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Salivary immunoglobulin A response at rest and after exercise following a 48 h period of fluid and/or energy restriction

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The aim was to investigate the effects of a 48 h period of fluid, energy or combined fluid and energy restriction on salivary IgA (s-IgA) responses at rest and after exercise. Thirteen healthy males (age 21 (SEM 1) years) participated in four randomised 48 h trials. In the control trial participants received their estimated energy (12 154 (SEM 230) kJ/d) and water (3912 (SEM 140) ml/d) requirements. On fluid restriction (FR) participants received their energy requirements and 193 (SEM 19) ml water/d to drink and on energy restriction (ER) participants received their water requirements and 1214 (SEM 25) kJ/d. Fluid and energy restriction (F + ER) was a combination of FR and ER. After 48 h, participants performed a 30 min treadmill time trial (TT) followed by rehydration (0–2 h) and refeeding (2–6 h). Unstimulated saliva was collected at 0, 24 and 48 h, post-TT, and 2 and 6 h post-TT. Saliva flow rate (sflw) and s-IgA (ELISA) remained unchanged in control conditions and on ER. However, 48 h on FR decreased sflw (64 %) which most probably accounted for the increase in s-IgA concentration ($P<0{\cdot}01$). Despite a decrease in sflw (54 %), s-IgA concentration did not increase on F + ER, resulting in a decreased s-IgA secretion rate by 24 h (0 h: 20 (SEM 2); 24 h: 12 (SEM 2) μg/min; $P<0{\cdot}01$). Post-TT s-IgA secretion rate was not lower compared with 48 h on any trial. s-IgA secretion rate returned to within 0 h values by 6 h post-TT on F + ER. In conclusion, a 24–48 h period of combined F + ER decreased s-IgA secretion rate but normalisation occurred upon refeeding.

Dietary restriction: Immune function: Saliva immunoglobulin: Exercise

A decrease in the saliva concentration of IgA has been implicated as a possible causal factor in the increased susceptibility to upper respiratory tract infection during heavy training in athletes (Gleeson *et al.* 1999) and military personnel (Carins & Booth, 2002). Saliva and mucosal secretions form an important first line of defence against pathogens invading the oral cavity. Saliva provides a mechanical washing effect to protect the oral mucosa, and the antiviral and antibacterial effects of IgA prevent viral replication and bacterial attachment to the mucosal surfaces (Tomasi *et al.* 1982; Mackinnon & Hooper, 1994; Dowd, 1999).

Episodes of forced or voluntary fluid and energy restriction, often lasting for a number of days, frequently occur in occupational and athletic settings, for example, in military recruits during survival training (Carins & Booth, 2002), athletes with eating disorders (Baum, 2006) and athletes making weight for competition (Brownell *et al.* 1987). Nutrient restrictions have the potential to weaken many aspects of immune function because macronutrients are involved in cell metabolism and protein synthesis and micronutrients are involved in immune cell replication and antioxidant defences (Chandra, 1997). Decreased fuel availability may also impair immune function through raised sympatho-adrenal activation in response to stress (for example, exercise) and resulting increases in stress hormones (for example, cortisol) known to have immuno-suppressive effects (Gleeson *et al.* 2004). Elevated plasma cortisol has been observed during prolonged exercise with fluid restriction (Bishop *et al.* 2004) and during severe energy restriction (Fichter *et al.* 1986). Cortisol has been shown to rapidly inhibit transepithelial transport of salivary IgA (s-IgA) in rodents (Sabbadini & Berczi, 1995) and glucocorticoid administration *in vivo* had a delayed inhibitory effect (taking several hours) on *in vitro* B lymphocyte antibody synthesis in human subjects (Saxon *et al.* 1978). In addition, dehydration evoked by a 24 h period without fluid or food has been shown to decrease saliva flow rate (Ship & Fischer, 1997) which might further impair oral health by limiting the availability of several proteins (for example, IgA, lysozyme and α-amylase) known to have important antimicrobial properties (Tenovuo, 1998). Indeed, saliva flow rate appears to be an influential factor in protection against oral pathogens and as such it has been recommended that s-IgA be reported as a

Abbreviations: BML, body mass loss; ER, energy restriction trial; F + ER, fluid and energy restriction trial; FR, fluid restriction trial; s-IgA, salivary IgA; TT, 30 min treadmill time trial.

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secretion rate (flow rate × concentration) to reflect the total availability of s-IgA at the oral surface and correct for changes in hydration status (Walsh *et al.* 1999). In line with this, individuals who suffer from dry mouth syndrome (xerostomia) are known to suffer an increased incidence of upper respiratory tract infection (Fox *et al.* 1985).

The effects of prolonged fluid and/or energy restriction on s-IgA responses remain unclear. s-IgA has been reported to be lower in moderately malnourished children compared with well-fed children (Watson *et al.* 1985; Johansson *et al.* 1994) but remained unaltered after an 8 d fast in healthy adults (Johansson *et al.* 1984). The discrepant findings may be partly explained by differences in saliva collection methods (for example, parotid *v.* whole and stimulated *v.* unstimulated), the method used to express s-IgA (for example, as a concentration or relative to protein) and the time of day when samples were collected. Studies either did not report the time of day when saliva samples were collected or gave a broad time frame for collection (for example, 09.00 to 13.00 hours (Johansson *et al.* 1994)). This is a limitation as s-IgA concentration has since been shown to exhibit diurnal variation, with a significant decrease during the morning hours and reaching a plateau 6 h after awakening (Hucklebridge *et al.* 1998). Intense military training involving severe fluid and energy restriction has been shown to decrease s-IgA concentration (Carins & Booth, 2002; Gomez-Merino *et al.* 2003; Tiollier *et al.* 2005) which possibly contributed to the raised upper respiratory tract infection incidence among recruits (Carins & Booth, 2002). However, the presence of other stressors previously shown to decrease s-IgA (for example, arduous exercise (Tomasi *et al.* 1982) and psychological stress (Jemmott *et al.* 1983)) may account, at least in part, for these findings. Furthermore, these studies (Carins & Booth, 2002; Gomez-Merino *et al.* 2003; Tiollier *et al.* 2005) did not report s-IgA as a secretion rate as recommended (Walsh *et al.* 1999) and the lack of experimental control is also a limitation. Whether prolonged fluid and/or energy restriction alters the s-IgA response to subsequent exercise remains unknown.

Therefore, the purpose of the present study was to determine the effects of a prolonged (48 h) period of fluid, energy or combined fluid and energy restriction on s-IgA responses at rest and after exercise. We hypothesised that fluid or energy restriction would decrease s-IgA responses both at rest and after exercise and that the effects of combined fluid and energy restriction would be additive.

Methods

Participants

Thirteen recreationally active healthy males (age 21 (SEM 1) years; height 179 (SEM 2) cm; body mass 74·7 (SEM 2·2) kg; body fat 16·8 (SEM 1·4) %; VO_{2max} 50·9 (SEM 1·2) ml/kg per min) volunteered to participate in the study. All participants gave written informed consent before the study, which received local ethics committee approval.

Preliminary measurements

Before the experimental trials, VO_{2max} was estimated by means of a continuous incremental exercise test on a treadmill. Criteria for attaining VO_{2max} included the participant reaching volitional exhaustion, a heart rate within 10 beats per min of age-predicted maximum heart rate and an RER $\geq$ 1.15 (Bird & Davison, 1997). From the VO_2–work rate relationship, the work rate equivalent to 50 % VO_{2max} was estimated and used for submaximal exercise during the experimental trials. On a separate day, 7 d before beginning the experimental trials, participants returned to the laboratory for individual energy expenditure estimation and familiarisation. Participants arrived euhydrated at 08.00 hours after an overnight fast, having consumed water equal to 40 ml/kg body mass the previous day. On arrival and after voiding, anthropometric measurements of height and nude body mass were collected. Following these measures, body composition was estimated using whole-body dual-energy X-ray absorptiometry (QDR1500 software version 5.72; Hologic Inc., Bedford, MA, USA) and RMR was estimated for 10 min using a portable breath-by-breath system (Metamax 3B; Biophysik, Leipzig, Germany). After breakfast, participants performed a 1.5 h treadmill walk at 50 % VO_{2max} during which energy expenditure was estimated (Cortex Metalyser 3B; Biophysik, Leipzig, Germany). For short periods during the day participants wore the portable breath-by-breath system (Metamax 3B; Biophysik, Leipzig, Germany) to estimate the energy expenditure incurred during habitual living in the laboratory environment. This additional energy expenditure data were used, along with the RMR data, to estimate the energy intake required for the experimental trials. Additionally, during this 24 h period fluid requirements were estimated by assessing changes in body mass at hourly intervals. Physical activity was standardised throughout the familiarisation and all experimental trials by recording 24 h step counts with pedometers (Digi-walker SW-200; Yamax, Tokyo, Japan).

Experimental trials

Separated by 7–10 d, participants were required to complete four experimental trials in a random order (Table 1). The four dietary interventions included a control trial, a fluid restriction trial (FR), an energy restriction trial (ER) and a combined fluid and energy restriction trial (F + ER). Dietary composition was estimated using software (Dietmaster, version 4.0; Swift Computer Systems, Camberley, Surrey, UK). On the day before the experimental trial, to control nutritional and hydration status, participants were provided with their estimated energy requirements (11 346 (SEM 197) kJ/d of which 49, 36 and 15 % were carbohydrate, fat and protein respectively) and water equal to 40 ml/kg body mass (Fig. 1). Participants were also instructed to refrain from exercise. Participants arrived at the laboratory at 22.00 hours the evening before each trial. On the evening before the completion of each trial participants slept for 8 h in a temperate laboratory (19·7 (SEM 0·3) °C; 58·8 (SEM 1·9) % relative humidity). The intervention began at 08.30 hours the following morning after participants had voided and a nude body mass was obtained. Thereafter during waking hours nude body mass was recorded at two-hourly intervals. Euhydration was verified by ensuring all participants' urine specific gravity was less than 1·020 g/ml (Atago Uricon-NE; NSG Precision Cells, Farmingdale, NY, USA; Casa *et al.* 2005). Following nude body mass and 10–15 min seated, baseline (0 h) blood and

Table 1. Nutrient intake for a 24 h period*
(Mean values with their standard errors; *n* 13)

	Control		FR		ER		F + ER	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Fluid								
Fluid consumed (ml)	3912	140	960	15	3893	136	962	16
Water to drink (ml)	3145	134	193	19	3816	135	885	15
Water in food (ml)	767	11	767	11	77	1	77	1
Energy								
Energy consumed (kJ)	12 154	230	12 154	230	1214	25	1214	25
Carbohydrates (g)	387	8	387	8	39	1	39	1
Fat (g)	119	3	119	2	12	0	12	1
Protein (g)	104	2	104	2	10	0	10	1

FR, fluid restriction trial; ER, energy restriction trial; F + ER, fluid and energy restriction trial.
*Macronutrient composition was the same across all trials and equalled 50, 36 and 14 % for carbohydrate, fat and protein respectively.

saliva samples were obtained. Further collections of first morning blood and saliva were made after 24 h (08.30 hours on day 2) and 48 h (08.30 hours on day 3). To simulate active populations (for example, military recruits on field exercise) participants performed a 1.5 h treadmill walk at a set workload equivalent to 50 % VO_{2max} after breakfast on day 1 and 2. During the 1.5 h walks water was consumed equal to fluid losses in the control trial and ER whereas no fluids were provided on FR and F + ER. Following lunch and evening meals, participants also completed a 20 min walk. After providing a 48 h sample, participants performed a self-paced 30 min treadmill time trial (TT), the data from which are presented elsewhere (Oliver *et al.* 2007). Participants were instructed to 'run as far as possible in 30 minutes' and to control the speed of the treadmill (gradient set at 1 %) as and when they felt appropriate. No fluids were consumed during the TT. Further blood and saliva samples were obtained immediately post-TT, 2 h post-TT and 6 h post-TT. During the first 2 h of recovery, fluid was provided as a citrus-flavoured electrolyte-only solution (50 mmol Na/l; Science in Sport, Blackburn, Lancs, UK). The rehydration solution was divided evenly across the 2 h and consumed equal to 100 % body mass loss (BML) or up to 29 ml/kg body mass, which reflects the approximate maximal gastric emptying rate for this solution (Mitchell *et al.* 1994). During 2–3 and 4–5 h of recovery, participants consumed a total of 8164 (SEM 155) kJ (49, 36 and 15 % were carbohydrate, fat and protein respectively) divided equally into two meals. Water was available *ad libitum* during these two meals (Fig. 1).

Sample collection and analysis

Saliva. Unstimulated whole saliva samples were collected using pre-weighed universal tubes. All saliva samples were collected while the participant sat quietly in the laboratory. After thoroughly rinsing the mouth with sterile water each participant was asked to swallow in order to empty the mouth before saliva was collected. The saliva sample was collected for exactly 2 min by the participant leaning forward and passively drooling into the tube with minimal orofacial movements. Saliva volume was estimated by weighing the universal tube immediately after collection to the nearest milligram, and saliva density was assumed to be 1 g/ml (Cole & Eastoe, 1988). From this, the saliva flow rate was determined by dividing the volume of saliva by the collection time. Saliva was then aspirated into eppendorfs and stored at −80°C for further analysis. After thawing, s-IgA concentration was determined by ELISA (Probiomics Ltd, Eveleigh, NSW, Australia) using IgA monomer from human serum as standard. The intra-assay CV was 3·2 %. s-IgA secretion rate was calculated by multiplying the saliva flow rate by s-IgA concentration.

Blood and urine. Blood samples were collected, without venestasis by venepuncture from an antecubital vein, into two separate vacutainer tubes (Beckton Dickinson, Oxford, UK), one containing lithium heparin and one containing K_3EDTA. Blood in the tube containing K_3EDTA was used to determine Hb concentration in triplicate using a haematology analyser (Beckman Coulter Gen S; Beckman Coulter, Inc., Fullerton, CA, USA). Packed cell volume (heparinised blood) was determined in triplicate using the capillary method and plasma volume changes were estimated (Dill &

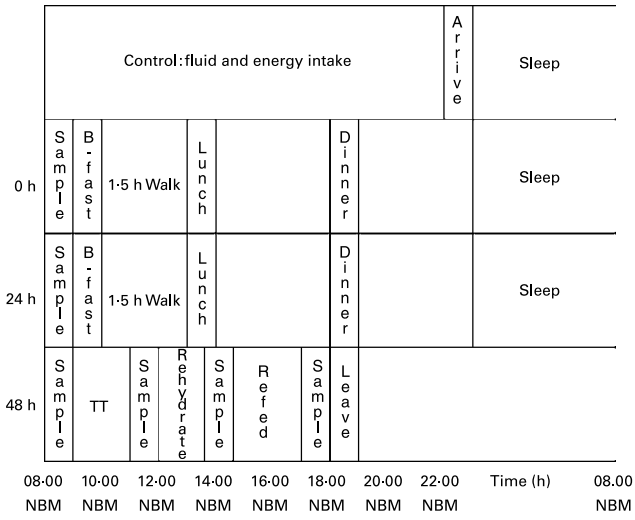

Fig. 1. Schematic of trial events. B-fast, breakfast; NBM, nude body mass. Adapted from Oliver *et al.* (2007).

Costill, 1974). The remaining blood in the lithium heparin tube and K_3EDTA tube was immediately centrifuged (1500 *g* for 10 min at 5°C) and the plasma aspirated and stored at −80°C for further analysis. Plasma concentrations of NEFA (K_3EDTA plasma) and glucose (heparinised plasma) were determined using spectrophotometric kits (Randox, Crumlin, County Antrim, UK). Plasma cortisol (heparinised plasma) was determined using ELISA (DRG Diagnostics, Marburg, Germany). Plasma NEFA, glucose and cortisol concentrations were corrected for changes in plasma volume. Urine samples were collected mid-flow into universal containers. Urine specific gravity was determined by a handheld refractometer (Atago Uricon-Ne; NSG Precision Cells, Farmingdale, NY, USA).

Statistical analysis

One-way fully repeated-measures ANOVA were performed on pre-experimental body mass measures and trial physical activity. Two-way fully repeated ANOVA were performed on BML, plasma and saliva parameters. Appropriate adjustments to the degrees of freedom were made in cases where the assumptions of sphericity and normality were violated. *Post hoc* Tukey's honestly significantly different and Bonferroni adjusted *t* tests were used where appropriate. Data in the text, tables and figures are presented as means and standard errors of the mean. Significance was accepted at $P<0{\cdot}05$. All analyses were performed using the Statistical Package for the Social Sciences (SPSS version 12; SPSS Inc., Chicago, IL, USA).

Results

Body mass loss, recovery fluid intake and physical activity

At baseline (0 h) body mass was not significantly different between trials (control, 73·4 (SEM 2·0); FR, 73·9 (SEM 2·0); ER, 73·9 (SEM 2·1); F + ER, 73·7 (SEM 2·1) kg; $P>0{\cdot}05$). After 48 h, BML on FR, ER, and F + ER was significantly greater than control ($P<0{\cdot}01$; Table 2). BML was also greater on F + ER than FR at 48 h ($P<0{\cdot}01$). Following the TT, BML was further increased compared with 48 h on all trials ($P<0{\cdot}01$). The 6 h recovery protocol re-established 0 h body mass in the control trial and on FR although significant BML was still evident on ER and F + ER ($P<0{\cdot}01$). Fluid provided during the 2 h rehydration period was 1363 (SEM 89) ml in the control trial, 2056 (SEM 56) ml on FR, 2048 (SEM 57) ml on ER and 2037 (SEM 53) ml on F + ER. The *ad libitum* water intake during recovery meals (2–3 h and 4–5 h post-recovery) was 830 (SEM 72) ml in the control trial, 1031 (SEM 95) ml on FR, 818 (SEM 129) ml on ER and 914 (SEM 123) ml on F + ER. The mean experimental trial physical activity ranged from 18 459 to 19 230 steps/d and was not significantly different between trials ($P>0{\cdot}05$).

Table 2. The effects of a 48 h period of fluid restriction (FR), energy restriction (ER) or combined fluid and energy restriction (F + ER) and a 30 min treadmill time trial (TT) on body mass loss

(Mean values with their standard errors of the mean; *n* 13)

	Body mass loss					
	48 h		Post-TT		6 h post-TT	
	Mean	SEM	Mean	SEM	Mean	SEM
Control (%)	0·6§	0·1	1·7§‖	0·1	−1·0§	0·1
FR (%)	3·2*§	0·1	4·1*§‖	0·1	−1·0§	0·2
ER (%)	3·4*§	0·1	4·3*§‖	0·1	0·9*†§	0·2
F + ER (%)	3·6*†§	0·2	4·4*§‖	0·1	0·5*†‡§	0·2

* Mean value within a column was significantly different from that for control ($P<0{\cdot}01$).
† Mean value within a column was significantly different from that for FR ($P<0{\cdot}01$).
‡ Mean value within a column was significantly different from that for ER ($P<0{\cdot}01$).
§ Mean value within a row was significantly different from that at 0 h ($P<0{\cdot}01$).
‖ Mean value within a row was significantly different from that at 48 h ($P<0{\cdot}01$).

Saliva responses

Due to insufficient saliva volume for s-IgA analysis at all time points in all participants the data presented is from eight participants where we have a complete data set.

s-IgA concentration, saliva flow rate and s-IgA secretion rate remained unaltered during the 48 h period in the control trial and on ER (Fig. 2). s-IgA concentration increased on FR by 48 h and was greater than all other trials at this time ($P<0{\cdot}01$; Fig. 2(A)). The increase in s-IgA concentration on FR is most probably attributable to the decrease in saliva flow rate ($P<0{\cdot}01$; Fig. 2(B)). In contrast, a similar decrease in saliva flow rate ($P<0{\cdot}01$) during 48 h on F + ER was not accompanied by a significant increase in s-IgA concentration; this resulted in a reduced s-IgA secretion rate at 24 and 48 h on F + ER ($P<0{\cdot}01$; Fig. 2(C)). Following the 30 min TT, saliva flow rate was significantly lower than 0 h on FR, ER and F + ER ($P<0{\cdot}01$) and lower than pre-TT (48 h) on ER ($P<0{\cdot}05$). Post-TT s-IgA concentration was significantly greater than 0 h on ER ($P<0{\cdot}05$), FR and F + ER ($P<0{\cdot}01$) and greater than pre-TT (48 h) on ER ($P<0{\cdot}05$) and F + ER ($P<0{\cdot}01$). s-IgA secretion rate was lower post-TT compared with 0 h on ER and F + ER ($P<0{\cdot}05$) but was not lower at any time post-TT compared with pre-TT (48 h) on any trial. This suggests a limited effect of a 30 min maximal bout of running on s-IgA secretion rate even under conditions of severe fluid and/or energy intake restriction.

The 2 h rehydration protocol re-established s-IgA concentration to within 0 h values on all trials; this is most probably attributable to the increase in saliva flow rate observed at 2 h post-TT. Following the 2 h rehydration period, s-IgA secretion rate had returned to within 0 h values on ER but remained lower than 0 h on F + ER ($P<0{\cdot}05$). s-IgA secretion rate returned to within 0 h values on F + ER by 6 h post-TT after rehydration and refeeding. The increased s-IgA secretion rate on FR at 6 h post-TT is probably attributable to the elevated saliva flow rate at this time ($P<0{\cdot}01$).

Plasma volume change, non-esterified fatty acids, glucose and cortisol

Plasma volume did not change significantly during the 48 h period (control: −1·4 (SEM 1·6); FR: −0·9 (SEM 1·4); ER: −5·2 (SEM 0·9) and F + ER: −5·1 (SEM 1·2) %). Compared with 48 h, plasma volume decreased as a result of the TT although this only reached significance on FR (control: −5·8 (SEM 1·1); FR: −6·3 (SEM 1·4); $P<0{\cdot}05$; ER: −4·9 (SEM 1·3) and F + ER: −4·8 (SEM 1·0) %). Plasma volume

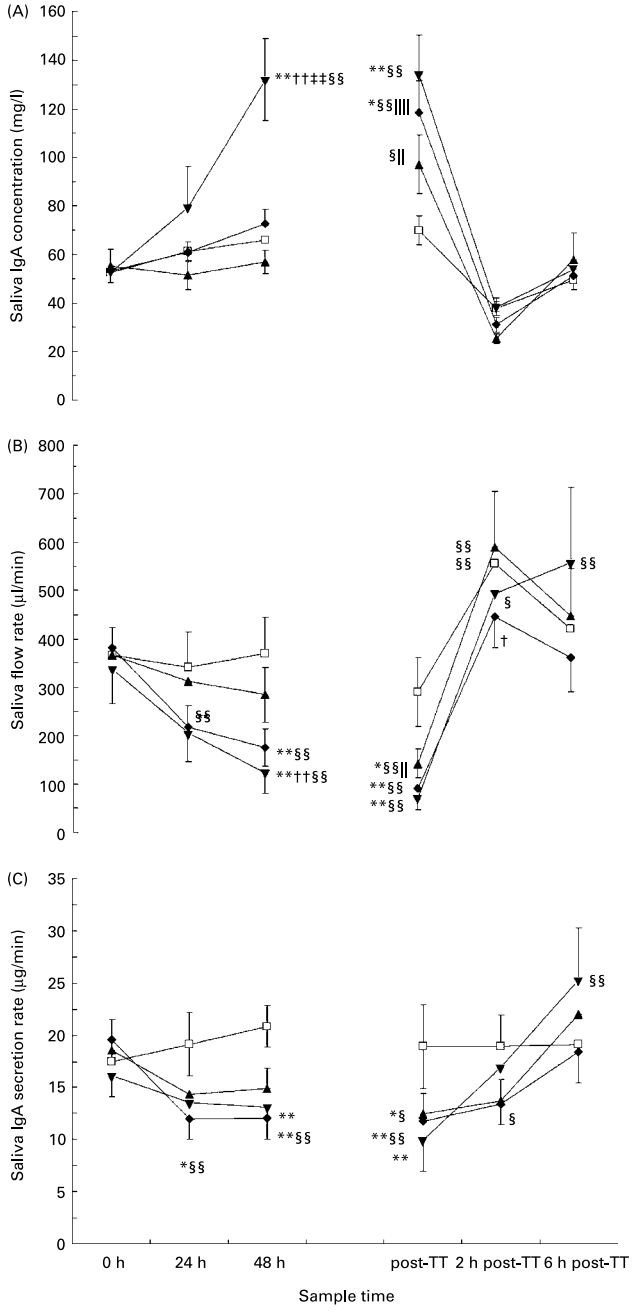

Fig. 2. The effects of a 48 h period of fluid restriction (▼), energy restriction (▲), fluid and energy restriction (◆) compared with control (□) on saliva IgA concentration (A), saliva flow rate (B) and saliva IgA secretion rate (C). Values are means for eight subjects, with standard errors represented by vertical bars. Mean value was significantly different from that for control: *$P<0{\cdot}05$, **$P<0{\cdot}01$. Mean value was significantly different from that for energy restriction: †$P<0{\cdot}05$, ††$P<0{\cdot}01$. Mean value was significantly different from that for fluid and energy restriction: ‡$P<0{\cdot}05$, ‡‡$P<0{\cdot}01$. Mean value was significantly different from that at 0 h: §$P<0{\cdot}05$, §§$P<0{\cdot}01$. Mean value was significantly different from that at 48 h: ‖$P<0{\cdot}05$, ‖‖$P<0{\cdot}01$. TT, 30 min maximal exercise bout.

was increased on all trials following the 2 h rehydration protocol (control: 6·4 (SEM 1·3); FR: 7·6 (SEM 1·2); ER: 9·0 (SEM 1·8) and F + ER: 10·5 (SEM 1·5) %; $P<0{\cdot}05$) and 6 h recovery period (control: 10·2 (SEM 1·3); FR: 13·4 (SEM 1·7); ER: 14·2 (SEM 1·4) and F + ER: 14·3 (SEM 1·6) %; $P<0{\cdot}01$). Plasma NEFA concentration increased on energy restriction trials by 24 h (ER and F + ER; $P<0{\cdot}05$; Table 3) and was significantly greater than control and FR from this point onwards ($P<0{\cdot}05$). Plasma glucose concentration decreased on ER and F + ER by 48 h compared with control and FR ($P<0{\cdot}05$). Following the TT, plasma glucose concentration was significantly greater on control and FR compared with 0 h, ER and F + ER ($P<0{\cdot}05$). Plasma cortisol concentration did not alter significantly throughout the 48 h period but was greater following the TT and the 2 h rehydration period on ER and F + ER compared with control and FR ($P<0{\cdot}05$; Table 3). Diurnal variation most probably accounts for the decrease in plasma cortisol concentration at 6 h post-TT (17.00 hours) compared with 0 h (08.30 hours) on all trials.

Discussion

The present results support the hypothesis that 48 h of combined fluid and energy restriction (about 10 000 kJ and about 2·9 litre fluid deficit per d) decrease s-IgA availability (s-IgA secretion rate). These results do not, however, support the hypothesis that 48 h of either fluid or energy restriction alone decreases the availability of s-IgA. s-IgA secretion rate was not lower at any time following the TT compared with pre-TT (48 h) which suggests a limited effect of a 30 min maximal exercise bout on s-IgA availability irrespective of prior nutritional status. s-IgA availability remained significantly depressed on F + ER following rehydration but normalised rapidly after refeeding.

s-IgA concentration was significantly increased following 48 h on FR compared with all other trials, which might be considered representative of an enhanced mucosal response. However, expressing s-IgA as a secretion rate indicates a 19 % reduction in s-IgA availability on FR, although this did not reach significance. This decrease in s-IgA secretion rate is most probably attributable to dehydration (3·2 % BML) and the associated decrease in saliva flow rate (Gregersen & Bullock, 1933; Ship & Fischer, 1997). Despite similar reductions in saliva flow rate on FR (64 %) and F + ER (54 %), s-IgA concentration was unaltered during 48 h on F + ER, resulting in a significant 39 % decrease in s-IgA secretion rate at 48 h. These results highlight the importance of expressing s-IgA as a secretion rate, thereby correcting for changes in hydration status (Walsh *et al.* 1999). Removal of concentrating and dilution effects of saliva flow rate ensures a true indication of s-IgA availability at the oral mucosa. The reduced s-IgA availability on F + ER at 48 h is probably attributable to a combination of decreased saliva flow rate and impaired local synthesis and/or secretion of s-IgA proteins; this may render the individual more susceptible to upper respiratory tract infection (Gleeson *et al.* 1999; Carins & Booth, 2002). In previous athletic and occupational studies the stressor(s) responsible for the reported decrease in s-IgA availability are unclear because any one stressor or a combination of stressors (for example, arduous exercise, psychological stress, sleep deprivation, fluid and energy restriction) may have impacted upon s-IgA availability (Gleeson *et al.* 1999; Carins & Booth, 2002). These results are the first to demonstrate, under controlled laboratory conditions, a reduction in s-IgA availability in active healthy individuals during combined fluid and energy restriction. The mechanism(s) by which fluid and energy restriction impairs local synthesis and/or secretion

Table 3. The effects of a 48 h period of fluid restriction (FR), energy restriction (ER) or combined fluid and energy restriction (F + ER) and a 30 min treadmill time trial (TT) on plasma non-esterified fatty acid, glucose and cortisol concentrations (Mean values with their standard errors; *n* 13)

	Control		FR		ER		F + ER	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
NEFA (mmol/l)								
0 h	0·4	0·0	0·4	0·0	0·3	0·0	0·4	0·0
24 h	0·6	0·0	0·5	0·0	1·6*†‡	0·2	1·7*†‡	0·1
48 h	0·6	0·1	0·6	0·1	1·8*†‡	0·2	2·0*†‡	0·2
Post-TT	1·1‡§	0·1	1·1‡§	0·1	2·2*†‡	0·1	2·4*†‡	0·2
Glucose (mmol/l)								
0 h	5·1	0·2	5·0	0·2	5·3	0·1	5·1	0·2
24 h	5·3	0·2	5·4	0·2	4·4	0·2	4·4	0·2
48 h	5·4	0·2	5·5	0·2	4·2*†	0·2	4·1*†	0·2
Post-TT	8·2‡§	0·6	7·9‡§	0·5	4·5*†	0·3	4·4*†	0·2
2 h post-TT	5·8	0·2	5·7	0·2	5·5	0·3	5·3	0·2
6 h post-TT	6·6‡§	0·2	6·4‡	0·2	7·2‡§	0·3	7·2‡§	0·4
Cortisol (mmol/l)								
0 h	494	24	455	16	479	17	502	16
24 h	528	24	502	23	554	25	544	17
48 h	516	22	500	24	559	32	564	21
Post-TT	459	29	448	27	571*†‡	41	556*†	38
2 h post-TT	263‡§	17	284‡§	17	377*†‡§	27	379*†‡§	34
6 h post-TT	246‡§	14	186‡§	14	247‡§	18	243‡§	26

* Mean value within a row was significantly different from that for control ($P<0·05$).
† Mean value within a row was significantly different from that for FR ($P<0·05$).
‡ Mean value within a column was significantly different from that at 0 h ($P<0·05$).
§ Mean value within a column was significantly different from that at 48 h ($P<0·05$).

of s-IgA remains unclear. The present results do not tend to support a role for decreased plasma glucose (Gleeson *et al.* 2004) or increased plasma cortisol (Saxon *et al.* 1978; Sabbadini & Berczi, 1995) in the impairment of s-IgA synthesis and/or secretion as both were not significantly altered (*v.* 0 h) during the 48 h period on the energy restriction trials. The restricted protein intake at least in part may be responsible for the impaired local synthesis and/or secretion of s-IgA, as protein deficiency is associated with impaired cell replication and the production of proteins (i.e. immunoglobulins and cytokines) (Chandra, 1997; Gleeson *et al.* 2004). Indeed, $CD4^+$ (helper) lymphocytes and IL-2 secretion have been reported to be lowered following energy restriction; both of these are thought to be essential for the induction and regulation of IgA synthesis (Savendahl & Underwood, 1997). Although we did not assess changes in IL-2 secretion we have reported a significant decrease in circulating $CD4^+$ lymphocytes during 48 h of ER and F + ER (Laing *et al.* 2005). The present study can not exclude other potent modulators of the immune system which include the sympathetic nervous system, catecholamines and other cytokines which are known to be altered with energy restriction and may potentially effect s-IgA production (Pequignot *et al.* 1980; Savendahl & Underwood, 1997; Gleeson *et al.* 2004).

The decrease in saliva flow rate during the 48 h period on FR and F + ER corresponds with previous reports of reductions in whole saliva flow rate after water deprivation lasting 48–72 h (Gregersen & Bullock, 1933) and reductions in parotid flow rate after a 24 h period without food and water (Ship & Fischer, 1997). In addition, the magnitude of the change in whole saliva flow rate compares favourably with the decrease (62 %) reported previously after similar fluid losses (about 3 % BML) during exercise in the heat (Walsh *et al.* 2004). Saliva flow rate did not decrease significantly during the 48 h in the control trial and on ER when participants received their estimated fluid requirements. In line with this, saliva flow rate remained unaltered when water was provided to offset fluid losses evoked by exercise and heat stress (Walsh *et al.* 2004). The mechanism(s) responsible for the reduction in saliva flow rate associated with dehydration is unclear. It has been hypothesised that an increase in plasma Na (osmolality) during dehydration may account for the production of smaller amounts of more concentrated saliva (Ship & Fischer, 1997). In order for water to move from plasma through acinar cells to form primary saliva a trans-acinar cell Na gradient must be generated (Ship & Fischer, 1997). Dehydration may cause the extracellular fluid to reflect an increased salt concentration and, accordingly, a greater salt concentration will have to be generated across the salivary acinar cell in order to drive fluid into the acinar lumen. Alternatively, other common responses to dehydration including an increase in circulating vasopressin and up regulated renin–angiotensin–aldosterone and sympathetic nervous system activity (Huch *et al.* 1998) may cause vasoconstriction of the blood vessels to the salivary glands, thereby limiting water availability to the saliva gland and ultimately reducing saliva flow rate. Besides decreasing the availability of s-IgA, decreases in saliva flow rate may further impair oral health by reducing the flushing of micro-organisms and their products into the gut and by limiting the availability of other proteins (for example, lysozyme and α-amylase) known to have important antimicrobial properties (Tenovuo, 1998; West *et al.* 2006). Future research should attempt to elucidate the mechanisms responsible for changes in saliva flow rate during dehydration and the decrease in s-IgA secretion rate following fluid and energy restriction.

In contrast to our proposed hypothesis, s-IgA secretion rate was not lower at any time following the TT, which suggests a limited effect of a 30 min maximal exercise bout and prior nutritional status on s-IgA availability. These results are in agreement with others reporting no change in s-IgA concentration after continuous exercise lasting <1 h in well-nourished individuals (McDowell *et al.* 1991). It remains unclear whether a similar dietary restriction affects the s-IgA response to more prolonged exercise (>30 min). The electrolyte solution provided during rehydration (0–2 h post-TT) returned s-IgA secretion rate to prior intervention values on ER but not on F + ER, as s-IgA secretion rate remained lower than 0 h at this time. Providing approximately two-thirds of individual daily energy requirements (8164 (SEM 155) kJ) normalised s-IgA availability on F + ER by 6 h post-TT. Although refeeding after F + ER has been shown to rapidly restore blood immune parameters (Walrand *et al.* 2001) the present investigation is the first to highlight the importance of refeeding to restore s-IgA availability after F + ER.

In conclusion, a 24–48 h period of combined fluid and energy restriction decreased s-IgA availability but normalisation occurred rapidly upon refeeding. In addition, nutrient restriction did not alter the s-IgA secretion rate response to a 30 min maximal exercise bout.

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