Relationship between measured aerobic capacity and total energy expenditure obtained by doubly labeled water in community-dwelling healthy adults aged 81-94 y: A preliminary study

Jun Yasukata 1,2 *, Yosuke Yamada 3 *, Hiroyuki Sagayama 4 and Yasuki Higaki 2, Hiroaki Tanaka †

1 Faculty of Human Sciences, Department of Sports and Health Sciences, University of East Asia, Yamaguchi, Japan; yasukata@toua-u.ac.jp
2 Fukuoka University Institute for Physical Activity and Faculty of Sports and Health Science, Fukuoka University, Fukuoka 814-0180, Japan; higaki@fukuoka-u.ac.jp
3 National Institute of Health and Nutrition, National Institutes of Biomedical Innovation, Health and Nutrition, Tokyo 162-8636, Japan; yamaday@nibiohn.go.jp
4 Faculty of Health and Sport Sciences, University of Tsukuba, Ibaraki 305-8577, Japan; sagayama.hiroyuki.ka@u.tsukuba.ac.jp
* Correspondence: Jun Yasukata, yasukata@toua-u.ac.jp and Yosuke Yamada, yamaday@nibiohn.go.jp
† Deceased on 23 April 2018.

Abstract: Adequate energy intake is critical for the healthy longevity of older adults, and the estimated energy requirement is determined by total energy expenditure (TEE). We aimed to identify the relationship between measured aerobic capacity and TEE, activity energy expenditure (AEE) or physical activity level (PAL) with the doubly labeled water (DLW) methods in the advanced older adults. A total of 12 physically independent older adults (10 males and 2 females) aged between 81 to 94 years participated in this study. Aerobic capacity was evaluated according to the lactate threshold (LT). TEE under free-living conditions was assessed using the DLW method, and self-reported physical activity was obtained through the Japanese version of the International Physical Activity Questionnaire (IPAQ). LT was significantly positively correlated with TEE, AEE, and PAL after adjustment for age and sex (ρ= 0.77 (P<0.01), 0.86 (p<0.01), and 0.86 (p<0.01), respectively). We found the LT as an aerobic capacity is positively and independently correlated with TEE, AEE or PAL. The present results suggest that maintaining aerobic capacity is an important factor for preventing frailty, although further research is needed to multisite studies and many samples.

Keywords: doubly labeled water; total energy expenditure; physical activity level; lactate threshold; physical fitness; International Physical Activity Questionnaire

1. Introduction

For older adults, healthy lifespan with good fit status without frailty is an important issue. Physiological markers of frailty include declines in muscle or fat-free mass (FFM), strength, endurance, walking ability, and physical activity in the Fried Phenotype [1]. Fried et al.[1] unified, theoretically, into a cycle of frailty associated with declining energetics and reserve (Figure 1). The cycle of frailty include decrease of total energy expenditure (TEE) and chronic undernutrition as well as loss of sarcopenia, aerobic capacity (e.g. VO2max), physical activity, walking speed, and muscle strength and power [2] (Fig. 1). The doubly labeled water (DLW) method is considered as a “gold standard” method to obtain the daily TEE, activity energy expenditure (AEE) and physical activity level (PAL) with the combination of measured or predicted resting energy expenditure [3,4]. TEE is also important to estimate the energy requirement for adequate energy intake and nutrition.

Frailty has been defined as a biologic syndrome of decreased reserve and resistance to stressors, resulting from cumulative declines across multiple physiologic systems, and causing vulnerability to adverse outcomes in this context [1,5]. Therefore, theoretically,
aerobic capacity and physical activity measured by objective and physiological methods may be preferable, but most previous studies used self-report exhaustion and/or physical activity as indices [1,6-8]. Previous studies indicated that the TEE and/or PAL obtained from self-report physical activity questionnaires (PAQ) have only poor-to-moderate accuracy and precision against DLW (e.g. [9]). Therefore, measurement of TEE with DLW is important in the old adults. Previous studies indicated that older people with frailty has lower TEE and PAL obtained by DLW than healthy or fit older people, and TEE and PAL are associated with several factors in the Fried’s frailty cycle [10-13]. However, to the best of our knowledge, no studies examined the relationship between TEE measured by DLW and measured aerobic capacity in the advanced older adults. Therefore, we examined the relationship between TEE measured by DLW and measured aerobic capacity in the Japanese healthy older adults aged 81-94y.

The primary aim of this present preliminary study was to identify the relationship between measured aerobic capacity and TEE, AEE or PAL with the DLW methods in the advanced older adults. We hypothesis that measured aerobic capacity is significantly and positively correlated with TEE, AEE or PAL in the healthy advanced older adults over 80 years even after controlling with age and sex and even with a small sample size.

Figure 1. Frail cycle modified from Fried et al. 2001. LT, lactate threshold.
2. Materials and Methods

2.1. Participants

A total of 12 physically independent older adults (10 males and 2 females) aged between 81 to 94 years participated in this study. They were voluntarily recruited from community-dwelling adults aged over 80 years old who living in a rural agricultural area, Nakatsue village, Hita City, Oita Prefecture, Japan (https://en.visit-oita.jp/files/en/Book/0/Book_10_file.pdf). No one was certificated by long-term care need, and all participants lived independently. After providing an explanation of the study requirements, each subject read and signed a consent form. The ethics committee of Fukuoka University approved all procedures used in the present investigation (15-11-01). Body weight was measured to the nearest 0.1 kg on an electronic scale, and height was measured to the nearest 0.1 cm.

2.2. Aerobic capacity

Aerobic capacity was evaluated according to the lactate threshold (LT). The LT was measured using the previously established bench gradually stepping test with blood lactate acid (LA) measurement [14,15]. Because this fitness test has several advantages for the individuals without any disorders of the lower extremities e.g. easy to use, low cost and can perform anywhere [14,15]. Step cadence was initially set at 40 steps/min and then it was increased to every 4 minutes by 10 steps/min with a 2 min rest interval until the LA reached 4 mmol/l, and a perceived exertion rate (RPE) of 13. The heart rate (HR) was determined at rest and during the last 30 seconds at each stage by HR monitor (Accurex Plus, Polar Electric, Finland). LA samples were obtained from the earlobe by a portable LA measuring device (Lactate Pro, Arkray Inc., Kyoto, Japan), while the RPE was obtained during rest and after the completion of each work stage immediately using the fifteen-point Borg category scale (Borg 1982). The first breaking point of the LA was assessed by five well trained technicians and the highest and lowest values were excluded. The mean of three values was accepted for the LT. Exercise intensity was converted to METs from the step height and the number of ascents/minute according to the formula described in the ACSM guidelines for exercise tests and prescription [16].

2.3. Total energy expenditure using DLW methods

TEE was measured over 16 days using DLW. Subjects were instructed to maintain normal activity, eating patterns, and body weight during the study. We collected urine samples at baseline (day 0) before the DLW dosing; the subjects received an oral dose of fluid containing $^{18}$O- and $^2$H-labeled water. The dose was approximately 1.25 g·kg$^{-1}$ estimated total body water (TBW) of $^{18}$O (20 atom% $^2$H$^{18}$O; Taiyo Nippon Sanso, Tokyo, Japan) and approximately 0.12 g·kg$^{-1}$ estimated TBW of $^2$H (99.9 atom% $^2$H$^2$O; Isotech Sigma-Aldrich, Miamisburg, OH). The TBW for the administration was estimated by bioelectrical impedance analysis (Tanita, DC-320, Tokyo, Japan).

After administering the doses, urine samples were collected at the following time points: before ingestion (day 0), the next morning (day 1), and the mornings of days 2, 8, 9, 15, and 16 to confirm the turnover slope and intercept. All samples were stored between $-30^\circ$C and $-5^\circ$C in internally threaded, polypropylene vials with a screw cap, incorporating a special silicone gasket for the best possible seal, wrapped tightly with Parafilm M (Bemis Co., Inc., Oshkosh, WI, USA).

We analyzed the urine samples by isotope ratio mass spectrometry (Hydra 20-20 Stable Isotope Mass Spectrometer; SerCon Ltd., Crewe, UK). The $^{18}$O and $^2$H dilution spaces (Nd and No) were determined using the intercept method [17]. It is because isotopic equilibration is delayed in elderly individuals, which influencing the accuracy of the DLW measurement [18,19]. Nd/No in the present study was 1.031 ± 0.004 (range 1.024 to 1.037), which is similar to reported previously values [20,21] and an acceptable value for DLW analysis. We calculated TBW as the mean of Nd and No divided by 1.041 and 1.007, respectively, for the dilution space. CO$_2$ production was determined with the modified two-
point DLW method, using equation A6 of Schoeller et al. [22], as modified by Racette et al. [20]. TEE (kcal/day) was calculated using the modified Weir’s formula based on the rCO2 (mOL/day) and 24-h estimated respiratory exchange ratio (RER) [23]: TEE (kcal/day) = 22.4 * (3.9 * (rCO2/RER) + 1.1 * rCO2), where 22.4 is the molar volume calculated from the dietary survey during the study period. We assumed perfect nourishment balance conditions, which determine that food quotient (FQ) must be equal to the RER [24].

The FQ was set at 0.87 for all participants, which was based on the previous study of community-dwelling older people [25,26]. PAL was obtained by dividing the calculated TEE by basal metabolic rate (BMR) predicted using the National Institute of Health and Nutrition, Japan’s equations for adult men and women [27]. The equation is highly correlated with measured resting metabolic rate with small standard error of estimation in Japanese older adults [28]. Activity energy expenditure was calculated as (0.9 * TEE) – BMR.

2.4. International Physical Activity Questionnaire

To assess the habitual physical activity of the participants, self-reported physical activity was obtained through the Japanese version of the International Physical Activity Questionnaire (IPAQ; the usual 7 days, short, self-administered version) [29,30]. The duration of vigorous and moderate physical activities and walking (min/wk) were obtained from IPAQ.

2.5. Statistical analysis

Results are presented as mean ± standard deviation (SD). The results of IPAQ are shown with median and quartiles [25% and 75%]. Pearson’s correlation coefficients were used to detect relationships among the metabolic and physiological parameters. Partial correlation coefficients were also obtained to examine the associations with age and sex as controlling variables. Alpha of 0.05 was employed to denote significant statistical deviation. We performed all analyses using IBM SPSS Statistics for Macintosh, version 23.0 (IBM Corp., Armonk, NY).

3. Results

The characteristics of the participants are shown in Table 1. The mean ± SD of LT, TEE, PAL, and AEE were 4.5 ± 0.8 METs, 2106 ± 372 kcal/day, 1.85 ± 0.29, and 761 ± 288 kcal/day, respectively. In this participants, self-report physical activity by IPAQ was following: Median [25%, 75%] of duration of vigorous activities was 0 [0, 60] min/wk, moderate activities was 150 [0, 420] min/wk, and walking was 90 [0, 315] min/wk. Total IPAQ was 1386 [247.5, 3546] MET·min/wk. All of the simple correlation between LT and TEE, AEE, and PAL were positively significant, and their coefficients (r) were 0.81 (p<0.01), 0.81 (p<0.01), and 0.68 (P<0.05) (Figure 2). All of the partial correlations between LT and TEE, AEE, and PAL were also positively significant, and the partial coefficients (ρ) were 0.77 (P<0.01), 0.86 (p<0.01), and 0.86 (p<0.01), respectively.

4. Discussion

Longevity and healthy life span is increasing in our society, it was necessary to conduct research to determine energy requirement for the healthy and independent advanced older people aged >80y. However, there was little data on TEE of the advanced older people over 80 years old, assessed by objective method of PAL and other physical abilities. Our main findings were the relationship between LT and TEE, PAL, and AEE determined by DLW method in advanced older aged 81-94 years.
Table 1. Physical characteristics, body composition, energy expenditure, physical activity, and aerobic capacity of the participants.

| Sex | Age (yrs) | Ht (cm) | Bw (kg) | %fat (%) | FM (kg) | FFM (kg) | TBW (kg) | TEE (kcal/day) | eBMR (kcal/day) | PAL (kcal/day) | AEE (kcal/day) | LT (METs) |
|-----|-----------|---------|---------|----------|---------|----------|----------|---------------|----------------|---------------|--------------|------------|
| Female | 85 | 151 | 59.3 | 38.8 | 23.0 | 36.3 | 26.6 | 1833 | 1013 | 1.81 | 637 | 3.7 |
| Female | 94 | 148 | 46.9 | 33.7 | 15.8 | 31.1 | 22.8 | 1673 | 824 | 2.03 | 682 | 3.5 |
| Male | 83 | 152 | 55.8 | 26.4 | 14.7 | 41.1 | 30.1 | 2144 | 1116 | 1.92 | 814 | 4.9 |
| Male | 82 | 163 | 58.0 | 31.7 | 15.8 | 42.1 | 33.7 | 2236 | 1239 | 1.80 | 773 | 4.7 |
| Male | 86 | 170 | 56.2 | 25.6 | 14.4 | 41.8 | 30.6 | 2487 | 1211 | 2.05 | 1027 | 4.7 |
| Male | 81 | 162 | 51.0 | 9.7 | 4.9 | 46.1 | 33.7 | 2346 | 1123 | 2.27 | 1168 | 5.5 |
| Male | 85 | 150 | 47.9 | 19.2 | 9.2 | 38.7 | 28.3 | 2288 | 1008 | 2.27 | 1051 | 5.5 |
| Male | 84 | 160 | 49.1 | 40.9 | 20.1 | 29.0 | 21.2 | 1396 | 1081 | 1.29 | 175 | 3.0 |
| Male | 83 | 156 | 57.5 | 25.3 | 14.5 | 43.0 | 31.4 | 2250 | 1158 | 1.94 | 867 | 4.8 |
| Male | 87 | 167 | 66.7 | 32.2 | 21.5 | 45.2 | 33.1 | 2149 | 1312 | 1.64 | 622 | 5.1 |
| Male | 83 | 158 | 83.8 | 37.3 | 31.3 | 52.5 | 38.5 | 2689 | 1471 | 1.83 | 949 | 4.5 |
| Male | 81 | 160 | 62.6 | 33.7 | 21.1 | 41.5 | 30.4 | 1788 | 1246 | 1.44 | 363 | 3.8 |

| Mean ± SD | 85 ± 4 | 158 ± 7 | 58.1 ± 10.2 | 38.8 ± 9.8 | 23.0 ± 7.2 | 36.3 ± 7.2 | 26.6 ± 5.3 | 1213 ± 166 | 1150 ± 166 | 1.86 ± 0.30 | 761 ± 288 | 4.5 ± 0.8 |

Ht, height; Bw, body weight; %fat, percent body fat; FM, fat mass; FFM, fat-free mass; TBW, total body water; TEE, total energy expenditure; eBMR, estimated basal metabolic rate using the equation of National Institute of Health and Nutrition for Japanese subjects (Ganpule et al. 2007); AEE, activity energy expenditure; PAL, physical activity level; LT, exercise intensity at LT.

Reduction of physical fitness, including muscular strength and physical function, is thought to be one of the factors in the frailty, as well as a decline in physical activity. We objectively assessed aerobic capacity using MET at LT because low LT is related to the exhaustion of the frail cycle in daily physical activity. Reduction of TEE and PAL is also considered a key factor in the frail cycle, but a few studies have examined the relationship between TEE and aerobic capacity in advanced older adults. Our data supports the frail cycle concept objectively. The present result indicate that increasing aerobic capacity is important for preventing frailty.

Previous studies indicated that older people with frailty has lower TEE and PAL obtained by DLW than healthy or fit older people [11], the older people using wheel chair has lower TEE measured by DLW than the older people not using wheel chair (Nishida et al. 2019), the older people who has lower activity of daily life (ADL) assessed by Barthel index has lower TEE obtained by DLW than the older people who has higher ADL (Nishida et al. 2019). Furthermore, previous study indicated that continuous scale of physical functional performance test score are positively associated with PAL obtained by DLW [10]. Another recent study showed that muscle power of lower extremity measured by vertical jump and sit-to-stand test are positively associated with PAL obtained by DLW [31]. Previous studies suggest that PAL obtained by DLW is positively associated with FFM, as a proxy of skeletal muscle mass, in older adults [10,13]. However, to the best of our knowledge, no studies examined the relationship between TEE measured by DLW and measured aerobic capacity in the advanced older adults. This study clearly indicated the measured aerobic capacity are associated with TEE, AEE, and PAL in advanced older adults even after controlling with age and sex.
Figure 2. Relationship between LT and TEE (A), AEE (B), or PAL (C) is shown in panel. R, Pearson’s correlation coefficients; ρ, Partial correlation coefficients after adjusting for sex and age.

We measured LT rather than VO\(_{2}\)max. The concept of aerobic capacity seems simple but rather complex in actual. VO\(_{2}\)max or VO\(_{2}\)peak was a commonly used index for aerobic capacity. However, the fact that many older adults are unable to satisfactorily complete a maximal exercise until exhaustion and the concerns regarding patient safety often limit direct measurement of VO\(_{2}\)max especially in the advanced-older adults [32]. The measurement of LA concentration during incremental exercise is another indicator of aerobic capacity. Several previous researches suggests that the exercise intensity of LT is a superior measurement of aerobic capacity that compares favorably with the VO\(_{2}\)max, the most representative index of aerobic capacity [33,34]. LT is probably the term most commonly used in the literature in association with estimates of the anaerobic threshold (AT) [35]. LT can be measured in light to moderate submaximal exercise, thus there is less concerns about safety problems in older adults. Additionally, LT is an important indicator for exercise prescription, and previous studies indicated that the exercise intervention at the intensity of LT increases skeletal muscle mass and gait speed in older adults [36] as well
as improving other health parameters, such as blood pressure, inflammatory cytokines levels and lipid profiles [15], with increase of LT.

There are several limitations in the present study. In addition to a small sample size, the participants were recruited from one community-dwelling of a rural area. There is variation in the body composition or nutrition status and physical activity levels between rural and urban areas, even in the same country. Since small-scale farming is the main work for advanced older adults who are living independently in the rural area of Japan, and they may not use large machines. Therefore, there is possible the TEE and PAL level could maintain high even over 80 years old. Such lifestyle may introduce to maintain their FFM and aerobic capacity, e.g. LT. To generalize these observations, multisite studies and many samples are needed.

5. Conclusions

We measured LT as an aerobic capacity and TEE, AEE or PAL with the DLW methods in the advanced older adults (80-94 years old) in community-dwelling of a rural area. As the results, we found the LT as an aerobic capacity is positively and independently correlated with TEE, AEE or PAL. We hope these findings could guide future clinical trials designed to evaluate the efficacy of aerobic exercises in the prevention and treatment of frailty.

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Conflicts of Interest: There is no conflicts of interest in this study.

References

1. Fried, L.P.; Tangen, C.M.; Walston, J.; Newman, A.B.; Hirsch, C.; Gottdiener, J.; Seeman, T.; Tracy, R.; Kop, W.J.; Burke, G., et al. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci 2001, 56, M146-156.
2. Fried, L.P.; Walston, J. Frailty and failure to thrive. In Principles of Geriatric Medicine and Gerontology. 4th ed., Hazzard WR, B.J., Ed. McGraw Hill: New York, 1998; Vol. 1387–1402.
3. Westerterp, K.R. Physical activity and physical activity induced energy expenditure in humans: measurement, determinants, and effects. Front Physiol 2013, 4, 90, doi:10.3389/fphys.2013.00090.
4. Speakman, J.R.; Yamada, Y.; Ainslie, P.N.; Andersen, L.F.; Anderson, L.J.; Arab, L.; Baddou, I.; Bedu-Addo, K.; Blaak, E.E.; Blanc, S., et al. A standard calculation methodology for human doubly labeled water studies. Submitted 2021.
5. Xue, Q.L.; Bandeen-Roche, K.; Varadhan, R.; Zhou, J.; Fried, L.P. Initial manifestations of frailty criteria and the development of frailty phenotype in the Women’s Health and Aging Study II. J Gerontol A Biol Sci Med Sci 2008, 63, 984-990.
6. Yamada, Y.; Nanri, H.; Watanabe, Y.; Yoshida, T.; Yokoyama, K.; Itoi, A.; Date, H.; Yamaguchi, M.; Miyake, M.; Yamagata, E., et al. Prevalence of frailty assessed by Fried and Kihon checklist indices in a prospective cohort study: Design and demographics of the Kyoto-Kameoka longitudinal study. JAMDA 2017, In press.
7. Watanabe, D.; Yoshida, T.; Nanri, H.; Watanabe, Y.; Date, H.; Itoi, A.; Goto, C.; Ishikawa-Takata, K.; Sagayama, H.; Ebine, N., et al. Association between the prevalence of frailty and doubly labeled water-calibrated energy intake among community-dwelling older adults. J Gerontol A Biol Sci Med Sci 2020, 10.1093/gerona/glaa133, doi:10.1093/gerona/glaa133.
8. Watanabe, D.; Yoshida, T.; Watanabe, Y.; Yamada, Y.; Kimura, M.; Group, K.S. Objectively Measured Daily Step Counts and Prevalence of Frailty in 3,616 Older Adults. J Am Geriatr Soc 2020, 68, 2310-2318, doi:10.1111/jgs.16655.
9. Sasai, H.; Nakata, Y.; Murakami, H.; Kawakami, R.; Nakae, S.; Tanaka, S.; Ishikawa-Takata, K.; Yamada, Y.; Miyachi, M. Simultaneous Validation of Seven Physical Activity Questionnaires Used in Japanese Cohorts for Estimating Energy Expenditure: A Doubly Labeled Water Study. Journal of epidemiology 2018, 28, 437-442, doi:10.2188/jea.JE20170129.
10. Frisard, M.I.; Faire, J.M.; Russell, R.D.; King, C.M.; DeLany, J.P.; Wood, R.H.; Ravussin, E. Physical activity level and physical functionality in nonagenarians compared to individuals aged 60-74 years. J Gerontol A Biol Sci Med Sci 2007, 62, 783-788, doi:10.1093/gerona/62.7.783.
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doi:10.20944/preprints202104.0195.v1

11. Yamada, Y.; Hashii-Arishima, Y.; Yokoyama, K.; Ito, A.; Adachi, T.; Kimura, M. Validity of a triaxial accelerometer and simplified physical activity record in older adults aged 64-96 years: a doubly labeled water study. *Eur J Appl Physiol* 2018, 118, 2133-2146, doi:10.1007/s00421-018-3944-6.

12. Nishida, Y.; Nakae, S.; Yamada, Y.; Kondo, E.; Yamaguchi, M.; Shirato, H.; Hirano, H.; Sasaki, S.; Tanaka, S.; Katsukawa, F. Validity of One-Day Physical Activity Recall for Estimating Total Energy Expenditure in Elderly Residents at Long-Term Care Facilities: CLinical EVAluation of Energy Requirements Study (CLEVER Study). *Journal of nutritional science and vitamnology* 2019, 65, 148-156, doi:10.3177/jrvs.65.148.

13. Takae, R.; Hatamoto, Y.; Yasukata, J.; Kose, Y.; Komiyama, T.; Ikenaga, M.; Yoshimura, E.; Yamada, Y.; Ebine, N.; Higaki, Y., et al. Physical Activity and/or High Protein Intake Maintains Fat-Free Mass in Older People with Mild Disability; the Fukuoka Island City Study: A Cross-Sectional Study. *Nutrients* 2019, 11, doi:10.3390/nu11112595.

14. Mori, Y.; Ayabe, M.; Yahiro, T.; Tobina, T.; Kiyonaga, A.; Shimino, M.; Yamada, T.; Tanaka, H. The effects of home-based bench step exercise on aerobic capacity, lower extremity power and static balance in older adults. *International Journal of Sport and Health Science* 2006, 4, 570-576.

15. Nishida, Y.; Tanaka, K.; Hara, M.; Hirao, N.; Tanaka, H.; Tobina, T.; Ikeda, M.; Yamato, H.; Ohta, M. Effects of home-based bench step exercise on inflammatory cytokines and lipid profiles in elderly Japanese females: A randomized controlled trial. *Arch Gerontol Geriatr* 2015, 61, 443-451, doi:10.1016/j.archger.2015.06.017.

16. Martin, S.B.; Morrow, J.R., Jr.; Jackson, A.W.; Dunn, A.L. Variables related to meeting the CDC/ACSM physical activity guidelines. *Med Sci Sports Exerc* 2000, 32, 2087-2092, doi:10.1097/00005768-200012000-00019.

17. Coward, W.A. Calculation of pool sizes and flux rates. In *The Doubly-Labeled Water Method for the Measurement of Energy Expenditure*, Prentice, A.M., Ed. International Atomic Energy Agency: Vienna, Austria, 1990; pp. 48-65.

18. Blanc, S.; Colligan, A.S.; Trabulsi, J.; Harris, T.; Everhart, J.E.; Bauer, D.; Schoeller, D.A. Influence of delayed isotopic equilibration in urine on the accuracy of the H-2(2)O-18 method in the elderly. *J. Appl. Physiol. Physiol*. 2002, 92, 1036-1044.

19. Blanc, S.; Schoeller, D.A.; Bauer, D.; Danielson, M.E.; Tylavsky, F.; Simonsick, E.M.; Harris, T.B.; Kritchevsky, S.B.; Everhart, J.E. Energy requirements in the eighth decade of life. *Am. J. Clin. Nutr.* 2004, 79, 303-310.

20. Racette, S.B.; Schoeller, D.A.; Luke, A.H.; Shay, K.; Hnilicka, J.; Kushner, R.F. Relative dilution spaces of 2H- and 18O-labeled water in humans. *Am J Physiol Endocrinol Metab* 1994, 267, E585-590.

21. Sagayama, H.; Yamada, Y.; Racine, N.M.; Shriver, T.C.; Schoeller, D.A. Dilution space ratio of 2H and 18O of doubly labeled water method in humans. *J Appl Physiol* 1985 (1985), 120, 1349-1354, doi:10.1152/japplphysiol.01037.2015.

22. Schoeller, D.A.; Ravussin, E.; Schultz, Y.; Acheson, K.J.; Baertschi, P.; Jequier, E. Energy expenditure by doubly labeled water: validation in humans and proposed calculation. *Am. J Physiol Regul Integr Comp Physiol* 1986, 250, R823-830.

23. Weir, J.B. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol 1949*, 109, 1-9.

24. Black, A.E.; Prentice, A.M.; Coward, W.A. Use of food quotients to predict respiratory quotients for the doubly-labelled water method of measuring energy expenditure. *Human Nutrition: Clinical Nutrition* 1986, 40, 381-391.

25. Ishikawa-Takata, K.; Tabata, I.; Sasaki, S.; Ramanantantsoa, H.H.; Okazaki, H.; Okubo, H.; Tanaka, S.; Yamamoto, S.; Shirata, T.; Uchida, K., et al. Physical activity level in healthy free-living Japanese estimated by doubly labelled water method and International Physical Activity Questionnaire. *Journ Clin Nutr* 2011, 60, 1256-1261, doi:10.1038/jijoe.1602645.

26. Ito, A.; Yamada, Y.; Yokoyama, K.; Adachi, T.; Kimura, M. Validity of predictive equations for resting metabolic rate in healthy older adults. *Clinical nutrition ESPEN* 2017, 22, 64-70, doi:10.1016/j.clnesp.2017.08.010.

27. Craig, C.L.; Marshall, A.L.; Sjöström, M.; Bauman, A.E.; Booth, M.L.; Ainsworth, B.E.; Pratt, M.; Ekelund, U.; Yngve, A.; Sallis, J.F., et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 2003, 35, 1381-1395, doi:10.1249/01.mss.0000078924.61453.fb.

28. Saji, A.; Nakane, K.; Saiki, K.; Tomioka, K.; Ishii, M.; Okamoto, N. Reliability and validity of the International Physical Activity Questionnaire (IPAQ) in elderly adults: the Fukuoka-kyo Study. *Journal of epidemiology* 2011, 21, 459-465, doi:10.2188/jea.je20110003.

29. Nakane, K.; Ishii, M.; Okamoto, N. Reliability and validity of the International Physical Activity Questionnaire (IPAQ) in elderly adults: the Fukuoka-kyo Study. *Journal of epidemiology* 2011, 21, 459-465, doi:10.2188/jea.je20110003.
35. Svedahl, K.; MacIntosh, B.R. Anaerobic threshold: the concept and methods of measurement. *Can J Appl Physiol* **2003**, *28*, 299-323, doi:10.1139/h03-023.

36. Ikenaga, M.; Yamada, Y.; Kose, Y.; Morimura, K.; Higaki, Y.; Kiyonaga, A.; Tanaka, H. Effects of a 12-week, short-interval, intermittent, low-intensity, slow-jogging program on skeletal muscle, fat infiltration, and fitness in older adults: randomized controlled trial. *Eur J Appl Physiol* **2017**, *117*, 7-15, doi:10.1007/s00421-016-3493-9.