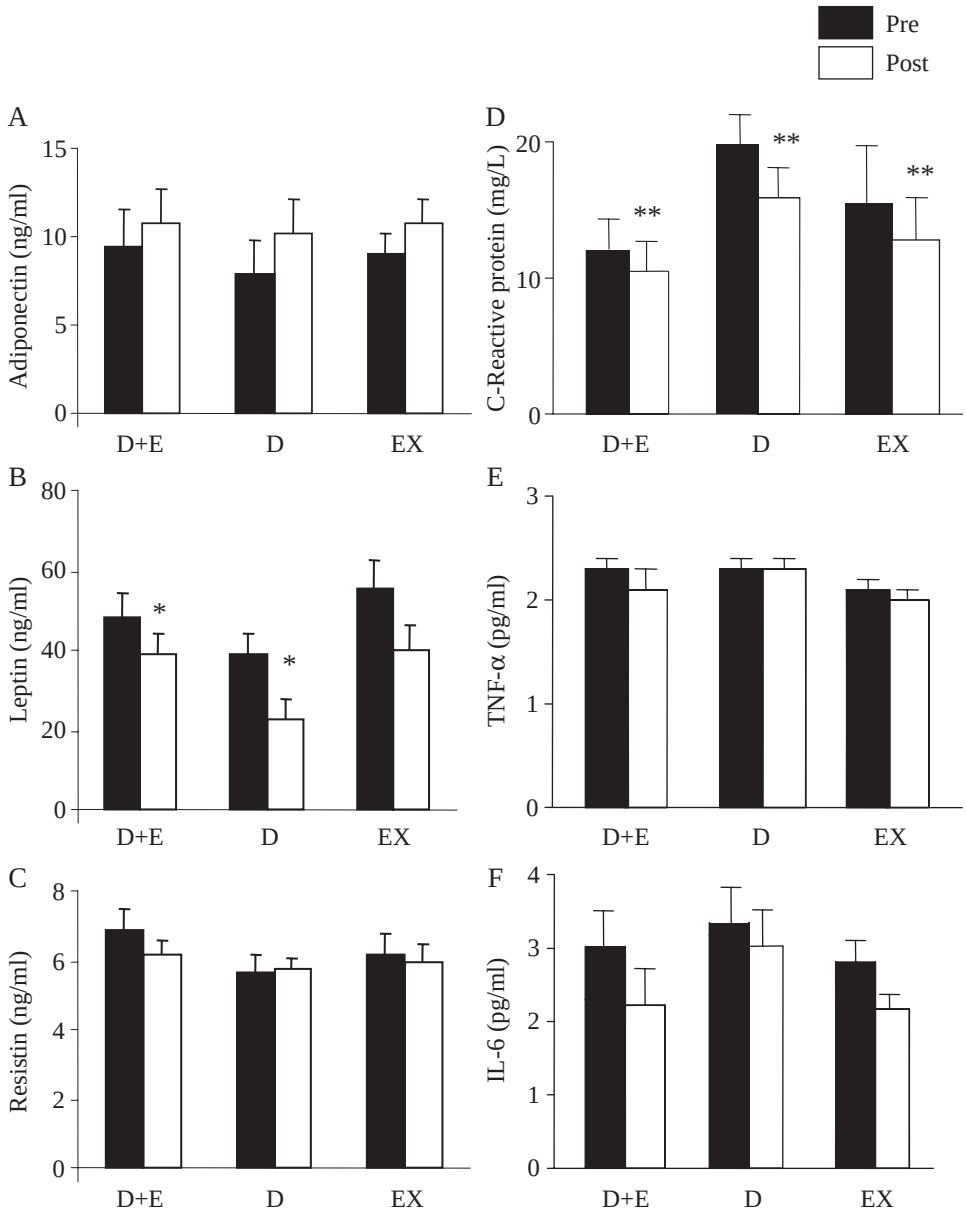

Fig. 1. Plasma adiponectin (A), leptin (B), resistin (C), reactive protein (D), tumor necrosis factor α (TNF-α) (E), and inter leukin-6 (IL-6) (F), before and after the D + E, D, and EX interventions in postmenopausal women with type 2 diabetes. Asterisk indicates $P < .05$ from pre; double asterisk, $P < .01$ from pre.

and statistically analyzed but no significant differences were found with the log-transformed data; data not shown.) A Pearson correlation was used to quantify the relationship between baseline plasma adipocytokines, inflammatory markers, and metabolic variables. Partial correlations, adjusting for fat loss, were run to determine the unique relationship between changes in adipocytokines and inflammatory cytokines and changes in body fat and metabolic status. The variables with significant correlations were entered into a multiple regression analysis with the enter mode to examine independent predictors for these variables. Statistical analysis was performed with SPSS for Windows, version 11 (SPSS, Inc, Cary, NC). All values are presented as mean ± SEM. If a value was found greater than ±3 standard deviation from the mean it was considered an outlier and excluded from the analysis.

3. Results

Of the 40 postmenopausal women recruited for the study, 33 women completed all aspects of the study; 1 woman dropped out because of noncompliance with the dietary protocol, 1 woman failed to return for posttesting, and 5 women discontinued their participation because of illness or injuries unrelated to the study. The data presented reflect the 11 women studied in each intervention group. The lifestyle intervention groups were similar in age, body weight, and height at baseline (Table 1). All women were obese as defined by BMI (34.6 ± 1.07 kg/m^2) and percentage of body fat (36.3 ± 3.6%). No significant differences were found in any of the baseline anthropometric variables. Although no differences were found at baseline between groups for fasting glucose concentrations and HbA_{1c}, group differences were found for fasting insulin concentrations and IR. These group differences occurred despite random assignment to the groups. Because of the intervention, body weight was reduced by 5.4 ± 1.3 kg with D + E, 4.6 ± 1.4 kg with D, and 1.7 ± 0.8 kg with EX. Percentage body fat decreased by 5.4% ± 1.1%, 5.2% ± 1.2%, and 2.3% ± 1.4%, respectively. Fasting glucose levels decreased in all groups but were only significant in the D + E and D groups ($P < .05$).

3.1. Adipocytokines

Fig. 1 illustrates the changes in adiponectin, leptin, and resistin levels with all 3 interventions. Plasma leptin levels were reduced ($P < .05$) with the D and D + E interventions from pre- to post-intervention (D: pre = 48.7 ± 6.0, post = 38.9 ± 5.0 ng/mL; D + E: pre = 38.5 ± 6.0, post = 22.9 ± 5.0 ng/mL), with no differences between these interventions. There was a tendency for EX to decrease plasma leptin levels from pre- to post-intervention ($P = .06$). Adjusting leptin concentrations for body weight did not alter the aforementioned significant findings ($P < .05$). The diet and/or exercise interventions resulted in small, but not statistically significant, elevations in plasma adiponectin concentrations (D: pre = 9.5 ± 2.0, post = 10.7 ± 1.9 ng/mL; D + E: pre = 7.8 ± 2.0, post = 10.2 ± 1.9 ng/mL; EX: pre = 8.9 ± 1.2, post = 10.7 ± 1.5 ng/mL; $P > .05$). Plasma resistin levels were not significantly altered with any of the lifestyle interventions (D: pre = 6.9 ± 0.6, post = 6.2 ± 0.4 ng/mL; D + E: pre = 5.6 ± 0.6, post = 5.7 ± 0.4 ng/mL; EX: pre = 6.2 ± 0.6, post = 5.9 ± 0.6 ng/mL; $P > .05$). Log transformation of the data and adjusting these values for total body fat mass or abdominal fat mass did not alter these findings ($P > .05$).

3.2. Inflammatory markers

Close inspection of the CRP data revealed that there was 1 outlier, which was removed before data analysis. C-reactive protein significantly decreased in all groups after the interventions ($P < .01$), but there were no differences between groups. There was a trend for IL-6 concentrations to decrease ($P = .07$) in all groups, with no differences found between groups (Fig. 1). No significant changes were found in the TNF-α levels with any of the 3 interventions. Again, log transformations of the data did not alter our findings.

3.3. Correlations

At baseline, significant correlations between CRP, IL-6, TNF-α levels, and the anthropometric and metabolic characteristics of the women were observed (data not shown). Plasma CRP levels were positively correlated with body weight ($r = 0.36$, $P = .04$), BMI ($r = 0.42$, $P = .02$), total body fat mass ($r = 0.49$, $P = .005$), SAT ($r = 0.37$, $P = .04$), and IL-6 levels ($r = 0.39$, $P = .03$) and negatively correlated with $\dot{V}O_2$ peak ($r = -0.38$, $P = .03$). No correlation between plasma CRP levels and VAT was found. Plasma IL-6 levels were only related to $\dot{V}O_2$ peak ($r = -0.38$, $P = .03$). Plasma TNF-α levels were negatively correlated with TRIG and HDL levels only ($r = -0.35$, and $r = -0.36$, respectively; $P < .05$). No significant association was found between baseline cytokine levels and fasting insulin levels or calculated IR.

Table 2
Partial correlations between the absolute changes of CRP, TNF-α, and IL-6 (adjusted for weight loss) with changes in metabolic, abdominal fat distribution, and anthropometric variables in all subjects

Absolute change	CRP	TNF-α	IL-6	Adiponectin
Fat mass	−0.03	0.07	0.16	−0.44*
Total abdominal fat	0.29	0.30	0.53*	−0.46*
Subcutaneous adipose tissue	0.18	−0.12	0.52*	−0.42*
Visceral adipose tissue	0.18	0.42	0.22	−0.23
HbA_{1c}	−0.21	0.14	0.60*	0.01
Glucose	0.05	0.51*	0.35	−0.43*
HDL	−0.36	−0.49*	0.20	−0.20
CRP	–	0.67*	0.17	−0.28
TNF-α	0.67*	–	0.02	−0.21
IL-6	0.17	0.02	–	−0.43*

Mean ± SEM; n = 33.
* $P < .05$.

Table 3
Regression results for the prediction of changes in IR using changes in abdominal fat distribution and adipocytokines

Dependent variable	Independent variable	β	B	P	Multiple R	R^2	sr^2	Overall P
Insulin Resistance Δ	Visceral adipose tissue Δ	0.002	.35	.026	0.658	0.433	0.103	.000
	TNF-α Δ	4.277	.435	.007			0.16	

Insulin resistance indicates area under the curve insulin/area under the curve glucose [46].

The diet and/or exercise-induced reductions in cytokine levels were related to changes observed in abdominal fat as well as the metabolic status of the women after adjusting for weight loss (Table 2). Significant partial correlations were found between the changes in IL-6 levels and the reductions observed in adiponectin levels, total abdominal adipose tissue, SAT, and HbA_{1c}. The reduction in TNF-α levels was related ($P < .05$) to the changes in fasting glucose ($r = 0.51$), HDL ($r = -0.49$), and CRP levels ($r = 0.67$). The absolute change in IL-6 levels was found to be positively associated with the baseline IL-6 concentration ($r = 0.71$, $P < .01$), such that those with the highest IL-6 concentrations at baseline demonstrated greater changes in IL-6 levels.

Significant correlations were found between the change in plasma adiponectin levels and changes in IL-6 levels ($r = -0.45$, $P < .01$), total body fat mass ($r = 0.37$, $P < .01$), total abdominal fat ($r = -0.47$, $P < .01$), SAT ($r = -0.43$, $P < .05$), and IR ($r = -0.39$, $P < .05$). The change in leptin levels was associated with the change in calculated IR ($r = -0.54$, $P < .05$). After adjusting the correlations for total body fat loss the changes in adiponectin and leptin levels were correlated only with the changes in calculated IR ($r = -0.45$, and $r = 0.57$, respectively, $P < .05$).

3.4. Regression

The multiple regression analysis revealed that the change in IR was predicted by the changes in VAT and TNF-α. Together these variables accounted for 43% of the variability (Table 3). Changes in the adipocytokine levels had no impact on the regression model.

4. Discussion

Recently there have been numerous reports on changes in adipocytokine concentrations with diet and/or exercise interventions in obese individuals [29-39], but only a few reports examine these changes in individuals with type 2 diabetes. This is also one of the first controlled studies to examine the impact of weight loss on resistin levels in human beings. Our data clearly demonstrate that lifestyle interventions of diet and/or exercise had no effect on the resistin levels in women with type 2 diabetes in agreement with the findings of Monzillo et al [41]. These authors reported no change in the resistin levels of obese individuals with IR after 6 months of a hypoenergetic diet and moderate physical activity [41]. Our findings and those of Monzillo taken together suggest that resistin may not play an important role in the improvements often observed in IR with diet and/or exercise in this population. In animals, studies have shown an inhibitory effect of resistin on insulin-stimulated glucose uptake [47], and in human beings resistin is proposed to link obesity and IR. In the present study, baseline resistin concentrations were not associated with any measure of adiposity or IR or with any changes in these variables. In addition, Heilbronn et al [6] has noted that resistin concentrations are not correlated with glucose disposal during a hyperinsulemic clamp and that only in non-obese subjects were resistin concentrations related to insulin sensitivity. This suggests that circulating levels of resistin do not play a major role in IR or energy homeostasis in human beings [5-9].

In obese individuals, numerous studies have been conducted examining changes in adiponectin levels with lifestyle interventions and have reported either no change [40,42] or an increase in adiponectin levels [32-34,38,41]. We found a small, nonsignificant increase (~17%) in plasma adiponectin levels in the D and D + E groups, respectively. Supporting our lack of statistical change in adiponectin levels in individuals with type 2 diabetes is the work by Engeli et al [48] who noted that changes in adiponectin levels with increased obesity and IR are best described with a logarithmic function implying that adiponectin concentrations deteriorate early in obesity development and decrease with further weight gain. This suggests that a greater weight loss may be needed to increase adiponectin levels in this population. This potentially explains the discrepancy between our findings and those of Hotta et al [33] who noted a ~ 65% increase in adiponectin levels in men and women with type 2 diabetes after 2 months of a very low-energy diet and a 10% decrease in BMI compared with our ~5% decrease in BMI. Although our weight loss was not sufficient to alter the adiponectin levels, the D and D + E interventions were sufficient to result in a ~ 30% reduction in leptin levels, in agreement with previous work [32,49]. Thus, metabolic changes were occurring in these women but did not result in statistical changes in adiponectin levels.

We included an exercise-alone group because the independent effect of exercise alone on adipocytokines has not been investigated [32,33,41,48,49], yet changes in glucose concentrations and insulin sensitivity have been shown with exercise without weight loss [44]. Improvements in IR were found in the present study, but the slight increase in adiponectin levels was not associated with these changes. Yokoyana et al [50] also reported that improvement in insulin sensitivity with aerobic exercise is not mediated by changes in plasma adiponectin. Furthermore,

exercise alone had no significant impact on the leptin levels of our diabetic women. The lack of change in leptin concentrations with exercise is probably because of the fact there was minimal weight loss in this group, which is consistent with previous studies [51-53].

In the present study, modest reductions (10%-18%) in CRP concentrations were found with all interventions in agreement with earlier work conducted in obese individuals [35]. Exercise alone also resulted in similar CRP reductions to those induced by the D and D + E interventions consistent with the findings of previous studies [54,55]. Our findings, however, contrast those of Youn et al [9] who noted that diet alone did not decrease chronic inflammation in obese postmenopausal women and that D + E was required. This discrepancy may be because we used subjects with type 2 diabetes or because of differences in the dietary intervention. Nevertheless, our findings indicate that individuals with type 2 diabetes with very high CRP levels respond favorably to the typically prescribed diet and/or exercise intervention; however, in a 14-week period these changes are small and the high CRP levels still leave these subjects at high risk of cardiovascular disease.

Slight but not significant reductions in IL-6 levels were observed with the D + E and D interventions, respectively, similar to findings from other studies [56]. The reductions in IL-6 appeared to be related to the initial IL-6 levels of these women, as those individuals with the highest baseline IL-6 levels had the most change. Other researchers have speculated that reductions in total body fat mass, particularly SAT, may play a role in decreases in IL-6 levels [20,22,57]. In vitro research has shown that large amounts of IL-6 are expressed and secreted by SAT [20,22,57]. Likewise, we found a strong association between the changes in IL-6 levels and the changes in total abdominal fat and SAT. Exercise alone without weight loss produced marginal reductions in IL-6 levels in these diabetic women. To date, no study has reported the chronic effects of exercise alone, without dietary control, on IL-6 levels in healthy or diseased individuals. How exercise alone affects inflammation is not clear. Previous studies in healthy, athletic individuals have reported increased local inflammation with intense exercise, possibly attributed to microdamage of the active muscle tissue [58]. In contrast, recent studies by Febbraio and Pedersen [59] have shown a marked increase in IL-6 with exercise that originates primarily from the contracting skeletal muscles. The increased IL-6 levels have been proposed to aid in maintaining metabolic homeostasis and to have anti-inflammatory effects by down-regulating the production of TNF-α [59]. Chronic endurance exercise training on the other hand has been reported to suppress the increased production of inflammatory proteins that takes place with intense exercise [54]. Furthermore, exercise training has been shown to augment the production of cytokines with cardioprotective properties such as IL-10, suppressing the production of cytokines with atherogenic properties such as TNF-α, in obese healthy individuals [55]. Our data reveal that 14 weeks of training did not have a deleterious effect on cytokine levels and there was a trend for improvement in these levels in this population.

Adiponectin and leptin are secreted almost exclusively by the adipose tissue. In particular, abdominal VAT and SAT have been reported to be the predominant fat depots to secrete these cytokines [60]. Changes in leptin levels have been correlated with both changes in VAT and SAT with weight loss in healthy individuals [39], but in the present study we did not find any association between changes in leptin levels and VAT and SAT loss in our diabetic women.

Both leptin and adiponectin have been posited to play a key role in mediating insulin action and sensitivity in the human body [61]. Previous observations suggest that IR is a consequence rather than a determinant of increased adiponectin expression in adipose tissue [48]. We found improvements in the calculated IR with diet and/or exercise that were associated with the changes observed in VAT and TNF-α levels. Although adiponectin did not explain any of the variables in the change in IR in our study, low levels of adiponectin have been associated with IR possibly through the inhibitory effect of adiponectin on TNF-α function [11,12]. Incubation of adipose tissue in vitro with TNF-α decreased adiponectin mRNA levels, linking the cytokines to IR [62]. The loss of VAT has been shown previously to affect IR and our finding supports this further.

This study demonstrates the difficulty in altering the levels of adipocytokines and cytokines in individuals with type 2 diabetes. The different findings reported between studies using individuals with type 2 diabetes and obese individuals may be attributed to the different metabolic status of these populations. As part of type 2 diabetes, there are metabolic disturbances at the level of the adipose tissue, the skeletal muscle, as well as the pancreatic beta cell. Potentially, the more disturbed hormonal and metabolic milieu of the type 2 diabetic individuals may result in more resistance of the adipose tissue to weight loss and hence alter the response in adipocytokine or cytokine levels. It is likely that a substantially larger weight loss, a *longer* experimental period, or greater improvement in IR needs to occur before changes in the adipocytokines are evident. Other factors may play a larger role in altering the adipocytokine levels (eg, cardiovascular disease, alterations in energy balance, free fatty acid metabolism, etc). Yet the changes in leptin concentrations observed in our study indicate that lifestyle interventions are impacting the adipose tissue hormonal milieu.

There are important experimental considerations regarding the present study that should be acknowledged. To date, primarily correlational or cross-sectional study designs have been most frequently used to provide insight into the effects of obesity and type 2 diabetes on adipocytokines/cytokines. It is possible that genetic or other constitutional factors, independent of obesity or type 2 diabetes, influence these results. Secondly, because we studied only obese women with type 2 diabetes, we may

have overestimated the potential effect of our intervention in this population. For example, inclusion of less obese women may have resulted in more favorable results, as has been seen in other studies [63,64]. Furthermore, our women with type 2 diabetes may have microvascular damage or chronic inflammation for other reasons that cannot be altered at all or only minimally in a short period and/or without substantial weight loss.

In the present study, we found no effect of hormone replacement therapy (HRT) on CRP, adiponectin, leptin, and resistin levels before and after intervention. Similarly, one other study [65] also noted no influence of HRT on the leptin levels after exercise training in healthy women. In addition, because 22 of our subjects were on oral hypoglycemic agents for type 2 diabetes, we analyzed the data to see if there were any potential effects of these medications on the adipocytokine concentrations of our subjects. We found no effect of medication on the baseline concentrations, or on the diet and/or exercise-induced changes on adipocytokines. Thus, HRT and oral hypoglycemic drugs were not used as a covariate. Because the present study was not designed to specifically test such hypotheses and our diabetic women were on varied drug treatments that may have had some effect on the results of this study, no certain conclusions can be drawn on the effects of HRT or oral hypoglycemic drugs on adipocytokine levels. To our knowledge no other study has investigated the effects of HRT or oral hypoglycemic drugs on the adiponectin and resistin levels of healthy and diabetic postmenopausal women.

In conclusion, a typical diet and exercise plan (~2510 kJ/d) resulted in no change in resistin levels and only a few changes in the adipocytokines *of* older women with type 2 diabetes. This implies that either a more dramatic weight loss or other clinical interventions are needed. The change in IR was once again most closely associated with changes in VAT; however, the small change in TNF-α levels was associated with improvements in IR. In a population with type 2 diabetes even small changes in adipocytokine levels may help improve the metabolic status.

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