Online supplement

Methods

Exclusion criteria

None of the patients were participating in any rehabilitation programs during the study period or experienced any exacerbation within 1 month prior to the study. Exclusion criteria were as follows: patients with infectious diseases, diabetes mellitus being treated with medication, dysphagia, or other serious diseases; patients treated with drugs affecting energy expenditure (thyroid hormone, beta-blocker, GLP-1 receptor agonist) or water balance (SGLT2 blocker); patients with weight loss more than 5% of body weight during the previous 3 months. All patients were treated with long-acting bronchodilators with the exception of two GOLD 0 patients.

Chest CT-analysis

Chest CT scans (Somatom Emotion 16, Siemens Healthcare, Erlangen, Germany) were performed with the use of 16 × 1.2 mm collimation, rotation time of 600 ms/rot, pitch factor 1.05, 130 kV peak, and automatic exposure control (Quality Reference mAs 100). Routine calibration of the CT scanner was conducted using air and water phantoms. For quantitative analysis of pulmonary emphysema, whole-lung CT images with 1.5 mm-thickness were obtained using a reconstruction kernel B41s medium+, with the percentage of the low attenuation area (LAA%) determined using a cutoff value of -950 Hounsfield units with a Synapse Vincent volume analyzer (Fujifilm Medical Co., Ltd., Tokyo, Japan).

Weight, body composition and grip strength

Body weight (BW) was measured without shoes and with light clothing using an electronic scale (BF-220, Tanita, Tokyo, Japan). BMI was calculated as BW (kg)/height $(m)^2$. Fat-free mass (FFM) and skeletal muscle mass (SMM) were measured by a bioelectrical impedance analyzer (SFB7, ImpediMed, Queensland, Australia). Fat-free mass index (FFMI) and skeletal muscle mass index (SMI) were calculated as FFM (kg)/height $(m)^2$ and SMM (kg)/height $(m)^2$. Grip strength (GS) of both hands was measured twice using a dynamometer (GRIP-D, Takei Scientific Instruments, Co., Ltd., Niigata, Japan) and the mean of the best efforts in each hand was used for analysis.

DLW method

An oral dose of 0.1 g $^{2}H_2O$ and 2.0 g $H_2{}^{18}O$ per kg of estimated total body water was given on visit 1. Baseline urine (BLU) and blood (BLB) samples were collected before a dose of DLW. Post-dose urine samples were collected at 2, 3 and 4 h (PD2U, PD3U and PD4U, respectively), while the post-dose blood samples were collected only at 4 h (PD4B). On the morning of visit 2, end-of-day samples were collected twice for urine (ED1U and ED2U) with an interval of 1 h, and once for blood (ED1B). Isotope analysis of the urine and blood samples was performed in duplicate using an isotope-ratio mass spectrometer (Hydra 20-20 Stable Isotope Mass Spectrometer, Sercon, Crewe, UK). The ^{2}H:^{1}H ratio was analyzed by hydrogen gas equilibration using a platinum catalyst. The ^{18}O:^{16}O ratio was analyzed after carbon dioxide equilibration. Isotope analyses were carried out at ESTech Kyoto (Kyoto, Japan). The average standard deviations for the analyses were 1.4±1.7 ‰ for ^{2}H and 0.13±0.15 ‰ for ^{18}O. Among the collected samples, the representative value of TEE was calculated by the average TEEs obtained from the urine samples (BLU, PD4U and ED2U) and blood samples (BLB, PD4B and ED1B).

Measurement and prediction of BMR

The patients were instructed to ingest only water for 12 h before the measurement. All tests were conducted between 8:30 and 10:00. The patients rested on a bed for 30 min before the measurement. A steady state was achieved for more than 5 min by the Quark BMR after 10-15 min of breathing while the patient lay awake in a supine position. BMR was calculated as the volume of oxygen consumed and the volume of carbon dioxide expired using the modified Weir equation [19]. We also used predicted BMR values that were obtained using the Ganpule equation as described below [20].

BMR_G = ((0.1238 + (0.0481 × BW kg) + (0.0234 × height cm)−(0.0138 × age)-sex*[1])) × 1000/4.186

*[1]; male = 0.5473 × 1, female = 0.5473 × 2

The equation, which was developed by the National Institute of Health and Nutrition in Japan, has been reported to be able to better predict BMR in Japanese populations as compared to other equations such as the Harris-Benedict equation [21].

Results

Multiple regression analyses

In order to predict TEE_{DLW}, the best two parameters, GS and FFMI (Table 3) were added to TEE_{ACC} as predictor variables. Considering the number of patients (n=36), the number of variables was set to be at most 3 in this analysis. Calculated sample size for the multiple regression analysis was 36, when the conditions were as follows: $\alpha=0.05$, $1-\beta=0.80$, number of variables=3, and effect size $f^2=0.35$.

Table S1 Multiple regression analyses for predicting TEE_{DLW}

Model	Regression coefficient	Standardized coefficient	t	P-value	Corrected coefficient of determination
1 (constant)	138.082	138.082	0.37	0.714	0.525
GS(kg)	29.170	0.457	3.01	0.005	
FFMI(kg/m^2)	68.743	0.361	2.38	0.023	
2 (constant)	-242.019	-242.019	-0.94	0.354	0.734
TEE_{ACC}(kcal/day)	1.017	0.720	5.99	<0.0001	
GS(kg)	12.435	0.195	1.62	0.114	
3 (constant)	-494.291	-494.291	-1.66	0.106	0.746
TEE_{ACC}(kcal/day)	0.940	0.665	5.44	<0.0001	
GS(kg)	7.241	0.114	0.89	0.382	
FFMI(kg/m^2)	35.204	0.185	1.60	0.120	
4 (constant)	-534.562	-534.562	-1.83	0.077	0.747
TEE_{ACC}(kcal/day)	1.016	0.719	6.78	<0.0001	
FFMI(kg/m^2)	42.990	0.226	2.13	0.041	

TEE: total energy expenditure, DLW: doubly labeled water, ACC: accelerometer, FFMI: fat free mass index, GS: grip strength.

To predict PAL_{DLW}, the best two parameters, 6MWD and GS (Table 3) were added to PAL_{ACC} as predictor variables. The number of variables was also set to be at most 3.

Table S2 Multiple regression analyses for predicting PAL_{DLW}

Model	Regression coefficient	Standardized coefficient	t	P-value	Corrected coefficient of determination
1 (constant)	1.043	1.043	6.89	<0.0001	0.404
6MWD(m)	0.000911	0.424	2.95	0.006	
GS(kg)	0.0105	0.361	2.51	0.017	
2 (constant)	0.332	0.332	1.09	0.283	0.467
PAL_{ACC}	0.712	0.472	3.31	0.002	
6MWD(m)	0.000725	0.337	2.36	0.024	
3 (constant)	0.250	0.250	0.83	0.413	0.468
PAL_{ACC}	0.760	0.504	3.70	0.001	
GS(kg)	0.00944	0.324	2.38	0.023	
4 (constant)	0.323	0.323	1.10	0.278	0.505
PAL_{ACC}	0.601	0.399	2.79	0.009	
6MWD(m)	0.000573	0.266	1.87	0.071	
GS(kg)	0.00750	0.257	1.89	0.068	

PAL: physical activity level, DLW: doubly labeled water, ACC: accelerometer, MWD: minute walking distance, GS: grip strength