British Journal of Nutrition (2022), 128, 311–323 doi:10.1017/S0007114521003147

Acute feeding has minimal effect on the validity of body composition and metabolic measures: dual-energy X-ray absorptiometry and a multi-compartment model

Abbie E. Smith-Ryan[1,2]*, Gabrielle Brewer[1], Lacey M. Gould[1], Malia N.M. Blue[1,2], Katie R. Hirsch[3], Casey E. Greenwalt[1], Courtney Harrison[1,2], Hannah E. Cabre[1,2] and Eric D. Ryan[1,2]

[1]*Applied Physiology Laboratory, Department of Exercise and Sport Science, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA*

[2]*Human Movement Science Curriculum, Department of Allied Health Science, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA*

[3]*Department of Geriatrics, Donald W. Reynolds Institute on Aging, Center for Translational Research in Aging & Longevity, University of Arkansas for Medical Sciences, Little Rock, AR, USA*

(Submitted 31 March 2021 – Final revision received 19 July 2021 – Accepted 12 August 2021 – First published online 16 August 2021)

Abstract

Understanding the effects of acute feeding on body composition and metabolic measures is essential to the translational component and practical application of measurement and clinical use. To investigate the influence of acute feeding on the validity of dual-energy X-ray absorptiometry (DXA), a four-compartment model (4C) and indirect calorimetry metabolic outcomes, thirty-nine healthy young adults (*n* 19 females; age: 21·8 (SD 3·1) years, weight; 71·5 (SD 10·0) kg) participated in a randomised cross-over study. Subjects were provided one of four randomised meals on separate occasions (high carbohydrate, high protein, ad libitum or fasted baseline) prior to body composition and metabolic assessments. Regardless of macronutrient content, acute feeding increased DXA percent body fat (%fat) for the total sample and females (average constant error (CE):–0·30 %; total error (TE): 2·34 %), although not significant ($P = 0·062$); the error in males was minimal (CE: 0·11 %; TE: 0·86 %). DXA fat mass (CE: 0·26 kg; TE: 0·75 kg) and lean mass (LM) (CE: 0·83 kg; TE: 1·23 kg) were not altered beyond measurement error for the total sample. 4C %fat was significantly impacted from all acute feedings (avg CE: 0·46 %; TE: 3·7 %). 4C fat mass (CE: 0·71 kg; TE: 3·38 kg) and fat-free mass (CE: 0·55 kg; TE: 3·05 kg) exceeded measurement error for the total sample. RMR was increased for each feeding condition (TE: 1666·9 kJ/d; 398 kcal/d). Standard pre-testing fasting guidelines may be important when evaluating DXA and 4C %fat, whereas additional DXA variables (fat mass and LM) may not be significantly impacted by an acute meal. Measuring body composition via DXA under less stringent pre-testing guidelines may be valid and increase feasibility of testing in clinical settings.

Key words: **Four-compartment model: Dual-energy X-ray absorptiometry: Protein: carbohydrate: Sex differences**

Body composition and metabolic assessments are utilised in a variety of research, clinical and applied settings to assess health outcomes and track effects of diet, exercise and surgical interventions. Dual-energy X-ray absorptiometry (DXA) technology is one of the more commonly used laboratory methods to assess fat mass (FM), lean mass (LM), and bone mineral content (BMC) or bone mineral density[1] when gold standard multi-compartment methods are not available, such as a four-compartment (4C) model. A multi-compartment 4C model has improved accuracy compared with single two- and three-compartment models due to the measurement of additional body compartments, such as BMC and total body water (TBW)[2]. In a laboratory setting, assessments of RMR and RQ are often collected in conjunction with body composition measures, via indirect calorimetry, to understand energetic expenditure and substrate utilisation.

Previous studies have observed that body composition and metabolic estimations are influenced by acute intake of food and water, hydration status, and physical activity[3–5]. To ensure valid measurements, recommended pre-assessment guidelines for body composition and metabolic testing typically require an overnight fast of 8–12 h, refraining from moderate to vigorous exercise 24 h prior, and suggest that subjects arrive to the

Abbreviations: BMC, bone mineral content; CE, constant error; CHO, carbohydrate; DXA, dual-energy X-ray absorptiometry; FM, fat mass; FFM, fat-free mass; ICC, intra-class coefficient; MX, mixed; PRO, protein; TBW, total body water; TE, total error; TFM, trunk FM.

* **Corresponding author:** Abbie E. Smith-Ryan, email abbsmith@email.unc.edu

laboratory in an euhydrated state. Although these recommendations are ideal for minimising measurement error, abiding by pre-assessment guidelines, particularly fasting, may not always be feasible for various populations. Complying with 8–12 h fasting recommendations may be compromised in clinical populations, where testing may occur around several other appointments; in working adults, who may arrive to the laboratory after the workday; in athletes, who have to work around practice schedules; or in populations where long fasting periods would not be recommended, such as individuals with type 1 diabetes. Consequences of failing to adhere to pre-assessment guidelines are not entirely understood but may alter validity and reliability of outcomes[5,6]. Manufacturer pre-testing guidelines for many devices, including the DXA, are vague and warrant well-controlled research studies to provide optimal fasting and pre-assessment recommendations.

While data are limited, previous studies suggest that acute food consumption may significantly alter DXA and 4C outcomes, but results are conflicting regarding macronutrient type, meal size and potential differences between sexes[3,5,7]. It has been reported that carbohydrate (CHO) intake prior to testing will increase DXA-derived total body and regional LM and decrease FM and trunk FM (TFM), with no notable differences between sexes[5]. Another study demonstrated that a breakfast meal altered body mass (BM), LM and trunk LM (TLM) with greater changes in males compared with females[3]. The size of a meal and amount of fluid ingested is also likely to influence DXA[6] and 4C[7] validity.

Obtaining accurate body composition and metabolic measurements is critical for tracking changes and evaluating health; however, adherence to strict pre-assessment guidelines (i.e. extended fasting) may not be possible in most settings. Evaluating the impact of acute feeding of various macronutrients, such as high CHO, high protein (PRO) or a mixed (MX) meal on the validity of body composition and metabolic measures would allow researchers, practitioners and coaches to make more informed pre-testing recommendations in situations where long fasting periods are not feasible. Therefore, the primary purpose of this study was to investigate the influence of different acute feeding conditions varying in macronutrient content on the validity of DXA and 4C body composition measures of FM, LM, fat-free mass (FFM), and %fat and to evaluate divergent effects for sex. A secondary aim was to evaluate the influence of acute feeding on indirect calorimetry measures of RMR and RQ. It was hypothesised that acute feeding, particularly CHO, would increase estimates of LM, TLM and FFM and result in a decrease in FM, TFM and %fat for DXA and 4C outcomes. Additionally, we hypothesised that the thermic effect of food[8] would significantly increase RMR in all feeding conditions, and that RQ would reflect the macronutrient content of the meal[9,10].

Materials & methods

Subjects

One hundred and twenty-three people expressed interest in the study through email; 50 participants were screened for eligibility (Fig. 1). Forty-eight young adults were enrolled, prior to randomisation/measurement six individuals dropped out, resulting in forty-two participating in testing: (*n* 19 females; total sample age: (mean ± standard deviation) 21·8 (SD 3·1) years; height: 174·0 (SD 9·1) cm; weight: 71·5 (SD 10·0) kg; BMI: 23·6 (SD 2·0) kg/m^2); (males age: 22·3 (SD 3·2) years; height: 180·7 (SD 6·1) cm; weight: 77·8 (SD 9·4) kg; BMI: 23·8 (SD 2·1) kg/m^2); (females age: 21·3 (SD 3·1) years; height: 166·9 (SD 5·7) cm; weight: 65·0 (SD 5·5) kg; BMI: 23·3 (SD 2·0) kg/m^2). Three subjects dropped out due to time constraints, and one subject dropped out due to unknown circumstances; data were analysed for thirty-nine participants (*n* 20 males and *n* 19 females). One additional subject was unable to return for the PRO condition due to COVID-19 shutdown, leaving *n* 38 in that group. Sample size was determined based on an average effect size of 0·66, 80 % power at an α level of 0·05, resulting in an *n* 36 required[5,7]. All subjects were healthy and free of disease (metabolic, neuromuscular or cardiovascular), as determined by a health history questionnaire, and had a BM) between 18·5 and 29·9 kg/m^2. Subjects were excluded from the study if they had lost or gained 3·6 kg or more in the previous two months or were participating in a diet that would influence hydration or muscle mass (i.e. ketogenic). Females were not pregnant or planning to become pregnant, were not taking hormonal contraceptives and had regular menstrual cycles. All procedures used in the study were in accordance with the Helsinki Declaration of 1975 as revised in 1983 and were approved by the University's Biomedical Institutional Review Board, and all subjects provided written informed consent.

Experimental design and protocol

In a randomised cross-over design, all subjects completed four testing sessions. At each testing session, subjects consumed one of four randomly assigned, using Random Allocation Software, feeding treatments: fasted (FAST; no food or energetic drink for 12 h prior); high CHO (77·5 g of food and 39 g of fluid CHO; Meal: 86 % CHO), high PRO (44 g of food and 19·5 g of fluid PRO, Meal: 58 % PRO); MX meal (ad libitum meal of choice), followed by metabolic and body composition assessments. Females completed the PRO, CHO, MX and FAST conditions within a 7-d window post-menstruation during the follicular phase confirmed through self-reported tracking of the menstrual cycle. The average time it took to complete the study for males and females was 37 d and 66 d, respectively. Subjects were asked to arrive to the laboratory 12 h fasted, euhydrated (encouraged to consume ≤ 1 cup of water prior to their visit) and having refrained from exercise 24 h prior. All visits began with confirmation of hydration status through urine-specific gravity assessment (1·002–1·025) and a pregnancy test for females. Height and weight were collected, and assigned meals were distributed by study staff. Subjects were allotted up to 20 min to consume the feeding assignment, followed by a 30-min resting period to allow for digestion to occur. Upon completion of the digestion window, subjects proceeded with indirect calorimetry and body composition measures. A minimum of 24 h separated all visits, and all subjects were asked to arrive to the laboratory within a ± 1-h window from their first visit.

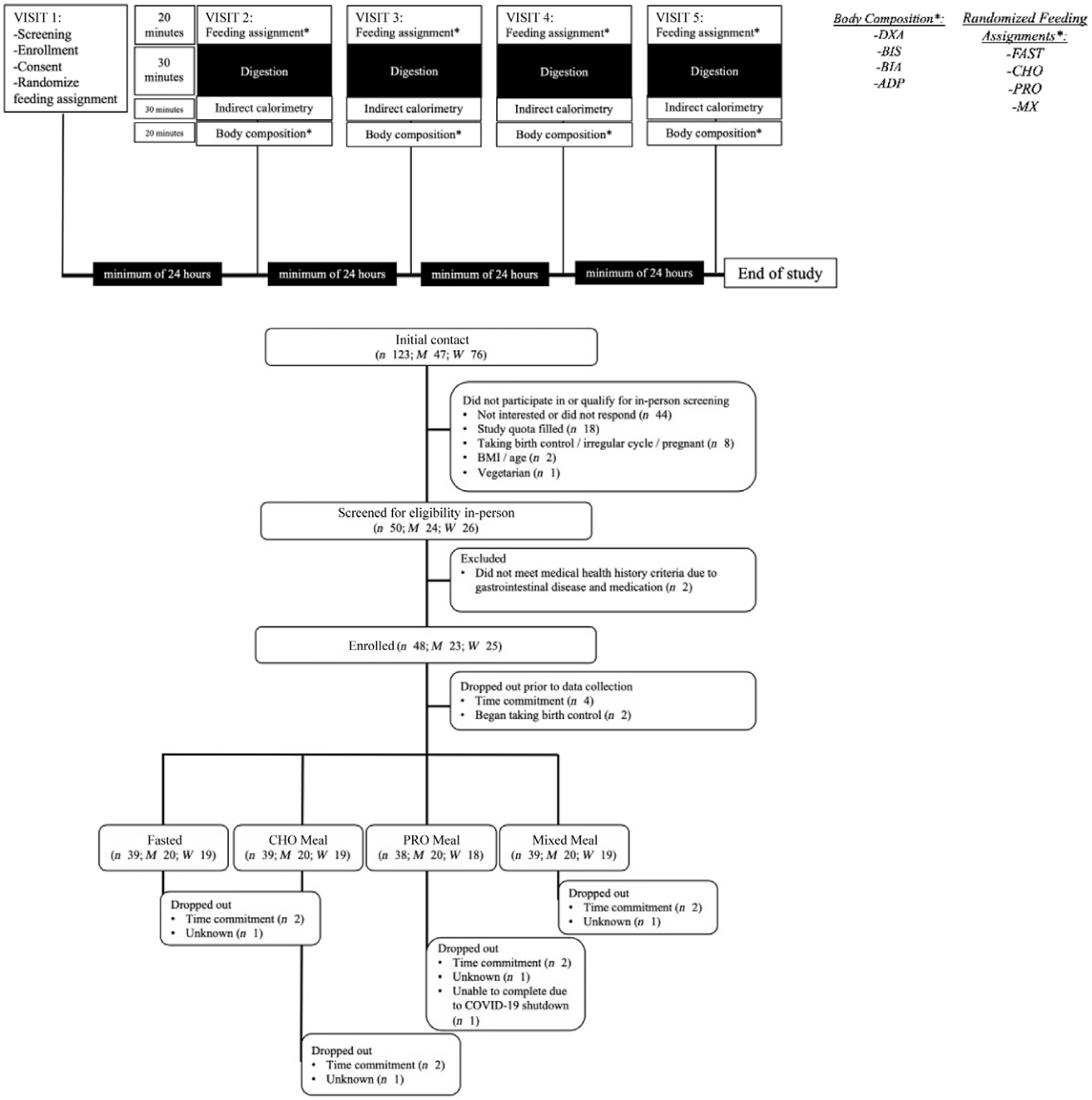

Fig. 1. Experimental design protocol and CONSORT (Consolidated Standards of Reporting Trials) diagram.

Feeding procedures

Feeding conditions (CHO, PRO, MX and FAST) were randomly assigned in using Random Allocation Software. Randomised treatment sequences included all permutations for the four treatment arms. Meals were prepared and distributed by study staff in the laboratory (Table 1). For the MX feeding assignment, subjects brought in a meal of their choice; research assistants recorded the composition and weighed the MX meal using a diet log. (Table 1). All subjects were provided 3·5 cups (about 828 ml) of fluids across all feeding conditions, excluding FAST. All meals were consumed in 20 min or less.

Body composition

Dual-energy X-ray absorptiometry. A full body DXA (Lunar iDXA; General Electric Medical Systems Ultrasound & Primary Care Diagnostics, enCORE Software version 16) scan was used to assess total body FM, TFM, LM, TLM, %fat and BMC. After removing shoes and any clothing or jewelry containing metal or hard plastic, subjects laid supine in the centre of the scanning table. Styrofoam boards were placed between the hands and hips to establish a consistent distance between the limbs for all subjects. To standardise foot position, styrofoam boards were also strapped to the subject's feet while they remained

Table 1. a) High carbohydrate meal and macronutrient information. b) High protein meal and macronutrient information. c) Average ad libitum mixed meal macronutrient information

	Total (g)	Calories/Energy (kcal)	CHO (g)	FAT (g)	PRO (g)
a) High carbohydrate meal					
Pop-Tarts – strawberry (1 tart)	52	200	38	5	2
Bagel – plain (1/2)	47·5	130	26·5	0·5	4·5
Jelly – grape (1 tbsp)	50	50	13	0	0
Orange juice (1·5 cups)	360	165	39	0	3
Water (2 cups)	373	0	0	0	0
Total	882·5	545	116·5	5·5	9·5
b) High protein meal					
Dymatize whey protein isolate (1 scoop)	32	120	2	0·5	25
Egg whites (6 tbs)	92	50	0	0	10
Turkey deli meal (2 oz)	56	50	1	1	9
Fairlife milk (1·5 cup)	360	225	9	0	19·5
Water (2 cups)	373	0	0	0	0
Total	913	445	9	1·5	63·5
c) Ad libitum mixed meal					
Males	404·4	652·3	74·7	27·0	34·1
Females	250·0	411·1	58·6	13·9	16·4
Total sample	329·2	534·8	66·9	20·6	25·5

CHO, carbohydrate; PRO, protein.

dorsiflexed. The scans were automatically analysed by the software and specific regions of interest were confirmed by the same technician. Between-day test–retest reliability for DXA from our laboratory is as follows: LM: intra-class coefficient (ICC) = 0·99, SEM = 1·97 kg; FM: ICC = 0·98 kg, SEM = 0·85 kg; %fat: ICC = 0·96 %, SEM = 1·28 %.

Four compartment model. A 4C model, described by Wang et al.[11] (Equation 1–3), was used to determine FM, FFM and %fat. Variables utilised in the equation included BV = body volume, TBW, Mo = total body bone mineral content and BM[11]. BV was collected from air displacement plethysmography, TBW from bioelectrical impedance spectroscopy and BM from a scale. BMC was collected from DXA and multiplied by a constant to attain total bone body mineral (Mo = BMC × 1·0436)[11]. Between-day test–retest reliability for the 4C is as follows: FM: ICC = 0·995 kg, SEM = 0·831 kg = 2·30 kg; %fat: ICC = 0·982 %, SEM = 0·960 %; FFM: ICC = 0·996 kg; SEM = 0·999 kg.

Equation 1: FM (kg) = 2·748 (BV) – 0·699 (TBW) + 1·129 (Mo) – 2·051 (BM)

Equation 2: FFM (kg) = BM – FM

Equation 3: %fat (%) = (FM/BM) × 100

Equation 4: Mo (kg) = BMC × 1·0436

Air displacement plethysmography. Air displacement plethysmography (BodPod; Cosmed, Software version 4.2+) was used to assess BV. Subjects were seated upright inside of the testing chamber wearing spandex or a swimsuit with all metal removed. A swim cap was placed over their head to minimise the effect of isothermal air. Subjects were asked to sit still to obtain measures of BV. Values were automatically computed by the software and averaged from two tests. BV was measured by a minimum of two trials that were within 150 ml of each other. Thoracic gas volume was estimated by the software's standard prediction equations. Previous investigations have reported no significant differences between predicted and measured lung volume in adults[12,13].

Bioelectrical impedance spectroscopy. TBW was collected from bioelectrical impedance spectroscopy (SFB7; ImpediMed) using standard coefficients to be utilised in the 4C model. This method has been previously validated against ^{2}H and provides good agreement but may result in additional error in TBW assessments[14–16]. Prior to measurement, subjects removed all metal to avoid interference with data collection accuracy. Height, sex and age were entered into the device. Subjects removed their right sock and laid supine on a table with their arms separated from the torso and legs separate from each other. Four electrodes were placed on the right side of the body: two between the malleoli of the ankle, five centimetres away from this electrode (proximal to the third metatarsal–phalangeal joint) and two between the distal end of the radius and ulna, and five centimetres away from this electrode (proximal to the third metacarpal–phalangeal joint). The average of two measurements was used for TBW outcomes.

Indirect calorimetry. RMR (kcal/d) and RQ (a.u.) were determined from indirect calorimetry (TrueOne 2400 Metabolic Measurement System, Parvo Medics). Subjects wore a chest strap heart rate monitor (Polar; Polar Electro) and laid supine with their head under a clear ventilated hood[17]. Resting measurements were collected for 30 min, with the first 5 min discarded. RMR and RQ were computed internally using the following equations[18,19]:

Equation 5: $\text{RMR (kcal/d)} = [(3{\cdot}9 \times (\text{VO}_2\ (\text{L} \times \text{min}^{-1}))) + (1{\cdot}1 \times (\text{VCO}_2\ (\text{L} \times \text{min}^{-1})))] \times 1440$ min

Equation 6: $\text{RQ (a.u.)} = (\text{VCO}_2)/(\text{VO}_2)$

The test–retest reliability of RMR (kcal/d) and RQ (a.u.) has been tested in our lab: ICC = 0·94 kcal/d; 0·83 a.u., SEM = 125·6 kcal/d; 0·03 a.u., MD = 224·3 kcal/d; 0·05 a.u.

Statistical analyses. Descriptive statistics are presented in Tables 2 and 3. To assess body composition and metabolic outcomes, validity statistics and Bland–Altman plots[20] were utilised to identify the agreement between outcomes from the FAST

Table 2. DXA and 4C descriptive statistics for all feeding conditions for the a) total sample (mean ± SD), b) males and c) females

	DXA								4C							
	FAST		CHO		PRO		MX		FAST		CHO		PRO		MX	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
a) Total Sample																
Body mass (kg)	74·7	10·6	72·8	9·9*	73·0	10·1**	72·8	10·1***	72·5	9·9	72·6	10·0*	72·3	10·0**	72·5	10·1***
Fat mass (kg)	15·6	5·0	15·8	5·1*	15·6	5·2	15·8	5·0	12·2	5·0	12·3	5·2	12·7	5·8	12·8	5·2
Lean mass (kg)	53·1	10·8	54·0	10·7*	54·0	10·5**	53·7	10·8***	–		–		–		–	
Fat-free mass (kg)	–		–		–		–		59·3	11·5	60·5	11·6	59·8	11·7	59·7	11·5
%fat	22·4	7·9	22·1	7·6	22·1	7·9	22·4	7·6	17·5	7·8	17·3	7·7	17·9	8·5	18·1	7·7
Trunk fat mass (kg)	6·8	2·5	6·9	2·5	6·9	2·6	7·0	2·5	–		–		–		–	
Trunk lean mass (kg)	24·0	4·7	24·7	4·8*	24·8	4·5**	24·7	4·7***	–		–		–		–	
Bone mineral content (kg)	2·9	0·6	2·9	0·6	2·9	0·6	2·9	0·6	–		–		–		–	
Body volume (l)	–		–		–		–		61·7	9·0	69·0	9·0*	67·9	9·1**	67·9	9·1***
Total body water (l)	–		–		–		–		41·1	7·9	41·2	7·9	40·8	8·0	41·0	7·9
Intracellular fluid content (L)	–		–		–		–		24·6	4·7	24·6	4·7	24·4	4·7	24·4	4·7
Extracellular fluid content (l)	–		–		–		–		16·6	3·3	16·6	3·3	16·5	3·3	16·6	3·3
b) Males																
Fat mass (kg)	12·8	3·8	13·0	3·7*	12·9	3·9	13·0	4·1	9·2	3·8	9·4	3·6	9·2	4·2	9·7	4·0
Lean mass (kg)	62·0	7·2	62·3	7·0*	62·4	6·5**	63·0	6·7***	–		–		–		–	
Fat-free mass (kg)	–		–		–		–		68·6	7·8	69·1	8·6	69·2	7·0	69·4	7·6
%fat	16·2	3·9	16·4	3·7	16·2	3·7	16·3	4·1	11·6	4·1	11·9	4·1	11·5	4·4	12·1	4·2
Trunk fat mass (kg)	7·1	6·3	6·0	2·1	5·9	2·2	6·1	2·3	–		–		–		–	
Trunk lean mass (kg)	27·7	3·6	28·3	3·5*	28·2	3·2**	28·6	3·1***	–		–		–		–	
Bone mineral content (kg)	3·3	0·5	3·3	0·5	3·3	0·5	3·3	0·5	–		–		–		–	
c) Females																
Fat mass (kg)	18·6	4·3	19·1	4·5	18·9	4·5	18·7	4·0	15·4	4·0	15·7	4·8	16·9	4·7	15·9	4·3
Lean mass (kg)	43·6	3·4	44·2	3·0*	44·2	2·9**	44·4	3·2***	–		–		–		–	
Fat-free mass (kg)	–		–		–		–		49·5	4·4	50·5	4·0	48·9	3·4	49·9	3·9
%fat	29·0	5·3	28·8	5·1	29·0	5·5	28·6	4·6	23·6	5·1	23·5	5·9	25·4	5·3	24·0	5·4
Trunk fat mass (kg)	7·8	2·5	8·0	2·7	8·0	2·7	7·9	2·4	–		–		–		–	
Trunk lean mass (kg)	20·1	1·7	20·5	1·4*	20·8	1·4**	20·7	1·6***	–		–		–		–	
Bone mineral content (kg)	2·5	0·2	2·5	0·3	2·5	0·2	2·5	0·2	–		–		–		–	

DXA, dual-energy X-ray absorptiometry; 4C, four compartment, FAST, fasted; CHO, carbohydrate; MX, mixed.
Statistical significance ($P < 0·05$) from the *post hoc* pairwise comparisons is indicated.
* For CHO compared with FAST.
** For PRO compared with FAST.
*** For MX compared with FAST.

Table 3. Indirect calorimetry descriptive statistics for all feeding conditions for the a) total sample (mean ± SD), b) males and c) females

	FAST		CHO		PRO		MX	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
a) Total sample								
RMR (kcal/d)	1797	258	2110	367*	2222	374**	2081	409***
RQ (a.u.)	0·75	0·04	0·87	0·08*	0·75	0·05	0·80	0·06***
b) Males								
RMR (kcal/d)	1986	190	2324	314*	2465	266**	2392	299***
RQ (a.u.)	0·76	0·04	0·88	0·06*	0·76	0·04	0·80	0·06***
c) Females								
RMR (kcal/d)	1598	146	1860	249*	1938	170**	1771	227***
RQ (a.u.)	0·73	0·03	0·87	0·09*	0·75	0·05	0·80	0·06***

FAST, fasted; CHO, carbohydrate; MX, mixed.
Statistical significance ($P < 0·05$) from the *post hoc* pairwise comparisons is indicated by
* For CHO compared with FAST.
** For PRO compared with FAST.
*** For MX compared with FAST.

Table 4. DXA, 4C and indirect calorimetry validity statistics for the a) total sample, b) males and c) females

	FAST *v.* CHO			FAST *v.* PRO			FAST *v.* MX		
Absolute error	CE	TE	SEE	CE	TE	SEE	CE	TE	SEE
a) Total sample									
DXA Fat mass	0·33	0·79	0·72*	0·23	0·80	0·76*	0·23	0·65	0·62*
DXA lean mass	0·63	1·10	0·40*	0·90	1·25	0·78*	0·96	1·33	0·93*
DXA %fat	–0·03	1·58	1·59*	–0·09	1·92	1·94*	–0·09	1·71	1·73*
DXA trunk fat mass	0·23	0·57	0·53	0·15	0·55	0·51	0·17	0·49	0·46
DXA trunk lean mass	0·66	0·94	0·67	0·82	1·02	0·57	0·82	1·15	0·81
4C fat mass	0·31	2·84	2·71†	0·84	2·81	2·37‡	0·55	2·36	2·21‡
4C fat-free mass	0·82	2·72	2·60†	0·40	2·58	2·54†	0·70	2·62	2·56†
4C %fat	0·14	4·04	3·95†	0·82	3·77	3·39†	0·42	3·40	3·31†
RMR	300·05	364·05	146·44	431·45	464·20	126·14	293·13	367·08	126·06
RQt	0·12	0·15	0·04	0·00	0·05	0·04	0·05	0·08	0·04
b) Males									
DXA fat mass	0·36	0·80	0·74*	0·29	0·75	0·67*	0·27	0·77	0·67*
DXA lean mass	0·62	1·14	0·98*	0·73	1·26	0·85*	1·03	1·51	0·85*
DXA %fat	0·29	0·91	0·89*	0·11	0·85	0·86*	0·02	0·83	0·86*
DXA trunk fat mass	0·27	0·60	0·55	0·16	0·51	0·47	0·22	0·57	0·47
DXA trunk lean mass	0·85	1·06	0·65	0·76	1·03	0·64	0·98	1·37	0·64
4C fat mass	0·44	1·89	1·85*	0·31	1·51	1·39*	0·57	1·84	1·69*
4C fat-free mass	0·70	2·28	1·92*	0·81	1·95	1·75*	0·75	2·32	2·25*
4C %fat	0·51	2·63	2·49*	0·16	0·20	1·92*	0·51	2·43	2·29*
RMR	329·50	386·94	118·34	479·70	507·92	118·43	405·65	456·97	136·91
RQ	0·11	0·14	0·04	0·00	0·04	0·04	0·05	0·08	0·04
c) Females									
DXA fat mass	0·30	0·78	0·70*	0·18	0·87	0·83*	0·18	0·50	0·83*
DXA lean Mass	0·63	1·05	0·81*	1·15	1·27	0·66*	0·89	1·10	0·66*
DXA %fat	–0·36	2·06	2·07*	–0·32	2·67	2·59‡	–0·22	2·29	2·59‡
DXA trunk fat mass	0·18	0·54	0·51	0·15	0·62	0·57	0·13	0·39	0·57
DXA trunk lean mass	0·46	0·80	0·64	0·93	1·03	0·54	0·65	0·86	0·54
4C fat mass	0·17	3·58	3·05†	1·42	3·76	2·94†	0·53	2·81	2·53‡
4C fat-free mass	0·95	3·12	2·98‡	–0·05	3·13	3·24†	0·65	2·91	2·86‡
4C %fat	–0·24	5·12	4·30†	1·54	5·05	4·17†	0·32	4·19	3·76†
RMR	269·05	338·29	125·75	377·83	410·21	124·62	174·68	238·29	103·92
RQ	0·13	0·15	0·03	0·01	0·06	0·03	0·06	0·08	0·03

DXA, dual-energy X-ray absorptiometry; 4C, four compartment, FAST, fasted; CHO, carbohydrate; MX, mixed; CE, constant error (kg or %); TE, total error (kg or %); SEE, standard error of the estimate (kg or %).
Subjective ratings for FM and %fat as defined by Heyward and Wang[21].
* Ideal/excellent.
† Fairly good/fair.
‡ Very good/good.

condition compared with each feeding condition (CHO *v.* PRO *v.* MX). Validity statistics are presented in Table 4. Validity statistics included constant error (CE) determined as: $CE = \Sigma(\text{feeding condition-FAST})/n$; total error (TE) = $\sqrt{\Sigma}$(feeding condition-FAST)$^2/n$; and standard error of the estimate (SEE) determined as: (FAST)$\sqrt{1 - r^2}$. Validity analyses and Bland–Altman plots were completed using a customised spreadsheet in Microsoft Excel (version 16.20, Microsoft Corporation). Validity values

British Journal of Nutrition

were interpreted for %fat and FFM using published criteria from Heyward and Wagner[21]. Agreement between feeding conditions to FAST measures received subjective ratings ranging from ideal/excellent, very good/good, to fairly good/fair and poor based on the Standards for Evaluating Prediction Errors.

A one-way ANOVA was also used to evaluate the effects of feeding (FAST *v.* CHO *v.* PRO *v.* MX) on body composition (FM, LM, TFM, TLM, %fat and BMC) from DXA, body composition from 4C (FM, FFM and %fat), and RMR and RQ for the total sample. To evaluate an effect of sex, separate one-way ANOVA stratified by sex were completed. Normality of the data was confirmed via visual histogram assessments due to the relatively small sample size. If sphericity was violated (i.e. Mauchly's test of Sphericity $P < 0{\cdot}05$), the Greenhouse–Geisser *P*-values were used and reported. Bonferroni *post hoc* analyses were performed to identify differences between feeding sessions. Statistical significance for the ANOVA and Bonferroni *post hoc* pairwise comparisons was determined *a priori* at an alpha level of 0·05 ($P < 0{\cdot}05$). These analyses were completed using SPSS software (version 25, IMB Corporation).

Results

Dual-energy X-ray absorptiometry outcomes

Similar validity statistics were observed with all three feeding conditions for FM for PRO (CE = 0·23 kg; TE = 0·80 kg), CHO (CE = 0·33; TE = 0·79 kg) and MX (CE = 0·23 kg; TE = 0·65 kg), when compared with the FAST condition. All errors were considered acceptable, with validity as ideal/excellent for all conditions (Table 4). From the ANOVA, there was a significant main effect of acute feeding on FM for the total sample ($P = 0{\cdot}049$; η^2:0·610). *Post hoc* comparisons demonstrated a significant increase in FM for CHO *v.* FAST (mean difference (MD) ± SE: 0·36 (SD 0·73) kg; $P = 0{\cdot}031$). There were no other significant treatment differences ($P > 0{\cdot}05$). In males, FM was significantly higher in CHO *v.* FAST (0·36 (SD 0·74) kg; $P = 0{\cdot}041$). In females, acute feeding did not significantly impact FM ($P > 0{\cdot}05$). However, the magnitude of the increase in FM for females from CHO *v.* FAST was higher (0·5 kg) on average.

Validity statistics for LM for CHO (CE = 0·63 kg; TE = 1·10 kg), PRO (CE = 0·90 kg; TE = 1·25 kg) and MX (CE = 0·96 kg; TE = 1·33 kg) all produced significant error when compared with the FAST condition, as depicted in Bland–Altman plots (Fig. 2). Qualitatively, these errors are acceptable, with ideal/excellent validity (Table 4). There was a significant main effect of acute feeding on LM ($P = 0{\cdot}001$; η^2:0·99). LM in all feeding conditions was significantly greater than FAST ($P = 0{\cdot}001$), with no other differences between meals. Specifically, LM was increased following CHO (0·60 (SD 0·88) kg; $P = 0{\cdot}001$), PRO (0·91 (SD 0·87) kg; $P =$ 0,001) and MX (0·97 (SD 0·93) kg; $P = 0{\cdot}001$) feeding. In males, LM was significantly increased following CHO (0·62 (SD 0·98) kg; $P = 0{\cdot}011$), PRO (0·73 (SD 1·05) kg; $P = 0{\cdot}006$) and MX (1·02 (SD 1·14) kg; $P = 0{\cdot}001$) compared with FAST. Similarly, in females, LM was increased by CHO (0·53 (SD 0·77) kg; $P = 0{\cdot}009$), PRO (1·11 (SD 0·56) kg; $P = 0{\cdot}001$) and MX (0·89 (SD 0·67) kg; $P = 0{\cdot}001$).

Validity statistics for %fat demonstrated notable, but acceptable effects of CHO (CE = –0·03 %; TE = 1·58 %), PRO (CE = –0·09 %; TE = 1·92 %) and MX (CE = –0·09 %; TE = 1·71 %) when compared with the FAST condition (Fig. 3; Table 4). For the total sample, from the ANOVA, there was no significant effect of acute feeding on %fat ($P = 0{\cdot}956$; η^2:0·062). Acute feeding did not significantly impact %fat in males ($P = 0{\cdot}396$; η^2:0·254) or females ($P = 0{\cdot}909$; η^2:0·069).

Validity statistics for TFM demonstrated small error for all conditions when compared with the FAST condition (Table 4). There was no significant effect of acute feeding on TFM for the total sample ($P = 0{\cdot}489$; η^2:0·108). Acute feeding did not significantly impact TFM in males ($P = 0{\cdot}422$; η^2:0·124) or females ($P = 0{\cdot}413$; η^2:0·160).

Validity statistics for TLM demonstrated similar error for all conditions (Table 4; Fig. 2). There was a significant effect of feeding on TLM for the total sample ($P = 0{\cdot}001$, η^2:0·999). *Post hoc* comparisons demonstrated a significant increase of trunk TLM for CHO (0·67 (SD 0·63) kg; $P = 0{\cdot}001$), PRO (0·83 (SD 0·61) kg; $P = 0{\cdot}001$) and MX (0·83 (SD 0·83) kg; $P = 0{\cdot}001$) conditions compared with FAST. For males, TLM was significantly increased by CHO (0·85 (SD 0·65) kg; $P = 0{\cdot}001$), PRO (0·76 (SD 0·71) kg; $P = 0{\cdot}001$) and MX (0·98 (SD 0·98) kg; $P = 0{\cdot}001$) compared with FAST. Similar findings resulted within the females for CHO (0·39 (SD 0·62) kg; $P = 0{\cdot}015$), PRO (0·91 (SD 0·48) kg; $P = 0{\cdot}001$) and MX (0·66 (SD 0·57) kg; $P = 0{\cdot}001$).

There was no significant acute effect of feeding on BMC for the total sample ($P = 0{\cdot}567$; η^2:0·152). Acute feeding did not significantly impact BMC in males ($P = 0{\cdot}406$; η^2:0·178) or females ($P = 0{\cdot}878$; η^2:0·074).

Four-compartment model outcomes

For the 4C model, validity error for FM of CHO (CE = 0·31 kg; TE = 2·84 kg), PRO (CE = 0·84 kg; TE = 2·81 kg) and MX (CE = 0·55 kg; TE = 2·36 kg) were all moderate when compared with the FAST condition. Validity error for FFM was also moderate: CHO (CE = 0·82 kg; TE = 2·72 kg), PRO (CE = 0·40 kg; TE = 2·58 kg; SEE = 2·54 kg) and MX (CE = 0·70 kg; TE = 2·62 kg). Validity for %fat appeared to result in the largest error: CHO (CE = 0·14 %, TE = 4·04 %), PRO (CE = 0·82 %; TE = 3·77 %) and MX (CE = 0·42 %; TE = 3·40 %) as depicted in Bland–Altman plots (Fig. 4). Qualitatively, these errors are considered good/fair, suggesting body composition when measured by the 4C will be notably altered with food consumption, less from a high PRO meal. For the total sample, there was no main effect for feeding for FM ($P = 0{\cdot}142$; η^2:0·433), FFM ($P = 0{\cdot}248$; η^2:0·362) or %fat ($P = 0{\cdot}392$; η^2:0·236). For the males, there was no main effect for feeding for FM ($P = 0{\cdot}524$; η^2:0·184), FFM ($P = 0{\cdot}343$; η^2:0·289) or %fat ($P = 0{\cdot}716$; η^2:0·123). For the females, there was no main effect for feeding for FM ($P = 0{\cdot}162$; η^2:0 350), FFM ($P = 0{\cdot}389$; η^2:0 261) or %fat ($P = 0{\cdot}252$; η^2:0 273).

Compartments of the 4C were evaluated (Table 2); BM was significantly different across all treatments compared with fast ($P < 0{\cdot}001$); BV was significantly altered by each acute feeding condition, Fast *v.* CHO (MD: 0·93 (SD 1·60) l; $P = 0{\cdot}001$), Fast *v.* PRO (MD: 1·20 (SD 1·38) l; $P < 0{\cdot}001$); Fast *v.* MX (MD:

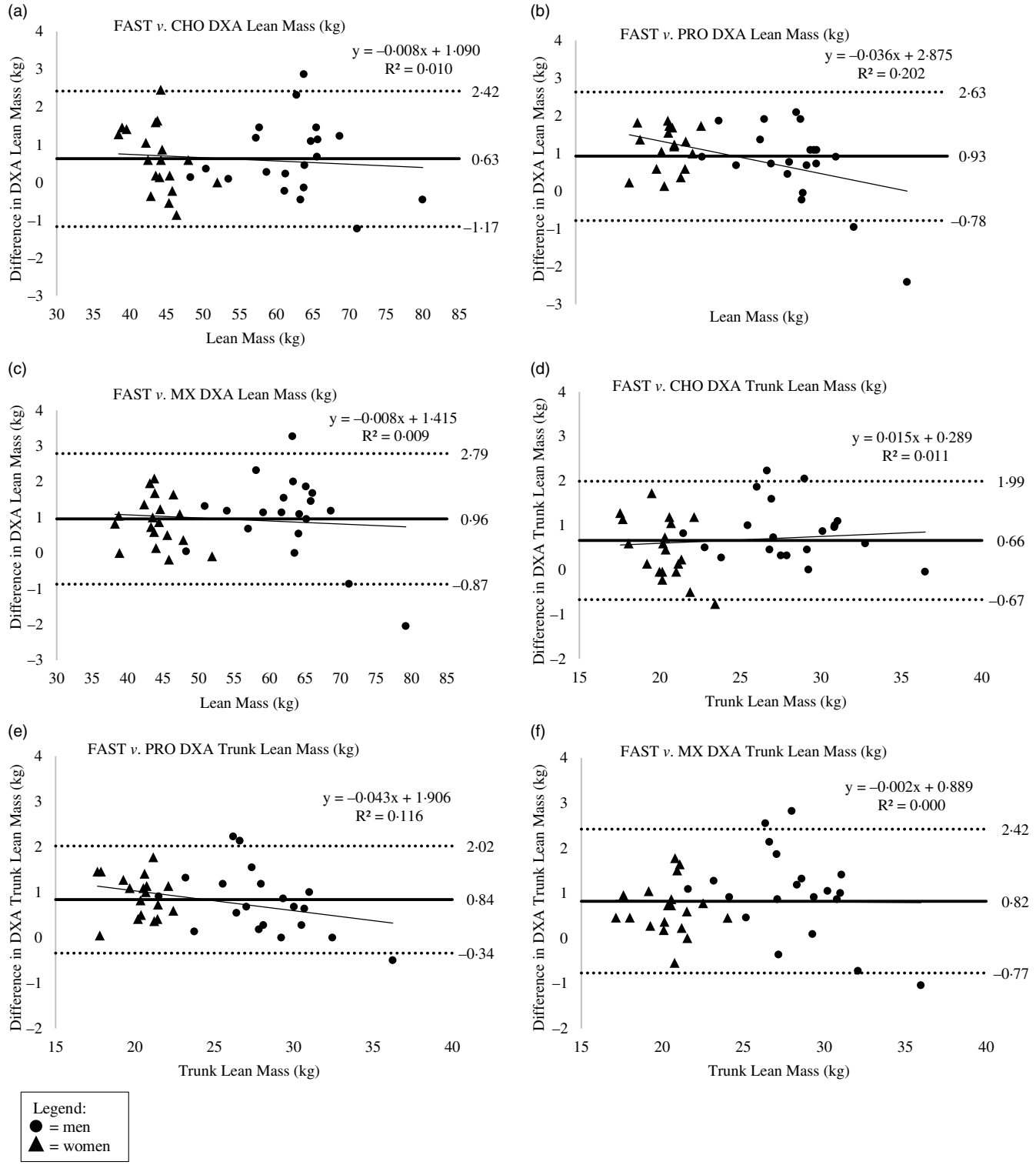

Fig. 2. a) Difference between DXA, LM, CHO and FAST (diff = CHO – FAST) for the entire sample. b) Difference between DXA, LM, PRO and FAST (diff = PRO – FAST) for the entire sample. c) Difference between DXA, LM, MX and FAST (diff = MX – FAST) for the entire sample. The constant error (CE) is represented by a solid line; upper and lower limits are represented by dashed lines. The line of regression is represented by a solid line. d) Difference between DXA, TLM, CHO and FAST (diff = CHO – FAST) for the entire sample. e) Difference between DXA, TLM, PRO and FAST (diff = PRO – FAST) for the entire sample. f) Difference between DXA, TLM, MX and FAST (diff = MX – FAST) for the entire sample. The CE is represented by a solid line; upper and lower limits are represented by dashed lines. The line of regression is represented by a solid line. DXA, dual-energy X-ray absorptiometry; LM, lean mass; CHO, carbohydrate; FAST, fasted; MX, mixed; TLM, trunk LM.

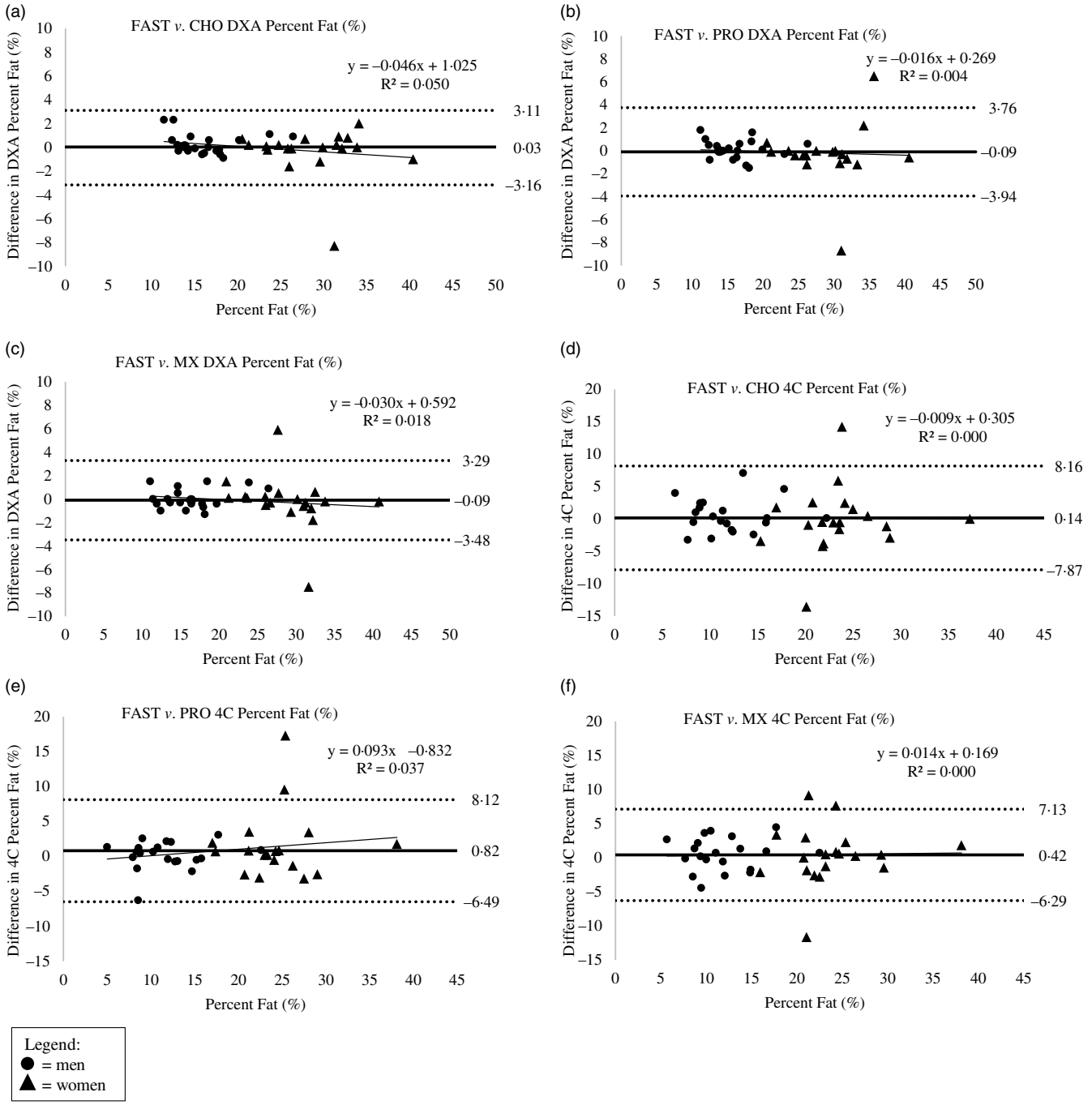

Fig. 3. a) Difference between DXA, %fat, CHO and FAST (diff = CHO – FAST) for the entire sample. b) DXA, %fat, PRO and FAST (diff = PRO – FAST) for the entire sample. c) Difference between DXA, %fat, MX and FAST (diff = MX – FAST) for the entire sample. The constant error (CE) is represented by a solid line; upper and lower limits are represented by dashed lines. The line of regression is represented by a solid line. d) Difference between 4C, %fat, CHO and FAST (diff = CHO – FAST) for the entire sample. e) Difference between 4C, %fat, PRO and FAST (diff = PRO – FAST) for the entire sample. f) Difference between 4C, %fat, MX and FAST (diff = MX – FAST) for the entire sample. The CE is represented by a solid line; upper and lower limits are represented by dashed lines. The line of regression is represented by a solid line. DXA, dual-energy X-ray absorptiometry; CHO, carbohydrate; FAST, fasted; MX, mixed; 4C, four compartment.

1·13 (SD 1·23) l; $P < 0·001$) (Table 2). When stratified by sex, the only condition that did not elicit a significant change in BV was FAST *v.* CHO for the females (MD: 0·86 l (SD 2·02), $P = 0·089$). No significant effect for TBW, extracellular fluid or intracellular fluid of the total sample, males or females ($P > 0·05$) were identified.

Indirect calorimetry outcomes

Validity statistics for RMR yielded similar variability for CHO (CE = 300·1 kcal; TE = 364·1 kcal), PRO (CE = 431·5 kcal; TE = 464·2 kcal) and MX (CE: 293·1 kcal; TE = 367·1 kcal) when compared with the FAST condition (Table 4). There was a significant main effect for feeding on RMR for the total

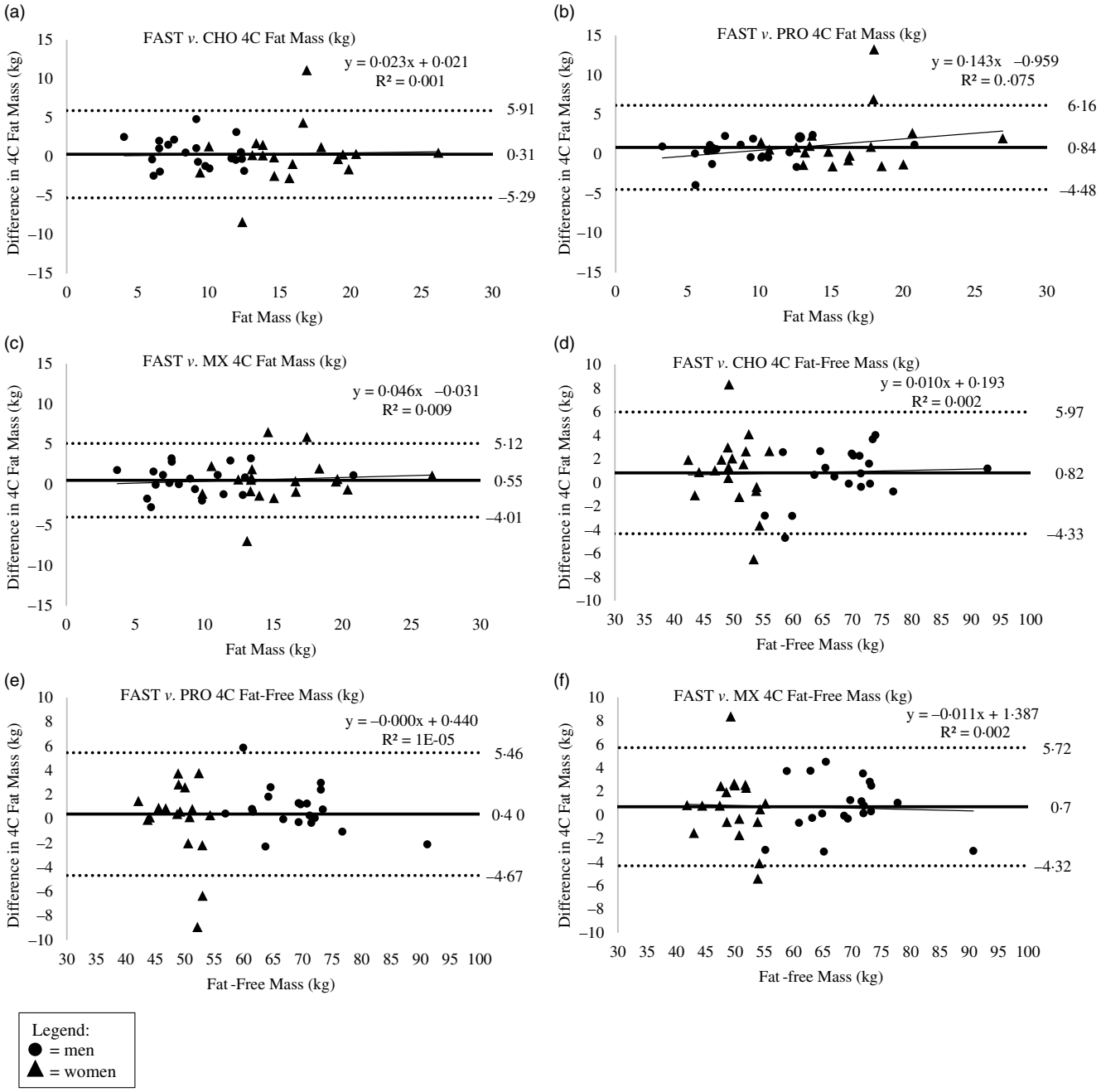

Fig. 4. a) Difference between 4C, FM, CHO and FAST (diff = CHO – FAST) for the entire sample. b) Difference between 4C, FM, PRO and FAST (diff = PRO – FAST) for the entire sample. c) Difference between 4C, FM, MX and FAST (diff = MX – FAST) for the entire sample. The constant error (CE) is represented by a solid line; upper and lower limits are represented by dashed lines. The line of regression is represented by a solid line. d) Difference between 4C, FFM, CHO and FAST (diff = CHO – FAST) for the entire sample. e) Difference between 4C, FFM, PRO and FAST (diff = PRO – FAST) for the entire sample. f) Difference between 4C, FFM, MX and FAST (diff = MX – FAST) for the entire sample. The CE is represented by a solid line; upper and lower limits are represented by dashed lines. The line of regression is represented by a solid line. 4C, four compartment; FM, fat mass; CHO, carbohydrate; FAST, fasted; MX, mixed; FFM, fat-free mass.

sample ($P = 0{\cdot}001$; η^2:0·999). *Post hoc* comparisons demonstrated a significant increase in RMR after all feeding conditions compared with FAST. Specifically, RMR was elevated after CHO (293·3 (SD 149·5) kcal; $P = 0{\cdot}001$), PRO (425·9 (SD 122·0) kcal; $P = 0{\cdot}001$) and MX (303·0 (SD 149·4) kcal; $P = 0{\cdot}001$). PRO resulted in a significantly greater RMR compared with CHO (132·6 (SD 99·3) kcal; $P = 0{\cdot}001$) and MX (122·9 (SD 136·6) kcal; $P = 0{\cdot}003$). CHO was not different than MX (9·7 (SD 147·2) kcal; $P = 0{\cdot}999$) (Table 3). When stratified by sex, males demonstrated significant differences for CHO (329·5 (SD 208·1) kcal; $P = 0{\cdot}001$), PRO (479·7 (SD 171·3) kcal; $P = 0{\cdot}001$) and MX (405·7 (SD 215·9) kcal; $P = 0{\cdot}001$) compared with FAST. The same was found in females for CHO (265·33 (SD 216·2) kcal; $P = 0{\cdot}001$), PRO (362·6 (SD 155·8) kcal; $P = 0{\cdot}001$) and MX (174·6 (SD 166·5) kcal; $P = 0{\cdot}001$) compared with FAST.

Validity statistics for RQ demonstrated similar error for PRO (CE = 0·00 a.u., TE = 0·05 a.u) and MX (CE = 0·05 a.u., TE = 0·08 a.u.) when compared with the FAST condition (Table 4). TE for the CHO group was nearly double that of the other conditions in compared with FAST (CHO TE = 0·15 a.u.). There was a significant main effect for feeding on RQ ($P = 0·001$; η^2:0·99). *Post hoc* comparisons demonstrated a significantly greater RQ for CHO (0·12 a.u. (SD 0·01)) and MX (0·05 a.u. (SD 0·01)) compared with FAST. CHO was significantly greater than PRO (0·12 a.u. (SD 0·01)) and MX (0·07 a.u. (SD 0·01)), and PRO was significantly lower than MX (–0·05 a.u. (SD 0·01)). CHO significantly increased RQ in males (0·11 a.u. (SD 0·08)) and females (0·13 a.u. (SD 0·09)) compared with FAST. There was no significant effect of PRO on RQ compared with FAST in either males ($P = 0·916$) or females ($P = 0·531$); the MX meal had a similar, significant, effect in males (0·05 a.u. (SD 0·06)) and females (0·06 a.u. (SD 0·05)) compared with FAST.

Discussion

A common barrier to body composition and metabolic research lies in the feasibility of testing subjects in a fasted, euhydrated state, in order to obtain valid measures. To further understand the influence of food consumption on testing outcomes and to increase the accuracy of practical application, evaluating the effects of acute feeding on body composition measurement is warranted. Findings from this study suggest that acute CHO, PRO and MX feeding may have minimal effects on body composition measured via DXA, with greater effects resulting for 4C estimates. Changes in body composition (FM, LM and %fat) from the DXA did not exceed the standard error associated with measurement, suggesting that the clinical interpretation of these changes may not be as significant. Effects on 4C estimates were more notable and are likely to be more impacted from acute food intake; according to TE, the acute CHO meal had the greatest impact on 4C outcomes. The 4C results demonstrate the importance of maintaining pre-testing guidelines for this method and notes the sensitivity of the 4C model to detect acute alterations to body compartments. For DXA and 4C outcomes, the greatest influence on validity was seen on %fat for all feeding sessions, with the largest contributions resulting from PRO and MX meals. The greatest sex-based differences were reported in %fat 4C and DXA outcomes with females being more impacted by acute feeding compared with males. All acute feeding sessions significantly influenced RMR, with no differences between groups.

When evaluating the influence of acute feeding on DXA FM, previous data are conflicting. A previous study reported a small underestimation of FM by 0·2 kg following both high and low CHO feeding[5], with no significant differences between sexes in healthy young adults[5]. Additional studies have found no significant changes in FM following acute feeding of small meals[3,6,7,22,23]. Validity statistics from the present study demonstrated an overestimation of FM from all feeding conditions, for CHO (CE = 0·33 kg; TE = 0·79 kg), PRO (CE = 0·23 kg; TE = 0·80 kg) and MX (CE = 0·23 kg; TE = 0·65 kg). Although this is an overestimation, these errors fall within the standard measurement error for DXA FM (0·85 kg), thus it may not be considered clinically relevant.

The influence of acute feeding on DXA LM has been evaluated in previous studies[3,5,7,22]; findings from the present study are consistent with these findings. Results from the current study report that all feeding conditions influenced LM, with significant sex differences. In the total sample, an overestimation of LM resulted from CHO (CE: 0·63 kg, TE: 1·1 kg) and PRO (CE: 0·90 kg, TE: 1·25 kg), and MX (CE: 0·96 kg, TE: 1·33 kg). Comparable to the influence of the present study's CHO and PRO feeding conditions, Tinsley et al.[5]. reported an overestimation of LM by 0·80 kg following both high (9 g/kg) and low CHO (1–1·5 g/kg) feeding, with no differences between sexes[5]. Additionally, a study in males reported that consumption of a 500-g meal significantly increased LM by 0·4 kg, and a 500-g meal with 1 litre of water increased LM by 1·6 kg, further illustrating that a meal greater than 500 g impacts DXA measures[6,7,23]. Increases in LM (MD: 1·039 kg) were also reported by Thomsen et al.[22]. following consumption of an about1300-g meal with 1 litre of water. These findings are further solidified by the present study consisting of meals exceeding this amount (average 898 grams and 0·72 l of fluid). Although acute feeding resulted in greater overestimation of LM compared with FM, changes still fell within DXA measurement error of LM (1·97 kg). Thus, differences may not be as clinically impactful as we originally hypothesised. The overestimations appear to be more skewed for heavier individuals, according to the Bland–Altman interpretations (Fig. 2); the impact of a meal may result in a greater overestimation of LM for individuals of higher weight.

Measurements across all condition testing days for %fat produced a similar increase in %fat for the total sample ranging from −0·03 to −0·09 %. %fat estimates resulted in the largest divergent responses for males and females, with females reporting greater average TE, across all feeding conditions (avg TE: 2·3 %, CE: −0·30 %) compared with males (avg TE: 0·86 %, CE: 0·11 %). Tinsley et al.[5]. also reported greater influence of feeding on females (–0·6 % fat) compared with males (–0·3 % fat), although much smaller than reported in the present study and within the measurement error. The error presented from the present study is qualitatively ideal/excellent[21], with an average underestimation of %fat across all conditions.

It was predicted that the trunk region would be significantly impacted by acute feeding, specifically for TLM, due to the addition of food mass within the digestive tract. Similar to the FM outcomes, the influence of acute feeding did not significantly alter TFM beyond the measurement error, and significant sex-based differences were not identified. Validity statistics showed small increases in TFM resulting from CHO (TE: 0·57 kg), PRO (TE: 0·55 kg) and MX (TE: 0·49 kg) conditions for the total sample. Males and females responded similarly all feeding conditions (Table 4). Tinsley *et al.*[5] reported a similar decrease in TFM by 0·1 kg as a result of acute feeding. Additionally, no substantial changes in TFM were found by Nana *et al.*[3] following the ingestion of a small breakfast meal. Contrary to the effects on TFM, TLM was slightly altered by all feeding conditions (average TE: 1·0 kg). The present study did not necessarily allow for complete

digestion of food, thus, as digestion progresses, changes in trunk mass may vary.

The influence of acute feeding on 4C FM and FFM has only been evaluated in one previous study completed in males[7]. Kerr *et al.*[7] reported that a 500-g meal with 1 litre of water altered FM by 1·14 kg exceeding the measurement error of 0·43 kg, with no significant changes seen beyond measurement error for FFM. In the same study, a 500-g meal consumed without fluid did not substantially alter FM or FFM, emphasising the impact of acute fluid intake on body composition estimations. Results of the current study showed that under all feeding conditions, 4C estimates of body composition (FM, FFM and %fat) were significantly altered beyond measurement error, with little difference between conditions. The validity of %fat was altered the most by acute feeding with similar increases %fat as a result of acute CHO (TE: 4·04 %), PRO (TE:3·77 %) and MX (TE:3·40 %) feeding. Similar to DXA %fat, there was a greater impact of acute feeding on females, with an average TE of 4·79 % across all conditions, compared with an average (Table 4). TE for FM was moderate and similar between all conditions ranging from an increase of 2·36–2·84 kg following consumption. Acute feeding produced similar TE for FFM with values ranging from 2·58 to 2·72 kg. Compared with the study by Kerr *et al.*[7], the PRO and CHO meals in the current study were almost twice as large (882–913 g), while less fluid was consumed (0·83 l). Similar to DXA, the significant increase in total BM is also likely to be the main contributor of 4C %fat error; however, in contrast to DXA, the greater number of compartmental variables used for 4C outcomes may have impacted results. BV was significantly altered by each acute feeding condition, but this increase was minimal for the total sample (MD: 0·93–1·20 l). When stratified by sex, the only condition that did not elicit a significant change in BV was FAST *v.* CHO for the females (MD: 0·86 l (SD 2·02), $P = 0·089$). No significant changes were found for any condition for bone, TBW, extracellular fluid or intracellular fluid of the total sample, males or females ($P > 0·05$).

As hypothesised, RMR and RQ were altered following acute food ingestion. RMR was elevated during all conditions (Table 3). On average, the impact of acute feeding increased RMR by 340 kcal for the total sample. Validity error for the total sample was similar between all feeding conditions; PRO resulted in the greatest TE (431·5 kcal). Males and females had a similar metabolic response, with the PRO feeding resulting in the greatest TE. In addition to RMR, RQ increased significantly as a result of the CHO (0·87 a.u.) and MX (0·80 a.u.) conditions, with no significant effect on RQ from PRO. Validity errors for RQ were relatively consistent across conditions; with CHO having the largest influence. The current study suggests that the acute feeding of an about 900-g meal will likely significantly elevate RMR; with CHO resulting in an acute increase in RQ. When conducting metabolic testing, particularly RQ, it would be advisable to adhere to more stringent fasting procedures. Understanding the effects of feeding on these outcomes are important for calculating energetic requirements and expenditure estimates. Following an 8–12 fast for metabolic assessments is important for accurate energy and fuel oxidation evaluations.

Limitations exist with all studies; in the present study, verbal confirmation was utilised to acknowledge that subjects adhered to pre-assessment guidelines: arriving to testing 12 h fasted and refraining from moderate and vigorous physical activity 24 h prior. Additionally, while hydration was assessed via urine-specific gravity, exact volume of liquid consumed was not tracked; deuterium oxide is considered the gold standard for hydration, using bioelectrical impedance spectroscopy for TBW estimates may have provided some inaccuracies. Although females were tested during the follicular phase, based on self-reported menstrual bleeding, hormones were not assessed to verify or evaluate variability in oestrogen concentrations. Due to subject availability, testing for some females (n 12) occurred over multiple months which may have allowed for minor changes in body composition. Moreover, although assessing body composition prior to and following feeding sessions would have been cumbersome for participants, this study design would have been more ideal in order to remove the possibility of day-to-day variability in measurements. Lastly, conclusions from this study should only be applied to healthy, young adults, and further research may be needed in elderly, elite performers and clinical populations.

Conclusion

In healthy young adults, acute feeding of varied macronutrients (CHO, PRO and MX) does not appear to have large implications for the validity of DXA outcomes. Acute feeding 1 hour prior to body composition testing, regardless of macronutrient content, resulted in an overestimation of DXA-derived %fat, FM, LM, TFM and TLM. When evaluating individuals, these changes could be more relevant, resulting in more significant differences. When using 4C model, feeding appears to significantly alter all body composition outcomes. For females, %fat was elevated by 4·0 %, while FM and FFM were elevated by 2·9 kg. Data in males showed similar, but smaller overestimations for %fat (2·2 %), FM and FFM (1·8 kg). For metabolic measurements, acute feedings increased RMR by an average of 121·3 kcal and RQ by 0·035 a.u, which supports the fasting guideline prior to metabolic assessments. Standard pre-testing fasting guidelines may be particularly important when evaluating %fat, whereas DXA-derived FM and LM outcomes may not be largely influenced by a small meal prior to measurement. Due to the greater sensitivity of the methodology, 4C estimates of body composition will likely be altered from an acute meal. Altogether, measuring body composition via DXA under less stringent pre-testing guidelines may retain validity and increase feasibility of testing in clinical and applied settings, while adhering to fasted pre-assessment guidelines remains important for 4C estimates and metabolic outcomes. However, if the recommended pre-testing guidelines (8–12 h fast) cannot be followed, consumption of food and water prior to body composition and metabolic testing will result in some error compared with fasting. This might be more important for athletes or when establishing energetic needs.

Acknowledgement

The authors would like to acknowledge and thank Gabrielle Brewer as a senior author for her leadership and dedication to the data collection and analysis of this project.

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

A. E. S-R.: conceptualisation, methodology, analysis, resources, data curation, writing – original draft, reviewing and supervision; G. J. B.: project administration, data curation, investigation, analysis and writing original draft; L. M. G.: data curation, investigation, analysis and writing – review and editing; M. N. M. B.: conceptualisation, methodology and writing – review and editing; K. R. H.: conceptualisation, methodology, analysis, writing – review and editing, and supervision; C. E. G.: data curation, investigation and writing – review and editing; C. E. H.: data curation, investigation, supervision, writing – reviewing and editing; H. E. C.: data curation, investigation, supervision, writing – reviewing and editing; E. D. R.: methodology, investigation, supervision, writing – reviewing and editing.

The authors certify that there is no conflict of interest with any financial organisation regarding the material discussed in the manuscript. The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation.

References

1. Nana A, Slater GJ, Stewart AD, *et al.* (2015) Methodology review: using dual-energy X-ray Absorptiometry (DXA) for the assessment of body composition in athletes and active people. *Int J Sport Nutr Exerc Metab* **25**, 198–215.
2. Smith-Ryan AE, Mock MG, Ryan ED, *et al.* (2017) Validity and reliability of a 4-compartment body composition model using dual energy X-ray absorptiometry-derived body volume. *Clin Nutr* **36**, 825–830.
3. Nana A, Slater GJ, Hopkins WG, *et al.* (2012) Effects of daily activities on dual-energy X-ray absorptiometry measurements of body composition in active people. *Med Sci Sports Exerc* **44**, 180–189.
4. Nana A, Slater GJ, Hopkins WG, *et al.* (2013) Effects of exercise sessions on DXA measurements of body composition in active people. *Med Sci Sports Exerc* **45**, 178–185.
5. Tinsley GM, Morales E, Forsse JS, *et al.* (2017) Impact of acute dietary manipulations on DXA and BIA body composition estimates. *Med Sci Sports Exerc* **49**, 823–832.
6. Horber FF, Thomi F, Casez JP, *et al.* (1992) Impact of hydration status on body composition as measured by dual energy X-ray absorptiometry in normal volunteers and patients on haemodialysis. *Br J Radiol* **65**, 895–900.
7. Kerr A, Slater GJ & Byrne N (2017) Impact of food and fluid intake on technical and biological measurement error in body composition assessment methods in athletes. *Br J Nutr* **117**, 591–601.
8. Haugen HA, Melanson EL, Tran ZV, *et al.* (2003) Variability of measured resting metabolic rate. *Am J Clin Nutr* **78**, 1141–1145.
9. Coyle EF (1995) Substrate utilization during exercise in active people. *Am J Clin Nutr* **61**, 968S–979S.
10. Gorostiaga EM, Maurer CA & Eclache JP (1989) Decrease in respiratory quotient during exercise following L-carnitine supplementation. *Int J Sports Med* **10**, 169–174.
11. Wang ZM, Pierson RN & Heymsfield SB (1992) The five-level model: a new approach to organizing body-composition research. *Am J Clin Nutr* **56**, 19–28.
12. Demerath EW, Guo SS, Chumlea WC, *et al.* (2002) Comparison of percent body fat estimates using air displacement plethysmography and hydrodensitometry in adults and children. *Int J Obes Relat Metab Disord* **26**, 389–397.
13. McCrory MA, Molé PA, Gomez TD, *et al.* (1998) Body composition by air-displacement plethysmography by using predicted and measured thoracic gas volumes. *J Appl Physiol* **84**, 1475–1479.
14. Buendia R, Seoane F, Lindecrantz K, *et al.* (2015) Estimation of body fluids with bioimpedance spectroscopy: state of the art methods and proposal of novel methods. *Physiol Meas* **36**, 2171–2187.
15. Moon JR, Tobkin SE, Roberts MD, *et al.* (2008) Total body water estimations in healthy men and women using bioimpedance spectroscopy: a deuterium oxide comparison. *Nutr Metab* **5**, 7.
16. Earthman C, Traughber D, Dobratz J, *et al.* (2007) Bioimpedance spectroscopy for clinical assessment of fluid distribution and body cell mass. *Nutr Clin Pract* **22**, 389–405.
17. Bray GA, Whipp BJ & Koyal SN (1974) The acute effects of food intake on energy expenditure during cycle ergometry. *Am J Clin Nutr* **27**, 254–259.
18. Brooks GA & Fahey TD (2005) *Exercise Physiology: Human Bioenergetics and Its Applications*, 4th ed. New York, NY, USA: McGraw-Hill Education.
19. Weir J (1949) New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* **109**, 1–9.
20. Bland JM & Altman DG (1999) Measuring agreement in method comparison studies. *Stat Meth Med Res* **8**, 135–160.
21. Heyward VH & Wagner DR (2004) *Use of Regression Analysis in Body Composition. Applied Body Composition Assessment*, 2nd ed. Champaign: Human Kinetics Publishers, Inc.
22. Thomsen TK, Jensen VJ & Henriksen MG (1998) *In vivo* measurement of human body composition by dual-energy X-ray Absorptiometry (DXA). *Eur J Surg* **164**, 133–137.
23. Vilaca KH, Ferriolli E, Lima ND, *et al.* (2009) Effect of fluid and food intake on the body composition evaluation of elderly persons. *J Nutr Health Aging* **13**, 183–186.