ORIGINAL RESEARCH

Association of plasma adiponectin levels with cellular hydration state measured using bioelectrical impedance analysis in patients with COPD

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Background: It is widely recognized that chronic obstructive pulmonary disease (COPD) includes a variety of extra pulmonary complications and comorbidities. Recently, adiponectin was shown to regulate cellular metabolism in humans. Cellular hydration state is affected by a variety of hormonal factors and regulates cellular metabolic state. Therefore, this study was designed to determine whether adiponectin is a possible factor involved in cellular hydration state in COPD.

Methods: Thirty patients with COPD and 41 age-matched controls participated in the study. Plasma levels of total and high molecular weight (HMW) adiponectin were measured and anthropometry and pulmonary function tests were conducted. Intracellular water (ICW), extracellular water (ECW), and ECW/ICW ratio, which are parameters of cellular hydration state, were measured using bioelectrical impedance analysis.

Results: Higher levels of total and HMW adiponectin in plasma were found in patients with COPD compared with levels in controls. A significant inverse correlation was observed between body mass index and plasma levels of total and HMW adiponectin in the control group. However, this significant correlation was not observed in patients with COPD. The plasma levels of total and HMW adiponectin were also not significantly correlated with any pulmonary function parameters in patients with COPD. Regarding the state of cellular hydration, the plasma levels of total adiponectin were inversely correlated with the ECW/ICW ratio and positively with ICW values in patients with COPD. Moreover, closer correlations were found between these parameters and plasma HMW adiponectin levels.

Conclusion: The results of the present study suggest a novel association of the plasma adiponectin with cellular hydration state in patients with COPD. Accordingly, lower adiponectin levels may result in cellular shrinkage, leading to metabolic malfunction at a cellular level. Thus, our findings provide new insights regarding the preventive roles of adiponectin in the progression of comorbidities in COPD.

Keywords: adiponectin, bioelectrical impedance analysis (BIA), cellular hydration, COPD, extra pulmonary phenotype

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Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by airflow limitation that is not fully reversible, typically progressive, and associated with an abnormal inflammatory response of the lung.[1] To date, it is widely recognized that COPD includes a variety of extra pulmonary complications and comorbidities.[2] For example, patients with COPD often show weight loss associated with muscle atrophy,[3] which

is thought to have adverse effects on physical performance and to be one of independent risk factors for mortality.[4–6] Although metabolic perturbation and systemic inflammation are thought to contribute to the progression of muscle atrophy,[7–11] current information of the cellular mechanisms remains elusive. Many studies reported the extent of muscle atrophy assessed using computed tomography and magnetic resonance imaging scans, and demonstrated long-term quantitative reduction in muscle mass in COPD.[12–15] However, muscle atrophy originates from functional alterations resulting from accumulative effects of various biological abnormalities in protein synthesis and oxidative enzyme activities.[3] Accordingly, qualitative assessment of functional alterations should be performed to elucidate the mechanism of muscle atrophy in COPD.

It is known that cellular hydration state (cell shrinkage or swelling) is dynamic and changes within minutes, which can be triggered by a variety of factors such as hormonal and metabolic alterations.[16] In turn, the changes serve as a signal which triggers production or breakdown of protein and glycogen and modifies gene expression of gluconeogenic enzymes to regulate cellular catabolic and anabolic systems. An increase in cellular hydration (cell swelling) acts as an anabolic and proliferative signal, whereas cell shrinkage acts as a catabolic and antiproliferative signal.[17] Thus, the measurement of cellular hydration state does not simply provide quantitative information regarding water content of tissues and organs, but enables qualitative assessment of cellular functions. The cellular hydration state can be indirectly but easily measured as the compartmental distribution of body fluid by bioelectrical impedance analysis (BIA).[18–20] BIA is a non-invasive technique for studying cellular water content in the human body through measurements of body impedance based on the conduction of an applied electrical current in the body at multiple frequencies followed by using empirically derived formulas validated in earlier studies. BIA can provide estimates of intracellular water (ICW) and extracellular water (ECW). A large proportion of the ICW in the entire body is likely to originate in the skeletal muscle. Therefore, a lower ICW and a higher ECW/ICW ratio indicate that skeletal myocytes are likely to shrink.

Adiponectin, a cytokine synthesized exclusively by adipocytes,[21] regulates lipid and glucose metabolism at a cellular level for functional maintenance of various tissues including skeletal muscle.[22,23] Recently, adiponectin is thought to be a promising biomarker for respiratory outcomes and other comorbidities in COPD.[24] Cellular hydration state is affected by a variety of hormonal factors and regulates cellular metabolic state. On the basis of these findings, we speculated that adiponectin may also act as a biomarker for cellular hydration in COPD. Therefore, the aim of the present study was to assess the plasma levels of adiponectin and cellular hydration state by BIA as well as other anthropometric parameters and pulmonary function and to examine the relationship among these parameters in patients with COPD.

Methods

All male patients with COPD, who had a history of former smoking (>20 pack-years) randomly enrolled in the present study from the outpatient clinic of our university hospital. All patients underwent physical examinations, anthropometric measurements including body mass index (BMI), assessment of lung function, and blood sampling. A spirometer (Chestac-25F; Chest CO, Tokyo, Japan) was used to obtain all spirometric measurements. COPD was diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria.[25] The diffusing capacity of the lung for carbon monoxide (DL_{CO}) was measured using the single-breath carbon monoxide method at least twice. No patients received medication during the 12-hour period preceding the lung function test and blood sampling. Age-matched males without respiratory diseases were also recruited into the study, as control subjects. All subjects in both groups were >40 years of age, and had no history of asthma, allergic rhinitis, or atopy. Study subjects with concomitant confounding diseases such as malignant disorders, cardiovascular, and gastrointestinal abnormalities, recent surgery, or severe endocrine disorders were excluded. No subjects suffered from a recent respiratory tract infection or an exacerbation for at least 4 weeks prior to the study. The study was approved by the institutional review board of Osaka City University (approval number: 1537) and all patients gave written informed consent. All procedures were conducted according to the research ethics of the Declaration of Helsinki.[26]

BIA was measured using Inbody 3.0 (Biospace, Tokyo, Japan). For this instrumentation, impedance is measured with the subject standing and holding hand grips. The subject's age, gender, and height were entered on the machine. Subjects were then asked to stand barefoot on the metal foot-plates of the machine while holding the handles for approximately 1 min. Impedances were measured at multiple frequencies, including 5, 50, 250, and 500 kHz, and used to estimate the volume of ICW (L) and ECW (L). In addition, the ECW/ICW ratio was expressed as a percentage. Plasma levels of total and high-molecular-weight (HMW) adiponec-

tin and TNF-α were measured using the enzyme-linked immunosorbent assay method (Quantikine; R&D Systems; Minneapolis, MN).

Statistical analyses were performed using SPSS ver. 18 for Windows (SPSS Inc, Chicago, IL). All values are expressed as the median (IQR; interquartile range). When comparisons of non-parametric data were performed between groups, a Mann-Whitney U test was used. The significance of correlations was evaluated by determining Spearman's rank correlation coefficients. In all statistical analysis, a P value < 0.05 was considered significant.

Results

Thirty patients with COPD and 41 control subjects were recruited into the study. Table 1 presents the clinical characteristics of study subjects. All COPD patients were ex-smokers (mean (SD); 48 (15) pack-years) and all controls had never been smokers. BMI between two study groups was not significantly different. Regular medication in patients with COPD consisted of inhaled anticholinergic drugs (n = 24) or β_2-adrenergic receptor agonists (n = 4). Three patients had received inhaled corticosteroids.

Despite the similar BMI between the two study groups, higher levels of total adiponectin in plasma were observed in patients with COPD compared with those in controls. Similarly, HMW adiponectin levels were significantly higher in patients with COPD than in controls. Though plasma TNF-α levels between COPD patients and controls were not significant, TNF-α levels were closely correlated with total adiponectin levels in COPD patients ($r = 0.75$, $P < 0.001$) (Figure 1). A significant inverse correlation was observed
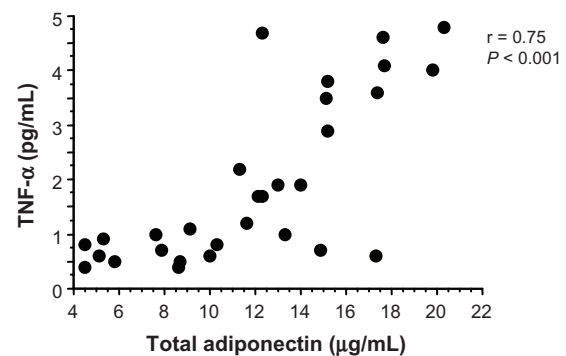
Figure 1 Correlation of plasma levels of total adiponectin with TNF-α levels in patients with COPD.
Abbreviations: COPD, chronic obstructive pulmonary disease; TNF, tumor necrosis factor.

between BMI and plasma levels of total adiponectin in the control group ($r = -0.41$, $P = 0.009$) (Figure 2A). However, this significant correlation was not observed in COPD patients (Figure 2B). Similarly, while a significant inverse correlation was observed between BMI and plasma levels of HMW adiponectin in the control group ($r = -0.50$, $P = 0.002$) (Figure 2C), there was no significant correlation between these parameters in the COPD group (Figure 2D). We evaluated the correlations of plasma levels of total and HMW adiponectin with lung function in patients with COPD (Table 2). The plasma levels of total and HMW adiponectin were not significantly correlated with any pulmonary function parameters.

The parameters of cellular hydration state, including ECW, ICW, and ECW/ICW, in patients with COPD were 10.5% [10.1%–10.9%], 20.1% [19.1%–20.6%], and 53.1% [49.2–55.8], respectively. Regarding the state of cellular hydration, the plasma levels of total adiponectin were inversely correlated with the ECW/ICW ratio and positively with ICW values in patients with COPD (ECW/ICW ratio: $r = -0.39$, $P = 0.04$; ICW: $r = 0.43$, $P = 0.02$) (Figure 3A and B). Interestingly, closer correlations were observed between these parameters and plasma HMW adiponectin levels in the COPD group (ECW/ICW: $r = -0.46$, $P = 0.01$; ICW: $r = 0.49$, $P = 0.008$) (Figure 3C and D).

Discussion

In the present study, we found that patients with COPD had higher levels of plasma adiponectin compared with age-matched control subjects. Additionally, a significant correlation of plasma adiponectin levels with BMI was found in control subjects, while this relationship was not observed in patients with COPD. An increased number of studies has demonstrated elevated plasma levels of adiponectin in chronic inflammatory disorders such as chronic heart failure

Table 1 Clinical characteristics of study subjects

	Control (n = 41)	COPD (n = 30)	P
Age (years)	68 [65–69]	67 [63–69]	0.76
BMI (kg/m²)	21.7 [19.5–23.6]	20.9 [19.4–22.8]	0.50
FEV₁ (% predicted)	90 [84–92]	51 [42–64]	<0.001
FEV₁/FVC (%)	80 [75–84]	44 [39–49]	<0.001
DL_CO (% predicted)	ND	41 [37–49]	–
Total adiponectin (μg/mL)	7.6 [6.5–8.2]	12.2 [8.6–15.2]	0.001
HMW adiponectin (μg/mL)	3.3 [2.2–3.7]	5.1 [3.6–6.6]	<0.001
TNF-α (pg/mL)	1.0 [0.7–1.4]	1.2 [0.7–3.5]	0.24
ECW (%)	ND	10.5 [10.1–10.9]	–
ICW (%)	ND	20.1 [19.1–20.6]	–
ECW/ICW ratio (%)	ND	53.1 [49.2–55.8]	–

Note: Values are presented as median [IQR].
Abbreviations: BMI, body mass index; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; DL_CO, diffusing capacity of the lung for carbon monoxide; HMW, high molecular weight; ICW, intracellular water; ECW, extracellular water; ND, not determined; IQR, interquartile range.
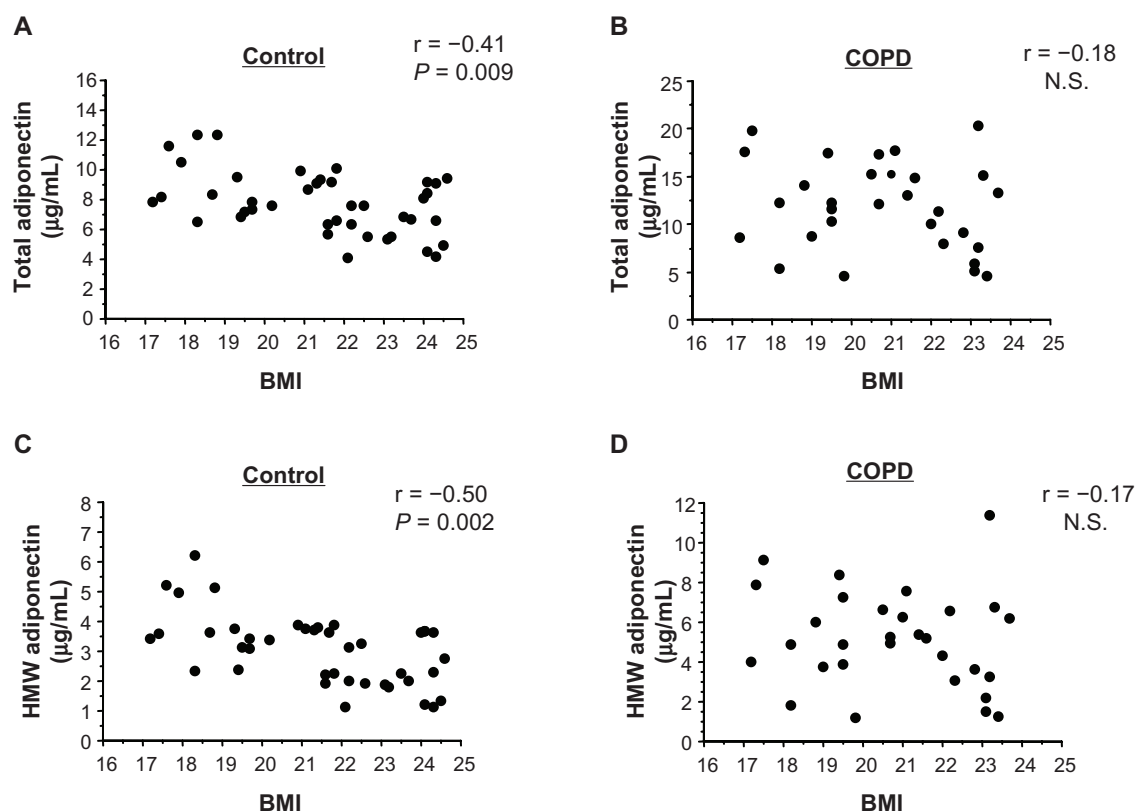
Figure 2 Correlation of plasma levels of total and HMW adiponectin with BMI in controls and patients with COPD.
Abbreviations: COPD, chronic obstructive pulmonary disease; BMI, body mass index; HMW, high-molecular-weight.

and COPD and has emerged as an independent risk factor for morbidity and mortality in these diseases.[27,28] These results are consistent with the present findings. Though differences in BMI may affect plasma adiponectin levels, control subjects in the current study had similar BMIs as those of COPD patients. In general, it is known that the circulating levels of adiponectin are inversely correlated with BMI as observed in control group of the present study.[29,30] Nevertheless, we did not observe this inverse correlation in patients with COPD. Moreover, we also found that plasma adiponectin levels in patients with COPD were not significantly correlated with any parameters of pulmonary function. Therefore, higher levels of plasma adiponectin in patients with COPD are

Table 2 Correlation of total and HMW adiponectin levels with lung function in patients with COPD

	Total adiponectin		HMW adiponectin	
	r	P	r	P
FEV$_1$ (% predicted)	−0.142	0.454	−0.098	0.606
FEV$_1$/FVC	−0.078	0.684	0.009	0.962
DL$_{CO}$ (% predicted)	−0.186	0.326	−0.167	0.379

Abbreviations: FEV$_1$, forced expiratory volume in 1 second; FVC, forced vital capacity; DL$_{CO}$, diffusing capacity of the lung for carbon monoxide; HMW, high molecular weight.
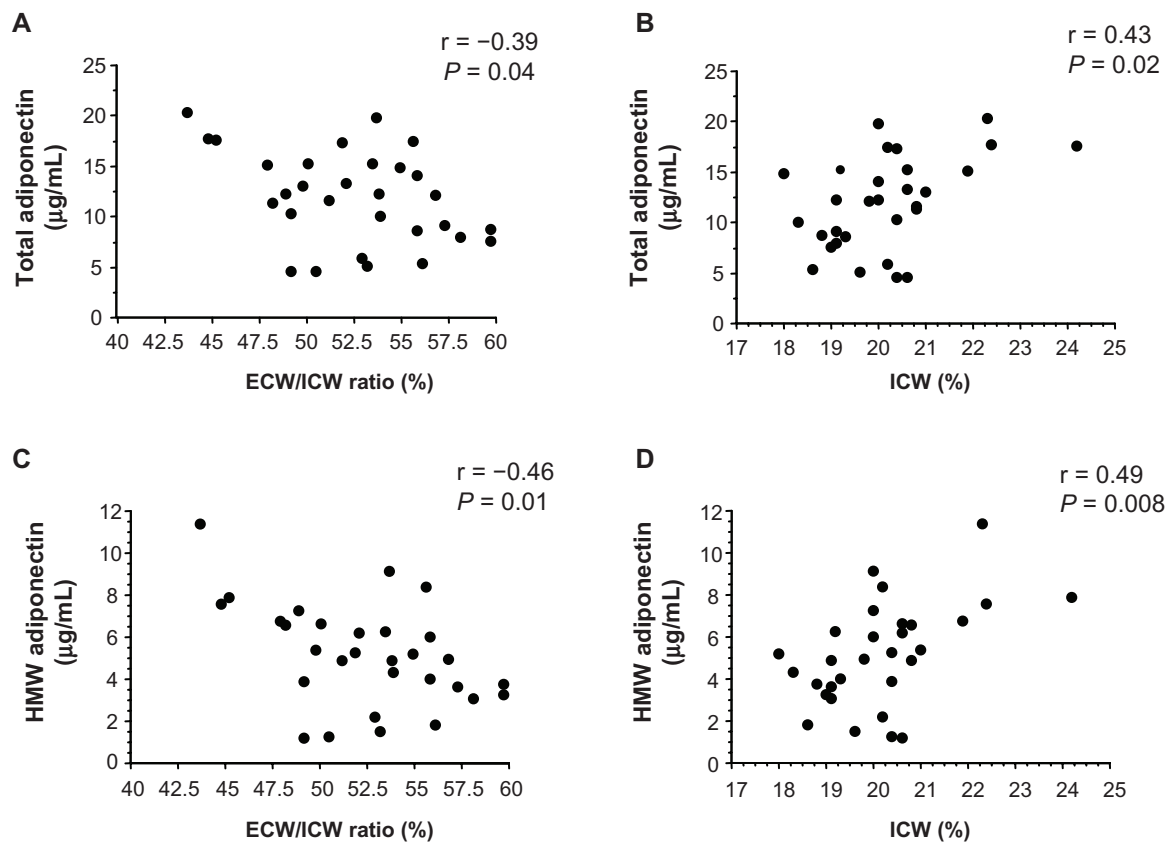
likely to be regulated by factors other than BMI and severity of airway obstruction. Tomoda and colleagues reported that plasma adiponectin levels are inversely correlated with BMI.[28] However, they found that the plasma adiponectin level in normal-weight patients was approximately twice that of normal-weight control subjects with a similar fat mass. They suggested that the elevation of plasma adiponectin level may be associated with other pathophysiologic findings besides BMI in COPD. However, potential factors involved in the up-regulation of circulating levels of adiponectin in COPD are unclear. One possible explanation is the compensatory roles of adiponectin in chronic inflammatory disorders. We found that plasma adiponectin levels were closely associated with plasma TNF-α level in COPD patients. In this regard, high levels of plasma adiponectin may play an important role in the counter-regulation of pro-inflammatory cytokines accompanied by systemic inflammation.

A novel outstanding finding in this study is the linear correlation of the circulating adiponectin levels with the ECW/ICW ratio and ICW value, which are known to be indirect parameters of cellular hydration state. Patients with lower levels of plasma adiponectin tended to have higher ECW/ICW ratio and lower ICW values, which represents

Figure 3 Correlation of plasma levels of total and HMW adiponectin with ECW/ICW ratio and ICW value assessed using bioelectrical impedance analysis in patients with COPD.
Abbreviations: COPD, chronic obstructive pulmonary disease; ECW, extracellular water; HMW, high-molecular-weight; ICW, intracellular water.

cellular shrinkage. Thus, adiponectin may be involved in cellular hydration state, which can regulate cellular metabolism in COPD. Adiponectin induces extracellular Ca^{2+} influx through adiponectin receptor 1 (AdipoR1) with subsequent activation of AMP-activated protein kinase in skeletal myocytes. In contrast, suppression of AdipoR1 in skeletal myocytes decreases mitochondrial content, oxidative type I myofibers, and oxidative stress-detoxifying enzymes.[31,32] Thus, it is possible that the decrease in adiponectin signaling may cause dysregulation in microenvironments in skeletal myocytes such as ion content and osmolality, myofiber composition, and oxidative stress. A recent study demonstrated that adiponectin-knockout mice displayed smaller skeletal muscle fibers in the quadriceps with nuclei located in the center of the muscle cells, indicating the presence of skeletal muscle atrophy due to adiponectin deficiency.[33] Collectively, adiponectin may play a pivotal role in maintenance of the long-term homeostasis of skeletal myocytes, preventing the development of muscle atrophy in COPD.

Adiponectin exists in three forms: low-molecular-weight trimers, medium-molecular-weight hexamers, and HMW multimers. In particular, HMW adiponectin is thought to have

more biological activity than other forms of adiponectin.[34] Therefore, the involvement of HMW adiponectin in the pathophysiology of COPD is worthy of discussion. First, only HMW adiponectin can induce activation of AMP-activated protein kinase in skeletal muscle.[34] Second, changes in serum HMW adiponectin, but not total adiponectin, have been associated with improvement in hepatic insulin sensitivity after treatment with thiazolidinedione.[35] Third, a previous study showed lower levels of HMW adiponectin in patients with type 2 diabetes with coronary artery diseases than those without coronary artery disease, particularly in male patients.[36] In this study, we found that the plasma levels of HMW adiponectin were more closely correlated with cellular hydration state than those of total adiponectin. These points indicate the role of the adiponectin in the cellular hydration state in COPD.

Based on these findings, we speculate that the assessment of cellular hydration state may represent the net effect of cellular activities triggered by adiponectin in patients with COPD. Interestingly, while the cellular hydration state fluctuates within minutes under influence of cellular microenvironments, changes of cell hydration and cell

volume play important roles in potent signals for cellular metabolism and gene expression, including protein turnover, carbohydrate and fatty acid metabolism, plasma membrane transport and oxidative stress.[17] Accordingly, adiponectin may participate in the cellular processes of growth and maintenance in parallel with the fluctuation of cellular hydration state. One interventional study of nutritional support for patients with COPD demonstrated a significant inverse relationship between baseline ECW/ICW ratio and fat-free mass index (fat-free mass/height[2]), suggesting that cell shrinkage was associated with weight loss.[37] Interestingly, the ECW/ICW ratio was selected as an independent, significant parameter contributing to increase in body weight after nutritional therapy. A tendency towards a higher ECW/ICW ratio was observed in patients who failed to respond to nutritional support. This finding is consistent with our hypothesis. It is plausible that cellular metabolic changes by reduced plasma adiponectin levels can induce cellular shrinkage and/or such cellular shrinkage might inhibit cellular anabolic process in parallel with the reduced actions of adiponectin. Thus, this data revealed that cellular shrinkage may represent disadvantages for the development and maintenance of individual cells and tissues through nutritional support and hormonal activities. Measuring cellular hydration state not only allows evaluation of anabolic processes, but also the integrated effect by other various hormonal and metabolic responses to adiponectin at a cellular level.

A limitation of the present study is the small number of subjects recruited. Despite the small sample size, our approach is novel. Second, conclusions concerning cause-and-effect relationships between adiponectin and cellular hydration cannot be drawn due to the cross-sectional nature of our data. Future prospective cohort studies will be needed to confirm any cause-and-effect relationships. Third, COPD patients enrolled in the present study had lower BMIs compared with those in the Caucasian population. A recent report stated that distribution of the body size as assessed by BMI in patients with COPD differs between the Caucasian and Japanese populations, suggesting some variability of biochemical and physiological background among different ethnicities.[38] Thus, it will be necessary to examine whether the association of the adiponectin levels with BMI in Caucasian population differ from that in Japanese population. Since the body size and composition in patients with COPD appear to vary among ethnicities, it is necessary to compare the association of the adiponectin levels with cellular hydration state between ethnicities.

The results of the present study suggest a novel association of the plasma adiponectin with cellular hydration state in patients with COPD. In particular, lower adiponectin levels correlated with cellular shrinkage, leading to hormonal and metabolic malfunction at a cellular level, resulting in the progression of muscle atrophy. Accordingly, the present findings provide new insights regarding the preventive roles of adiponectin in the progression of comorbidities in COPD.

Disclosure

The authors declare no conflict of interest relevant to the submitted work.

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