Effect of Metformin on Handgrip Strength, Gait Speed, Myostatin Serum Level, and Health-related Quality of Life: A Double Blind Randomized Controlled Trial among Non-diabetic Pre-frail Elderly Patients

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ABSTRAK

Latar belakang: *sarkopenia berkontribusi terhadap terjadinya sindrom frailty. Sindrom frailty berpotensi membaik dengan memodifikasi faktor inflamasi, resistensi insulin, dan miostatin. Penelitian ini bertujuan mempelajari pengaruh metformin terhadap kekuatan genggam tangan, kecepatan berjalan, konsentrasi miostatin serum, dan kualitas hidup terkait kesehatan pada pasien lanjut usia (lansia) non-diabetes dengan pre-frail.* ***Metode:*** *studi ini merupakan uji klinis acak tersamar ganda yang dilakukan pada pasien rawat jalan berusia ≥ 60 tahun dengan status pre-frail berdasarkan kriteria fenotip dan/atau indeks (Cardiovascular Health Study dan/atau Frailty Index 40 items) di Rumah Sakit Cipto Mangunkusumo yang direkrut dari bulan Maret 2015 sampai Juni 2016. Subjek yang memenuhi kriteria penelitian dirandomisasi menjadi grup metformin (3 x 500 mg) atau grup plasebo (amilum 3 x 500 mg). Luaran penelitian diukur pada awal studi dan 16 minggu setelah intervensi.* ***Hasil:*** *dari 120 subjek, 43 subjek dari grup metformin dan 48 subjek dari grup plasebo yang menyelesaikan penelitian. Terdapat peningkatan kecepatan berjalan pada kelompok metformin sebesar 0,39 (0,77) detik atau 0,13 (0,24) meter/detik yang tetap bermakna setelah disesuaikan dengan faktor prognostik penting (p=0,024). Tidak didapatkan perbedaan bermakna kekuatan genggam tangan, konsentrasi miostatin serum, dan kualitas hidup terkait kesehatan antara kedua kelompok perlakuan.* ***Kesimpulan:*** *pemberian metformin 3 x 500 mg selama 16 minggu secara bermakna meningkatkan kecepatan berjalan sebagai salah satu dimensi kualitas hidup terkait kesehatan, namun tidak meningkatkan secara bermakna skor indeks EQ-5D, kekuatan genggam tangan, dan konsentrasi miostatin serum.*

Kata kunci: *kecepatan berjalan, kekuatan genggam tangan, kualitas hidup, metformin, miostatin, pre-frail, lanjut usia.*

ABSTRACT

Background: *sarcopenia contributes to the development of frailty syndrome. Frailty syndrome is potentially improved by modifying insulin resistance, inflammation, and myostatin level. This study is aimed to investigate the effect of metformin on handgrip strength, gait speed, myostatin serum level, and health-related quality of life (HR-QoL) among non-diabetic pre-frail elderly patients.* ***Methods:*** *a double blind randomized controlled trial was conducted on non-diabetic elderly outpatients aged ≥ 60 years with pre-frail status based on phenotype and/ or index criteria (Cardiovascular Health Study and/ or Frailty Index 40 items) consecutively recruited from March 2015 to June 2016 at Cipto Mangunkusumo Hospital. One-hundred-twenty subjects who met the research criteria were randomized and equally assigned into 3 x 500 mg metformin or placebo group. The study outcomes were measured at baseline and after 16 weeks of intervention.* ***Results:*** *out of 120 subjects, 43 subjects in metformin group and 48 subjects in placebo group completed the intervention. There was a significant improvement on the mean gait speed of metformin group by 0.39 (0.77) second or 0.13 (0.24) meter/second that remained significant after adjusting for important prognostic factors (p = 0.024). There was no significant difference on handgrip strength, myostatin serum level, and HR-QoL between both groups.* ***Conclusion:*** *3 x 500 mg metformin for 16 weeks was statistically significant and clinically important in improving usual gait speed as one of the HR-QoL dimensions, but did not significantly improve the EQ-5D index score, handgrip strength, nor myostatin serum level.*

Keywords: *elderly, gait speed, handgrip strength, health-related quality of life, metformin, myostatin serum, pre-frail.*

INTRODUCTION

Sarcopenia, a progressive loss of muscle mass and function with advancing age, leads to decreased metabolic rate, muscle strength, and maximal VO_2 resulting in clinical/ phenotypic manifestations of frailty syndrome (i.e. physical frailty) that is characterized by weight loss, exhaustion, weakness, slowness, and low physical activity level.[1] Sarcopenia is considered to be related to myostatin, a transforming growth factor-β (TGF-β) that trigger muscle protein degradation which leads to muscle growth inhibition.[2,3]

Frailty syndrome is a continuum spectrum of normal/ robust, pre-frail, and frail states with dynamic transition from robustness to frailer state and vice versa.[4] Inflammation, insulin resistance, diabetes mellitus (DM), low vitamin D concentration and protein intake, poly-pharmacy (> 4 medications), and depression have significant association with the incidence of frailty syndrome.[5–11] Administration of metformin potentially improves frailty syndrome by modifying insulin resistance, hyperglycemia, inflammation, and myostatin level. Not only does metformin activate cellular metabolic Adenosine Monophosphate-Activated Protein Kinase (AMPK), it also inhibits Nuclear Factor-κB (NF-κB) and mammalian Target of Rapamycin (mTOR).[12–14] Metformin also improves Na^+K^+ATPase activity and increases circulating nitric oxide which optimize cellular energy production.[15] On the other hand, previous studies indicated that AMPK may trigger muscle protein degradation and down-regulate muscle protein synthesis by stimulating myostatin expression and mTOR signal.[16,17]

Bulcao *et al*[18] study showed that 16-weeks administration of 2 x 850 mg metformin for pre-diabetic subjects significantly decreased body mass index (BMI), inflammation mediators (C-reactive protein/ CRP and interleukin-6/ IL-6), and fasting blood glucose as well as improved insulin resistance (evaluated by HOMA IR). Esteghamati *et al*[19] reported that administration of 1000 mg metformin/day for 12 weeks in newly diagnosed type-2 diabetes mellitus (DM) patients significantly improved oxidative stress. Furthermore, previous *in vitro* study showed that metformin increased the myostatin expression and myostatin protein level in C2C12 myotubes at low concentration (0.5 mM), but down-regulated them at higher concentration (1.5 and 2.0 mM).[17]

A case-control study indicated the protective effect of metformin against frailty syndrome. This study showed significant difference of frailty status between metformin-treated and non-metformin-treated type-2 DM patients.[20] However, there is no randomized, double blind, clinical trial on the effect of metformin on frailty syndrome, especially its effect on physical components of frailty syndrome and myostatin serum level. The aim of this study was to investigate the effects of metformin with the dose of 500 mg three times daily for 16 weeks on handgrip strength, gait speed, myostatin serum level, and health-related quality of life (HR-QoL) among non-diabetic pre-frail elderly. To the best of authors' knowledge, this was the first study that investigated the effect of metformin administration in order to prevent physical frailty in non-diabetic pre-frail elderly. It was also the first study that investigated the effect of metformin on myostatin serum level in human.

METHODS

This was a double blind randomized controlled trial. Subjects were allocated in each treatment group using permuted block randomization with block size of four and the code lists were concealed. Investigators, doctors, and subjects were blinded to treatment allocation (double blind).

Study Participants

Elderly outpatients aged 60 years and older with pre-frail status based on Cardiovascular Health Study (CHS)[1] and/ or Frailty Index 40 items (FI 40 items) score,[21] were consecutively recruited from March 2015 to June 2016 at Geriatric and Internal Medicine Clinic in Cipto Mangunkusumo Hospital, Jakarta, Indonesia. Exclusion criteria were unwillingness to participate in this study, malnutrition (body mass index/ BMI < 18.5 kg/m^2 or Mini Nutritional Assessment/ MNA full form score < 17), diabetes mellitus, cognitive impairment (Abbreviated Mental Test/AMT score < 8), depression (Geriatric Depression Scale/ GDS score ≥ 10), acute phase of disease(s), and contraindication(s) to metformin. The minimum sample size for gait speed outcome was 29 subjects, whereas for handgrip strength, myostatin serum level, and HR-QoL outcomes were 60 subjects for each treatment group.

Intervention Protocol

After giving written consent, eligible subjects were randomly assigned to metformin (3 x 500 mg) or placebo (amylum 3 x 500 mg) group for 16 weeks of intervention. Both metformin and placebo capsules prepared by the hospital's pharmacy unit were indistinguishable. The allocated treatment was dispensed to the subjects every four weeks.

The collected data consist of subjects' demographic data (age, sex, income, level of education), clinical data (illnesses and medications history), functional status (Barthel index Basic Activity of Daily Living/ B-ADL and Lawton Instrumental Activity of Daily Living/ L-IADL), mental status (GDS), cognitive status (AMT), frailty status (CHS and FI 40 items), level of activities (Physical Activity Scale for the Elderly/ PASE), sarcopenia status (Asian Working Group of Sarcopenia/AWGS criteria),[22] anthropometry measurements, nutritional status (MNA full form as well as food record of two weekdays and one holiday), and body composition (Bioelectrical Impedance Analysis/ BIA Tahita SC 330). Fasting venous blood samples were collected for myostatin serum level, oral glucose tolerance test (OGTT) as well as liver and renal function test.

The measurement of study outcomes was conducted at baseline and after 16 weeks of intervention. Handgrip strength of dominant hand was assessed using JAMAR hydraulic handheld dynamometer model J00105 and was conducted in accordance with American Society of Hand Therapist (ASHT) recommended procedure.[23] The 15-feet walk test was performed to measure usual gait speed. Myostatin serum level was measured using ELISA kit Immundiagnostik AG, Bensheim, Germany Cat #K1012. Health-related quality of life was assessed using Euro Quality of life-5 Dimensions (EQ-5D) questionnaire with 3 Likert scale. Adverse events, drug's side effects, compliance, level of activities, consumption of other medications, and co-morbidities were evaluated every four weeks.

Ethics

This study was approved by the Ethical Committee of Faculty of Medicine Universitas Indonesia/ Cipto Mangunkusumo Hospital (No. 69/UN2.F1/ETIK/2015) and registered in clinical trial database www.clinicaltrials.gov with identifier number NCT02325245. The study procedure was performed in accordance with the Helsinki, Guideline for Good Clinical Practice from ICH Tripartite Guideline (ICH-GCP).

Statistical Analysis

Data analysis were done using SPSS 20. Drop-out subjects were excluded from the analyses (per protocol analysis). Intention-to-treat (ITT) analysis was used to estimate the treatment's efficacy in order to get the number needed-to-treat (NTT). Ancova statistic test was used to analyze the study outcomes of the two assigned groups, since some important prognostic factors of the baseline subjects' characteristics (including numerical and categorical data) were unequal.

RESULTS

Despite recruiting 153 elderly patients to participate in the study, as many as 33 subjects were excluded from this clinical trial (of which 16 patients declined to participate and 17 did not meet the research criteria) which resulted in 120 subject who were randomized and equally assigned into metformin or placebo group (60 subjects in each group). There were 43 subjects in metformin group and 48 subjects in placebo group who completed the intervention (**Figure 1**).

From 91 subjects who completed the intervention, the mean age was 68.97 (5.34) years old and more than half (62.64%) were female. The top four co-morbidities in both groups were hypertension, dyslipidemia, knee osteoarthritis (OA), and stable coronary artery disease. There was no subject with sarcopenia based on AWGS criteria. Compared to subjects in placebo group, subjects in metformin group were less likely to have dyslipidemia, knee OA, and CIRS score >5, but more likely to be

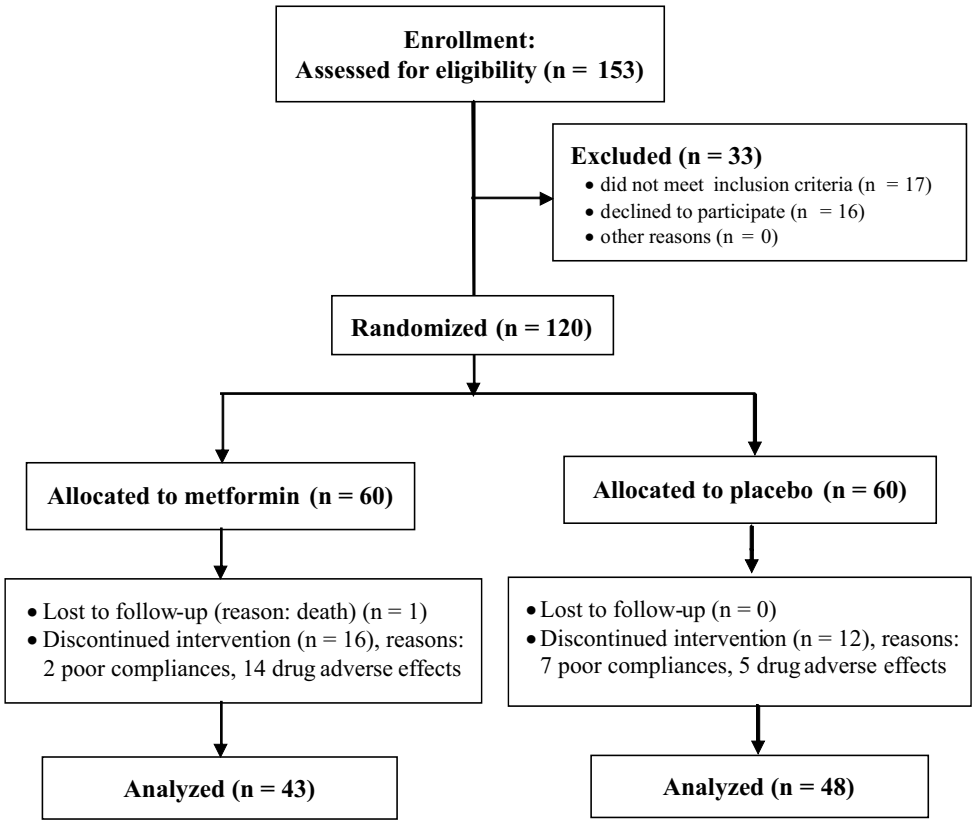

Figure 1. Flow diagram of randomized clinical trial

younger, consume acetylsalicylic acid (ASA), as well as to have higher fat mass, BMI, upper arm muscle circumference, calf circumference, skeletal muscle index, and handgrip strength (**Table 1** and **Table 2**).

Table 1. Baseline demographic characteristic of the subjects

Characteristics	Metformin (n=43)	Placebo (n=48)
Age (years), median (min–max)	67.77 (5.14)	70.04 (5.34)
Sex, n (%)		
- Female	24 (55.8)	33 (68.8)
- Male	19 (44.2)	15 (31.2)
Level of Education, n (%)		
- Low	3 (7)	5 (10.4)
- Moderate	10 (23.2)	13 (27.1)
- High	30 (69.8)	30 (62.5)
Co-morbidity, n (%)		
- Hypertension	38 (88.4)	39 (81.3)
- Dyslipidemia	24 (55.8)	37 (77.1)
- Knee osteoarthritis	23 (53.5)	30 (62.5)
- Coronary artery disease	17 (39.5)	11 (22.9)
CIRS score, n (%)		
- ≤ 5	22 (51.2)	17 (35.4)
- > 5	21 (48.8)	31 (64.6)
Poly-pharmacy, n (%)		
- Yes	31 (72.1)	38 (79.2)
- No	12 (27.9)	10 (20.8)
Medications used, n (%)		
- Statin	36 (83.7)	42 (87.5)
- Proton pump inhibitor (PPI)	25 (58.1)	31 (64.6)
- Angiotensin receptor blocker (ARB)	28 (65.1)	24 (50)
- Acetylsalicylic acid (ASA)	24 (55.8)	13 (27.1)
- Calcium-vitamin D supplement	9 (20.9)	16 (33.3)
- ACE inhibitor	5 (11.6)	7 (14.6)

Table 3 shows that the baseline mean walking time in metformin group was 4.05 (0.93) seconds which represented the mean usual gait speed of 1.18 (0.26) meter/second, whereas the mean walking time in placebo group was 4.29 (1.38) seconds which represented the mean usual gait speed of 1.14 (0.26) meter/second. The

Table 2. Baseline characteristic of subjects' nutritional status parameter

Characteristics	Metformin (n=43)	Placebo (n=48)
Anthropometry Measurements, mean (SD)		
Mid-arm circumference (cm)	31.65 (3.3)	28.35 (2.93)
- Male	30.08 (2.98)	28.00 (3.09)
- Female	32.89 (3.06)	28.51 (2.88)
Upper-arm Muscle Circumference (cm)	24.37 (2.33)	22.41 (1.88)
- Male	24.79 (2.54)	22.73 (1.40)
- Female	24.09 (2.15)	22.27 (2.07)
Waist circumference (cm)	94.44 (9.56)	87.35 (9.71)
- Male	93.23 (10.24)	88.02 (11.35)
- Female	95.39 (9.09)	87.05 (9.04)
Thigh circumference (cm)	51.88 (4.35)	46.95 (4.69)
- Male	50.44 (4.57)	46.46 (3.44)
- Female	53.00 (3.89)	47.17 (5.19)
Calf circumference (cm)	37.22 (3,20)	34.74 (3.90)
- Male	36.76 (2,78)	35.87 (3.67)
- Female	37.59 (3,51)	34.22 (3.93)
BMI (kg/m^2)	27.40 (3.15)	23.90 (3.10)
- Male	26.17 (3.28)	23.91 (2.85)
- Female	28.37 (2.73)	23.90 (3.25)
Body composition		
Muscle mass (kg), median (min–max)	39.8 (34.0–62.8)	37.4 (29.3–59.7)
Muscle mass Index (kg/m^2), median (min-max)	16.23 (14.69–20.35)	15.42 (12.77–20.66)
- Male, mean (SD)	18.89 (1.38)	18.14 (1.19)
- Female, mean (SD)	15.79 (0.51)	14.79 (0.97)
Fat Mass (kg), mean (SD)	22.92 (8.25)	17.63 (6.81)
- Male	16.52 (6.32)	13.16 (5.13)
- Female	27.99 (5.7)	19.66 (6.55)
Dietary Intake		
Energy (Kcal), mean (SD)	1,434.60 (320.87)	1,418.27 (265.32)
Protein (gram), mean (SD)	47.53 (11.80)	46.56 (12.09)
Vitamin D (mcg), median (min–max)	4.3 (0.1–20.8)	2.8 (0.1–17.10)
Calcium (mg), median (min–max)	304.5 (66.3-2,451.5)	372.25 (79.7–2,848.7)
OGTT, mean (SD)		
Fasting (mg/dL)	89.47 (9.26)	88.81 (8.85)
Post 75 gr glucose load (mg/dL)	127.67 (30.48)	136.31 (30.68)
Pre-diabetes, n (%)	18 (41.9)	21 (43.8)

Table 3. Baseline characteristics of subjects' level of activity, frailty status, and study outcomes

Characteristics	Metformin (n=43)	Placebo (n=48)
B-ADL Score, n (%)		
- Independence	38 (88.4)	43 (89.6)
- Mild Dependency	5 (11.6)	5 (10.4)
PASE Score (Kcal per week), mean (SD)	1,200.69 (619.11)	1,206.82 (585.71)
FI 40 items score, mean (SD)	0.147 (0.040)	0.151 (0.040)
Handgrip strength (kg), med (min–max)	24 (12–45)	20 (14–38)
- Male, mean (SD)	32.53 (5.65)	27.27 (5.00)
- Female, median (min–max)	20 (12–26)	18 (14–38)
Walking Time (second), mean (SD)	4.05 (0.93)	4.29 (1.38)
- Male	3.68 (0.78)	3.79 (0.69)
- Female	4.35 (0.94)	4.52 (1.56)
Gait Speed (meter/second), mean (SD)	1.18 (0.26)	1.14 (0.26)
- Male	1.29 (0.25)	1.24 (0.23)
- Female	1.10 (0.25)	1.09 (0.27)
Myostatin Serum Level (ng/mL), median (min–max)	35.72 (17.77–56.85)	34.83 (18.33–133.95)
Health-Related Quality of Life: EQ-5D Index Score, median (min–max)	0.77 (0.57–1.0)	0,77 (0.59–1.0)
Health-Related Quality of Life: EQ-5D VAS Score, median (min–max)	80 (40–90)	75 (48–100)
Drug compliance (%), mean (SD)	91.05 (5.67)	91.74 (5.96)

baseline median handgrip strength in metformin group was 24 (12–45) kg, whereas in placebo group was 20 (14–38) kg. The baseline median myostatin serum level of all subjects was 35.26 (17.77–133.95) ng/mL. The baseline median EQ-5D index score in metformin group was 0.77 (0.57–1.0) with EQ-5D VAS score of 80 (40–90), whereas the median EQ-5D index score in placebo group was 0.77 (0.59–1.0) with EQ-5D VAS score of 75 (48–100). The compliance rate of both groups was good, which was 91.05 (5.67)% in metformin group and 91.74 (5.96)% in placebo group.

Ancova statistic test showed that at the end of intervention there was a significant difference in usual gait speed between metformin and placebo group, which remained statistically significant even after adjusting for age, sex, knee OA, acetylsalicylic acid consumption, as well as baseline handgrip strength, calf circumference, and BMI (**Table 4**). The mean walking time in metformin group became 0.39 (0.77) seconds shorter than baseline. In other words, there was a significant improvement on usual gait speed by 0.13 (0.24) meter/second.

Although there was a significant difference in handgrip strength between metformin and placebo group in unadjusted model, the difference was statistically insignificant after adjusting for important prognostic factors. Moreover, there were also no significant difference in myostatin serum level and HR-QoL between the two groups (**Table 4**).

Until the end of intervention, the dietary intake as well as physical activity level of the

Table 4. The effect of metformin on handgrip strength, walking time, myostatin serum level, and health-related quality of life

Study Outcomes	Unadjusted		p	Adjusted∞		p
	Metformin mean (95%CI)	Placebo mean (95%CI)		Metformin mean (95%CI)	Placebo mean (95%CI)	
Handgrip Strength (kg)	25.47 (23.51–27.42)	21.90 (20.04–23.75)	0.010	23.39 (22.28–24.49)	23.50 (22.57–24.44)	0.877
Walking Time (second)	3.66 (3.32–3.99)	4.25 (3.94–4.57)	0.012	3.72 (3.37–4.06)	4.23 (3.94–4.52)	**0.024**
Myostatin Serum Level (ng/mL)	35.77 (32.65–38.89)	36.58 (33.62–39.53)	0.711	34.82 (31.69–37.95)	37.43 (34.48–40.38)	0.244
EQ-5D Index Score	0.83 (0.79–0.87)	0.82 (0.78–0.86)	0.761	0.85 (0.80–0.89)	0.83 (0.79–0.87)	0.660

∞ Ancova Test

metformin and placebo group were similar (data not shown). Gastrointestinal symptoms, such as diarrhea, nausea, bloated, and epigastric pain were the side effects of metformin commonly reported in this study. There were five serious adverse events (SAEs) in metformin group: death after heart attack, recurrent stroke, malleolus ulcers, diarrhea, and melena.

DISCUSSION

According to the AWGS criteria, the median handgrip strength in both intervention groups were within normal limit. Similarly, the mean usual gait speed of both groups were also good, which were above 1 meter/second.[22]

At the end of intervention, the mean walking time in metformin group improved significantly by 0.39 (0.77) seconds which represented the mean gait speed improvement by 0.13 (0.24) meter/second. This finding is consistent with Lee *et al*[24] cohort study which reported that the decrease in gait speed among diabetic patients who received insulin sensitizer drugs (metformin or thiazolidinedione) was not only lesser than diabetic patients who received other types of oral anti-diabetic drugs but also lesser than non-diabetic patients.

Previous study indicated that the minimum improvement of gait speed by 0.05 meter/second is considered significant, whereas a 0.10 meter/second change in gait speed is considered a substantial improvement.[25] The age-adjusted relative risk ratio per 0.1 meter/second greater speed for B-ADL dependence was 0.68 (95%CI 0.57–0.81) among male and 0.74 (95%CI 0.66–0.82) among female.[26] Every 0.1 meter/second decrease in gait speed was also associated with a 7% increase in risk for falls.[27] Meta-analysis of 9 cohorts concluded that gait speed was associated with survival with pooled HR 0.88 (95%CI 0.87–0.90) per 0.1 meter/second improvement.[28] Hence, the 0.13 (0.24) meter/second improvement of usual gait speed found in our study was not only statistically significant but also clinically important.

Insulin resistance state decreases muscle mass and muscle contractility due to cytokine, increased myostatin expressions, and ineffective insulin activity which result in reduction of blood flow and skeletal muscle glucose uptake, as well as muscle protein degradation.[17,29] Kuo *et al*[30] study reported that among non-diabetic elderly patients, every 1 standar deviation increment of HOMA-IR value was parallel with a decrease in gait speed of 0.04 meter/second (p=0.003). The significant gait speed improvement in metformin group might be caused by the improvement in insulin resistance state, inflammation, oxidative stress, and nitric oxide. Our study did not assess the laboratory parameter of inflammation nor insulin resistance. However, the significant decrease in BMI and waist circumference among subjects in metformin group (data not shown) might represent the improvement in their insulin resistance state.

Our study showed that handgrip strength was not a suitable parameter to investigate the effects of metformin. The purpose of handgrip strength measurement is to evaluate the isometric hand muscle contraction which is a sudden, fast, and high force activity. The muscle fibers that are particularly involved in this kind of activity are type-II muscle fiber (fast twitch) whose source of energy comes from anaerobic metabolism of ATP and creatine phosphate stored in the muscle.[31,32] It seems that metformin has no important role in the utilization of stored ATP and creatine phosphate to produce that kind of energy. Furthermore, metformin was not found to increase muscle mass (data not shown), thus the stored ATP and creatine phosphate in the muscle which are parallel with higher muscle mass was probably not elevated.

In contrast to handgrip strength test, the 15-feet (~4.57 meter) walking test is a dynamic, constant, and rhythmic muscle contraction without inflicted fatigue on oxygen transport system. The source of energy for this kind of activity may not only derive from anaerobic metabolism of creatinine phosphate, but also from glycogen and glucose aerobic metabolism. Metformin administration increases the glucose and calcium uptake of the skeletal muscle by improving the insulin resistant state.[13,29,32,33] Therefore, gait speed improvement occurred in the metformin group. However, further investigation on the mechanism of how metformin improves gait speed is needed, especially regarding the

muscle energy metabolism.

Intention-to-treat analysis on the absence or presence of increased gait speed of > 0.1 meter/second showed that there were 40 events out of 60 subjects (66.7%) in the metformin group and 45 events out of 60 subjects (75%) in the placebo group. Absolute risk reduction (ARR) was 8.3% (95%CI -7.9–24%; p = 0.422) which resulted in NNT of 12. It was suggested that the administration of metformin to 12 pre-frail elderly patients was needed to add one gait speed improvement of > 0.1 meter/second.

The myostatin serum levels in this study was higher than what was reported in Ryan *et al*[34] study but almost similar to the mean plasma myostatin level in Hittel *et al*[35] study. The varying results in myostatin serum/ plasma level among studies are assumed to be related to distinct ELISA assays of different antigen and antibody combinations with variances in the sensitivity and specificity, whether it measures both the mature active C-terminal dimer of myostatin and the N-terminal propeptide or it specifically measures the active C-terminal dimer. Moreover, the mean and higher proportion body mass index of our subjects were classified as overweight–obese (based on Asia-Pacific criteria[36]) and 42.9% of the subjects were in pre-diabetic state. It has been reported that mRNA myostatin expression in muscle and serum/ plasma myostatin level are increased in insulin resistant state and obesity.[2,3,35,37] Therefore, those factors also contributed to the higher myostatin serum level in our study.

At the end of observation, our study did not find significant difference in myostatin serum level between metformin and placebo group. There was also no significant difference in myostatin serum level before and after intervention among subjects in metformin group. Previous *in vitro* study showed inconclusive result whether metformin increases or decreases myostatin level.[17] In contrast to our study, Ryan *et al*[34] and Hittel *et al*[35] reported that exercise, which is another AMPK activator, significantly affected myostatin serum/ plasma level. However, those studies were not randomized controlled trials.

Myostatin level can be evaluated by measuring its concentration in serum or plasma and detecting mRNA expression of myostatin in muscle. mRNA expression of myostatin in the muscle represents biological activity of myostatin in the body. Unfortunately, muscle biopsy to measure mRNA expression of myostatin was not conducted in our clinical trial. Furthermore, changes in mRNA expression of myostatin in muscle are not always followed by changes in myostatin serum or plasma level. Brandt *et al*[38] reported that although mRNA myostatin expressions in muscle were 1.4 times higher in type 2 DM subjects compared to normal subjects, there was no significant difference in plasma myostatin level between the two groups. Therefore, whether metformin affected mRNA expression of myostatin in skeletal muscle or not was still unknown.

The median score of EQ-5D index in our study was quite good since most subjects had good functional status with B-ADL score ranged 19–20 and our study excluded subjects with depression and cognitive impairment. Administration of metformin for 16 weeks did not show improvement in HR-QoL among elderly outpatients. It seems that metformin did not directly enhance the overall HR-QoL, but rather improved the mobility which is considered as one of the many dimensions of HR-QoL. In the future, improvement in mobility is expected to increase patient's capability in daily life activities.

Out of 17 subjects in the metformin group who drop-out, 14 subjects experienced the adverse effects of metformin, 1 subject died, and 2 subjects had poor compliance. In placebo group, there were 5 drop-out subjects due to drug adverse effects and 7 drop-out subjects due to poor compliance. The number needed to harm (NNH) was -12, which indicated that subjects in the placebo group had fewer risk of experiencing adverse effects compared to metformin group. However, this NNH value did not seem to represent the NNH of metformin in daily clinical practice which has been widely used as the first line treatment for type 2 DM. Subject drop out due to gastrointestinal symptoms occurred mostly in the first week of the intervention. Although re-education and counselling had been

re-applied to them, those subjects still refused to continue the study. A review by Scheen *et al*[39] suggested that the elderly have relatively good tolerance to metformin and its safety is more related to the documented contraindications rather than age per se.

Although there were five serious adverse events (SAEs) reported in the metformin group (e.g. death after heart attack, recurrent stroke, malleolus ulcers, diarrhea, and melena), further investigation showed that all of those subjects had initially possessed the risk factors for those events. Therefore, it can be concluded that SAEs were not related to metformin administration.

There were several limitations of this study. The proportion of drop-out subjects was 24% due to drug side effects, poor compliance, and death. However, the important prognostic factors of the subjects who completed the study were similar to those drop-out subjects.

The data analysis can only be applied to those who completed the study (per protocol analysis). However, due to logistic limitations, despite the prolonged subjects' recruitment period for 15 months, this study did not meet the minimum sample size of 60 subjects who completed the study in each treatment group. Nevertheless, the minimum sample size for the study outcome on gait speed was already met, which was a minimum of 29 subjects in each treatment group. Therefore, the result analysis for gait speed was valid and has good statistical power (80%).

Objective measurement of insulin resistance and inflammation mediators, as well as measurement of lower extremities strength were not conducted in this study. Therefore, this study cannot fully explain the mechanism of metformin in improving the study outcome. Muscle biopsy to evaluate the mRNA expression of myostatin was not conducted in this study. Hence, it remains undetermined whether metformin affected mRNA expression of myostatin in skeletal muscle or not. Moreover, the measurement of body composition in this study used BIA which is not as accurate as Dual-Energy X-Ray Absorptiometry (DXA).

CONCLUSION

Our results suggested that administration of metformin with the dose of 3 x 500 mg for non-diabetic pre-frail elderly subjects for 16 weeks was statistically significant and clinically important in improving usual gait speed, but it did not significantly increase handgrip strength nor decrease myostatin serum level. Although there was an improvement of mobility as one of HR-QoL dimensions, it did not increase the overall score of EQ-5D index.

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