British Journal of Nutrition (2020), 124, 1035–1043 doi:10.1017/S0007114520002238
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Comparison of whole egg *v.* egg white ingestion during 12 weeks of resistance training on skeletal muscle regulatory markers in resistance-trained men

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(Submitted 11 March 2020 – Final revision received 2 June 2020 – Accepted 12 June 2020 – First published online 24 June 2020)

Abstract

Eggs are considered a high-quality protein source for their complete amino acid profile and digestibility. Therefore, this study aimed to compare the effects of whole egg (WE) *v.* egg white (EW) ingestion during 12 weeks of resistance training (RT) on the skeletal muscle regulatory markers and body composition in resistance-trained men. Thirty resistance-trained men (mean age 24·6 (SD 2·7) years) were randomly assigned into the WE + RT (WER, *n* 15) or EW + RT (EWR, *n* 15) group. The WER group ingested three WE, while the EWR group ingested an isonitrogenous quantity of six EW per d immediately after the RT session. Serum concentrations of regulatory markers and body composition were measured at baseline and after 12 weeks. Significant main effects of time were observed for body weight (WER 1·7, EWR 1·8 kg), skeletal muscle mass (WER 2·9, EWR 2·7 kg), fibroblast growth factor-2 (WER 116·1, EWR 83·2 pg/ml) and follistatin (WER 0·05, EWR 0·04 ng/ml), which significantly increased ($P < 0·05$), and for fat mass (WER −1·9, EWR −1·1 kg), transforming growth factor-β1 (WER −0·5, EWR −0·1 ng/ml), activin A (WER −6·2, EWR −4·5 pg/ml) and myostatin (WER −0·1, EWR −0·06 ng/ml), which significantly decreased ($P < 0·05$) in both WER and EWR groups. The consumption of eggs absent of yolk during chronic RT resulted in similar body composition and functional outcomes as WE of equal protein value. EW or WE may be used interchangeably for the dietary support of RT-induced muscular hypertrophy when protein intake is maintained.

Key words: Resistance training: Egg consumption: Body composition: Skeletal muscle mass: Whole eggs

Resistance training (RT) is acknowledged as an effective modality for enhancing muscular strength and hypertrophy[1]. The mechanical tension and metabolic stress imposed on skeletal muscle via RT modalities, when combined with proper nutritional factors, can result in a net anabolic response in myofibrillar protein metabolism, leading to myofiber hypertrophy and muscular growth over time[2]. The stimuli for myofibrillar protein synthesis (MPS) are multifactorial and include hormones, growth factors, cytokines, mechano-transduction and the constituents of dietary protein known as amino acids.

Post-exercise protein ingestion is suggested to increase MPS through the provision of sufficient amino acids, particularly leucine, thus activating the key anabolic signalling mechanism of the mechanistic target of rapamycin (mTOR)[3,4]. Consequently, dietary protein intake is considered an essential component in the optimisation of skeletal muscle adaptations to RT. Eggs are also considered a high-quality protein source by most standards. According to the Digestible Indispensable Amino Acid Score[5], whole egg (WE) is comparable to other high-quality protein sources that often accompany RT programmes, such as whey, casein and soy protein[6–9]. In addition, WE has a nitrogen protein utilisation evaluation of 98 %, which is comparable to whey and casein. Finally, the biological value of egg rates between 88 and 100, with only whey and casein being

Abbreviations: 1RM, one-repetition maximum; ACVA, activin A; BW, body weight; EW, egg white; FGF-2, fibroblast growth factor-2; FLST, follistatin; MPS, myofibrillar protein synthesis; MSTN, myostatin; mTOR, mechanistic target of rapamycin; mTORC1, mTOR complex 1; RT, resistance training; SMM, skeletal muscle mass; TGF-β1, transforming growth factor-β1; WE, whole egg.

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British Journal of Nutrition

https://doi.org/10.1017/S0007114520002238 Published online by Cambridge University Press

higher[10]. Accordingly, WE may be a viable alternative to the previously mentioned supplemental proteins given its similar ratings on various measures of dietary protein quality.

It is commonplace, however, for individuals to remove the egg yolk in an attempt to limit the ingestion of energy-dense dietary lipids and cholesterol, especially when multiple eggs are ingested[11]. There is an unsubstantiated notion that the cholesterol and fat content of egg yolk dramatically increases the risk of dyslipidaemia and CVD[12]. However, the egg yolk contains approximately 40 % of the total protein contained in WE[13]. Accordingly, the removal of yolk would greatly decrease the protein content of the egg, and either fails to provide a sufficient amino acid stimulus or requires a greater quantity of eggs to be consumed per sitting, especially in RT populations[14]. Additionally, even when matched for protein, there is evidence that WE is more effective than egg white (EW) alone at promoting molecular changes associated with muscular adaptions. Specifically, it has been previously observed that WE consumption promotes greater stimulation of post-exercise MPS compared with EW in young men, presumably due to non-protein nutrients found within the egg yolk[11,15]. While these acute findings are notable, no information is currently available to determine if the variability in MPS responses would result in measurable tissue changes over time. Additionally, whether or not WE *v.* egg yolk consumption produces differential effects on skeletal muscle regulatory markers in response to chronic RT has not been previously evaluated. Since active populations are often recommended to consume high-protein nutrient-dense foods, it is important to define how the ingestion of such foods modulates protein metabolism when combined with RT. Therefore, the aim of this investigation was to compare the effects of WE *v.* EW ingestion (daily and after training sessions) during 12 weeks of RT on the skeletal muscle regulatory markers in resistance-trained men. We hypothesised that RT with WE ingestion would differentially influence skeletal muscle regulatory markers compared with the protein-matched ingestion of EW due to the presence of non-protein nutrients in egg yolk.

Experimental methods

Participants

Thirty resistance-trained men (mean age 24·6 (SD 2·7) years and height 174·4 (SD 5·7) cm) were recruited to participate in this study. Participants were considered trained if they had performed RT at least three times a week for 1 year prior to the start of the study. Additionally, participants were not taking any dietary supplements or medications for at least 1 year prior to enrolment in the study, had no known medical issues, did not use alcohol or smoke or had egg allergy/sensitivity. Possible participants were excluded from participating through failure to meet any of the previously stated criteria. Additional exclusions were non-willingness to continue nutritional or exercise protocols, participation in exercise other than the prescribed RT programme throughout the length of the investigation, consumption of any dietary supplement during the study period and missing more than one RT session or post-exercise egg consumption throughout the study period. A physician evaluated all these criteria using PAR-Q and the medical health/history questionnaire. All the protocols were approved by the Institutional Review Board of Tehran University and the study was registered in Clinicaltrials.gov (NCT04381390). Participants provided informed consent before study enrolment, and all procedures were carried out in accordance with the Declaration of Helsinki.

Design

Before baseline measurements, participants were fully familiarised with all testing and experimental procedures. Participants were randomly assigned to one of two groups: WE + RT (WER, *n* 15) or EW + RT (EWR, *n* 15). Measurements were collected at two time-points – baseline and 12 weeks post-RT. Final assessments took place approximately 72 h after the last exercise bout in order to minimise potential acute influences of RT on outcome variables. All measurements were recorded at the same time of day (within about 1 h) and under the same environmental conditions (approximately 20°C and 55 % humidity). Participants were instructed not to alter their regular lifestyle and dietary habits during the study. A schematic design of the study is shown in Fig. 1.

Egg ingestion

The WER group ingested three WE (with yolk), while the EWR group ingested an isonitrogenous quantity of six EW (no yolk) immediately after each RT session and in the morning on non-training days. The ingredients of three WE and six EW[16] are shown in Table 1. Eggs were scrambled in a skillet until solid with no visible liquid remaining[15]. Including the eggs supplied (≈20 g protein), participants were instructed to consume approximately 1·4–1·5 g of protein per kg of body weight (BW) per d, which was based on the recommendations of the Academy of Nutrition and Dietetics, Dietitians of Canada and the American College of Sports Medicine (1·2–1·7 g of protein per kg of body mass per d) for individuals aiming to increase skeletal muscle mass (SMM) in combination with physical activity[17]. As our participants were students living in a dormitory, we visited them every day and monitored their cooking and egg ingestion to ensure accuracy. In addition, participants were monitored regularly through the use of a group application (WhatsApp and Telegram).

Anthropometry and body composition

Upon arriving at the laboratory, participants were asked to urinate (void) completely within 30 min of the test. We instructed the participants to fast for 12 h (an overnight fast, with at least 8 h of sleep) and refrain from exercising and consuming alcohol for 48 h before the test. Participants were also asked to appear for testing in a normally hydrated state after an overnight fast. BW was measured with a digital scale (Lumbar) to the nearest 0·1 kg. The participant's stature was measured with a stadiometer (Race Industrialization) to the nearest 0·1 cm. BMI, fat mass (FM) and SMM were evaluated using a multi-frequency bioelectrical impedance device (Inbody 720)[1]. Prior to the measurement, the participant's palms and soles were cleaned with an electrolyte tissue.

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https://doi.org/10.1017/S0007114520002238 Published online by Cambridge University Press

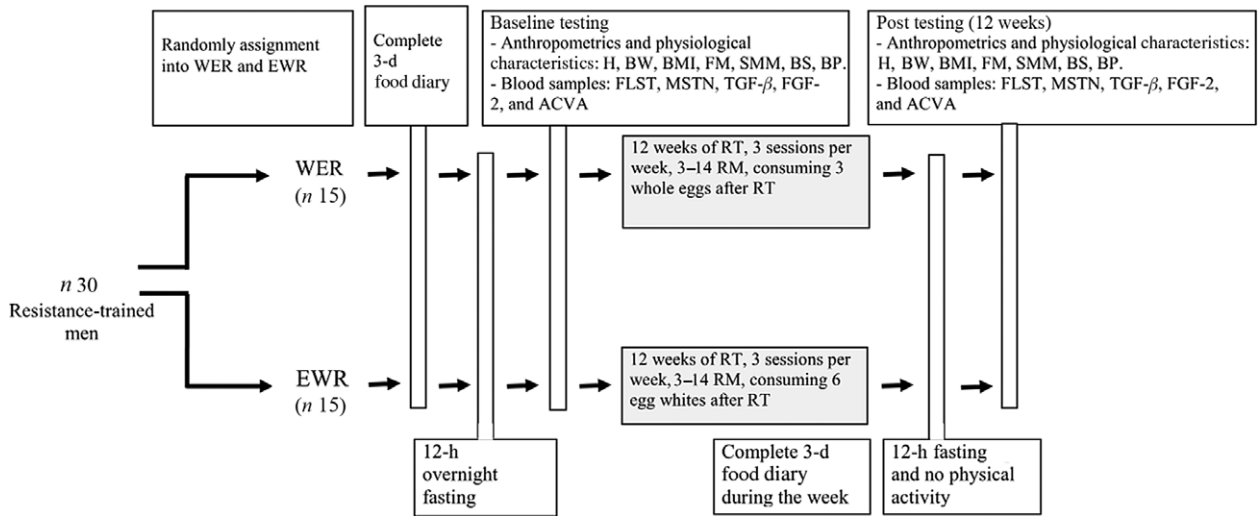

Fig. 1. Schematic of the study design. Both baseline and post-testing (12 weeks) were conducted between 08.00 and 08.30 hours after a 12-h overnight fast and avoidance of exercise. Three-day food diaries were recorded before and at study end in both groups. WER, whole egg + resistance training; EWR, egg white + resistance training; RT, resistance training; RM, repetition maximum; BW, body weight; H, height; FM, fat mass; SMM, skeletal muscle mass, BS, back squat; BP, bench press; FLST, follistatin; MSTN, myostatin; TGF-β, transforming growth factor-β; FGF-2, fibroblast growth factor-2; ACVA, activin A.

Table 1. Energy and nutrient composition of three whole eggs and six egg whites

Content per dose	Three whole eggs (≈160 g)	Six egg whites (≈190 g)
Energy (kcal)*	246·4	89·3
Total fat (g)	19	0·2
SFA (g)	7	–†
Unsaturated fatty acids (g)	11·2	–
Cholesterol (mg)	672	0
Carbohydrates (g)	1·1	1·5
Protein (g)	19·7	20·5
Leucine (mg)	1056	1064
Isoleucine (mg)	461	456
Valine (mg)	304	323
Vitamin D (μg)	2·4	0
Vitamin A (μg)	240	0
Thiamine (mg)	1·4	0·02
Riboflavin (mg)	715	817
Niacin equivalents (mg)	126	171
Fe (mg)	2·7	0·2

* To convert energy values from kcal to kJ, multiply by 4·184.
† Undetectable.

Participants then stood on InBody 720, placing the soles of their feet on the electrodes. Age and sex were manually entered into the display by a researcher, and the instrument derived each participant's BW. To establish FM and SMM, participants then grasped the handles of the unit ensuring that the palm and fingers of each hand made direct contact with the electrodes, with arms fully extended and abducted at approximately 20°[18].

Blood sampling and analysis

Fasting blood samples (5 ml) were collected from the antecubital vein using standard procedures. Following blood sampling, the samples were centrifuged at 3000 rpm for 10 min, and serum was stored at –80°C for a future analysis of fibroblast growth factor-2 (FGF-2; Abcam), transforming growth factor-β1 (TGF-β1; Cusabio), activin A (ACVA; Cusabio), follistatin (FLST; Cusabio) and myostatin (MSTN; Cusabio). Intra- and inter-assay CV for ACVA, TGF-β1, FGF-2, FLST and MSTN was <8 and 10 %, respectively.

Strength testing

Strength testing was performed 24 h following body composition and blood sampling assessment. One-repetition maximum (1RM) testing for the back squat and bench press was implemented according to procedures outlined by the National Strength and Conditioning Association[19]. The 1RM value was also used to determine the relative training intensity of the RT programme[19]. Participants were instructed to refrain from caffeinated drinks for 12 h and food intake for 2 h prior to the testing session; however, water consumption was allowed[1].

Resistance training programme

Participants were familiarised with all testing and training procedures before the onset of the study to minimise the influence of learning effects on dependent measures. The RT programme consisted of a 12-week (thirty-six sessions) non-linear periodised programme. The RT programme stressed all major muscle groups and included the following exercises, or variations of these, in each session: bench press, back squat, lunges, shoulder press, arm curls, stiff-leg deadlift, lat pull down, seated row, calf raises and sit-ups. RT volume and intensity progressed during the training programme according to previous recommendations[19,20]. We used a planned non-linear periodisation RT programme with varying daily training prescriptions during the week. Briefly, training days were split into 'light', 'moderate' and 'heavy' days in a non-linear periodised manner. RM zones were used to progress intensity. 'Light' days consisted of a 12–14 RM zone (three sets), 'moderate' days consisted of an 8–10 RM zone (three sets), and 'heavy' days consisted of a 3–5 RM zone (three sets but five sets for back squat and bench

British Journal of Nutrition

https://doi.org/10.1017/S0007114520002238 Published online by Cambridge University Press

press exercises). Weight was increased systematically if the prescribed amount of repetitions was completed. All training sessions were performed at the University of Tehran under the supervision of certified strength and conditioning specialists by the National Strength and Conditioning Association who were blinded to the dietary assignment. Make-up sessions were allowed if participants missed a regularly scheduled training session; therefore, participant compliance in this study was 100 % for the RT programme[21].

Nutrient intake and dietary analysis

Study participants were instructed not to alter their habitual diet during the study. To minimise dietary variability, participants submitted 3-d (2 weekdays and 1 weekend) food records at baseline and at 12 weeks of the assigned intervention. Each item of food was individually entered into Diet Analysis Plus, version 10 (Cengage), and total energy consumption and the amount of energy derived from proteins, fats and carbohydrates were assessed[1].

Statistical analysis

An *a priori* sample size calculation was conducted using the G*Power analysis software[22]. Our rationale for sample size was based on previous data[1,23,24]. Based on an α value of 0·05 and a power $(1-\beta)$ value of 0·85, the analysis revealed that a total sample size of at least thirty participants (n 15 per group) was needed to have sufficient power to detect significant changes in MSTN and FLST concentrations. All analyses were performed using SPSS (version 25.0). Baseline data were compared between groups using an independent t test. Data were assessed for normality using the Shapiro–Wilks test, and any non-normal data (MSTN) were corrected with the use of logarithmic transformation to ensure that kurtosis and skewness were within normal bounds. Mean differences in BW, FM, SMM, 1RM bench press, 1RM back squat, FGF-2, TGF-β1, ACVA, FLST and MSTN were evaluated with two-way (group (WER *v.* EWR) × time (pre-intervention, post-intervention)) repeated-measures ANOVA. When appropriate, follow-up procedures included paired and independent samples t test. The η^2 statistic, which typifies the amount of variation attributable to a given factor when partialling out other factors from total non-error variation, was used to evaluate the effect size for each ANOVA. Cohen's d effect sizes were also calculated as part of follow-up to significant ANOVA interactions. An α level of 0·05 was used to determine statistical significance for all analyses.

Results

Body composition

Participant's body composition and muscular strength are presented in Table 2. There were no significant differences between groups at baseline for any body composition marker. Significant main effects of time were observed for BW (WER 1·7 kg (95 % CI 1·1, 2·5, $d=-2·2$) and EWR 1·8 kg (95 % CI 1·2, 2·1, $d=-1·4$)), FM (WER –1·9 kg (95 % CI –2·4, –1·5, $d=2·3$) and EWR –1·1 kg (95 % CI –1·3, –0·8, $d=2·2$)), SMM (WER 2·9 kg (95 % CI 2·3, 3·6, $d=-4·8$) and EWR 2·7 kg (95 % CI 2·2, 3·2, $d=-3·1$)) and total body water (WER 1·7 l (95 % CI –1·3, –2, $d=-2·8$) and EWR 1·5 l (95 % CI –1·3, –1·7, $d=-4·6$)). A significant group × time interaction was observed for FM ($P=0·020$, $\eta^2=0·291$). Follow-up paired samples t test indicated both groups decreased FM from pre- to post-intervention. However, follow-up independent samples t test indicated the groups were not significantly different at pre- or post-intervention in FM. No significant group × time interactions were noted for BW ($P=0·863$, $\eta^2=0·001$) or SMM ($P=0·589$, $\eta^2=0·011$).

Dietary analysis and compliance to exercise training

Baseline parameters were not significantly different between groups ($P>0·05$). There were also no significant group differences in mean daily energy as well as the amount of

Table 2. Physiological characteristics of participants
(Mean values and standard deviations)

		Pre-training		Post-training		*P*		
Variables	Group	Mean	SD	Mean	SD	T	G × T	G
BW (kg)	WER	83·3	8·9	85·1*	8·6	0·00	0·8	0·6
	EWR	82·0	5·5	83·8*	5·4			
FM (kg)	WER	17·2	1·5	15·2*	1·2	0·00	0·002	0·9
	EWR	16·7	1·4	15·6*	1·4			
SMM (kg)	WER	37	3·7	40·4*	3·8	0·00	0·5	0·6
	EWR	37·6	4·4	40·4*	3·8			
Absolute back squat (kg)	WER	125·8	17·5	139·7*	17·2	0·00	0·06	0·4
	EWR	132·1	16	143·4*	13·4			
Absolute bench press (kg)	WER	97·3	11·1	107·2*	10·4	0·00	0·2	0·7
	EWR	96·9	9·7	105·46*	9·6			
Relative back squat (kg/kg of BW)	WER	1·5	0·2	1·6*	0·2	0·00	0·06	0·5
	EWR	1·6	0·3	1·7*	0·2			
Relative bench press (kg/kg of BW)	WER	1·1	0·1	1·2*	0·1	0·00	0·2	0·7
	EWR	1·1	0·2	1·2*	0·1			
Total body water (litres)	WER	49·6	6·5	51·3*	6·5	0·00	0·3	0·7
	EWR	48·8	6·3	50·4*	6·1			

T, time; G × T, group × time; G, group; BW, body weight; WER, whole egg + resistance training; EWR, egg white + resistance training; FM, fat mass; SMM, skeletal muscle mass.
* Different from baseline ($P<0·05$).

British Journal of Nutrition

https://doi.org/10.1017/S0007114520002238 Published online by Cambridge University Press

Table 3. Energy, macronutrients and micronutrients
(Mean values and standard deviations)

Variables	Group	Pre-training		Post-training		*P*		
		Mean	SD	Mean	SD	T	G × T	G
Energy (kcal/d)‡	WER	2809	71·2	2840	74·9	0·6	0·2	0·07
	EWR	2767·8	121·1	2754·5	96·4			
Energy (kcal/kg per d)‡	WER	34·3	2·5	34·0	2·4	0·1	0·3	0·3
	EWR	33·5	4·0	32·7	3·8			
Carbohydrate (g/d)	WER	348·2	14·2	344·7	14·4	0·06	0·7	0·2
	EWR	341·2	13·7	338·7	14·4			
Carbohydrate (g/kg per d)	WER	4·2	0·3	4·1	0·3	0·07	0·08	0·4
	EWR	4·1	0·5	4·0	0·4			
Carbohydrate (% of energy)	WER	49·5	15·3	48·5	12·9	0·4	0·8	0·4
	EWR	49·3	21·5	49·1	18·2			
Dietary fibre (g/d)	WER	21·4	5·2	22·1	5·0	0·3	0·2	0·6
	EWR	18·7	4·8	19·6	4·7			
Sugar (g/d)	WER	67·4	37·7	66·1	39·8	0·5	0·6	0·1
	EWR	69·2	29·0	68·7	31·4			
Protein (g/kg per d)	WER	1·4	0·1	1·4	0·1	0·8	0·3	0·5
	EWR	1·4	0·2	1·4	0·1			
Protein (g/d)	WER	120·3	8·0	121·2	8·2	0·1	0·3	0·5
	EWR	116·8	12·3	120·6	10·7			
Protein (% of energy)	WER	17·1	1·2	17·0	1·1	0·8	0·3	0·5
	EWR	16·8	1·9	17·5	1·7			
Leucine (g/d)	WER	8·5	2·7	8·9	2·2	0·08	0·4	0·3
	EWR	7·9	0·8	8·2	1·0			
Isoleucine (g/d)	WER	7·7	1·4	7·9	1·3	0·1	0·3	0·3
	EWR	7·8	0·9	8·1	1·1			
Valine (g/d)	WER	6·8	1·0	7·1	1·2	0·2	0·4	0·5
	EWR	6·2	0·9	6·8	0·8			
Branched-chain amino acids (g/d)	WER	23·0	5·2	23·9	5·1	0·07	0·8	0·7
	EWR	21·9	3·3	23·1	3·6			
Fat (g/d)	WER	103·8	5·8	106·2	4·6	0·7	0·2	0·6
	EWR	106·5	8·1	105·2	5·2			
Fat (g/kg per d)	WER	1·2	0·1	1·2	0·07	0·2	0·2	0·9
	EWR	1·2	0·1	1·2	0·1			
Fat (% of energy)	WER	33·4	4·1	34·5	5·0	0·2	0·2	0·9
	EWR	33·9	5·2	33·4	0·1			
Saturated fat (% of total fat)	WER	35·2	6·7	36·8	6·5	0·08	0·2	0·07
	EWR	33·8	5·4	33·4	5·7			
Polyunsaturated fat (% of total fat)	WER	19·5	4·1	19·1	4·5	0·4	0·6	0·8
	EWR	21·0	5·4	20·4	5·6			
Monounsaturated fat (% of total fat)	WER	45·3	7·3	44·1	7·1	0·7	0·4	0·7
	EWR	45·2	6·6	46·2	6·5			
Cholesterol (mg/d)	WER	268·7	24·1	842·7*†	88·7	0·00	0·00	0·00
	EWR	279·1	20·3	285·2*	25·1			
Vitamin A equivalents (μg/d)	WER	1754	78	1832	70	0·07	0·3	0·1
	EWR	1802	62	1810	67			
Vitamin C (mg/d)	WER	174	12	179	12	0·2	0·6	0·2
	EWR	187	18	190	17			
Thiamine (mg/d)	WER	1·7	0·1	2·8*†	0·2	0·00	0·00	0·00
	EWR	1·5	0·1	1·6	0·1			
Riboflavin (mg/d)	WER	2·3	0·1	2·7*	0·1	0·00	0·2	0·09
	EWR	2·1	0·1	2·4*	0·1			
Niacin equivalents (mg/d)	WER	32	0·8	33*	0·7	0·00	0·7	0·1
	EWR	28	0·9	30*	1·0			
Ca (mg/d)	WER	984	25	1008	26	0·1	0·5	0·3
	EWR	949	17	966	17			
Na (mg/d)	WER	3292	1210	3227	1189	0·7	0·7	0·6
	EWR	3328	974	3301	957			
Fe (mg/d)	WER	10·9	0·2	11·5	0·2	0·2	0·8	0·3
	EWR	12·2	0·4	12·3	0·4			

T, time; G × T, group × time; G, group; WER, whole egg + resistance training; EWR, egg white + resistance training.
* Different from baseline.
† Between-group difference.
‡ To convert energy values from kcal to kJ, multiply by 4·184.

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https://doi.org/10.1017/S0007114520002238 Published online by Cambridge University Press

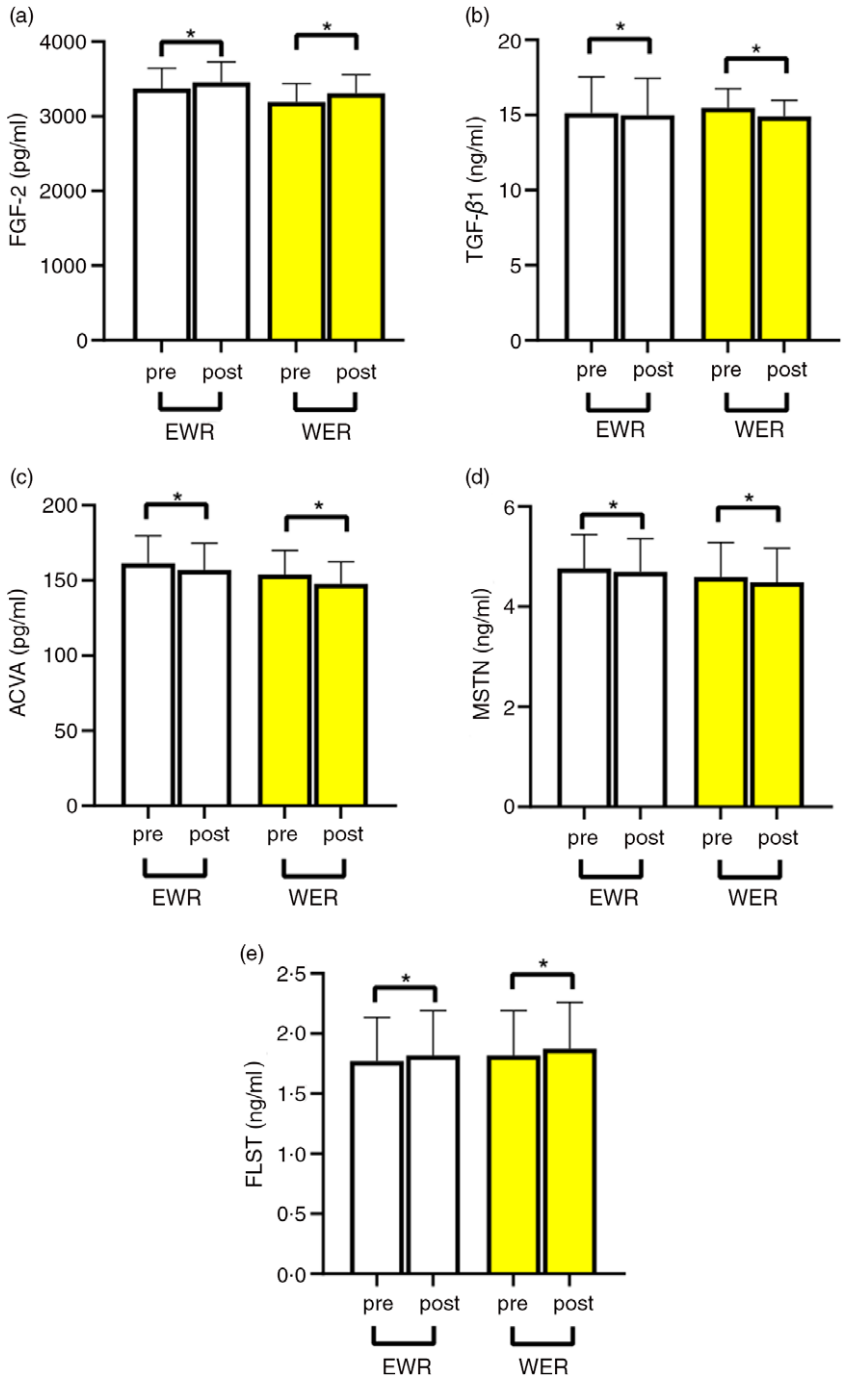

Fig. 2. Serum concentrations of fibroblast growth factor-2 (FGF-2) (a); transforming growth factor-β1 (TGF-β1) (b); activin A (ACVA) (c); myostatin (MSTN) (d) and follistatin (FLST) (e) from pre- to post-training in whole egg + resistance training (WER) and egg white + resistance training (EWR) groups. * $P < 0{\cdot}05$ different from baseline.

protein, carbohydrates and fats consumed per d (Table 3) after 12 weeks. Cholesterol was significantly increased after 12 weeks in the WER group. Participants in WER and EWR groups completed all training sessions over the 12-week period.

Maximal strength

There were no significant between-groups differences at baseline for any maximal strength values. Significant main effects of time were observed for absolute bench press (WER 9·8 kg (95 % CI 7·9, 11·8, $d = -2{\cdot}8$) and EWR 8·5 kg (95 % CI 7·1, 9·9, $d = -3{\cdot}4$)), relative bench press (WER 0·09 kg/kg of BW (95 % CI 0·06, 0·1, $d = -1{\cdot}8$) and EWR 0·07 kg/kg of BW (95 % CI 0·05, 0·09, $d = -2{\cdot}3$)), absolute back squat (WER 13·8 kg (95 % CI 12·1, 15·6, $d = -4{\cdot}4$) and EWR 11·3 kg (95 % CI 9·1, 13·4, $d = -2{\cdot}9$)), relative back squat (WER 0·1 kg/kg of BW (95 % CI 0·08, 0·12, $d = -3{\cdot}2$) and EWR 0·09 kg/kg of BW g (95 % CI 0·07, 0·1, $d = -1{\cdot}8$)) and

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1RM (Table 2). However, no significant group × time interactions were noted for bench press or back squat 1RM.

Skeletal muscle regulatory markers

The concentrations of skeletal muscle regulatory markers are shown in Fig. 2. Baseline circulating myokines were not significantly different between groups ($P > 0·05$). Significant main effects of time were observed for FGF-2 (WER 116·1 pg/ml (95 % CI 78·8, 153·4, $d = -1·7$) and EWR 83·2 pg/ml (95 % CI 32, 134·3, $d = -0·9$)), TGF-β1 (WER –0·5 ng/ml (95 % CI –1, 0·1, $d = 0·7$) and EWR –0·1 ng/ml (95 % CI –0·2, 0·05, $d = 0·9$)), ACVA (WER –6·2 pg/ml (95 % CI –8·5, –3·9, $d = 1·5$) and EWR –4·5 pg/ml (95 % CI –5·9, –3·2, $d = 1·8$)), FLST (WER 0·05 ng/ml (95 % CI 0·03, 0·06, $d = -1·6$) and EWR 0·04 ng/ml (95 % CI 0·03, 0·05, $d = -2$)) and MSTN (WER –0·1 ng/ml (95 % CI –0·2, 0·03, $d = 1·1$) and EWR –0·06 ng/ml (95 % CI –0·1, 0·05, $d = 1$)). However, no significant group × time interactions were noted for any marker ($P = 0·054$–$0·636$).

Discussion

This present study examined the influence of daily EW or WE consumption on skeletal muscle regulatory markers following 12 weeks of RT in resistance-trained men. Our findings suggest that the consumption of egg yolk throughout 12 weeks of RT had no bearing on any changes in FGF-2, TGF-β1, AVCA, FLST, MSTN, muscular strength development or alterations in body composition when protein intake was equalised.

Post-exercise egg protein ingestion has previously received much attention owing to its amino acid profile, that is, inclusion of all essential amino acids and relatively rapid postprandial aminoacidemia or leucinemia[11]. For instance, 100 g of raw WE or EW is estimated to contain approximately 11–12 g of protein[11] and 0·6 g of leucine[16]. Leucine is known to be an essential component for the stimulation of postprandial MPS. Prior investigations in young adults have indicated that 1 g of leucine supplementation concomitant with RT is adequate to stimulate MPS[25,26]. It has also been previously reported that approximately 20 g of egg protein stimulated MPS after RT[26]. To promote optimal muscular adaptations over time, it is prudent for athletes and active individuals to employ nutritional strategies to not only maximise acute MPS responses to RT but create net anabolic environments through the support of MPS and suppression of muscle protein breakdown via a complete and sufficient amino acid provision[27]. Given that the yolk contains a large portion of the total egg protein, its removal would conceivably blunt the postprandial MPS response, especially post-exercise where protein needs may be increased to reach maximal stimulation[3], as well as limit the amino acid support for anabolic protein metabolism. However, it appears from the present data that when controlling for total egg protein consumption and total daily protein intake, the removal of yolk has no influence on the body composition and functional adaptations to chronic RT.

Our longitudinal data contrast previous findings from a seminal study by Van Vliet and colleagues who experimented on the acute effects of WE *v.* EW consumption on postprandial MPS in young men following an acute bout of RT[11]. Specifically, researchers conducted a cross-over investigation on ten resistance-trained men who consumed either WE (18 g protein, 17 g fat) or isonitrogenous EW (18 g protein, 0 g fat) following an acute bout of RT. First, a more rapid appearance of egg protein-derived leucine in the plasma was found after EW consumption; however, the overall availability of circulating leucine was similar between treatments within the 5-h postprandial period of testing. Additionally, postprandial levels of muscle leucine enrichment and expression of skeletal muscle amino acid transporters after exercise did not differ between treatments. However, despite the lack of difference in the measures of amino acid or leucine availability, sensing and uptake, there was a greater stimulation of post-exercise MPS following WE consumption. Further, these differential MPS responses were not mediated by signalling mechanisms often linked to the control of protein anabolism, such as those relating to mitogen-activated protein kinase and mTOR complex 1 (mTORC1). Lastly, these results for MPS could not be explained by incongruent egg protein intake or the essential amino acid composition between the two egg conditions. The authors attributed the superior effects of WE on postprandial and post-exercise MPS to other nutrient constituents of the yolk that is lacking in EW, such as vitamin D[28], lipids[29] and total energy consumption. For instance, 100 g of WE contains 1·5 mcg vitamin D, 11·9 g fat and 644·336 kJ (154 kcal) energy, while EW had no vitamin D and only contains 0·1 g fat and 196·648 kJ (47 kcal) energy[16]. Although these acute outcomes may suggest a greater RT-induced outcome for SMM and muscular strength with WE consumption, our longitudinal data fail to support this hypothesis. The discrepancies between the findings of each study demonstrate the limited inferential value of acute data to predict long-term outcomes, that is, does greater acute MPS adequately suggest greater RT-induced outcomes in skeletal muscle?

Despite previous evidence suggesting that other non-protein constituents of egg yolk would provide additional stimulatory benefits to acute rises in MPS, it might be argued that the between-treatment similarities in total protein content, essential amino acid profile and subsequent leucine content are more decisive for the effects of egg consumption on RT-induced adaptations. Although the examination on skeletal muscle regulatory markers was more exploratory, a novel finding of this investigation was the lack of effect of type of egg protein consumption on circulating skeletal muscle regulatory markers.

These regulatory markers have demonstrated an ability to either stimulate or inhibit muscular hypertrophy[1]. For instance, MSTN is recognised as a strong negative regulator of SMM, which acts by binding to activin type II receptors, eliciting an inhibitory response for muscle hypertrophy[30]. A previous investigation by Hulmi *et al.*[23] demonstrated that following 21 weeks of RT, MSTN concentrations were only altered immediately post-exercise in groups not consuming protein after the workout. Furthermore, basal concentrations were not significantly different between groups despite chronic protein ingestion[23]. In contrast to MSTN, FLST is recognised as an anabolic myokine that blocks the MSTN receptor, subsequently facilitating skeletal muscle hypertrophy[1]. ACVA, a homodimeric glycoprotein produced by the decidua, placenta and fetal membranes, is involved

British Journal of Nutrition

https://doi.org/10.1017/S0007114520002238 Published online by Cambridge University Press

in cellular differentiation, proliferation, remodelling and morphogenesis[31]. FGF-2 is a heparin-binding protein that regulates cellular functions, including muscle regeneration, maintenance and skeletal muscle growth by increasing the proliferation of satellite cells[32]. Moreover, TGF-β1 is known as a multifunctional protein that acts as a skeletal muscle regenerator since it contributes to extracellular matrix reconstitution as well as muscle tissue remodelling[33]. Several investigations have evaluated the aforementioned myokines to determine their role in RT-induced muscular adaptations[1,30,34,35]. Accordingly, we showed that 8 weeks of whole-body RT increased circulating FLST and decreased MSTN concentrations in middle-aged men[1]. In addition, we recently indicated that 8 weeks of concurrent training increased circulating FLST and decreased MSTN concentrations in sarcopenic elderly men[24]. Moreover, a prior study revealed that a single bout of RT increased FGF-2, possibly in response to mechanical stimuli. The authors speculated that the observed effect was potentially one of the many connections between mechanical stress and muscle regeneration/hypertrophy[36]. Additionally, combined RT and endurance training increased TGF-β1 concentrations after 8 weeks in patients with type II diabetes[33]. However, other data have indicated that while TGF-β1 is initially elevated with RT, continued training will produce a subsequent decrease in concentrations of this myokine[37]. Thus, the resistance-trained status of participants in the present study could explain the observed decrease in this marker.

Ultimately, although non-protein nutrients in the egg yolk may acutely affect some aspects of the MPS response, they did not appear to exert any effect on the aforementioned skeletal muscle regulatory markers in the context of the present study. To the best of the authors' knowledge, no investigations have been completed examining the effect of isonitrogenous meals varying in non-protein nutrient combinations on the skeletal muscle regulatory markers examined in the present investigation. Although, since the overall protein intake seems to be a driving force behind many post-absorptive alterations in anabolic signalling proteins when combined with sufficient RT stimulus, it is possible that there would not be a difference between groups. Ultimately, the lack of differences between groups in any of the skeletal muscle regulatory markers in the present study aligns with the comparable whole-body outcomes between groups.

The present study is limited by the absence of measuring upstream and downstream proteins of the mTORC1 signalling pathway, which would determine anabolic and catabolic conditions better than only measuring skeletal muscle regulatory markers. Further, the present study only examined basal concentrations of the previously mentioned regulatory markers. Therefore, changes in responses to training and nutrient ingestion could not possibly be examined. However, since the overall adaptations failed to differ between conditions, it is unlikely that any changes in acute responses would be practically relevant to long-term outcomes. A further limitation of our investigation is the use of bioelectrical impedance, which is not as accurate as dual-energy X-ray absorptiometry (the 'gold standard' technique for body composition measurement); however, previous studies have shown that it is a valid and reliable method[38,39]. In addition, we did not measure the lipid profile of our participants, which may have revealed important cardiometabolic changes after our WER intervention. Another limitation of our methods was the use of self-reported dietary intake (via food records), which has been shown to be vulnerable to social desirability bias with underreporting of energy intake and overreporting of fruit and vegetable intake[40].

From a practical application standpoint, it appears, at least within the limits of the present study, that the consumption of eggs absent of yolk during chronic RT would result in similar body composition and functional outcomes as WE of equal protein value. Our data suggest that EW or WE may be used interchangeably for the dietary support of RT-induced muscular hypertrophy when total protein intake is maintained. However, the effects of long-term consumption of three WE per d on cardiometabolic health outcomes in our study cohort are unknown. Therefore, future research warrants the evaluation of cardiometabolic health parameters in resistance-trained men following chronic WE consumption.

Acknowledgements

The authors wish to thank all the participants in this research project.

This study was financially supported by the University of Tehran.

R. B. and B. H. M. conceived and designed the research. B. H. M., R. B. and M. E. conducted experiments. A. W. contributed new reagents or analytical tools. R. B. analysed data. R. B., E. J., G. M. T. and M. T. S. drafted the manuscript. G. M. T., A. W. and D. A.-L. revised the manuscript. All authors read and approved the manuscript.

The authors declare that there are no conflicts of interest.

References

1. Bagheri R, Rashidlamir A, Motevalli MS, *et al.* (2019) Effects of upper-body, lower-body, or combined resistance training on the ratio of follistatin and myostatin in middle-aged men. *Eur J Appl Physiol* **119**, 1921–1931.
2. Damas F, Libardi CA & Ugrinowitsch C (2018) The development of skeletal muscle hypertrophy through resistance training: the role of muscle damage and muscle protein synthesis. *Eur J Appl Physiol* **118**, 485–500.
3. Macnaughton LS, Wardle SL, Witard OC, *et al.* (2016) The response of muscle protein synthesis following whole-body resistance exercise is greater following 40 g than 20 g of ingested whey protein. *Physiol Rep* **4**, 4–15.
4. Moro T, Brightwell CR, Velarde B, *et al.* (2019) Whey protein hydrolysate increases amino acid uptake, mTORC1 signaling, and protein synthesis in skeletal muscle of healthy young men in a randomized crossover trial. *J Nutr* **149**, 1149–1158.
5. Wolfe RR, Rutherfurd SM, Kim I-Y, *et al.* (2016) Protein quality as determined by the Digestible Indispensable Amino Acid Score: evaluation of factors underlying the calculation. *Nutr Rev* **74**, 584–599.
6. Asensio-Grau A, Peinado I, Heredia A, *et al.* (2018) Effect of cooking methods and intestinal conditions on lipolysis, proteolysis and xanthophylls bioaccessibility of eggs. *J Funct Foods* **46**, 579–586.

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https://doi.org/10.1017/S0007114520002238 Published online by Cambridge University Press

7. Wilson J & Wilson GJ (2006) Contemporary issues in protein requirements and consumption for resistance trained athletes. *J Int Soc Sports Nutr* **3**, 7.
8. Hu FB, Stampfer MJ, Rimm EB, *et al.* (1999) A prospective study of egg consumption and risk of cardiovascular disease in men and women. *JAMA* **281**, 1387–1394.
9. Jäger R, Kerksick CM, Campbell BI, *et al.* (2017) International Society of Sports Nutrition Position Stand: protein and exercise. *J Int Soc Sports Nutr* **14**, 20.
10. Hoffman JR & Falvo MJ (2004) Protein–which is best? *J Sports Sci Med* **3**, 118.
11. van Vliet S, Shy EL, Abou Sawan S, *et al.* (2017) Consumption of whole eggs promotes greater stimulation of postexercise muscle protein synthesis than consumption of isonitrogenous amounts of egg whites in young men. *Am J Clin Nutr* **106**, 1401–1412.
12. Kanter MM, Kris-Etherton PM, Fernandez ML, *et al.* (2012) Exploring the factors that affect blood cholesterol and heart disease risk: is dietary cholesterol as bad for you as history leads us to believe? *Adv Nutr* **3**, 711–717.
13. Réhault-Godbert S, Guyot N & Nys Y (2019) The golden egg: nutritional value, bioactivities, and emerging benefits for human health. *Nutrients* **11**, 684.
14. Phillips SM, Fulgoni VL III, Heaney RP, *et al.* (2015) Commonly consumed protein foods contribute to nutrient intake, diet quality, and nutrient adequacy. *Am J Clin Nutr* **101**, 1346S–1352S.
15. Abou Sawan S, van Vliet S, West DW, *et al.* (2018) Whole egg, but not egg white, ingestion induces mTOR colocalization with the lysosome after resistance exercise. *Am J Physiol Cell Physiol* **315**, C537–C543.
16. Seuss-Baum I (2007) Nutritional evaluation of egg compounds. In *Bioactive Egg Compounds*, pp. 117–144. Berlin: Springer.
17. Thomas DT, Erdman KA & Burke LM (2016) Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: nutrition and athletic performance. *J Acad Nutr Diet* **116**, 501–528.
18. Schoenfeld BJ, Nickerson BS, Wilborn CD, *et al.* (2020) Comparison of multifrequency bioelectrical impedance vs. dual-energy X-ray absorptiometry for assessing body composition changes after participation in a 10-week resistance training program. *J Strength Cond Res* **34**, 678–688.
19. Alver BA, Sell K, Deuster PA (2017) *NSCA's Essentials of Tactical Strength and Conditioning*. Champaign, IL: Human Kinetics.
20. Haff GG & Triplett NT (2015) *Essentials of Strength Training and Conditioning*, 4th ed. Champaign, IL: Human Kinetics.
21. Kraemer WJ, Hatfield DL, Volek JS, *et al.* (2009) Effects of amino acids supplement on physiological adaptations to resistance training. *Med Sci Sports Exerc* **41**, 1111–1121.
22. Faul F, Erdfelder E, Lang A-G, *et al.* (2007) G* Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* **39**, 175–191.
23. Hulmi JJ, Tannerstedt J, Selanne H, *et al.* (2009) Resistance exercise with whey protein ingestion affects mTOR signaling pathway and myostatin in men. *J Applied Physiol* **106**, 1720–1729.
24. Bagheri R, Moghadam BH, Church DD, *et al.* (2020) The effects of concurrent training order on body composition and serum concentrations of follistatin, myostatin and GDF11 in sarcopenic elderly men. *Exp Gerontol* **133**, 110869.
25. Tang JE, Manolakos JJ, Kujbida GW, *et al.* (2007) Minimal whey protein with carbohydrate stimulates muscle protein synthesis following resistance exercise in trained young men. *Appl Physiol Nutr Metab* **32**, 1132–1138.
26. Moore DR, Robinson MJ, Fry JL, *et al.* (2009) Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am J Clin Nutr* **89**, 161–168.
27. Churchward-Venne TA, Burd NA, Mitchell CJ, *et al.* (2012) Supplementation of a suboptimal protein dose with leucine or essential amino acids: effects on myofibrillar protein synthesis at rest and following resistance exercise in men. *J Physiol* **590**, 2751–2765.
28. Capiati D, Benassati S & Boland RL (2002) 1,25(OH)$_2$-vitamin D$_3$ induces translocation of the vitamin D receptor (VDR) to the plasma membrane in skeletal muscle cells. *J Cell Biochem* **86**, 128–135.
29. Yasuda M, Tanaka Y, Kume S, *et al.* (2014) Fatty acids are novel nutrient factors to regulate mTORC1 lysosomal localization and apoptosis in podocytes. *Biochim Biophys Acta* **1842**, 1097–1108.
30. Bagheri R, Rashidlamir A & Attarzadeh Hosseini SR (2018) Effect of resistance training with blood flow restriction on follistatin to myostatin ratio, body composition and anaerobic power of trained-volleyball players. *Med Lab J* **12**, 28–33.
31. Silva R, Bueno P, Avó L, *et al.* (2014) Effect of physical training on liver expression of activin A and follistatin in a nonalcoholic fatty liver disease model in rats. *Braz J Med Biol Res* **47**, 746–752.
32. Kim J-S, Yoon DH, Kim H-J, *et al.* (2016) Resistance exercise reduced the expression of fibroblast growth factor-2 in skeletal muscle of aged mice. *Integr Med Res* **5**, 230–235.
33. Touvra A-M, Volaklis KA, Spassis AT, *et al.* (2011) Combined strength and aerobic training increases transforming growth factor-β1 in patients with type 2 diabetes. *Hormones* **10**, 125–130.
34. Motevalli MS, Dalbo VJ, Attarzadeh RS, *et al.* (2015) The effect of rate of weight reduction on serum myostatin and follistatin concentrations in competitive wrestlers. *Int J Sports Physiol Perform* **10**, 139–146.
35. Negaresh R, Ranjbar R, Baker JS, *et al.* (2019) Skeletal muscle hypertrophy, insulin-like growth factor 1, myostatin and follistatin in healthy and sarcopenic elderly men: the effect of whole-body resistance training. *Int J Prev Med* **10**, 10–20.
36. Breen E, Johnson E, Wagner H, *et al.* (1996) Angiogenic growth factor mRNA responses in muscle to a single bout of exercise. *J Appl Physiol* **81**, 355–361.
37. Hering S, Jost C, Schulz H, *et al.* (2002) Circulating transforming growth factor β1 (TGFβ1) is elevated by extensive exercise. *Eur J Appl Physiol* **86**, 406–410.
38. Ling CH, de Craen AJ, Slagboom PE, *et al.* (2011) Accuracy of direct segmental multi-frequency bioimpedance analysis in the assessment of total body and segmental body composition in middle-aged adult population. *Clin Nutr* **30**, 610–615.
39. Jackson A, Pollock ML, Graves JE, *et al.* (1988) Reliability and validity of bioelectrical impedance in determining body composition. *J Appl Physiol* **64**, 529–534.
40. Schoeller DA (1995) Limitations in the assessment of dietary energy intake by self-report. *Metabolism* **44**, 18–22.

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https://doi.org/10.1017/S0007114520002238 Published online by Cambridge University Press