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Weight Loss Intervention and Early Biomarker Search in Middle-Aged and Elderly Women: Early Signs of Body Composition Changes Indicated by Blood Cholinesterase and Cystatin C

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Abstract

Background: This study aimed to comprehensively examine the effects of participation in a dietary intervention program on body composition and multiple hematological and biochemical markers in middle-aged and older women, particularly identifying the biomarkers that fluctuate in conjunction with changes in body composition.

Methods: A 6-month dietary intervention program was conducted involving 33 middle-aged and older women (mean age 60.9 ± 11.0 years, mean body mass index (BMI) 24.8 ± 3.2 kg/m^2). The body composition and nonfasting blood parameters were measured before and after the intervention. In this study, pre- or post-intervention changes and correlated changes in body composition were compared with blood parameters.

Results: Participation in the dietary program led to significant reductions in body weight, BMI, body fat mass, body fat percentage, and waist circumference. The blood parameters showing significant decreases were white blood cell count, red blood cell count, aspartate aminotransferase, alanine aminotransferase, and cholinesterase. The change in body fat mass (Δbody fat mass) was positively correlated with the change in cholinesterase (Δcholinesterase, r = 0.483). Multiple regression analysis identified Δcholinesterase (β = 0.505) as an independent explanatory variable for Δbody fat mass. In addition, Δcystatin C (β = 0.500) was considered an independent explanatory variable for the change in waist circumference (Δwaist circumference).

Conclusions: The findings of this study indicate that cholinesterase and cystatin C may capture fluctuations in body composition earlier than conventional inflammatory markers. Consequently, they may be useful, simple, and objective indicators for evaluating the effectiveness of lifestyle interventions.

Keywords: Cholinesterase; Cystatin C; Obesity; Early biomarker of body composition change

Introduction

The increasing prevalence of noncommunicable diseases has become a major social problem, which is largely attributable to obesity. Middle-aged and older women face a higher risk because of hormonal influences that promote fat accumulation and increase susceptibility to lifestyle-related diseases [1, 2]. Obesity is more than simple weight gain; the accumulation of excess body fat induces profound physiological and biochemical changes. This excessive fat leads to macrophage infiltration into adipose tissue and the release of inflammatory cytokines, such as tumor necrosis factor-α, which initiate a chronic inflammatory state. Therefore, obese individuals typically exhibit higher C-reactive protein (CRP) and white blood cell (WBC) counts compared with nonobese individuals [3]. Furthermore, excessive body fat causes adipocyte hypertrophy and dysfunction, resulting in increased efflux of free fatty acids (FFAs) from adipose tissue. This excess FFA accumulates as ectopic fat in nonadipose tissues such as the liver and skeletal muscle. This phenomenon, which is known as lipotoxicity, primarily causes insulin resistance and organ dysfunction [4]. Based on the biomarkers of liver and adipose dysfunction, metabolic dysfunction-associated steatotic liver disease is a key manifestation of ectopic fat accumulation, showing extremely high prevalence in obese populations [5]. Liver

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Table 1. Subject Characteristics

Age, years	60.9 ± 11.0 (37–83)
Height, cm	157.0 ± 5.4 (147.3–167.7)
Body weight, kg	61.3 ± 8.8 (42.5–81.8)
BMI, kg/m^2	24.8 ± 3.2 (18.5–30.6)
Diabetes, n (%)	2 (6.1)
Hypertension, n (%)	6 (18.2)
Dyslipidemia, n (5)	13 (30.3)

Data are presented as means ± SD (Min–Max). BMI: body mass index; SD: standard deviation; Min: minimum; Max: maximum.

damage resulting from fat accumulation is clinically indicated by elevated blood levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase [6]. However, butyrylcholinesterase (BChE), which is the primary form of blood cholinesterase measured clinically, is a nonspecific esterase mainly synthesized in the liver [7]. As an indicator of liver protein synthesis, BChE correlates positively with metabolic disorders, including body mass index (BMI), hepatic fat content, and insulin resistance [8]. Therefore, analyzing BChE fluctuations provides valuable insights into the early evaluation of metabolic intervention efficacy. Another emerging marker is cystatin C. Although primarily used as a renal function indicator because of its production by all nucleated cells and independence from muscle mass [9], recent studies have highlighted its metabolic roles. Cystatin C is also produced by adipocytes, and it correlates positively with visceral fat mass even in adults with normal renal function [10]. Therefore, cystatin C may serve as a novel marker reflecting visceral fat accumulation and an adipokine-like substance reflecting adipose tissue dysfunction and chronic inflammation. Despite the significance of these changes, studies that comprehensively examine the mechanism, by which these early metabolic and hepatic markers reflect body composition changes following lifestyle interventions, are limited. In particular, few investigations have focused on the combined fluctuations of cholinesterase (as an initial marker of metabolic abnormality) and cystatin C (as a marker of visceral fat). Therefore, this study aims to comprehensively examine the effects of participation in a dietary improvement program on body composition and multiple hematological and biochemical markers, focusing on identifying early biomarkers that correlate with changes in body composition.

Materials and Methods

Subjects

A total of 59 middle-aged and older women, who took part in the Mukogawa Women's University Dietary Improvement Program between 2018 and 2023, were enrolled in this study. Of these, 33 participants (mean age 60.9 ± 11.0 years) were selected for the study (Table 1), after excluding 11 participants who attended the online course, seven whose course was canceled midway because of the impact of coronavirus disease 2019 (COVID-19), and eight who had missing body composition and blood test results.

Dietary improvement program

The dietary improvement program was implemented once a month for 6 months. Each session included body composition measurement and individual counseling on diet. Blood tests were conducted before the program started and after 6 months.

The lecture lasted approximately 90 min and provided dietary guidance based on an original food constitute table. This food composition table was designed for 1,200 kcal/day, excluding the calories from seasonings, and it indicated the appropriate intake amount for each food group (protein-rich foods, vegetables, fruits, potatoes/starches, grains, and fats/oils).

In the program, the actual total daily energy intake would fall within the range of approximately 1,480–1,600 kcal/day by appropriately adding the energy from seasonings (sugar, soy sauce, miso, etc.) that are used in cooking, in addition to the 1,200 kcal shown in the table.

With regard to the individual dietary counseling, the focus of the guidance varied in accordance with the participant's status: energy optimization was emphasized for those who needed weight loss, whereas nutritional balance optimization was prioritized for those who did not require weight reduction. The lecture content included practical skills training, such as the basics of meal preparation following the food composition table, selection criteria for dining out, and simple cooking modifications for home use.

Anthropometric measurements

Height was measured using a stadiometer only before the program started. Body composition analysis was performed using a bioelectrical impedance analyzer (InBody 430; InBody Japan, Tokyo, Japan). BMI was calculated from the measured height and weight. Waist circumference was measured by a skilled registered dietitian at the umbilicus.

Blood test

The blood tests were conducted in the morning without fasting (nonfasting state). The collected blood was subjected to preprocessing required for each analysis and then sent to LSI Medience Corporation (Tokyo, Japan) for analysis. The estimated glomerular filtration rate (eGFR), which is an indicator of renal function, was calculated by applying the estimation formula for Japanese individuals based on the serum creatinine levels recommended by the Japanese Society of Nephrology [11].

$$\text{eGFR} = 194 \times \text{Cr}^{-1.094} \times \text{Age}^{-0.287} \times 0.739 \text{ (if female)}$$

Statistical analysis

Analysis was performed using IBM SPSS Statistics 25.0 for

Table 2. Body Composition and Changes Before and After the Course

	Pre-intervention	Post-intervention	P value
Age, years	60.9 ± 11.0 (37–83)		
Body weight, kg	61.3 ± 8.8 (42.5–81.8)	58.7 ± 7.7 (41.8–72.5)	< 0.001
BMI, kg/m^2	24.8 ± 3.2 (18.5–30.6)	23.8 ± 2.9 (18.2–28.7)	< 0.001
Body fat mass, kg	21.4 ± 6.0 (8.3–32.7)	19.2 ± 5.5 (8.3–28.1)	< 0.001
Body fat percent, %	34.3 ± 6.1 (19.6–43.9)	32.2 ± 6.5 (17.1–42.6)	< 0.001
Skeletal muscle mass, kg	21.5 ± 2.6 (17.9–27.0)	21.2 ± 2.6 (17.7–26.2)	0.010
Muscle mass, kg	37.6 ± 4.1 (32.1–46.3)	37.2 ± 4.0 (31.7–45.0)	0.017
Upper limb fat mass, kg	3.1 ± 1.1 (1.0–5.3)	2.7 ± 0.9 (1.0–4.6)	< 0.001
Trunk fat mass, kg	10.5 ± 3.1 (3.4–16.5)	9.3 ± 2.9 (3.3–14.0)	< 0.001
Lower limb fat mass, kg	6.7 ± 1.7 (3.0–9.6)	6.1 ± 1.6 (3.0–9.6)	< 0.001
Upper limb muscle mass, kg	3.8 ± 0.6 (2.8–5.4)	3.7 ± 0.6 (2.8–5.0)	0.002
Trunk muscle mass, kg	17.6 ± 2.0 (14.7–22.3)	17.2 ± 1.8 (14.6–21.4)	< 0.001
Lower limb muscle mass, kg	12.3 ± 1.9 (9.4–16.2)	12.2 ± 1.9 (9.4–16.3)	0.096
Waist circumference, cm	92.7 ± 8.8 (70.0–109.4)	89.0 ± 8.8 (68.7–105.0)	< 0.001

Comparison between the two groups was performed using the Mann–Whitney U test. Data are presented as means ± SD (Min–Max). BMI: body mass index; SD: standard deviation; Min: minimum; Max: maximum.

Windows (IBM Japan, Ltd., Tokyo, Japan). The level of significance was set at less than 5% ($P < 0.05$, two-sided test). The Wilcoxon signed-rank test was used for the before and after comparison of age and body composition. Spearman's rank correlation coefficient was calculated to examine the relationship between body composition and blood parameters. Multiple regression analysis was conducted to investigate the association between the change in body composition and the change in blood test items. In the multiple regression analysis model, the change in body composition was included as the dependent variable, and age was included as a covariate in addition to the change in blood test items.

Ethical considerations

The study was approved by the Ethics Committee of Mukogawa Women's University and was conducted in compliance with the ethical standards of the responsible institution on human subjects as well as with the Helsinki Declaration (Approval No. 22–63).

Prior to the study's implementation, all participants were fully informed, orally and in writing, regarding the purpose, content, duration, voluntary nature of participation, expected benefits and disadvantages, protection of personal information, and the right to withdraw from participation at any time. An informed consent was also obtained from all participants.

Results

The characteristics of the participants are shown in Table 1. In addition, the results of the body composition measurements before and after the program are shown in Table 2. Weight, BMI, body fat mass, body fat percentage, skeletal muscle mass, muscle mass, upper limb/trunk/lower limb fat mass, upper limb/trunk muscle mass, and waist circumference significantly decreased after the program.

The results of the blood parameters before and after the program are shown in Table 3. WBC counts, red blood cell (RBC) counts, AST, ALT, and cholinesterase significantly decreased after the program.

The associations between body composition and each blood parameter were examined for the preintervention values and the changes in values (Tables 4, 5 and Fig. 1). Detailed descriptions of the correlations for upper limb/trunk/lower limb fat mass and muscle mass were omitted, as they showed similar trends to the correlations for the total body fat mass, total muscle mass, and skeletal muscle mass.

Upon examining the correlation among weight, BMI, and various blood parameters at preintervention, significantly positive correlations were observed. In particular, weight was positively correlated with RBC count ($r = 0.517$), hemoglobin ($r = 0.540$), hematocrit ($r = 0.509$), and high-sensitivity CRP ($r = 0.360$). Similarly, BMI was significantly and positively correlated with RBC count ($r = 0.432$), hemoglobin ($r = 0.549$), hematocrit ($r = 0.524$), and high-sensitivity CRP ($r = 0.506$). Furthermore, weight was significantly and negatively correlated with inorganic phosphate ($r = -0.369$). BMI was also positively correlated with WBC count ($r = 0.374$), cholinesterase ($r = 0.427$), uric acid ($r = 0.466$), and glycated hemoglobin (HbA1c, $r = 0.362$). Based on the correlations involving body fat mass and body fat percentage with blood parameters, significantly positive associations were observed across multiple measures. These measures included WBC count (body fat mass $r = 0.376$, body fat percentage $r = 0.344$), RBC count (body fat mass $r = 0.627$, body fat percentage $r = 0.552$), hemoglobin (body fat mass $r = 0.660$,

Table 3. Blood Parameters Before and After the Course

	Pre-intervention	Post-intervention	P value
WBC, /mL	5,776 ± 1,291 (3,800–8,900)	5,503 ± 1,332 (3,700–8,700)	0.039
RBC, 10^4/mL	440.5 ± 29.3 (360–499)	438.9 ± 33.7 (341–514)	< 0.001
Hb, g/dL	13.3 ± 0.8 (11.7–15.0)	13.3 ± 1.0 (10.9–15.4)	0.800
Hematocrit, %	41.4 ± 2.3 (36.7–45.9)	41.3 ± 2.9 (33.7–47.6)	0.495
Platelets, 10^4/mL	26.5 ± 5.6 (13.5–41.9)	26.1 ± 5.2 (16.5–40.8)	0.235
MCV, fL	94.3 ± 3.5 (84–102)	94.2 ± 3.6 (85–100)	1.000
MCH, pg	30.2 ± 1.4 (26.2–32.5)	30.3 ± 1.5 (26.4–32.6)	0.530
MCHC, %	32.0 ± 0.7 (30.9–33.7)	32.1 ± 0.8 (30.5–33.7)	0.750
Total protein, g/dL	7.1 ± 0.3 (6.6–7.8)	7.2 ± 0.4 (6.4–8.2)	0.260
Albumin, g/dL	4.3 ± 0.2 (3.8–4.7)	4.3 ± 0.3 (3.9–4.8)	0.391
AST, U/L	23.1 ± 5.5 (17.0–44.0)	21.1 ± 6.5 (15.0–48.0)	0.011
ALT, U/L	21.3 ± 10.1 (10.0–53.0)	18.2 ± 9.3 (8.0–58.0)	0.005
GGT, U/L	30.6 ± 27.0 (10.0–146.0)	26.4 ± 17.4 (9.0–73.0)	0.283
Cholinesterase, U/L	349.4 ± 74.9 (155–598)	338.7 ± 76.4 (142–567)	0.007
Triglycerides, mg/dL	144.9 ± 88.2 (25–435)	129.2 ± 69.4 (39–338)	0.177
HDL-cholesterol, mg/dL	67.2 ± 15.4 (41.0–100.0)	68.5 ± 15.6 (42.0–116.0)	0.357
LDL-cholesterol, mg/dL	130.9 ± 34.4 (82.0–206.0)	132.8 ± 38.9 (77.0–243.0)	0.918
Calcium, mg/dL	9.3 ± 0.3 (8.7–10.0)	9.3 ± 0.3 (8.8–10.0)	0.595
Inorganic phosphate, mg/dL	3.7 ± 0.5 (2.9–4.8)	3.8 ± 0.5 (2.9–4.8)	0.218
Creatinine, mg/dL	0.7 ± 0.1 (0.5–0.9)	0.7 ± 0.1 (0.5–0.9)	0.180
Urea nitrogen, mg/dL	15.0 ± 3.7 (7.2–24.4)	15.1 ± 3.0 (8.2–21.9)	0.986
Uric acid, mg/dL	4.8 ± 1.0 (1.6–7.2)	4.9 ± 1.3 (1.8–7.3)	0.444
eGFR, mL/min/1.73 m^2	75.7 ± 12.7 (49.4–106.7)	74.7 ± 12.9 (50.9–108.0)	0.213
Cystatin C, mg/dL	0.77 ± 0.17 (0.51–1.15)	0.77 ± 0.18 (0.50–1.27)	0.961
HbA1c, %	5.5 ± 0.3 (5.0–6.3)	5.6 ± 0.3 (5.1–6.2)	0.274
High-sensitivity CRP, mg/dL	0.07 ± 0.08 (0.004–0.361)	0.07 ± 0.08 (0.009–0.329)	0.144

Data are presented as means ± SD (Min–Max). Comparison between the two groups was performed using the Mann-Whitney U test. P values are for comparisons between pre- and post-intervention measurements. WBC: white blood cell; RBC: red blood cell; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transpeptidase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; eGFR: estimated glomerular filtration rate; HbA1c: glycated hemoglobin; CRP: C-reactive protein; Min: minimum; Max: maximum.

body fat percentage r = 0.618), hematocrit (body fat mass r = 0.663, body fat percentage r = 0.662), uric acid (body fat mass r = 0.453, body fat percentage r = 0.445), and high-sensitivity CRP (body fat mass r = 0.568, body fat percentage r = 0.690). In addition, body fat mass was significantly and positively correlated with HbA1c (r = 0.362), and body fat percentage was correlated with cholinesterase (r = 0.376). Finally, waist circumference and blood parameters were significantly and positively associated with WBC count (r = 0.456), RBC count (r = 0.565), hemoglobin (r = 0.539), hematocrit (r = 0.593), cholinesterase (r = 0.542), uric acid (r = 0.534), and high-sensitivity CRP (r = 0.609). A significantly negative correlation was observed between waist circumference and inorganic phosphate (r = −0.366).

In addition, the changes in body composition were correlated with the changes in blood parameters. The changes in body weight and BMI were significantly and positively correlated with the changes in AST (body weight r = 0.480, BMI r = 0.505), ALT (body weight r = 0.450, BMI r = 0.461), cholinesterase (body weight r = 0.382, BMI r = 0.353), and high-sensitivity CRP (body weight r = 0.355, BMI r = 0.369). The changes in body fat mass and body fat percentage were positively correlated with RBC count (body fat mass r = 0.356,% body fat r = 0.431), platelet count (body fat mass r = 0.348,% body fat r = 0.374), total protein (body fat mass r = 0.523,% body fat r = 0.604), cholinesterase (body fat mass r = 0.483,% body fat r = 0.511), and cystatin C (body fat mass r = 0.445,% body fat r = 0.420). Furthermore, cystatin C was significantly and positively correlated with waist circumference (r = 0.501).

In this study, multiple regression analysis was performed using the forced entry method to identify the factors influ-

Table 4. The Relationship Between Baseline Body Composition and Blood Parameters

	Body weight	BMI	Body fat mass	Body fat percent	Skeletal muscle mass	Muscle mass	Waist circumference
WBC	0.328	0.374*	0.376*	0.344	0.113	0.140	0.456**
RBC	0.517**	0.432*	0.627**	0.552**	0.160	0.168	0.565**
Hb	0.540**	0.549**	0.660**	0.618**	0.146	0.155	0.539**
Hematocrit	0.509**	0.524**	0.663**	0.662**	0.068	0.082	0.593**
Platelets	0.215	0.148	0.223	0.235	0.143	0.173	0.221
MCV	–0.122	0.018	–0.069	0.038	–0.167	–0.167	–0.120
MCH	–0.010	0.124	–0.041	–0.027	0.070	0.062	–0.131
MCHC	0.108	0.150	0.034	–0.049	0.222	0.221	–0.043
Total protein	0.058	0.066	0.185	0.130	–0.118	–0.119	0.188
Albumin	–0.047	–0.093	0.001	–0.120	0.041	0.028	0.044
AST	0.219	0.175	0.185	0.097	0.126	0.098	0.115
ALT	0.295	0.266	0.276	0.224	0.145	0.127	0.250
GGT	0.226	0.251	0.329	0.284	0.018	0.010	0.241
Cholinesterase	0.323	0.427*	0.341	0.376*	0.058	0.057	0.542**
Triglycerides	0.109	0.074	0.077	0.043	0.078	0.078	0.323
HDL-cholesterol	–0.296	–0.207	–0.228	–0.136	–0.259	–0.245	–0.280
LDL-cholesterol	0.106	0.077	0.158	0.197	–0.010	–0.015	0.173
Calcium	–0.247	–0.138	–0.121	–0.011	–0.348*	–0.323	0.048
Inorganic phosphate	–0.369*	–0.245	–0.381*	–0.234	–0.262	–0.230	–0.366*
Creatinine	0.209	0.294	0.131	0.049	0.221	0.244	0.253
Urea nitrogen	–0.104	0.017	–0.190	–0.154	0.058	0.060	–0.025
Uric acid	0.329	0.466**	0.453**	0.445**	0.018	–0.005	0.534**
eGFR	–0.209	–0.294	–0.131	–0.049	–0.221	–0.244	–0.253
Cystatin C	0.072	0.241	0.148	0.227	–0.116	–0.044	0.334
HbA1c	0.321	0.362*	0.362*	0.252	0.073	0.089	0.264
High-sensitivity CRP	0.360*	0.506**	0.568**	0.690**	–0.164	–0.154	0.609**

Values are presented as Spearman's rank correlation coefficient (r). *P < 0.05; ** P <0.01. WBC: white blood cell; RBC: red blood cell; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transpeptidase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; eGFR: estimated glomerular filtration rate; HbA1c: glycated hemoglobin; CRP: C-reactive protein.

encing the changes in body composition (body weight, BMI, body fat mass, skeletal muscle mass, muscle mass and waist circumference), particularly body fat mass (Table 6). The independent variables included age and the change amounts of various blood parameters (RBC count, total protein, AST, ALT, cholinesterase, cystatin C, and high-sensitivity CRP). The coefficients of determination (R^2) for each model were as follows: $R^2 = 0.506$ ($P = 0.016$) for the change in body weight, $R^2 = 0.513$ ($P = 0.014$) for the change in BMI, and $R^2 = 0.558$ ($P = 0.005$) for the change in body fat mass, all of which were statistically significant. The change in body weight was significantly influenced by AST ($\beta = 0.475$, $P = 0.047$) and cholinesterase ($\beta = 0.483$, $P = 0.007$). The change in BMI was influenced by AST ($\beta = 0.485$, $P = 0.042$) and cholinesterase ($\beta = 0.451$, $P = 0.011$). The change in body fat mass was influenced by cholinesterase ($\beta = 0.505$, $P = 0.003$). These results indicate that AST is a factor influencing the changes in body weight and BMI, and that cholinesterase is a factor influencing the changes in body weight, BMI, and body fat mass.

In addition, stepwise multiple regression analysis was performed using the same variables for changes in muscle mass, skeletal muscle mass, and waist circumference. A significant model was constructed only for changes in waist circumference ($R^2 = 0.250$, $P = 0.003$), and cystatin C ($\beta = 0.500$, $P = 0.003$) was extracted as a significant explanatory variable for changes in waist circumference (Table 7).

When the effects of multicollinearity in these regression models were examined, the variance inflation factor (VIF) for each variable was less than 10. Furthermore, when correlations between each blood parameter were examined, no significant association was found between cholinesterase and

Table 5. Correlation Between Changes in Body Composition and Changes in Blood Parameters

	ΔBody weight	ΔBMI	ΔBody fat mass	ΔBody fat percent	ΔSkeletal muscle mass	ΔMuscle mass	ΔWaist circumference
ΔWBC	0.102	0.102	0.181	0.241	–0.280	–0.290	0.231
ΔRBC	0.190	0.166	0.356*	0.431*	–0.245	–0.325	0.187
ΔHb	0.215	0.188	0.333	0.391*	–0.195	–0.253	0.035
ΔHematocrit	0.118	0.094	0.263	0.382*	–0.240	–0.329	0.031
ΔPlatelets	0.210	0.233	0.348*	0.374*	–0.211	–0.245	0.293
ΔMCV	–0.149	–0.144	–0.263	–0.248	0.101	0.084	–0.310
ΔMCH	–0.083	–0.079	–0.184	–0.238	0.117	0.141	–0.348*
ΔMCHC	0.107	0.116	0.041	–0.098	0.121	0.166	0.074
ΔTotal protein	0.329	0.343	0.523**	0.604**	–0.316	–0.313	0.234
ΔAlbumin	0.196	0.217	0.263	0.306	–0.156	–0.155	0.165
ΔAST	0.480**	0.505**	0.357*	0.120	0.261	0.272	0.313
ΔALT	0.450**	0.461**	0.315	0.128	0.188	0.180	0.235
ΔGGT	0.337	0.359*	0.225	0.081	0.195	0.167	0.126
ΔCholinesterase	0.382*	0.353*	0.483**	0.511**	–0.084	–0.147	0.201
ΔTriglycerides	0.273	0.249	0.286	0.247	0.075	0.112	0.258
ΔHDL–cholesterol	–0.076	–0.064	–0.163	–0.202	0.182	0.092	–0.086
ΔLDL–cholesterol	0.150	0.134	0.207	0.257	–0.040	–0.116	–0.126
ΔCalcium	0.164	0.186	0.119	0.034	0.112	0.069	0.532**
ΔInorganic phosphate	0.168	0.148	0.181	0.276	–0.064	–0.155	0.249
ΔCreatinine	0.022	0.001	0.163	0.171	–0.362*	–0.349*	–0.191
ΔUrea nitrogen	0.163	0.157	0.144	0.221	0.041	0.057	0.271
ΔUric acid	0.223	0.175	0.194	0.203	0.107	0.057	0.024
ΔeGFR	0.049	0.072	–0.088	–0.158	0.355*	0.361*	0.278
ΔCystatin C	0.321	0.331	0.445**	0.420*	–0.146	–0.164	0.501**
ΔHbA1c	–0.029	–0.046	–0.030	–0.104	–0.080	–0.003	–0.031
ΔHigh–sensitivity CRP	0.355*	0.369*	0.316	0.192	0.179	0.233	0.300

Values are presented as Spearman's rank correlation coefficient (r). $^*P < 0.05$; $^{**}P < 0.01$. WBC: white blood cell; RBC: red blood cell; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transpeptidase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; eGFR: estimated glomerular filtration rate; HbA1c: glycated hemoglobin; CRP: C-reactive protein.

cystatin C, confirming the independence of each explanatory variable (Table 8).

Discussion

Obesity and an increase in body fat affect various hematological and biochemical markers. Thus, this study aimed to clarify how participation in a dietary improvement program influences these markers and body composition. We focused on the impact of short-term intervention on fluctuations in blood indicators and the identification of indices that could serve as early markers for changes in body composition, which we discuss for each item. Participation in the dietary improvement program resulted in significant changes in multiple blood indicators, including WBC count, RBC count, and liver function-related markers. Notably, the changes in serum cholinesterase and serum cystatin C were strongly correlated with the fluctuations in body composition, indicating the usefulness of these markers in capturing changes in hepatic lipid metabolism and body fat.

WBC counts of the subjects significantly decreased after the program. When examining the relationship with body composition, a significantly positive correlation was observed between the preprogram WBC count and body weight, BMI, body fat mass, and waist circumference, but no correlation was found with their changes. Based on previous reports, an increase in fat may directly increase CD4 count and total lymphocyte count [12, 13]. However, high-sensitivity CRP, which is an indicator of inflammatory response, showed a positive correlation with preprogram body composition, similar to WBC, but no correlation with the amount of change. These results indicate that a short-term reduction in body fat mass may

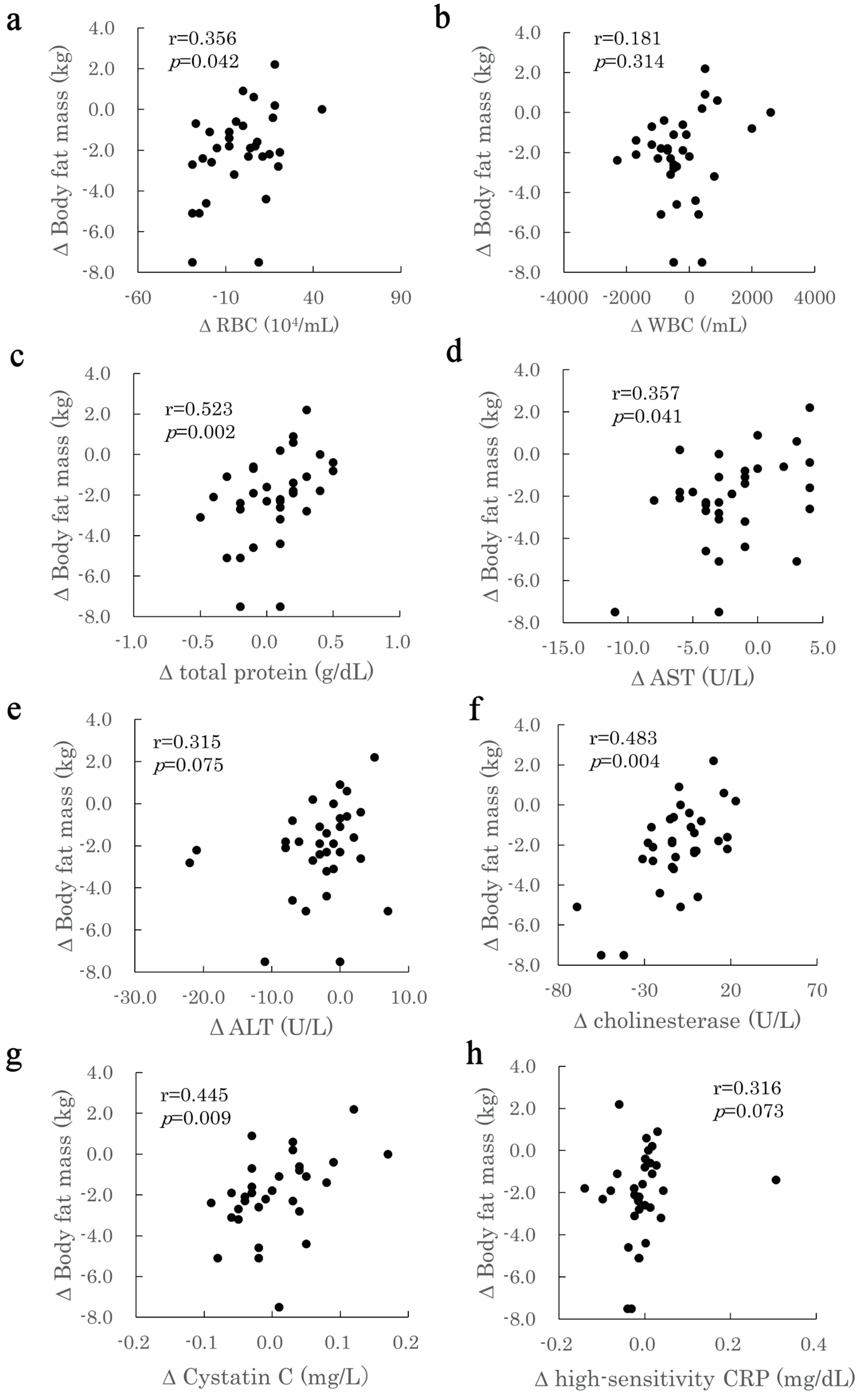

Figure 1. Correlation between changes in body fat mass and changes in blood parameters. The relationship between the change (Δ) in body fat mass (y) and the change (Δ) in various blood parameters (x) in study participants is shown. (a) ΔRed blood cell count (RBC); (b) ΔWhite blood cell count (WBC); (c) ΔTotal protein; (d) ΔAspartate aminotransferase (AST); (e) ΔAlanine aminotransferase (ALT); (f) ΔCholinesterase; (g) ΔCystatin C; (h) ΔHigh-sensitivity C-reactive protein (CRP).

Table 6. Relationship Between Body Composition Change and Blood Parameter Change

Independent variable	Dependent variable											
	ΔBody weight		ΔBMI		ΔBody fat mass		ΔSkeletal muscle mass		ΔMuscle mass		ΔWaist circumference	
	β	P value	β	P value	β	P value	β	P value	β	P value	β	P value
(Constant)		0.117		0.099		0.190		0.382		0.435		0.128
Age	0.238	0.277	0.245	0.260	0.166	0.419	0.194	0.462	0.167	0.516	0.157	0.508
ΔRBC	–0.156	0.533	–0.187	0.453	–0.025	0.914	–0.215	0.479	–0.295	0.324	–0.147	0.591
ΔTotal protein	–0.230	0.340	–0.217	0.363	–0.043	0.850	–0.485	0.103	–0.449	0.122	–0.403	0.130
ΔAST	0.475	0.047	0.485	0.042	0.262	0.235	0.548	0.059	0.589	0.039	0.296	0.246
ΔALT	–0.159	0.492	–0.135	0.556	–0.021	0.922	–0.297	0.293	–0.339	0.223	–0.099	0.696
ΔCholinesterase	0.483	0.007	0.451	0.011	0.505	0.003	0.046	0.821	0.053	0.789	0.254	0.170
ΔCystatin C	0.295	0.229	0.322	0.188	0.266	0.251	0.068	0.818	0.095	0.741	0.712	0.012
ΔHigh-sensitivity CRP	0.079	0.614	0.096	0.540	0.066	0.658	0.063	0.741	0.075	0.687	0.068	0.689
	R^2	P value	R^2	P value	R^2	P value	R^2	P value	R^2	P value	R^2	P value
	0.506	0.016	0.513	0.014	0.558	0.005	0.274	0.378	0.304	0.286	0.410	0.078

Multiple linear regression analysis shows independent predictors of anthropometric and body composition changes (Δ). BMI: body mass index; RBC: red blood cell; AST: aspartate aminotransferase; ALT: alanine aminotransferase; CRP: C-reactive protein.

not be sufficient to fully resolve the inflammatory response.

Similar to WBC count, RBC count also significantly decreased after the program. When the relationship with body composition was examined, a significant correlation was found between the preprogram values and body weight, BMI, body fat mass, percent body fat, and waist circumference. Obese individuals have a higher RBC count than normal-weight individuals [13] because of an increase in blood volume, as the metabolic demands of various tissues increase [14, 15], and because severe obesity causes a physical reduction in lung capacity, leading to chronic hypoxia (obesity hypoventilation syndrome) that secondarily increases RBC count [16]. Although the subjects in this study were not severely obese as reported in prior research, they may be experiencing a minor hypoxic state even before reaching obese hypoventilation syndrome, given that this condition can occur in Asians with lower BMIs compared with other races [17]. Obese hypoventilation syndrome has been reported to improve with weight loss [18], and in the current study, a correlation was observed between the change in RBC count and the change in body fat.

Table 7. Association Between Changes in Body Composition and Changes in Blood Parameters (Stepwise Method)

Independent variable	Dependent variable	
	ΔWaist circumference	
	β	P value
ΔCystatin C	0.500	0.003
	R^2	P value
	0.250	0.003

Stepwise multiple linear regression analysis was conducted to identify the independent predictors of age, RBC, total protein, AST, ALT, cholinesterase, cystatin C, and high-sensitivity CRP. RBC: red blood cell; AST: aspartate aminotransferase; ALT: alanine aminotransferase; CRP: C-reactive protein.

Table 8. Correlation of Cholinesterase and Cystatin C With Changes in Blood Parameters

	ΔWBC	ΔRBC	ΔHb	ΔHematocrit	ΔTotal protein	ΔAlbumin	ΔAST	ΔALT	ΔHigh-sensitivity CRP	ΔCholinesterase	ΔCystatin C
ΔCholinesterase	0.094	0.360*	0.337	0.295	0.383*	0.436*	0.090	0.121	–0.086	–	0.213
ΔCystatin C	0.318	0.405*	0.181	0.287	0.624**	0.555**	0.142	0.048	0.047	0.213	–

Values are presented as Spearman's rank correlation coefficient (r). *P < 0.05; **P < 0.01. WBC: white blood cell; RBC: red blood cell; Hb: hemoglobin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; CRP: C-reactive protein.

This correlation results from the reduction in body fat, which can ameliorate the minor hypoxic state.

AST and ALT significantly decreased after the program, but all values remained within the standard range, and no subjects were suspected of having fatty liver. Furthermore, no significant correlation was found between the preprogram body composition and AST or ALT. However, a positive correlation was found between the changes in body weight and BMI and the changes in AST and ALT. Moreover, a positive correlation was found between the change in AST and the change in body fat mass, whereas no relationship was observed between the change in ALT and the change in body fat mass. Therefore, the mild improvement in liver inflammation caused by the reduction in body fat may have been reflected in AST, which has a shorter half-life than ALT.

In addition, the results of multiple regression analysis indicated that the change in AST was associated with changes in body weight, BMI, muscle mass, and fat-free mass. Apart from the liver, AST also exists in the skeletal muscle, heart, and kidney. Based on previous reports, muscle mass can decrease because of weight loss resulting from dietary restrictions [19], and the association revealed by multiple regression analysis of this study indicates the possibility that changes in muscle mass are reflected in AST.

Serum cholinesterase significantly decreased after the program. When the relationship between preprogram serum cholinesterase and body composition was examined, a significantly positive correlation was found with preprogram BMI and percent body fat. Furthermore, a significantly positive correlation was observed between the changes in body composition (body weight, BMI, body fat mass, and percent body fat) and the change in serum cholinesterase. In addition, serum cholinesterase was positively correlated with blood lipid parameters such as total cholesterol, low-density lipoprotein, and very low-density lipoprotein [8], and it is thought to increase during active lipid metabolism [20]. The positive correlation found in this study between preprogram BMI and percent body fat, as well as the positive correlation observed between changes in body composition and serum cholinesterase, are considered results that support this view. Moreover, although a positive correlation was found between the change in body fat mass and the change in serum cholinesterase, no correlation was observed between the change in ALT and the change in body fat mass. ALT is an indicator of fatty liver, which increases when liver cells are damaged, but the ALT values of the subjects in this study were within the standard range, indicating that they were not in a state severe enough to show liver cell damage. Therefore, the change in serum cholinesterase may be an initial sign of a change in hepatic lipid metabolism.

No significant change was observed in serum cystatin C concentration when comparing the pre- and post-program values, but a trend toward a positive correlation with preprogram waist circumference was found (P = 0.057). Based on previous reports, serum cystatin C is positively correlated with visceral fat in women [21], and waist circumference is an indicator of visceral fat [22]. The trend of a positive correlation between cystatin C and waist circumference observed in this study is consistent with these previous reports. Furthermore, a positive correlation was found between the change in cystatin C and the changes in body fat mass, percent body fat, and waist circumference. Considering that cystatin C is also produced in adipose tissue, it has been reported that an increase in adipose tissue may contribute to the increase in circulating serum cystatin C concentration [10], and the results of this study support this finding. No relationship was observed between preprogram body composition and serum cystatin C, but a correlation was found when comparing the amounts of change post program. Therefore, although the blood concentration of cystatin C may vary because of the original cell number or cell size of an individual, it is an indicator that fluctuates when cell number or cell size changes. Moreover, although inflammatory response markers such as high-sensitivity CRP and WBC count are indicators related to changes in body fat mass, no association with cystatin C was found in the results of this study (Table 8). Therefore, cystatin C could serve as an indicator to capture fluctuations in body fat more quickly.

This study has also some limitations. First, the small sample size of 33 participants and the restriction to middle-aged and elderly women limit the generalizability of these findings, thereby requiring caution when applying the results to other genders or age groups. Second, because blood tests in this study were conducted under nonfasting conditions, caution is required in interpreting glucose metabolism indicators. However, it has been reported that fasting is not necessarily required for the assessment of lipid metabolism [23]. Furthermore, the main indicators in this study, cystatin C and cholinesterase, are known to exhibit minimal short-term fluctuations due to dietary intake [24, 25]. For these reasons, the impact of food intake on the main results of this study was limited, and assessment using random blood samples is considered appropriate. In addition, visceral fat mass was not obtained from image diagnostics such as computed tomography (CT) scans or magnetic resonance imaging (MRI). Although waist circumference is a valid simple indicator of visceral fat, caution is warranted, especially in women, because of the typically high volume of subcutaneous fat. Furthermore, we were unable to identify which specific dietary component contributed most to the changes in the biomarkers. Based on previous reports, increased *de novo* lipogenesis from carbohydrates or increased availability of FFAs may lead to an increase in BChE activity [26]; the association with these factors requires future investigation.

Conclusions

The results of this study indicate that the 6-month dietary improvement program for middle-aged and older women significantly reduced body weight, body fat mass, and waist circumference, while significantly decreasing the concentrations of serum cholinesterase, AST, and ALT, as well as RBC count. Notably, the change in cholinesterase was independently identified as an early biomarker explaining the change in body fat mass, and the change in cystatin C was identified as an early biomarker explaining the change in waist circumference. These markers can detect fluctuations in body composition earlier than conventional inflammatory markers (high-sensitivity CRP and WBC), and they can be useful indicators for a simple and objective evaluation of the efficacy of lifestyle interventions.

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Financial Disclosure

None to declare.

Conflict of Interest

None to declare.

Informed Consent

Written informed consent was obtained from all subjects who were enrolled in the study.

Author Contributions

YT and EY contributed to the analysis design, acquisition and interpretation of data and reviewed/edited the manuscript. MH, EO, RN, YO and KF contributed to the acquisition and interpretation of data and reviewed/edited the manuscript. All authors read and approved the final manuscript.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Abbreviations

WBC: white blood cell; RBC: red blood cell; CRP: C-reactive protein; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transpeptidase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; eGFR: estimated glomerular filtration rate

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