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**Energy replacement diminishes the postprandial triglyceride-lowering effect from
accumulated walking in older women**

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25   **Abstract (250/150-250 words)**

26   **[Purpose]** Dietary replacement of an acute exercise-induced energy deficit offsets the
27   postprandial triglyceride (TG)-lowering effect of exercise in young boys and
28   middle-aged men. It is unclear whether these findings are observed when exercise is
29   accumulated in older adults. This study examined the effect of accumulating short bouts
30   of exercise, with and without dietary replacement of an exercise-induced energy deficit,
31   on postprandial TG in older women. **[Methods]** Seventeen older women (≥ 65 years)
32   underwent three, 8-h trials: 1) control, 2) accumulated walking and 3) accumulated
33   walking with energy replacement. During the control trial, participants rested for 8 h.
34   The accumulated walking trials comprised twenty 1.5 min brisk walking bouts
35   performed at a pre-determined self-selected pace separated by 15 min seated rest. In
36   each trial, participants consumed a standardised breakfast and lunch. The breakfast in
37   the accumulated walking with energy replacement trial included replacement of the
38   energy deficit (0.62 MJ, 149 kcal) induced by exercise. Venous blood samples were
39   collected fasted and at 2, 4, 6 and 8 h after breakfast. **[Results]** Time-averaged
40   postprandial serum TG concentrations over 8 hours were lower after accumulated
41   walking than control and accumulated walking with energy replacement (mean $\pm$ SD:
42   1.46 ± 0.93 vs 1.71 ± 1.01 vs 1.60 ± 0.98 mmol/L respectively: main effect of trial $p =$

0.017). There was little difference between control and accumulated walking with energy replacement. **[Conclusions]** Replacing the energy expenditure induced by accumulating 30 min of brisk walking in short (1.5 min) bouts diminishes the postprandial TG-lowering effect in older women.

Keywords

Energy replacement • Postprandial triglyceride • Lipid metabolism • Accumulated walking • Older adults

Introduction

Repeated daily episodes of elevated non-fasting triglyceride (TG) and prolonged residence in the circulation of TG-rich lipoproteins are a risk factor for cardiovascular disease and all-cause mortality in men and women [1,2]. Thus, it is important to consider lifestyle modifications which may be effective in reducing diurnal exaggerations in postprandial TG.

Ample evidence supports the notion that an acute bout of aerobic exercise reduces postprandial TG concentrations (for a review of relevant studies see [3]). Most

61 randomised, cross-over studies in this area investigate participants whilst they are in a
62 state of energy deficit induced by exercise, which may at least partly mediate the
63 exercise-stimulated reduction in postprandial TG [3]. It is difficult to translate these
64 study findings to free living environments outside the laboratory where individuals with
65 normal free access to food and drink may replace the energy expended during exercise.
66 Thus, it is important to consider energy status when studying metabolic responses to
67 exercise [4].

68
69 To date, seven laboratory-based studies have examined the effects of an acute bout of
70 exercise, with and without dietary replacement of the exercise-induced energy deficit,
71 on postprandial TG concentrations with disparate findings [5-11]. Some studies note
72 that acute replacement of the energy deficit diminishes the exercise-induced reduction
73 in postprandial TG [5-8, 10, 11] whereas others report lower postprandial TG
74 concentrations even after the energy expended during exercise was replaced
75 immediately after exercise [7, 9-11]. The amount of energy expended during exercise,
76 the intensity of exercise, and the type of replacement meal/drink are possible reasons for
77 the inconsistent findings among studies. Furthermore, most of the participants of these
78 seven previous studies were males ranging in age from adolescent boys to middle-aged

men with only a few young, healthy adult women included in the analysis of two studies alongside men [7, 11]. Investigations in older women (≥ 65 years) represent an important gap in current understanding considering excursions in TG concentrations after meals are likely to be exaggerated in these individuals [12]. In addition, while accumulated exercise can reduce postprandial TG concentrations in young adults [3], more data for its effects are needed in older adults who may have a limited capacity to perform exercise for long periods because of low fitness or pre-existing disease. Thus, accumulated activity may be easier to incorporate into the daily life of older adults as activities that are intermittent in nature often involve bouts lasting less than a few minutes [13].

Therefore, the purpose of this study was to investigate the effect of accumulating short bouts of brisk walking throughout the day, with and without dietary replacement of the exercise-induced energy deficit, on postprandial TG in older (≥ 65 years) women.

Methods

Participants

After approval from the Ethics Committee on Human Research of Waseda University

(Approval number: 2017-103), 17 older women gave written informed consent to participate in this study. Exclusion criteria were currently smoking, reporting a major illness/disease, or physical problems (acute or chronic) limiting the ability to perform activities of daily living. The physical characteristics of the participants are provided in Table 1.

Insert Table 1 near here

Anthropometry

Body mass was measured to the nearest 0.1 kg using a digital scale (Inner Scan 50; Tanita Corporation, Tokyo, Japan). Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (YS-OA; AS ONE Corporation, Osaka, Japan). Body mass index was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured to the nearest 0.1 cm at the level of the umbilicus using a flexible plastic tape. Arterial blood pressure was measured from the right arm after 5 min of seated rest by a standard mercury sphygmomanometer (605P; Yagami Co Ltd, Yokohama, Japan). Two measurements were taken, and the mean of these values was recorded.

115

116 **Preliminary test**

117 After familiarisation with the treadmill (JOG NOW 700; Technogym, Milan, Italy),
118 each participant was asked to walk “briskly” for three minutes to determine their
119 walking speed and estimate the energy cost of the main exercise trial. “Brisk” walking
120 was defined as feeling slightly out of breath while walking but still able to hold a
121 conversation. Oxygen uptake, carbon dioxide production and respiratory exchange ratio
122 (RER) were measured breath-by-breath through a stationary gas analyser (Quark CPET,
123 COSMED, Rome, Italy). Energy expenditure was estimated from these using
124 standardised equations [14].

125

126 **Standardisation of dietary intake and physical activity**

127 Participants weighed and recorded all food and drink consumed the day before each trial
128 and refrained from drinking alcohol during this period. Participants replicated their
129 dietary intake from the first trial in all subsequent trials to ensure that dietary intake was
130 standardised across trials. Food diaries were analysed by a registered dietitian to
131 determine energy intake and macronutrient content. In addition, participants were asked
132 to remain inactive the day before each main trial and wore a uniaxial accelerometer

(Lifecoder-EX; Suzuken Co Ltd, Nagoya, Japan) on the hip to monitor their daily activity objectively during this period. The accelerometer defined 11 levels of activity intensity (0, 0.5, and 1-9), with 0 indicating the lowest intensity and 9 being the highest intensity. A level of 4 corresponds to an intensity of ~3 metabolic equivalents [15]. In addition, total step count (steps per day) was recorded and calculated from the accelerometer using computerised software (Lifelyzer 05 Coach; Suzuken Co Ltd).

Experimental design and protocol

Participants underwent three, one-day laboratory-based trials in a random order: 1) control, 2) accumulated walking and 3) accumulated walking with energy replacement. The interval between trials was at least 7 days. A schematic illustration of the study protocol is shown in Figure 1.

Insert Fig. 1 near here

Control trial. Participants reported to the laboratory at 0845 h after a 10-h overnight fast (no food or drink except water). Then, body mass was measured to the nearest 0.1 kg using a digital scale (Inner Scan 50; Tanita Corporation, Tokyo, Japan). After a 15-min

rest, a fasting venous blood sample was collected in a seated position by venipuncture at 0 h (0900 h). Participants then consumed a standardised meal for breakfast and rested thereafter in a seated position (reading or writing) for 8 h (1700 h). A second test meal was consumed for lunch 3 h after the initiation of breakfast. Further venous blood samples were collected by venipuncture at 2 h (1100 h), 4 h (1300 h), 6 h (1500 h), and 8 h (1700 h) after the initiation of breakfast for the measurement of circulating concentrations of TG, non-esterified fatty acids (NEFA), 3-hydroxybutyrate (3-OHB), insulin and glucose. NEFA was measured as a surrogate marker of substrate delivery to the liver for TG synthesis and 3-OHB as a marker of hepatic fatty acid oxidation affecting TG secretion. Furthermore, we used measures of insulin and glucose as indicators of carbohydrate metabolism, with the former also implicated in modulating lipoprotein lipase activity, the rate-limiting enzyme for the hydrolysis of TG.

Accumulated walking. The accumulated walking trial was identical to the control trial except participants were asked to complete 20 × 1.5 min bouts of treadmill walking throughout the day. The speed of walking was at a self-selected pace determined during the preliminary visit. Treadmill walks were completed at 1000, 1015, 1030, 1045, 1115, 1130, 1145, 1315, 1330, 1345, 1400, 1415, 1430, 1445, 1515, 1530, 1545, 1600, 1615,

and 1630 h. Most walking bouts were separated by 15 min of seated rest but three longer breaks were incorporated to allow for blood sampling and consumption of lunch. This timing was chosen because we have shown that a similar pattern of walking can reduce postprandial TG in healthy, older women [16] and older women with hypertriglyceridaemia [17]. Heart rate was monitored throughout the accumulated walking bouts using short-range telemetry (Polar RCX3, Polar Electro, Kempele, Finland), and ratings of perceived exertion (RPE) were assessed periodically [18].

Accumulated walking with energy replacement. The accumulated walking with energy replacement trial was identical to the accumulated walking trial except that the gross energy expended during accumulated walking (estimated during the preliminary visit) was provided at breakfast. The exercise-induced gross energy expenditure was replaced using white bread and was prescribed individually (0.62 ± 0.12 MJ (149 ± 28 kcal), 2.4 ± 0.4 g fat, 27.6 ± 5.1 g carbohydrate, 4.5 ± 0.8 g protein).

Test meals

Breakfast consisted of white bread with butter, a salad (made of lettuce, tomato, and ham with an Italian dressing), scrambled egg (one egg with tomato ketchup and soybean

oil), soup (made of whole milk with corn soup powder), apple, and yoghurt. In the control and accumulated walking trials, breakfast provided 0.34 g fat, 1.15 g carbohydrate, 0.35 g protein, and 38 kJ (9 kcal) energy per kilogram of body mass. In the accumulated walking with energy replacement trial, breakfast provided 0.39 ± 0.01 g fat, 1.68 ± 0.14 g carbohydrate, 0.44 ± 0.02 g protein, and 50 ± 3 kJ (12 ± 1 kcal) energy per kilogram of body mass including replacement of the estimated gross energy expenditure induced by exercise. For the control and accumulated walking trials, mean macronutrient content of the breakfast was 18.2 ± 2.4 g fat, 61.5 ± 8.2 g carbohydrate, and 18.7 ± 2.5 g protein, which provided 2.0 ± 0.3 MJ (486 ± 65 kcal) energy (34% from fat, 51% from carbohydrate, and 15% from protein). For the accumulated walking with energy replacement trial, mean macronutrient content of the breakfast was 20.6 ± 2.4 g fat, 89.1 ± 9.0 g carbohydrate, and 23.2 ± 2.5 g protein, which provided 2.7 ± 0.3 MJ (635 ± 67 kcal) energy ($29.1 \pm 0.9\%$ from fat, $56.2 \pm 1.1\%$ from carbohydrate, and $14.6 \pm 0.2\%$ from protein). Lunch consisted of a typical Japanese fish dish (made from grilled salmon), a bowl of white rice, soup (made with soybean curd, seaweed, soybean paste, and deep-fried soybean curd), and steamed vegetables (cabbage, carrot, cucumber, potato, and broccoli with a mayonnaise dressing) with ham and a cream cracker. It provided 0.34 g fat, 1.10 g carbohydrate, 0.35 g protein, and 38 kJ (9 kcal) energy per

kilogram of body mass in all trials. Mean macronutrient content of the lunch was 18.7 ± 2.5 g fat, 59.8 ± 7.8 g carbohydrate, and 18.2 ± 2.4 g protein, which provided 2.0 ± 0.3 MJ (486 ± 65 kcal) energy (35% from fat, 50% from carbohydrate, and 15% from protein). Participants were asked to consume each test meal within 30 min, and consumption time was recorded and replicated in subsequent trials. Mean time to consume breakfast and lunch was 20.1 ± 3.7 and 16.2 ± 5.9 min, respectively. None of the participants reported nausea or any gastrointestinal discomfort during or after either meal. Participants consumed water ad libitum during the first trial, and the pattern and volume ingested was replicated in subsequent trials. Average water intake was 904 ± 332 mL over 8 h.

Analytical methods

For serum TG, NEFA and 3-OHB measurements, venous blood samples were collected into tubes containing clotting activators for isolation of serum (Venoject 2; Terumo Corporation, Tokyo, Japan). Thereafter, samples were allowed to clot for 30 min at room temperature and then centrifuged at 1861g for 10 min at 4°C. Serum was removed, divided into aliquots, and stored at -80°C for later analysis. For plasma insulin measurements, venous blood samples were collected into tubes containing dipotassium

salt-EDTA (Venoject 2; Terumo Corporation, Tokyo, Japan). For plasma glucose measurements, venous blood samples were collected into tubes containing sodium fluoride-EDTA (Venoject 2; Terumo Corporation, Tokyo, Japan). Thereafter, both tubes were immediately centrifuged and the plasma supernatant was stored at -80°C for later analysis. Enzymatic, colorimetric assays were used to measure serum TG (Pure Auto S TG-N; Sekisui Medical Co Ltd, Tokyo, Japan), serum NEFA (NEFA-HR; Wako Pure Chemical Industries, Ltd, Osaka, Japan), serum 3-OHB (KAINOS 3-HB; Kainos Laboratories, Inc, Tokyo, Japan), and plasma glucose (GLU-HK(M); Shino-Test Corporation, Kanagawa, Japan). Enzyme-linked immunosorbent assays (ELISA) were used to measure plasma insulin (Mercodia Insulin ELISA; Mercodia AB, Uppsala, Sweden). Samples for each participant were analysed within the same run for each measure. Intra-assay coefficients of variation were 0.7% for TG, 0.8% for NEFA, 0.7% for 3-OHB, 4.3% for insulin, and 0.7% for glucose.

Statistical analysis

Data were analysed using the Predictive Analytics Software (PASW) version 25.0 for Windows (IBM SPSS Statistics 25.0, SPSS Japan Inc., Japan). The Shapiro-Wilk test was used to check for normality of distribution - all parameters were found to be

normally distributed. Time-averaged total area under the serum or plasma concentration versus time curves (AUC) were calculated using the trapezium rule. Repeated-measures one-factor analysis of variance (ANOVA) was used to assess differences among the three trials for fasting serum or plasma concentrations, AUC values, and dietary and physical activity data. Repeated measures, two-factor ANOVA was used to examine differences over time among the three trials for serum TG. Repeated measures generalised estimating equations were performed as a sensitivity analysis to examine between-trial differences in the time-averaged AUC values with the respective fasting serum or plasma concentration modeled as a covariate. Where significant trial-by-time interactions and trial effects were found, the data were subsequently analysed using post-hoc analysis and were adjusted for multiple comparisons using the Bonferroni method. The 95% confidence interval (95% CI) for the mean absolute pairwise differences between the trials was calculated using the t-distribution and degrees of freedom ($n - 1$). Absolute standardised effect sizes (ES) are provided to supplement the findings. An ES of 0.2 was considered the minimum important difference in all outcome measurements, 0.5 moderate and 0.8 large [19]. Data are expressed as means $\pm$ SD. Statistical significance was set at $p < 0.05$.

259   **Results**

260   **Dietary and body mass data**

261   Mean self-reported energy intake for the day prior to each trial was 7.5 ± 2.0 MJ (1796
262   ± 457 kcal). Energy intake equated to 18 ± 5 % (67.4 ± 29.7 g/day) from fat, 61 ± 5 %
263   (221.9 ± 58.8 g/day) from carbohydrate and 21 ± 5 % (74.9 ± 16.8 g/day) from protein.
264   Body mass did not differ on the morning of each main trial (53.2 ± 6.8 vs 53.0 ± 7.0 vs
265   52.7 ± 6.9 kg for the control, accumulated walking and accumulated walking with
266   energy replacement trials respectively; ES = 0.081, main effect of trial $p = 0.256$).

267

268   **Physical activity data**

269   The step counts recorded the day before the trials did not differ across trials ($8426 \pm$
270   3844 vs 8511 ± 3644 vs 7981 ± 3197 steps per day for the control, accumulated walking
271   and accumulated walking with energy replacement trials respectively; ES = 0.017, main
272   effect of trial $p = 0.755$). Accelerometer recorded frequencies for light (levels 1-3; $63 \pm$
273   30 vs 62 ± 22 vs 54 ± 26 min/day for the control, accumulated walking and accumulated
274   walking with energy replacement trials, respectively; ES = 0.123, main effect of trial p
275   = 0.159), moderate (levels 4-6; 31 ± 22 vs 28 ± 19 vs 22 ± 18 min/day for the control,
276   accumulated walking and accumulated walking with energy replacement trials,

respectively; ES = 0.211, main effect of trial $p = 0.105$), and vigorous (levels 7-9; 2 ± 2 vs 2 ± 1 vs 2 ± 3 min/day for the control, accumulated walking and accumulated walking with energy replacement trials, respectively; ES = 0.116, main effect of trial $p = 0.414$) activity did not differ across trials.

Responses during accumulated walking

Self-selected brisk walking speed during the walking trials was 4.1 ± 1.0 km/h. The mean heart rate and RPE did not differ between the accumulated walking and accumulated walking with energy replacement trials (heart rate, 91 ± 12 vs 90 ± 12 beats/min, respectively; 95% CI, -2 to 4 beats/min, ES = 0.243, $p = 0.546$; RPE, 10 ± 1 vs 10 ± 1 , respectively; 95% CI, -3 to 1, ES = 0.243, $p = 0.397$).

Fasting serum/plasma concentrations

Fasting plasma and serum concentrations for each trial are shown in Table 2. There were no differences across trials in serum TG, NEFA, 3-OHB, and plasma insulin and glucose concentrations (main effect of trial $p \geq 0.128$).

Insert Table 2 near here

295

296 **Postprandial serum/plasma concentrations**

297 Serum TG concentrations differed among trials (Fig. 2) (ES = 0.226, main effect of trial
298 $p = 0.017$) and were lower in the accumulated walking than control trial (95% CI -
299 0.471 to -0.320 mmol/L, $p = 0.022$). However, the accumulated walking with energy
300 replacement trial was not different from the accumulated walking (95% CI -0.043 to
301 0.325 mmol/L, $p = 0.172$) or control (95% CI -0.364 to 0.143 mmol/L, $p = 0.783$) trials.
302 Post-hoc analysis of an interaction effect ($p = 0.011$) revealed that TG concentrations
303 were lower in the accumulated walking than control trial at 4 h (95% CI -0.690 to
304 -0.075 mmol/L, $p = 0.013$), and 8 h after breakfast (95% CI -0.591 to -0.056 mmol/L, p
305 = 0.016). Moreover, TG was lower with accumulated walking than accumulated
306 walking with energy replacement (95% CI -0.442 to -0.040 mmol/L, $p = 0.017$) at 8 h.

307

308 The time-averaged TG AUC (ES = 0.207, main effect of trial $p = 0.024$) (Fig. 3) was
309 14% lower in the accumulated walking trial than the control trial (95% CI -0.491 to
310 -0.022 mmol/L h, $p = 0.030$). Accumulated walking with energy replacement values for
311 time-averaged TG AUC did not differ from values in the accumulated walking (95% CI
312 -0.057 to 0.331 mmol/L h, $p = 0.223$) or control (95% CI -0.396 to 0.157 mmol/L h, $p =$

0.795) trials. A sensitivity analysis using generalised estimating equations for the time-averaged TG AUC to adjust for fasting TG concentrations also revealed a main effect of trial ($p = 0.004$) with lower time-averaged TG AUC in the accumulated walking than control trial (95% CI -0.423 to -0.009 mmol/L, $p = 0.003$). However, the accumulated walking with energy replacement trial was not different from the accumulated walking (95% CI -0.275 to 0.001 mmol/L, $p = 0.052$) or control (95% CI -0.077 to 0.316 mmol/L, $p = 0.234$) trials. Please note that the results are similar to the ANOVA and therefore do not alter the interpretation of the data.

Insert Fig. 2 near here

Insert Fig. 3 near here

The time-averaged AUC values for NEFA, 3-OHB, insulin, and glucose are shown in Table 3. For NEFA (ES = 0.837, main effect of trial $p < 0.0005$) the AUC was lower on the accumulated walking with energy replacement trial than the control (95% CI -0.346 to -0.203 mmol/L h, $p < 0.0005$) and the accumulated walking (95% CI -0.381 to -0.246 mmol/L h, $p < 0.0005$) trials. The time-averaged 3-OHB and glucose AUC did not differ among trials. The time-averaged insulin (ES = 0.259, main effect of trial $p =$

0.018) AUC was lower on the accumulated walking than control (95% CI -26.47 to -0.36 pmol/L h, $p = 0.043$) and accumulated walking with energy replacement (95% CI -51.67 to -2.31 pmol/L h, $p = 0.030$) trials. A sensitivity analysis using generalised estimating equations for the time-averaged AUC values for NEFA, 3-OHB, insulin and glucose to adjust for the respective fasting serum or plasma concentrations did not alter the findings for any of the postprandial outcomes (data not shown). Mean values at each time-point in the three trials for NEFA, 3-OHB, insulin and glucose concentrations are provided in Supplementary Table 1.

Insert Table 3 near here

Discussion

The present study demonstrated that accumulating short bouts of brisk walking (1.5 min $\times$ 20) throughout the day reduced postprandial TG in older women but that dietary replacement of the exercise-induced energy deficit diminished this effect. This underscores the importance of maintaining an energy deficit in relation to exercise to augment the reduction in postprandial TG even when exercise is accumulated in short bouts during the day.

349

350 The diminished postprandial TG-lowering effect of accumulated walking with
351 replacement of the exercise energy expenditure is consistent with previous studies in
352 adolescent boys [8], young men and women [6, 7] and sedentary middle-age men [5]
353 when the postprandial measurements were conducted the day after exercise. The present
354 study extends these findings by demonstrating that the exercise-induced reduction in
355 postprandial TG is diminished on the same day exercise is performed, and this effect
356 persisted after adjusting for fasting concentrations. In contrast, other studies have
357 demonstrated that acute exercise reduced postprandial TG concentrations even when the
358 energy expended during exercise was replaced in young men and women [7, 9-11].
359 Differences in the intensity of exercise, and the composition and timing of the energy
360 replacement meals/drinks are possible reasons for the inconsistent findings among
361 studies. Since the intensity of exercise influences substrate partitioning during exercise
362 [20], the macronutrient composition of the energy replacement meal/drink may also
363 influence the subsequent postprandial TG response [9, 10]. Previous studies by Chiu
364 and colleagues [9] suggested that the exercise-induced fat deficit may be important,
365 whereas Trombold and colleagues [10] suggested the carbohydrate deficit may be
366 important in determining the reduction in postprandial TG after exercise. Alternatively,

another study has suggested that the type of carbohydrate, low or high glycemic index, is also an important factor [11]. It was not possible to isolate whether the exercise-induced substrate deficit or the energy deficit per se were more important for determining the reduction in postprandial TG in the present study and further work is required to differentiate this [4].

The present findings suggest that the effect of exercise on postprandial TG concentrations in these older women is dependent on an exercise-evoked energy deficit, at least on the same day exercise is performed. Nevertheless, it is important to note that an acute exercise-induced energy deficit appears more potent for provoking a reduction in postprandial TG concentrations than an isoenergetic diet-induced energy deficit [21, 22], suggesting the effect of exercise is not solely attributable to the resulting energy deficit.

One unique feature of the present investigation which extends previous studies is that the extra energy provided preceded exercise - at breakfast -, rather than being given as a post-exercise energy replacement. The walking accumulated throughout the day was unable to attenuate TG elevations brought about by this extra pre-exercise energy. The

ecological value of performing the experiment in this manner can be debated. Some individuals may compensate for an exercise-induced energy expenditure immediately afterwards. However, it also seems feasible that some individuals may perform compensatory eating beforehand e.g. eat a big breakfast when they realise that they have an active day ahead. Thus, the present study has novel features related to the order of energy intake that have not previously been addressed. Despite these arguments, the issue of compensatory eating in response to acute exercise has been challenged [23].

The reduction in postprandial TG concentrations observed in the accumulated walking trial may have been mediated via several mechanisms although it is difficult to ascertain which was acting or dominant. Reduced secretion of hepatic very-low density lipoproteins seems unlikely as postprandial serum 3-OHB concentrations, an indicator of hepatic fatty acid oxidation, were similar among trials, suggesting TG incorporation into these lipoproteins did not differ. Similarly, chylomicron appearance was unlikely affected with walking as blood flow to the splanchnic organs is not greatly compromised with moderate exercise [24]. The lower postprandial plasma insulin concentrations in the accumulated walking trial may have reduced insulin-mediated inhibition of skeletal muscle lipoprotein lipase activity and therefore enhanced TG

clearance at this site [25]. This effect is unlikely to have occurred in the accumulated walking with energy replacement trial where the extra energy intake with white bread increased insulin concentrations. Finally, increased skeletal muscle blood flow during and immediately after each accumulated walk may have enhanced TG clearance by increasing exposure of lipoprotein lipase to TG-rich lipoproteins. This effect may have been offset with higher TG concentrations in the energy replacement condition.

The reduction in postprandial TG after accumulating short bouts of walking is in accord with previous investigations in older women [16, 17]. However, the present study is the first to compare accumulating short bouts of walking with and without dietary replacement of the exercise-induced energy deficit on postprandial TG in older women. It is not known whether this population would regularly engage in this intermittent brisk walking pattern outside the laboratory setting. Nonetheless, we have previously found that encouraging older postmenopausal women to increase their weekend engagement in moderate-to-vigorous physical activity (average increase of 16 min in total) reduced postprandial TG concentrations under free-living conditions [26]. Moreover, another study has shown that middle-aged Japanese women typically engage in moderate-to-vigorous physical activity bouts lasting < 3 min [13]. Thus, the activity

pattern adopted in the present study has ecological relevance and may be attractive to many older adults as it can be easily incorporated into their day-to-day lives. Furthermore, although the clinical relevance of the present study is not known, it has been demonstrated recently that total moderate-to-vigorous physical activity of any bout duration is associated with a lower risk of all-cause mortality [27]. Nonetheless, it would be of future interest to examine the longer-term effects of accumulating short bouts of daily physical activity on postprandial TG and other clinical outcomes in a larger population.

The present study has several strengths. First, most studies examining the effects of exercise on postprandial TG have employed young individuals with few studies in individuals over 40 years (for a review of these, see [3]). The population recruited to the present study is important since a substantial increase in the risk of cardiovascular disease has been observed with higher concentrations of non-fasting TG and with increasing age [2]. Furthermore, in many Asian countries, like Japan, recent temporal shifts toward top-heavy elderly population structures [28] means that lifestyle interventions shown to mitigate disease risk in older adults are becoming more critical. Second, test meals of moderate-fat content (35% of total energy) were used to assess

postprandial metabolism in response to exercise with and without dietary replacement of the exercise-induced energy deficit. Most studies in this field have employed high-fat test meals (over 60% of total energy) (for a review of these, see [29, 30]). Thus, our test meals mimic real life settings where the macronutrient composition of the test meals reflects the background diet consumed in approximately one-third of Japanese adults (34 % of Japanese women aged over 60 years consume daily meals containing over 30 % of energy as fat) [31].

There are limitations to the experimental design employed. Expired air samples were not collected to quantify directly the energy expenditure during the walking trials for practical reasons given the short duration of the exercise bouts. This absence of energy expenditure quantification may under- or over-estimate replacement of the energy deficit in the accumulated walking with energy replacement trial. Furthermore, the replacement of the exercise gross energy expenditure may have overestimated the exercise-induced energy deficit. However, the difference between the net and gross exercise energy expenditure is likely to be relatively small given the short duration and low intensity of the exercise stimulus.

In conclusion, accumulating 30 min of brisk walking in short (1.5 min) bouts reduced postprandial TG but this effect was diminished when the exercise-induced energy expenditure was replaced in older postmenopausal women. The findings of the present study highlight the importance of maintaining an exercise-induced energy deficit on exercise days to maximise the reduction in postprandial TG in older postmenopausal women.

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Author contributions

M. M. conceived the study, obtained the funding and took the lead in writing the

manuscript. Y. H., K. F. and C. N. supervised the data collection, assisted with all aspects of the biochemistry and performed the data analysis. M. T., S. F. B., A. E. T. and D. J. S. performed the data interpretation and wrote the manuscript. All authors approved the final version of the manuscript.

Compliance with ethical standards

Ethical standard

This study was approved by the Ethics Committee on Human Research of Waseda University (Approval number: 2017-103). Written informed consent was obtained from all participants prior to the study.

Conflict of interest

All authors declare that there is no conflict of interest.

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Figure legends

Fig. 1 A schematic representation of the study protocol.

For the control trial, participants sat in a chair (reading and writing) in the laboratory between 0 h (0900 h) and 8 h (1700 h). For the accumulated walking and accumulated walking with energy replacement trials, participants rested for 40 minutes after consuming breakfast before performing twenty, 1.5-min bouts of brisk walking on a treadmill throughout the day. Rep.; replacement. 0.62 ± 0.12 MJ (149 ± 28 kcal).

Fig. 2 Fasting and postprandial serum triglyceride (TG) concentrations during the control, accumulated walking and accumulated walking with energy replacement trials.

Data are means $\pm$ SD for $n = 17$. The black rectangles indicate the times that the test meals were consumed. Data were analysed using 2-factor ANOVA. Post-hoc analysis was adjusted for multiple comparisons using the Bonferroni method. There was a significant main effect of trial ($p = 0.017$), main effect of time ($p < 0.0005$) and trial $\times$ time interaction ($p = 0.011$). * Significantly different between accumulated walking and control trials. † Significantly different between accumulated walking and accumulated walking with energy replacement trials.

611

612 **Fig. 3 The time-averaged serum triglyceride (TG) area under the curve (AUC)**
613 **values over 8 h after the consumption of the test meals in the control, accumulated**
614 **walking and accumulated walking with energy replacement trials.**

615 Data are means $\pm$ SD for n = 17. Means were compared using one-factor ANOVA.

616 Post-hoc analysis was adjusted for multiple comparisons using the Bonferroni method. *

617 Significantly different from the control trial, $p < 0.05$.

618 **Table 1.** Physical characteristics of the 17 participants.

Characteristic	Mean (SD)
Age (years)	70.2 (2.9)
Height (m)	1.55 (0.05)
Body mass (kg)	53.5 (7.1)
Body mass index (kg/m ²)	22.3 (3.0)
Waist circumference (cm)	79.2 (9.2)
Systolic blood pressure (mmHg)	133 (18)
Diastolic blood pressure (mmHg)	81 (11)

619 **Table 2.** Fasting concentrations of triglyceride (TG), non-esterified fatty acids (NEFA), 3-hydroxybutyrate (3-OHB), insulin and
620 glucose in the control, accumulated walking and accumulated walking with energy replacement trials.

Trial	Control	Accumulated walking	Accumulated walking with energy replacement	Control vs accumulated walking 95% CI ^a	Control vs accumulated walking with energy replacement 95% CI ^a	Accumulated walking with energy replacement vs accumulated walking 95% CI ^a
TG (mmol/L)	1.06 (0.60)	0.95 (0.59)	1.02 (0.56)	-0.26 to 0.03	-0.19 to 0.11	-0.21 to 0.07
NEFA (mmol/L)	0.62 (0.22)	0.65 (0.27)	0.64 (0.25)	-0.11 to 0.18	-0.11 to 0.15	-0.10 to 0.13
3-OHB (mmol/L)	0.10 (0.08)	0.11 (0.12)	0.11 (0.10)	-0.05 to 0.07	-0.06 to 0.07	-0.05 to 0.06
Insulin (pmol/L)	21.6 (11.5)	22.8 (15.1)	25.4 (20.0)	-6.44 to 8.77	-4.60 to 12.06	-10.60 to 5.44
Glucose (mmol/L)	5.28 (0.67)	5.27 (1.04)	5.29 (0.96)	-0.37 to 0.36	-0.22 to 0.25	-0.28 to 0.24

621
622 Values are mean (SD) for n = 17. Means were compared using one-factor ANOVA. Analysis revealed no main effect of trial for any

623 outcome (all $p \geq 0.128$). ^a 95% confidence interval (CI) of the mean absolute difference between the experimental conditions.

624 **Table 3.** The time-averaged serum non-esterified fatty acids (NEFA), 3-hydroxybutyrate (3-OHB), and plasma insulin and glucose area
625 under the curve (AUC) values over 8 h after the consumption of the test meals in the control, accumulated walking and accumulated
626 walking with energy replacement trials.

Trial	Control	Accumulated walking	Accumulated walking with energy replacement	Control vs accumulated walking 95% CI ^a	Control vs accumulated walking with energy replacement 95% CI ^a	Accumulated walking with energy replacement vs accumulated walking 95% CI ^a
NEFA AUC (mmol/L h)	0.31 (0.12)	0.35 (0.12)	0.04 (0.02)	-0.003 to 0.080	-0.346 to -0.203 **	0.246 to 0.381 ***
3-hydroxybutyrate AUC (mmol/L h)	0.03 (0.01)	0.04 (0.02)	0.03 (0.02)	-0.005 to 0.014	-0.008 to 0.008	-0.004 to 0.012
Insulin AUC (pmol/L h)	94.8 (75.4)	81.3 (73.5)	108.3 (76.9)	-26.47 to -0.36 *	-11.23 to 38.38	-51.67 to -2.31 ***
Glucose AUC (mmol/L h)	6.11 (1.00)	6.05 (1.20)	6.01 (1.01)	-0.43 to 0.32	-0.29 to 0.10	-0.32 to 0.39

627 Values are mean (SD) for n = 17. Means were compared using one-factor ANOVA and post-hoc analysis was adjusted for multiple
628 comparisons using the Bonferroni method. ^a 95% confidence interval (CI) of the mean absolute difference between the experimental

629 conditions. Analysis revealed a main effect of trial for NEFA ($p < 0.0005$) and insulin ($p = 0.018$). Post-hoc analysis of the main effect
630 of trial: * $p < 0.05$ between accumulated walking and control; ** $p < 0.05$ between accumulated walking with energy replacement and
631 control; *** $p < 0.05$ between accumulated walking and accumulated walking with energy replacement.

632